

Natural Product Extraction

Principles and Applications

RSC Green Chemistry

Series Editors:

James H Clark, *Department of Chemistry, University of York, UK*

George A Kraus, *Department of Chemistry, Iowa State University, Ames, Iowa, USA*

Andrzej Stankiewicz, *Delft University of Technology, The Netherlands*

Peter Siedl, *Federal University of Rio de Janeiro, Brazil*

Yuan Kou, *Peking University, People's Republic of China*

Titles in the Series:

- 1: The Future of Glycerol: New Uses of a Versatile Raw Material
- 2: Alternative Solvents for Green Chemistry
- 3: Eco-Friendly Synthesis of Fine Chemicals
- 4: Sustainable Solutions for Modern Economies
- 5: Chemical Reactions and Processes under Flow Conditions
- 6: Radical Reactions in Aqueous Media
- 7: Aqueous Microwave Chemistry
- 8: The Future of Glycerol: 2nd Edition
- 9: Transportation Biofuels: Novel Pathways for the Production of Ethanol, Biogas and Biodiesel
- 10: Alternatives to Conventional Food Processing
- 11: Green Trends in Insect Control
- 12: A Handbook of Applied Biopolymer Technology: Synthesis, Degradation and Applications
- 13: Challenges in Green Analytical Chemistry
- 14: Advanced Oil Crop Biorefineries
- 15: Enantioselective Homogeneous Supported Catalysis
- 16: Natural Polymers Volume 1: Composites
- 17: Natural Polymers Volume 2: Nanocomposites
- 18: Integrated Forest Biorefineries
- 19: Sustainable Preparation of Metal Nanoparticles: Methods and Applications
- 20: Alternative Solvents for Green Chemistry: 2nd Edition
- 21: Natural Product Extraction: Principles and Applications

How to obtain future titles on publication:

A standing order plan is available for this series. A standing order will bring delivery of each new volume immediately on publication.

For further information please contact:

Book Sales Department, Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge, CB4 0WF, UK

Telephone: +44 (0)1223 420066, Fax: +44 (0)1223 420247

Email: booksales@rsc.org

Visit our website at www.rsc.org/books

Natural Product Extraction Principles and Applications

Edited by

Mauricio A. Rostagno and Juliana M. Prado

University of Campinas, Brazil

Email: rostagno@fea.unicamp.br; tuska@fea.unicamp.br

RSC Green Chemistry No. 21

ISBN: 978-1-84973-606-0

ISSN: 1757-7039

A catalogue record for this book is available from the British Library

© The Royal Society of Chemistry 2013

All rights reserved

Apart from fair dealing for the purposes of research for non-commercial purposes or for private study, criticism or review, as permitted under the Copyright, Designs and Patents Act 1988 and the Copyright and Related Rights Regulations 2003, this publication may not be reproduced, stored or transmitted, in any form or by any means, without the prior permission in writing of The Royal Society of Chemistry or the copyright owner, or in the case of reproduction in accordance with the terms of licences issued by the Copyright Licensing Agency in the UK, or in accordance with the terms of the licences issued by the appropriate Reproduction Rights Organization outside the UK. Enquiries concerning reproduction outside the terms stated here should be sent to The Royal Society of Chemistry at the address printed on this page.

The RSC is not responsible for individual opinions expressed in this work.

Published by The Royal Society of Chemistry,
Thomas Graham House, Science Park, Milton Road,
Cambridge CB4 0WF, UK

Registered Charity Number 207890

For further information see our web site at www.rsc.org

Printed in the United Kingdom by Henry Ling Limited, Dorchester, DT1 1HD, UK

Preface

Natural products are a rich source of bioactive compounds with a wide range of potential applications. They have been used for centuries as popular medicines and in recent decades they have been a focus of the scientific community due to increasing evidence that associates them with health benefits and the prevention of several diseases. Their importance is also growing due to the concern of the negative effects of synthetic additives and their processing on human health and the environment. This makes natural products especially important to food, pharmaceutical and cosmetics industries. Several types of natural products are routinely used as functional foods, as components of products, as additives (colorants, antioxidants, etc.) or as final products (nutraceuticals and supplements). In several applications consumers are demanding the substitution of synthetic compounds by natural ones, since there is a popular belief that everything that is 'natural' is good. Thus, the importance of natural products has seen a progressive and steady increase in the last decades. Without doubt, consumer awareness, increasing quality demands and stricter regulations are driving the consolidation of natural products as part of productive processes of several industrial branches.

In general, the functional and/or technological properties of a natural product are associated with its components, their concentration and possible interactions. Many times, these bioactive components must be separated and/or concentrated from the raw material in order to be useable either as a sample or as a food or ingredient. Although analytical, semi-preparative and industrial separations of natural products have very different objectives and use different operational conditions and processes, all of these processes share the need of efficient extraction methods.

In the case of analytical applications, it is necessary to isolate target compounds to be later analyzed and they must be in sufficient concentration to allow their detection, identification and/or quantitation. When dealing with

quantitative analysis, it is critical to achieve an exhaustive and complete extraction of the target compounds from the sample. Usually in semi-preparative separations, the process is designed to produce small amounts of a highly purified extract containing only a few components. Purity is commonly considered a priority factor to the detriment of extraction efficiency. This type of separation may employ several additional steps when compared to analytical applications. In contrast, in industrial applications, the process is designed to manufacture a specific product with determined characteristics at a reasonable cost. The product may range from a very pure mixture of a few compounds (>98%) to a product that is commercialized as an extract with undefined concentration of bioactives present. As an example, a 1:10 extract indicates that it yielded 10% in relation to the raw material used, without any composition specifications. In most cases the final quality and the manufacturing cost of the product will assume decisive roles on determining the operational conditions.

Independently to the strategy adopted, the selective separation of specific components from such complex matrices is a difficult task that involves multiple steps and procedures. Obviously, the complexity of the process will depend on the raw material's natural characteristics, as the solute location, the characteristics of the target components, the desired concentration of target compounds in the final product, etc. Techniques for achieving these goals may range from the simple soaking of the ground-up material in a given solvent followed by filtration and evaporation, to a complex series of extraction and post-extraction processes using a combination of techniques on-line. Nevertheless, the technology currently in use by the natural product industry and most analytical laboratories is based on highly inefficient processes and outdated techniques. Without doubt, the technology used by the industry needs to be updated in order to increase the competitiveness of natural products. It is evident there is a need for more efficient processes that can increase yields and the overall quality of natural products at a feasible cost. Moreover, new products can be developed using the new extraction processes that allow controlling more variables than in conventional techniques, thus tuning the selectivity of the process.

Analytical and semi-preparative separations play a decisive role on the development of these extraction techniques. This development is fueled by a constant increase in the demand of higher sample throughput, higher selectivity and lower solvent consumption derived from the increasing performance of instrumental methods of analysis. For example, considering that a high-performance liquid chromatography separation can be achieved in a few minutes, it is likely that it will take longer to extract the sample than to analyze it! Furthermore, most semi-preparative and industrial processes are basically scaled-up from the processes developed at analytical scale. In this sense, several new technologies developed in the last decades have an enormous potential to be explored by the natural product industry and by analytical and semi-preparative laboratories in this field.

With these issues in mind, this book is intended to give a holistic, in-depth view of the state-of-the-art techniques for the extraction and processing of

natural products and the factors influencing the process performance. Each chapter was written by leading scientists in the specific field. Besides conventional extraction techniques, the use of ultrasound, microwaves, pressurized liquids and supercritical fluids are discussed in detail in specific chapters devoted to them. Each chapter gives a balanced outline of each technique's potential for the extraction of natural products. The principles and fundamentals of each extraction process are addressed and the factors influencing them are further discussed, including specific aspects of each technique. Each chapter will provide the reader with comprehensive information about the fundamentals of each technique, the parameters that affect the process and how to explore this knowledge to maximize the efficiency of the extraction method in order to obtain the products intended. The characteristics, advantages and disadvantages, and applications of these techniques are contextualized in maximizing their potential as an attractive alternative for the production of natural extracts. Examples and case studies are used to illustrate the application of each extraction method and to give a balanced outline of recent applications and potential uses of each technique for obtaining extracts from natural sources.

Chapter 1 presents the uses and potential applications of natural products. The following chapters (chapters 2–6) present both conventional and modern extraction techniques used to obtain them. Furthermore, in Chapters 7 and 8, the most recent trends on the extraction of natural products are discussed, including the combination and coupling of different techniques to maximize the production process and their applications for natural products purification, isolation and stabilization. Other relevant subjects, such as the elimination of the extraction solvent, the modification of the physicochemical characteristics and the improvement of functional characteristics of extracts using advanced techniques will be covered, including techniques for particle formation and encapsulation of the extracts. The isolation and purification techniques which may be used for further processing of the extracts are discussed in Chapter 9, giving special attention to chromatographic and non-chromatographic techniques. Further process design and optimization can be used to employ resources more effectively and to minimize costs. Chapter 10 is dedicated to the coupling of pressurized fluids to other post-extraction processes, assessing the interactions between different operations and units that can be used to optimize the overall process and which are used to illustrate that using a holistic approach leads to higher overall process efficiency.

Additionally, specific implications of scaling-up the process to industrial level are the focus of Chapter 11. Finally, in Chapter 12, we intend to provide the reader with a critical view about the economic aspects of the whole process and of scaling-up separations and why they are important as individual steps and procedures. These aspects are discussed in detail in terms of the factors involved in the cost of manufacturing natural products extracts and how to explore them to maximize extract production while minimizing costs. All this information will help when considering new less polluting extraction technologies when the decision comes to choosing the appropriate method for determined raw material, including scale-up to industrial level.

In general, this book is directed to researchers working both in academic and industrial sectors of chemistry, chemical and food engineering, food science and nutrition, among others. The information presented may be useful in a variety of fields, from the investigation about phytochemical composition to assisting in the assessment of biological activities of compounds present in natural products. Furthermore, the same principles also apply to large-scale separations and therefore this knowledge can be explored for industrial applications, especially by the food and pharmaceutical industries.

Mauricio Rostagno and Juliana Prado

Contents

Chapter 1	Uses and Applications of Extracts from Natural Sources	1
	<i>R. N. Cavalcanti, T. Forster-Carneiro, M. T. M. S. Gomes, M. A. Rostagno, J. M. Prado and M. A. A. Meireles</i>	
1.1	Introduction	1
1.2	Uses and Applications	3
1.2.1	Coloring Agents	4
1.2.2	Flavors and Fragrances: Essential Oils	19
1.2.3	Edible Fats and Oils	31
1.2.4	Functional Foods and Nutraceuticals	36
1.3	Conclusions	46
	Acknowledgements	46
	References	46
Chapter 2	Extraction of Natural Products: Principles and Fundamental Aspects	58
	<i>M. Palma, G. F. Barbero, Z. Piñeiro, A. Liazid, C. G. Barroso, M. A. Rostagno, J. M. Prado and M. A. A. Meireles</i>	
2.1	Introduction	58
2.2	Principles and Fundamentals of Extraction	59
2.3	Exhaustive Versus Non-exhaustive Extraction Methods	66
2.4	Conventional Extraction Techniques	67
2.4.1	Soaking	67
2.4.2	Soxhlet	69
2.4.3	Distillation with Water and/or Steam	73

RSC Green Chemistry No. 21

Natural Product Extraction: Principles and Applications

Edited by Mauricio A. Rostagno and Juliana M. Prado

© The Royal Society of Chemistry 2013

Published by the Royal Society of Chemistry, www.rsc.org

2.5	Main Extraction Variables	78
2.5.1	Preparation of the Solid	78
2.5.2	Solvent	79
2.5.3	Temperature	82
2.5.4	Time	82
2.5.5	Solvent to Feed Ratio	82
2.6	Case Study	83
2.7	Conclusions	85
	Acknowledgements	86
	References	86

Chapter 3 Ultrasound-assisted Extraction **89**

Daniella Pingret, Anne-Sylvie Fabiano-Tixier and Farid Chemat

3.1	Introduction	89
3.2	Ultrasound-assisted Extraction	90
3.2.1	Ultrasound Principles	90
3.2.2	Instrumentation	93
3.2.3	Important Parameters	96
3.2.4	Ultrasound-assisted Extraction: Applications in Food	102
3.3	Examples of Solvent-free Ultrasound-assisted Extraction of Carotenoids	105
3.3.1	Carotenoids Uses and Conventional Extraction	105
3.3.2	Solvent-free Ultrasound-assisted Extraction of β -Carotene	106
3.3.3	Analysis and Evaluation of UAE Process	107
3.4	Costs and Investment in Industrial Ultrasound	108
3.5	Conclusion	108
	References	109

Chapter 4 Microwave-assisted Extraction **113**

Emilie Destandau, Thomas Michel and Claire Elfakir

4.1	Introduction	113
4.2	Principles of Microwave-assisted Extraction	114
4.2.1	Microwave Heating Principle	114
4.2.2	Microwave Heating Applied to Plant Matrices	117
4.3	Microwave Instrumentation	118
4.3.1	Oven Design	119
4.3.2	Reactor Design	120

4.4	Parameter Influence on Microwave-assisted Extraction	121
4.4.1	Solvent	122
4.4.2	Temperature and Pressure	125
4.4.3	Extraction Time	126
4.4.4	Power	126
4.4.5	Nature of the Matrix	127
4.5	Trends in Microwave-assisted Extraction and Applications	128
4.5.1	Extraction of Sensitive Compounds	128
4.5.2	Extraction Methods Improved by Microwave Heating	130
4.5.3	Green Extraction without Solvent	135
4.6	Case Study	144
4.6.1	Optimization of the Pressurized Solvent-free Microwave Extraction (PSFME) Procedure	144
4.6.2	Influence of the Number of Cycles	145
4.6.3	Proposed Mechanism of PSFME	147
4.6.4	Comparison with other Extraction Methods	148
4.6.5	Advantages of PSFME	150
4.7	Conclusion	150
	List of Abbreviations	152
	References	152

Chapter 5 Accelerated Liquid Extraction 157

Feliciano Priego-Capote and María del Pilar Delgado de la Torre

5.1	Introduction	157
5.2	Static Accelerated Solvent Extraction (Static ASE)	158
5.2.1	Steps Involved in the Static ASE Process	158
5.2.2	Static ASE Commercial and Laboratory-designed Devices	161
5.3	Dynamic Accelerated Solvent Extraction (Dynamic ASE)	163
5.3.1	Steps Involved in the Dynamic ASE Process	163
5.3.2	Dynamic ASE Laboratory-designed Devices	164
5.4	Coupling ASE to Other Steps of the Analytical Process	165
5.5	Parameters Affecting Performance in ASE	167
5.5.1	Temperature	167
5.5.2	Pressure	169
5.5.3	Type of Solvent	169
5.5.4	Solvent to Feed Ratio	170
5.5.5	Sample Composition	171

5.5.6	Particle Size	171
5.5.7	Extraction Time	171
5.6	Comparison of ASE with other Extraction Techniques	172
5.7	Applications of ASE for the Isolation of Natural Products	176
5.7.1	Lipids	182
5.7.2	Volatile Compounds	183
5.7.3	Polar Compounds	184
5.8	Case Study	187
5.8.1	Optimisation of the Main Variables Involved in SHLE	187
5.8.2	Influence of Extraction pH	189
5.8.3	Comparison of SHLE with MAE and UAE for Extraction of Vine Shoots	189
5.9	Conclusions: Benefits and Limitations of ASE for Isolation of Natural Products	190
	Acknowledgements	190
	References	190

Chapter 6 Supercritical Fluid Extraction 196

Jose A. Mendiola, Miguel Herrero, María Castro-Puyana and Elena Ibáñez

6.1	Introduction	196
6.2	Fundamentals of Supercritical Fluid Extraction	197
6.2.1	Physical Properties of Supercritical Fluids	197
6.2.2	Supercritical Solvents	199
6.3	Instrumentation	201
6.4	Parameters Affecting the Extraction Process	203
6.4.1	Raw Material (Particle Size, Porosity, Location of the Solute, Moisture Content)	204
6.4.2	Solubility (Pressure and Temperature)	205
6.4.3	Use of Modifiers	208
6.4.4	Solvent Flow Rate (Solvent-to-Feed Ratio)	209
6.5	Applications	209
6.5.1	Plants	209
6.5.2	Marine Products	213
6.5.3	Agricultural and Food By-products	216
6.6	Case Study	220
6.6.1	Effect of Extraction Time	220
6.6.2	Effect of Pressure, Temperature and Modifier	221
6.6.3	Effect of Solvent	222
6.7	Future Trends and Conclusions	223
	References	225

Chapter 7	Recent Trends and Perspectives for the Extraction of Natural Products	231
	<i>M. E. M. Braga, I. J. Seabra, A. M. A. Dias and H. C. de Sousa</i>	
7.1	Introduction	231
7.2	Target Extracts/Compounds	236
7.3	Raw Materials	244
7.4	Extraction Methods	250
7.4.1	Microwave-assisted Extraction	253
7.4.2	Ultrasound-assisted Extraction	255
7.4.3	High-pressure Liquid Extraction	257
7.4.4	Supercritical Fluid Extraction	258
7.5	Extraction Solvents and Solvent Mixtures	261
7.5.1	Extraction Solvent Modification with Additives (Enzymes, H^+/OH^- , Surfactants)	263
7.5.2	Solvent Mixtures and Non-conventional Highly Hydrophobic Organic Solvents	267
7.5.3	Ionic Liquids	268
7.5.4	Aqueous Biphasic Systems (ABS)	269
7.5.5	Tunable Solvents	271
7.6	Conclusions and Future Perspectives	274
	References	275
Chapter 8	Post-extraction Processes: Improvement of Functional Characteristics of Extracts	285
	<i>Ángel Martín, Soraya Rodríguez-Rojo, Alexander Navarrete, Esther de Paz, Joao Queiroz and María José Cocero</i>	
8.1	Introduction	285
8.2	Purification of Extracts and Elimination of Solvents	286
8.2.1	Evaporation of Solvents	287
8.2.2	Freeze-drying	287
8.2.3	Reverse Osmosis	287
8.3	Particle Size Reduction	289
8.3.1	Top-down Methods	290
8.3.2	Bottom-up Methods	291
8.4	Formulation	298
8.4.1	Solvent Evaporation Method	300
8.4.2	Spray-drying Technique	301
8.4.3	High-pressure Emulsion Techniques	301
8.4.4	Supercritical Fluid Processes	303
8.4.5	Overview	305

8.5	Case Study: Formulation of β -carotene as a Natural Colorant	305
8.5.1	Formulation of β -carotene by Precipitation from Pressurized Organic Solvent-on-water Emulsions	307
8.5.2	Formulation of β -carotene with Soybean Lecithin by PGSS-drying	309
8.5.3	Co-precipitation of β -carotene with Polyethylene Glycol by Supercritical Anti-solvent Process (SAS)	309
8.5.4	Formulation of β -carotene by Supercritical Extraction from an Emulsion (SEE)	310
8.6	Conclusions	311
	References	311

Chapter 9 Isolation and Purification of Natural Products **314**

Wang Xiao, Fang Lei, Zhao Hengqiang and Lin Xiaojing

9.1	Introduction	314
9.2	Pre-isolation or Enrichment	315
9.2.1	Solvent Partitioning	316
9.2.2	Adsorption Enrichment	318
9.2.3	Membrane Separation	318
9.2.4	Solid Phase Extraction (SPE)	321
9.3	Purification	323
9.3.1	Chromatographic Techniques	323
9.3.2	Crystallization	339
9.4	Case Studies	340
9.4.1	Isolation of Saponins from <i>Clematis chinensis</i>	340
9.4.2	Isolation of Tritoniopsins A–D from <i>Cladiella krempfi</i>	341
9.4.3	Isolation of <i>cis</i> -Clerodane-type Furanoditerpenoids from <i>Tinospora crispa</i>	341
9.4.4	Isolation of Flavonoids from <i>Paeonia suffruticosa</i>	344
9.4.5	Isolation of Alkaloids from <i>Stephania kwangsiensis</i>	347
9.4.6	Isolation of Psoralen and Isopsoralen from <i>Psoralea corylitolia</i>	348
9.4.7	Isolation of Six Isoflavones from <i>Semen sojae praeparatum</i> by Prep-HPLC	348

<i>Contents</i>	xv
9.4.8 Isolation of Anthocyanins from Eggplant	352
9.4.9 Isolation and Purification of Flavonoid and Isoflavonoid from <i>Sophora japonica</i>	354
9.5 Conclusions	356
References	357
Chapter 10 Scale-up of Extraction Processes	363
<i>Julian Martínez and Luiz Paulo Sales Silva</i>	
10.1 Introduction	363
10.2 Fundamental Aspects of Scale-up Operations	364
10.2.1 What is Scale-up?	364
10.2.2 Scale-up Criteria	366
10.3 Factors Involved	372
10.3.1 Solubility	373
10.3.2 Solvent Flow Rate	374
10.3.3 Substrate Properties	374
10.3.4 Extraction Bed Geometry	375
10.4 State of the Art	376
10.4.1 Models for Extraction Processes	376
10.4.2 Some Examples of Scale-up Criteria in Extraction Processes	380
10.4.3 Scale-up Correlations	387
10.4.4 Configurations of Industrial Units	388
10.4.5 Some Published Works on Scale-up of Extraction Processes	390
10.5 Case Study: Supercritical CO ₂ Extraction from Red Pepper	391
10.5.1 Experimental Procedures	391
10.5.2 Results and Discussion	393
10.6 Conclusion	396
References	397
Chapter 11 Integration of Pressurized Fluid-based Technologies for Natural Product Processing	399
<i>Diego T. Santos, Maria T. M. S. Gomes, Renata Vardanega, Mauricio A. Rostagno and M. Angela A. Meireles</i>	
11.1 Introduction	399
11.2 Sequential Extraction using Different Process Conditions or Techniques	400
11.3 On-line Fractionation/Purification	404
11.3.1 On-line Separators: Fractionation by Changes in Temperature and Pressure	404

11.3.2	On-line Extraction and Adsorptive Purification Processes	406
11.3.3	On-line Coupling of Extraction and Membrane Processes for Purification	418
11.4	Integration of Pressurized Fluids to Different Technologies for Extract Stabilization	420
11.5	Case Study – Integrated Extraction and Encapsulation of Bixin from Annato Seeds	425
11.5.1	Materials and Methods	425
11.5.2	Results and Discussion	430
11.6	Conclusions	437
	Acknowledgements	438
	References	438
Chapter 12	Economic Evaluation of Natural Product Extraction Processes	442
	<i>Camila G. Pereira, Juliana M. Prado and M. Angela A. Meireles</i>	
12.1	Introduction	442
12.2	Cost Estimation of Industrial Processes	443
12.2.1	Costs Associated with the Raw Material	444
12.2.2	Costs Associated with the Operational Conditions	445
12.2.3	Costs Associated with the Industrial Requirements	445
12.3	Cost Estimation Procedures	446
12.3.1	Cost Estimate as a Function of Equipment Capacity	446
12.3.2	Lang Factor	448
12.3.3	Manufacturing Cost Estimation	448
12.4	Manufacturing Cost of Vegetable Extracts	450
12.4.1	Supercritical Extraction Process	450
12.4.2	Other Extraction Processes	464
12.5	Case Study	465
12.5.1	Introduction	465
12.5.2	Materials and Methods	465
12.5.3	Results and Discussion	466
12.6	Conclusion	469
	Acknowledgement	469
	References	469
	Subject Index	472

CHAPTER 1

Uses and Applications of Extracts from Natural Sources

R. N. CAVALCANTI, T. FORSTER-CARNEIRO,
M. T. M. S. GOMES, M. A. ROSTAGNO, J. M. PRADO AND
M. A. A. MEIRELES*

LASEFI/DEA/FEA, (School of Food Engineering)/UNICAMP (University of Campinas), R. Monteiro Lobato, 80, Campinas, 13083-862, SP, Brazil

*Email: meireles@fea.unicamp.br

1.1 Introduction

Current scientific evidence about physiological, nutritional, and medicinal benefits to human health provided by the use of natural products, as well as the potential harmful effects from the use of synthetic products and consequent legislative actions restricting their use, has motivated a significant increase in the consumption of natural products.¹ In this context, extracts from natural sources play an important role as natural additives or industrial inputs to food, cosmetic, textile, perfumery, and pharmaceutical industries (Figure 1.1), influencing many characteristics of the final product. Indeed, the majority of natural extracts have more than one or two functions. They have been used as natural colorants, nutraceuticals, functional foods, preserving agents, flavors and fragrances, edible oils and fats, drugs, vitamin supplements, chemical standards, and perfumes, among others. The major natural extracts are obtained from plant sources such as seeds, leaves, flowers, berries, barks, and roots, although some of them may be obtained from animal sources such as

RSC Green Chemistry No. 21

Natural Product Extraction: Principles and Applications

Edited by Mauricio A. Rostagno and Juliana M. Prado

© The Royal Society of Chemistry 2013

Published by the Royal Society of Chemistry, www.rsc.org

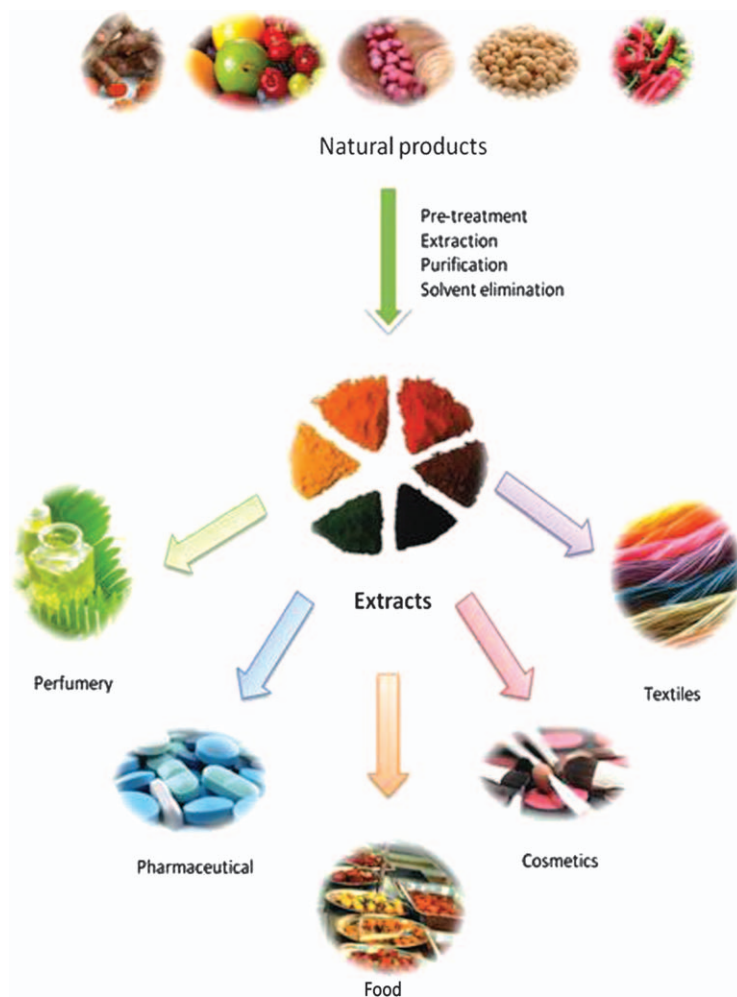


Figure 1.1 Uses and applications of extracts from natural sources.

carmine dye from female insect cochineal (*Dactylopius coccus*), honey from bees, squalene from shark liver, etc.

The applications of natural extracts are generally associated with the functionality derived from their active components. Usually, functional foods are obtained by enrichment with functional compounds, which are ingredients able to promote or provide a beneficial effect on human health. These compounds may also be concentrated, serving as nutritional supplements, known as nutraceuticals, which are commercialized as tablets and capsules.¹ They may also be used for technological roles, as coloring agents, conservation agents, etc., and for the production of chemicals.^{2,3}

Many of the bioactive properties assigned to functional foods and nutraceuticals are provided by compounds derived from the secondary metabolism

of plants, also called phytochemicals. 'Phytochemicals' literally means chemicals produced by plants; they play an important role in plant metabolism.⁴ Phytochemicals are not established as essential nutrients, but may have a great biological significance.⁵ In most cases they are ingested by humans as part of the diet, including in fruit, vegetables, beans, and grains, in beverages such as juices, green or black tea, coffee, etc. There are several phytochemical classes, including polyphenols (flavonoids, phenolic acids, tannins, stilbenes, coumarins, and lignans), carotenoids, phytosterols, alkaloids, terpenes, and sulfur-containing compounds (sulfides and glucosinolates).⁶ Although there is already sufficient scientific evidence pointing to the association between effects beneficial to human health and phytochemical intake, the mechanisms of action are not yet fully elucidated. Furthermore, it is believed that many of these beneficial effects are the result of additive and/or synergistic phenomena of these compounds, being attributed to the complex mixture of phytochemicals rather than to a single compound.^{7–10} Products with phytochemical compounds have many other applications in food and other industries, including pharmaceutical, cosmetics, perfumes, and textile industries. For example, many products of personal care include a wide variety of natural products in their formulation including soaps, shampoos, sunscreen, hair dye, make-up, toothpaste, deodorants, etc.^{11–14}

1.2 Uses and Applications

There are many uses of extracts from natural sources which can be grouped according to their technological role: coloring agents, functional food, nutraceuticals, preserving agents, flavors, fragrances, and edible oils.

Coloring agents or color additives are any pigment, dye, or substance that produces color when it is added to a product. The coloring agents may be found in liquid, solid, semi-solid, or gel forms. Due to the large availability of food coloring agents there are several other non-food applications that explore their properties, including cosmetics, pharmaceuticals, and medical devices. Natural colorants are extracted by various processes and classified according to their color, chemical composition/structure, biological function in plant/body (chlorophyll, hemoglobin, etc.), and physical properties (solubility). The main dyes from plant sources are red (Brazil wood, sugar, etc.), orange (saffron flower, *Crocus sativus*), yellow (chamomile, *Anthemis tinctoria*), green (ragweed, *Ambrosia artemisiifolia*), and blue (indigo, *Indigofera tinctoria*). The main food dyes from animal sources are sepia (cuttlefish bag), red (kermes lice), and purple (murex shellfish).¹⁵

Besides the technological function of several well-known natural coloring agents, the phytochemicals may have other biological functions and play a role on the prevention of diseases.^{16–19} Functional foods, nutraceuticals, food supplements, and antioxidants belong to an economically important sector of the global food market.^{20–24} Examples of potential applications include reducing the risk of cardiovascular disease, cancer, diabetes, inflammation, and

osteoporosis. Among the various functional effects, it is important to highlight the effects on gastrointestinal functions and hormonal modulation.^{25–27}

Furthermore, preserving agent activity, antibacterial activity, and antifungal activity also represent an economically important sector of the global natural products market. Certain types of food preservatives are needed to ensure the quality of the final product. Most chemical preservatives widely used are weak organic acids (e.g. ascorbic acid and benzoic acid) used in synergistic combinations.²⁸ In this case, the antimicrobial and antifungal properties of essential oils are considered to be the most important.²⁹

1.2.1 Coloring Agents

Highly conjugated systems which absorb electromagnetic radiation between wavelengths of 400 nm to 800 nm appear to be colored. Color can provide a pleasant aspect to the substrate as well as express emotions and ideas.³⁰ Color is often the first notable sensorial characteristic that influences the expectations of consumers and also influences quality-related decisions during visual inspections.^{31,32} Color plays an important role in quality perception indicating our expectations, perceptions, susceptibilities to, and preferences for products, as it is used to indicate good quality, to assist marketing, and to satisfy consumers.³³ The color of food, pharmaceutical, and cosmetic products can be the result of natural pigments present in the matrix used; coloration formed upon heating, processing, or storage; or the addition of natural or synthetic colorants.³² Colorants or color additives are the terms for all soluble or solubilized coloring agents (dyes or pigments), as well as insoluble pigments, employed to impart color to a material.³¹ The mechanism of color production is due to a molecule-specific structure (chromophore) of chemical compounds that absorbs light in the wavelength range of the visible region known as pigments. Those chromophores capture energy and the excitation of an electron from an external orbital to a higher orbital is produced; the non-absorbed energy is reflected and/or refracted to be captured by the eye, and neural impulses are generated, which are transmitted to the brain where they can be interpreted as a color.³⁴

Coloring agents can be defined by their origin as natural, synthetic, or inorganic colorants. Natural pigments are produced by living organisms. Synthetic colorants or dyes are synthesized by chemical reactions. Inorganic pigments can be found in nature or can be reproduced by synthesis.³⁴ Synthetic organic dyes have been recognized for many years as the most reliable and economical coloring agents because they are superior to natural pigments in tinctorial power, consistence of strength, range, and brilliance of shade, hue, stability, ease of application, and cost effectiveness, being the most applied source of color additives used in the food, pharmaceutical, and cosmetic industries.^{32,35} However, during the last few decades, the use of synthetic dyes is gradually receding due to an increased environmental awareness and to potential harmful effects of either toxic degraded products or their non-biodegradable nature.³⁰ Furthermore, the safety of synthetic dyes has been a

matter of concern since high levels of toxicity, allergic reactions, and carcinogenic potential have been identified following their consumption as coloring agents.³⁵ In this context, there is an increased interest in further use of colorants from natural sources instead of synthetic dyes, as a consequence of perceived consumer preferences as well as legislative actions.³²

Natural pigments (see Figure 1.2) are defined as dyes or colorants obtained from natural sources, such as plants, animals, and microorganisms. Nevertheless, the majority of commercial natural colorants currently used are extracted from plant sources such as roots, fruits, barks, leaves, wood, fungi, and lichens. Flavonoids, carotenoids, and chlorophyll are the major contributors to the natural colors of most plants, with betalines and curcumin playing a minor yet significant role.³⁶ However, there are some natural pigments derived from invertebrates, such as the cochineal pigments extracted from female coccid insects; the most well-known is the carminic acid obtained from the female *Dactylopius coccus* Costa.³⁶ All natural pigments are unstable and participate in different reactions, so the produced color is strongly dependent on storage and processing conditions. Natural colorants are much more unstable than synthetic dyes with respect to physical (temperature, light), chemical (oxidizing or reducing agents, acids, alkalis), and biological (enzymes, microorganisms) factors.^{32,33}



Figure 1.2 Main natural pigments and their colors.

This section discusses the major natural colorants commercially used and their application in food, pharmaceutical, and cosmetic industries. The main natural pigments are categorized according to their chemical structure as: isoprenoid derivatives (carotenoids); tetrapyrrole derivatives (chlorophylls and hemes); and benzopyran derivatives (anthocyanins, betalains, and curcuminoids).

1.2.1.1 Carotenoids

Carotenoids are the largest, most important, and most widespread group of pigments found in nature.³⁷ They are responsible for many of the brilliant red, orange, and yellow colors of fruits, vegetables, fungi, and flowers, and also of birds, insects, crustaceans, and trout.³⁴ They are usually fat soluble and associated with lipid fractions.³⁸ However, they can be synthesized only by plants and microorganisms. The chemical structure of carotenoids consist in a symmetrical polyisoprenoid structure formed by head-to-tail condensation of two C₂₀ units, which is modified by cyclization, addition, elimination, rearrangement, and substitution, as well as oxidation.^{34,39} Due to the presence of the conjugated double bonds, carotenoids can exist in *cis* and *trans* forms, but *cis* isomers are less stable than the *trans* form due to stoichiometric conformation; therefore the majority of natural carotenoids are in the all-*trans* configuration.³⁹ Based on their structure, carotenoids (Figure 1.3) are divided in two classes: (i) carotenes, which are pure polyene hydrocarbons; they contain only carbon and hydrogen atoms, including acyclic lycopene and bicyclic β - and α -carotene; (ii) xanthophylls, containing oxygen in the form of hydroxy (lutein), epoxy (violaxanthin), and oxo (canthaxanthin) groups.⁴⁰

Carotenoids perform important functions in plants as attractants for pollinators, as accessory light-harvesting pigments at wavelengths where chlorophyll does not absorb, and as photoprotective agents preventing photo-oxidative stress.⁴¹ The most common natural carotenoid extracts used as color additives for foodstuffs are obtained from annatto, paprika, and saffron. Many other sources, including alfalfa, carrot, tomato, citrus peel, and palm oil, are also used.³² Evidence of trends in looking for natural sources of carotenoids can be noticed from the patents that have been recently deposited worldwide on the subject.⁴²

Annatto. Annatto (E160b) is an yellow-red natural carotenoid coloring agent obtained from the seed coat of the tropical shrub *Bixa orellana* L.⁴³ The annatto tree is native to Central and South America, but it is also grown in Africa and Asia, being especially popular in Brazil, Peru, Bolivia, Ecuador, Jamaica, the Dominican Republic, East and West Africa, India, and the Philippines.⁴⁴ The major coloring component in annatto extract is bixin (>80%). This pigment is primarily present as the *cis*-bixin isomer, but other pigments derived from bixin as *trans*-bixin, *cis*-norbixin, and *trans*-norbixin are also present, although they may have different colors.⁴⁴ Bixin is a dicarboxyl monomethyl ester carotenoid with a C₂₅ skeleton called

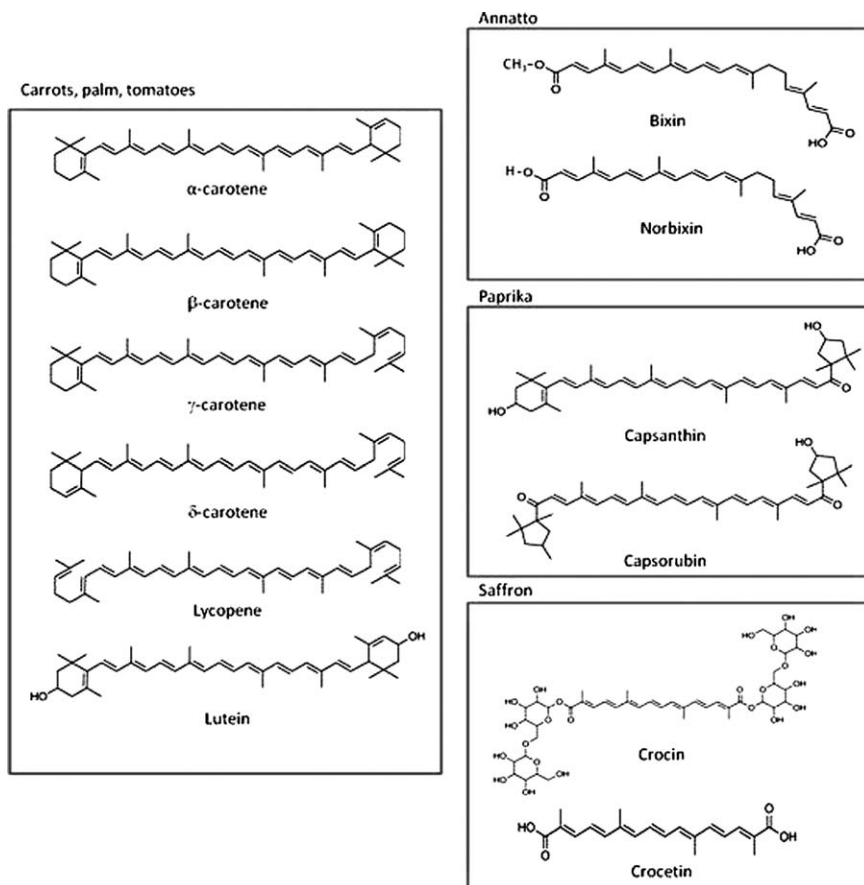


Figure 1.3 Chemical structure of main carotenoids.

apocarotenoid, whose biosynthesis has been suggested to take place by the oxidation of a normal C_{40} carotenoid such as lycopene.^{43,44} Annatto pigments can be separated from annatto seeds basically by two ways: (i) the method most used industrially consists of mechanical abrasion using a suitable suspending agent (e.g. vegetable oil, aqueous potassium hydroxide, or aqueous sodium hydroxide), followed by removal of the seeds (sieving); (ii) the second method consists of extraction with one or more organic solvents, which is also used as a means to produce annatto concentrates.^{44,45}

The most conventional extracts of annatto available are the bixin-rich oil extract and the water-soluble powder norbixin-rich extract.^{43,46} While the bixin-rich oil extract is an orange-red pigment, the norbixin-rich extract (a water-soluble powder) is a yellow-orange pigment. Annatto is used as a coloring agent in a wide range of foodstuffs such as butter, margarine, cheese, fats, cereals, baked goods, snacks, beverages, meat, and fish products.^{43,47} Annatto oil extract is one of the most common colorants used for high-fat food

products. Even though conventional methods are widely used extraction techniques, they present many drawbacks such as high energy costs, low selectivity, environmental concerns, toxicity, and the generation/retrieval of large quantities of solvent waste.⁴⁴ In this context, many researchers have been studying supercritical and ultrasound-assisted technologies as alternative extraction techniques to obtain annatto pigments.^{48–52}

Paprika. Paprika oleoresin (E160c) is the orange-red, oil-soluble extract generally obtained from dehydrated and milled fruit of certain varieties of red peppers (*Capsicum annum* L.).⁵³ Paprika oleoresin recovery uses hexane as extraction solvent, followed by miscella and meal disolventization, and finally oleoresin degumming. The paprika oleoresin is used in formulating nutraceuticals, colorants, and pharmaceuticals. It can become water soluble by microencapsulation in gelatin or Arabic gum.⁵⁴ Because of its high coloring capacity, and in some cases its peculiar pungency, paprika is one of the most widely used food colorants for culinary and industrial purposes; it is applied to modify the color and flavor of soups, sausage, cheese, snacks, salad dressing, sauces, pizza, and confectionary products.⁵³ Paprika oleoresin is recognized for its self-limiting use for technological and sensorial reasons; as with any other spice or flavor, too high levels can adversely impact the product's flavor profile balance.⁵⁵

The quality of paprika is evaluated through red color intensity and degree of pungency. The intense red color mainly originates from ketocarotenoids, capsanthin, and capsorubin (Figure 1.3), formed in the fruit during ripening. The yellow carotenoids of paprika, which are precursors of ketocarotenoids, mainly comprise zeaxanthin, violaxanthin, antheraxanthin, β -cryptoxanthin, β -carotene, and capsolutein.⁵³ The degree of pungency is originated from the group of pungent components called capsaicinoids, from which capsaicin and dihydrocapsaicin represent over 80%.⁵³

Saffron. Saffron, an extract from flowers of *Crocus sativus*, has been appreciated since Mesopotamian times for its biological, aromatic, and flavoring properties, but also particularly due to its color.⁵⁶ The sensorial properties of saffron extract are given by the presence of three carotenoid derivatives (crocin for color, picrocrocin for flavor, and safranal for aroma), mainly synthesized during flowering. These metabolites are produced by oxidative cleavage of zeaxanthin, followed by oxidative modifications and glycosilations.⁵⁷

Crocin, the major color component of saffron, is the digentiobioside ester of apocarotenic acid (crocetin). Crocetin, like bixin, is a dicarboxylic carotenoid.³⁶ The same pigment may be obtained from the flowers of *C. albifloris*, *C. luteus*, *Cedrela toona*, *Nyctasthes arborescens*, *Verbascum phlomoides*, and *Gardenia jasminoides*.³⁶ Other carotenoids have been found as a minor fraction of the total pigments of saffron, such as phytoene, phytofluene, tetra-hydrolycopene, β -carotene, ξ -carotene, zeaxanthin, and lycopene, but their color influence in saffron filaments has not been deeply studied as they are negligible compared to

crocin esters.⁵⁶ In addition to color, certain flavoring compounds (mainly picrocrocin and safranal) impart a distinct spicy flavor in saffron extract. Different studies have demonstrated that crocins have several nutraceutical properties including antioxidant, hypolipidemic, neuroprotective, anti-depressive, hypocholesterolemic, antitumor, and anticarcinogenic activities.⁵⁷

The saffron extract has many culinary and industrial applications, as a pigment in beverages, cakes, bakery products, curry products, soups, meat, and certain confectionery goods. However, the use of this colorant is restricted by its high price and pungency. Generally, it takes about 140 000 stigmas from saffron flowers to produce about 1 kg of powder. Combined with the high production cost, it makes saffron one of the most expensive coloring agents in the world.³⁶ The price of saffron depends on its quality, which is closely related to the area of production – in an analogous way to wine.⁵⁸ In the case of saffron, the best results for solid–liquid extraction of crocins are obtained with mixtures of ethanol:water and methanol:water.⁵⁷ The use of a green solvent, such as ethanol, rather than a toxic solvent, such as methanol, is, nevertheless, preferred.

β -Carotene. β -Carotene is one of the most widely used sources of pro-vitamin A and food colorant in the world, with a global market estimated to surpass USD 280 million in 2015.⁵⁹ The pro-vitamin A activity is the main nutritional function of β -carotene.⁵⁹ Furthermore, β -carotene is also used as coloring agent of fat-based products, in food, pharmaceutical, and cosmetic industries. It is authorized as a food ingredient, with extremely strong coloring properties, imparting the desired color to foods even at ppm content. β -Carotene can also act as antioxidant, cell communicator, UV skin protector, enhancer of the immune response, and reducer of the risk of degenerative diseases such as cancer, cardiovascular diseases, cataract, and macular degeneration.^{59–62}

The majority of the β -carotene commercially available in the world is synthetically produced from β -ionone.⁶³ Alternatively, the production of β -carotene can be reached on a biotechnological basis, using filamentous fungi, bacteria, microalgae, and yeasts as producers, or by extraction from vegetable sources. The β -carotene originated from vegetables is generally obtained by solvent extraction with organic solvents, e.g. hexane, acetone, ethyl acetate, ethanol, and ethyl lactate, from carrot and palm.⁵⁹ However, alternative techniques have been studied in order to improve the selectivity and quality of the extracts – such as ultrasound-assisted extraction, supercritical fluid extraction, and enzyme-assisted extraction, among others.⁶⁴

Carrot (*Daucus carota*) belongs to the Umbelliferae family and is one of the most important root crops. It is cultivated for its fleshy edible roots, which are consumed by both humans and animals.⁶⁵ Carrot is mostly a fresh-consumed food crop, and only a minor proportion of the whole production is processed for exploitation in agrofood, pharmaceutical, nutraceutical, and cosmetic industries (especially for making skin protective preparations).⁶⁶ Carrot extract is commonly obtained with organic solvents, and it is often commercialized as a

natural food colorant. Generally, it is mixed with jojoba, corn, sunflower, or other plant oils before marketing.⁶⁶ β -Carotene constitutes the largest portion (60–80%) of the carotenoids in carrot extracts, followed by α -carotene (10–40%), lutein (1–5%), and other minor carotenoids (0.1–1%) such as lycopene and zeaxanthin.⁶⁷

Fruits from the Arecaceae (palm) family, from the Amazon region, as buriti and palm oil, are the richest source of carotenoids among vegetable materials. Buriti is the richest source of carotenoids with 466 mg/kg raw material, from which about 75% is β -carotene.⁶⁸ Crude palm oil is a yellow-orange fat-soluble extract obtained from the mesocarp of the palm oil fruit (*Elaeis guineensis*); it also has a high content of β -carotene (500–700 ppm), and it is extensively cultivated, which makes it one of the richest sources of this carotenoid found in nature.⁶⁸ Commercial crude palm oil is obtained from the mechanical screw pressing of mesocarp of palm oil. The conventional milling process consists of: (1) sterilization of fresh fruit bunches for termination of enzymatic hydrolysis of oil; (2) stripping and digestion of fruits; (3) screw press for the extraction of crude oil; (4) screening of crude oil using a vibratory mechanism; (5) clarification of crude oil from water; and (6) centrifugation and vacuum drying of the oil.⁶⁹ The process to obtain carotene-rich palm oil basically involves two stages: a pretreatment step and a short path distillation. The pretreatment of the crude palm oil includes degumming and bleaching using conventional refining methods, while the short path distillation is carried out to deodorize and deacidify the crude palm oil. The existing technology is able to retain >80% of the carotenes present in the oil. The major drawbacks of the process is the necessity of several processing stages and the use of high temperatures which may contribute to carotene degradation.^{70,71}

Lycopene. Lycopene, a C_{40} polyisoprenoid compound containing 11 double bonds (Figure 1.3), is the most abundant carotenoid in ripe tomatoes (*Lycopersicon esculentum*), representing approximately 80–90% of the total pigment content.⁷¹ Low amounts of other carotenoids such as α -, β -, γ -, and ξ -carotenes, phytoene, phytofluene, neurosporene, and lutein are also present in tomatoes. Lycopene provides the bright red color to tomato, making it commercially important as a natural pigment.

The molecular structure of lycopene consists of a long chain of conjugated carbon–carbon double bonds, which make lycopene susceptible to chemical changes if exposed to light or heat.⁷¹ Lycopene exists in *cis* and *trans* isomeric forms, but occurs in nature primarily in the *trans* form, which is the most thermodynamically stable.⁷¹ However, when tomatoes are processed, some of the lycopene is isomerized into *cis* forms.⁷¹ Lycopene *cis* isomers have also been detected in plasma and tissue samples at significant levels, apparently being isomerized *in vivo*. In addition, *cis*-lycopene isomers are also found to be more bioavailable than the natural *trans* form.^{64,71,72}

Lycopene (E160) can be commonly obtained by chemical extraction, since it is soluble in highly toxic organic solvents such as hexane, benzene, chloroform, and methylene chloride.⁷³ In the last years, besides tomato, there has been an

increasing interest in recovering lycopene from the waste streams from the tomato-processing industry. Lycopene is also industrially produced by chemical synthesis in which C_{40} -carotenoids are efficiently produced by double Wittig olefination of the corresponding C_{15} -phosfonium salts with C_{10} -dialdehyde.⁷¹ Like β -carotene, it is authorized as a food ingredient with extremely strong coloring properties.

Besides its coloring application, lycopene acts on human health by preventing prostate, bladder, pancreas, and digestive tract cancer, and by its capacity to quench singlet oxygen, which is about three times higher than β -carotene's.^{74,75}

1.2.1.2 Chlorophylls

Chlorophyll is the most widely distributed natural plant pigment in nature; it is vital to the survival of both plant and animal kingdoms due to its critical light harvesting role in photosynthesis.^{32,72–77} Photosynthesis is a process that converts solar energy into chemical energy, using it together with water and carbon dioxide to produce oxygen and carbohydrates. The products from this chemical process reflect its significance, with carbohydrates being the primary building block for plants and oxygen being necessary for the survival of the animal kingdom.⁷⁸

Chlorophyll is a porphyrin pigment, made up of four pyrrole rings joined together via methine linkages. It is a dihydroporphyrin derivative chelated with a magnesium ion within the center of the porphyrin structure, held in position by two covalent and two coordinate bonds (Figure 1.4).^{32,36} The magnesium can be easily released from the molecule through acid-catalyzed hydrolysis to give olive-brown pheophytin.³⁶ Replacing Mg by Fe or Sn ions yields grayish-brown compounds, while Cu or Zn ions retain the green color.³² Chlorophylls are diesters: one carbonyl group is esterified with methanol and the other with phytol, a C_{20} monounsaturated isoprenoid alcohol.³² Upon removal of the phytol group by hydrolysis in dilute alkali, or by the action of chlorophyllase, green chlorophyllin is formed. Removal of Mg and the phytol group, which

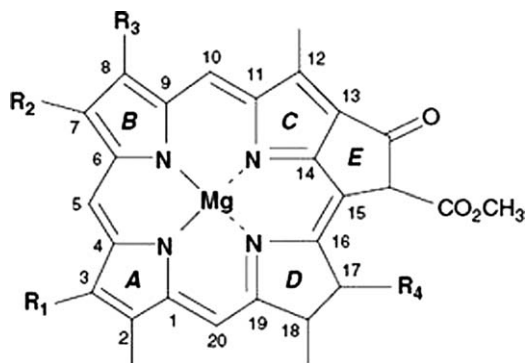


Figure 1.4 Chemical structure of chlorophyll a, b, c1, c2, c3 and d.^{69,77}

commonly occurs in conventional extraction, results in olive-brown pheophorbide formation.³²

Chlorophylls and pheophytins are lipophilic due to the presence of the phytol group (C₂₀), while chlorophyllins and pheophorbides without phytol are hydrophilic.³² The chlorophyll molecule is not isolated, but comprises a family of substances similar to each other, designated chlorophyll a, b, c, and d. Chlorophyll a (blue-green) and b (yellow-green) are the most abundant and important of this family, occurring in plants in a ratio about 3:1, while the chlorophyll c and d are commonly found in algae.⁷⁹ Chlorophyll b differs from chlorophyll a in that the methyl group on C₃ is replaced with an aldehyde.³²

Chlorophylls have received attention for a long time, not only because of their significance in living systems but also because of their potential relevance as natural pigments in a limited range of applications. The intense green color of natural chlorophylls suggests that they may be useful as oil-soluble color additives in food, pharmaceutical, and cosmetic products. However, in practice natural chlorophylls are rarely used as colorants for a range of reasons:⁷⁷

1. the co-extraction of carotenoids, phospholipids, and other oil-soluble substances results in products with diversified composition and variable levels of pigments, which makes subsequent purification steps indispensable;
2. the endogenous plant enzymes and extraction conditions employed can easily promote chemical modification of the sensitive chlorophylls, yielding unattractive brownish-green degradation products such as pheophytins and pheophorbides.

Consequently, it is more expensive and unstable than artificial coloring agents and therefore widespread application of natural chlorophylls as colorants is limited.³² To overcome some of these drawbacks, semi-synthetic, metal-chelated, and water-soluble chlorophyll derivatives, called chlorophyllins, have been produced as promising alternatives to generate colorants with a higher stability and tinctorial strength.⁷⁷ The most common stabilization process is chemical modification by replacing the magnesium center with a copper ion.³² Copper is much more stable than magnesium in relation to the aggressive conditions of processing and storage at low pH, high temperatures, and exposure to oxygen and light. Besides, the copper complex is not absorbed by the body and is removed in its entirety as an excretion product, being considered safe to be used in most countries as a food additive.⁷⁸

The commercial production of chlorophyll is generally carried out by two different ways: obtaining a water-soluble extract or an oil-soluble extract. The first and common step for both processes is the extraction of pheophytin using aqueous solvents, such as chlorinated hydrocarbons and acetone, from dried plant materials. The pheophytin crude extract is then further processed to give a more stable copper complex.³² Both the oil-soluble and water-soluble forms of chlorophyll are commercially available in the form of the stable copper complex (chlorophyllins, E141). Both forms are relatively stable towards light

and heat. However, unlike the water-soluble chlorophyll, the oil-soluble form is not very stable in acids and alkalis.

A major portion of the commercial chlorophyll is used in the food industry for coloring dairy products, edible oils, soups, chewing gum, sugar confectionery, and pet food. Chlorophyll preparations for the food colorant market are mainly obtained from alfalfa (*Medicago sativa*) and nettles (*Urtica dioica*). Brown seaweeds, which are the commercial source of alginates, are also an interesting source of chlorophyll because as in single-cell phytoplankton, they contain chlorophyll c, which is more stable than chlorophyll a and chlorophyll b.³²

The pharmaceutical and cosmetic industries also use chlorophyll and its derivatives.³⁶ Chlorophyll is similar in chemical structure to hemoglobin and, as such, is predicted to stimulate tissue growth in a similar way through the facilitation of rapid carbon dioxide and oxygen interchange. Due to its growth stimulation property, chlorophyll has been used to improve the healing process in the treatment of certain gastrointestinal diseases such as ulcers, oral sepsis, and proctologic disorders.⁷⁸ Additionally, chlorophyll was found to remove odors from the wound after a few applications. Its non-toxic nature, anti-bacterial property, and deodorizing function make chlorophyll a key product in the treatment of oral sepsis. Chlorophyll a and its derivatives also have potent antioxidant properties. Chlorophyll derivatives such as pheophorbide b and pheophytin b have always been known as strong antioxidants. However, these derivatives exist in very low concentrations in fruits and vegetables.⁷⁸

1.2.1.3 Anthocyanins

Anthocyanins (from the Greek *anthos*, a flower; and *kyanos*, dark blue) are the largest and most important group of water-soluble and vacuolar pigments in nature. They comprise a major flavonoid group that is responsible for cyanic colors ranging from orange/red to violet/blue of most flowers, fruits, and leaves of angiosperms commonly found in nature. They are sometimes present in other plant tissues such as roots, tubers, stems, bulbils, and are also found in various gymnosperms, ferns, and some bryophytes.⁸⁰ Anthocyanins are glycosylated polyhydroxy and polymethoxy derivatives of the flavylium cation (phenyl-2-benzopyrylium cation), also known as aglycone or anthocyanidin (Figure 1.5), which contains conjugated double bonds responsible for absorption of light around 500 nm causing the typical color of these pigments.⁸⁰ The sugar moieties are usually attached to the anthocyanidins via the 3-hydroxyl or 5-hydroxyl positions and to a lesser extent the 7-hydroxyl position. The anthocyanin sugars may be simple sugars, most commonly glucose, galactose, rhamnose, xylose, fructose, and arabinose, or complex sugars such as rutinose and sambubiose. These sugars may occur as monoglycosides, diglycosides, and triglycosides substituted directly on the aglycone. The sugar moieties may be acylated; the most common in order of occurrence are coumaric, caffeic, ferulic, *p*-hydroxy benzoic, synaptic, malonic, acetic,

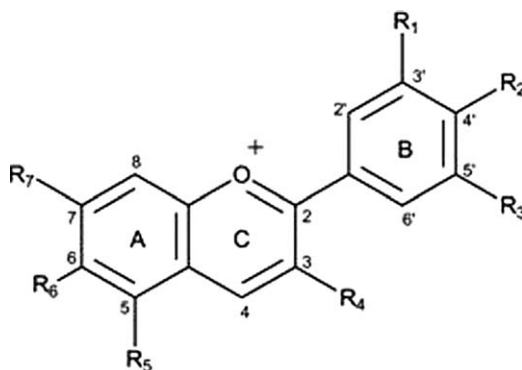


Figure 1.5 Chemical structure of the anthocyanidins or aglycones commonly found in nature.

succinic, oxalic, and malic. The first five are aromatic acids while the others are aliphatic acyl acids.^{36,81}

Chemically, the major factors that influence the color of these pigments are the degree of hydroxylation/methoxylation of the anthocyanidin B ring and the nature of sugar and/or acid conjugations. An increased number of hydroxyl and/or methoxyl groups on the B ring of an anthocyanidin results in a bathochromic shift of the visible absorption maximum, which has a bluing effect on the color produced. Substitutions on the R groups of the B ring may also affect the stability of the pigments; hydroxylation of the B ring has been reported to decrease the stability of the anthocyanin while methoxylation increases stability. Sugar substitution of the anthocyanidin may increase the visible absorption maximum of the pigment, producing a more red-orange color. Acylation of the sugar substitutions and/or individual anthocyanidins may also produce bathochromic (increased wavelength) and/or hyperchromic (increased absorption) shifts, altering the spectra of a compound.⁷⁷

The main drawback of the application of anthocyanins as natural colorants is their high instability and easily susceptibility to degradation during storage and processing. Anthocyanin color stability is strongly affected by pH, temperature, chemical structure, anthocyanin concentration, oxygen, light, enzymes, and other accompanying substances such as ascorbic acid, sugars, proteins, sulfites, co-pigments, and metallic ions, among others.⁸⁰

Anthocyanin color stability shows great susceptibility toward pH. At any given pH level, anthocyanins exist as an equilibrium of different chemical forms. They typically exhibit an absorption maximum at a pH of 1 when the anthocyanidin is in its most stable form, known as the flavylium cation. In this form, the pigment produces a bright orange-red to violet color, attractive for many applications. However, at a pH of 4.5 the flavylium cation suffers a hydration generating the carbinol pseudo-base (colorless) which due to its high instability converts to its chalcone form. As the pH approaches 6 the color becomes purple. In a pH environment of 7 the flavylium cation loses the proton producing the quinonoidal base form which is characterized by its dull blue to

green color.⁷⁷ The pigment turns into a deep blue when the pH is between 7 and 8. Further increase in pH sees the anthocyanin pigment turning from blue to green and then to yellow. Several studies reported a logarithmic course of anthocyanin degradation with an arithmetic increase in temperature, indicating that heating strongly accelerates anthocyanin pigment destruction; the magnitude and duration of heating have a strong influence on anthocyanin degradation.⁸²

Many studies have demonstrated that oxygen has a detrimental effect on anthocyanin stability, amplifying the impact of other factors on degradation processes.⁸⁰ Light also accelerates anthocyanin degradation. Some investigations have proved that light has a highly significant negative effect on anthocyanin stability during storage, especially in the presence of sugar.⁸⁰ The presence of enzymes in the plant matrix is also an important intrinsic factor on anthocyanin stability. The most common anthocyanin-degrading enzymes are glycosidases, which break the covalent bond between the glycosyl residue and the aglycone of an anthocyanin pigment, resulting in the degradation of the highly unstable anthocyanidin. Peroxidases and phenolases, such as phenol oxidases and polyphenol oxidases, which are both found naturally in fruits and berries, are also common anthocyanin-degradating enzymes.⁸⁰ Ascorbic acid may have a protective effect towards anthocyanins because it reduces the *o*-quinones formed before their polymerization.⁸⁰ Sugars, as well as their degradation products, are known to decrease anthocyanin stability; their effect depends on the anthocyanin structure, concentration, and type of sugar.⁸⁰ Sulfates and sulfites generally used as preserving agents in foodstuffs have a detrimental effect on anthocyanin stability, producing colorless sulfur derivative structures by replacement in positions 2 or 4 (Figure 1.5).

There are several mechanisms applied in the process of anthocyanin stabilization; the most common are encapsulation and co-pigmentation. Some studies suggest that the co-pigmentation of anthocyanins with other compounds is the main mechanism of stabilization of color in plants.⁸³ In this phenomenon the pigments and other colorless organic compounds, or metallic ions, form molecular or complex associations, generating a hyperchromic effect and a bathochromic shift in the absorption spectra of the UV visible region.⁸³ There are two types of co-pigmentation reactions:^{80–83}

1. intramolecular co-pigmentation with the aromatic groups of hydroxycinnamic acids;
2. intermolecular co-pigmentation with colorless substances such as flavonoids, alkaloids, amino acids, organic acids, nucleotides, polysaccharides, metals, or another anthocyanin.

The co-pigments are systems rich in π -electrons, which are able to associate with flavylium ions, which are rather poor in electrons. This association gives protection for the water nucleophilic attack in the 2 position of the flavylium ion and for other species such as peroxides and sulfur dioxide in the 4 position.⁸³

Grape extracts (*Vitis vinifera*) are the most widely used anthocyanin sources of natural colorants (E163). Nearly all the commercially available anthocyanins, known under the generic name of enocyanin or enocianina, are obtained from grape skin and other by-products of the wine industry.^{81,84} The generic product known as enocyanin is obtained by solvent extraction from the skins of wine grapes. Another source is lees, formed in the bottom of tanks of grape juice during fermentation. A precipitate formed by anthocyanins and proanthocyanins on the bottom of the tanks provides a rich source of pigments, which have been approved for food use by the FDA since 1981.⁸¹ Grape extracts are rich in anthocyanins complexed with other compounds, such as mono-, di-, or tri-acylated and di- or tri-glycosylated anthocyanins, which are much more stable under processing and storage conditions than monomeric anthocyanins due to co-pigmentation. Anthocyanins may be easily obtained in high quantities from grapes, as they represent about a quarter of the annual fruit crop worldwide.^{81,84}

Besides grapes, other fruits such as concentrated juice of blackcurrant,⁸⁵ elderberry,^{86,87} cranberry,⁸⁸ raspberry,⁸⁹ and cherry^{84,90,91} have been studied as potential sources of anthocyanins. Also, several vegetable extracts have been used as coloring agent sources, including red cabbage, purple sweet potato,^{92,93} radish,⁹⁴ and black carrot.^{95,96} These vegetable extracts have been shown to be rich in acylated anthocyanins, which improves the color stability during processing and storage.⁸⁴ Depending on the food matrix in which the anthocyanin extracts are intended to be used, other ingredients may be added in order to improve both solubility and color stability.

With a correct formulation of different ingredients, as well as adequate processing and storage conditions of the food product, a wide range of stable and attractive color hues may be obtained for several food matrices. Commercial applications of anthocyanins as food colorants include soft drinks, fruit preserves (jams, canned fruit), sugar confectionary (jellies), dairy products (mainly yogurts), dry mixes (acid dessert mixes and drink powders) and more rarely frozen products (ice cream) and a few alcoholic drinks.⁸⁴

1.2.1.4 Betalains

Betalains are water-soluble vacuolar nitrogen-containing pigments (Figure 1.6) with colors ranging from yellow-red to violet, which are commonly found in plants of the order Caryophyllales as well as in some Basidiomycota.⁹⁷ Chemically, they are immonium conjugates of betalamic acid; they are subdivided in two structural groups, the red-violet betacyanins (540 nm) and the yellow betaxanthins (480 nm).^{34,98} Betacyanins are derivatives of betanidin, an iminium adduct of betalamic acid and cyclo-DOPA (cyclic 3,4-dihydroxy-phenylalanine), whereas betaxanthins result from the condensation of α -amino acids or amines with betalamic acid.^{97,98} The major components found in betacyanins and betaxanthins are betanin and vulgaxanthine I and II, respectively.³⁶

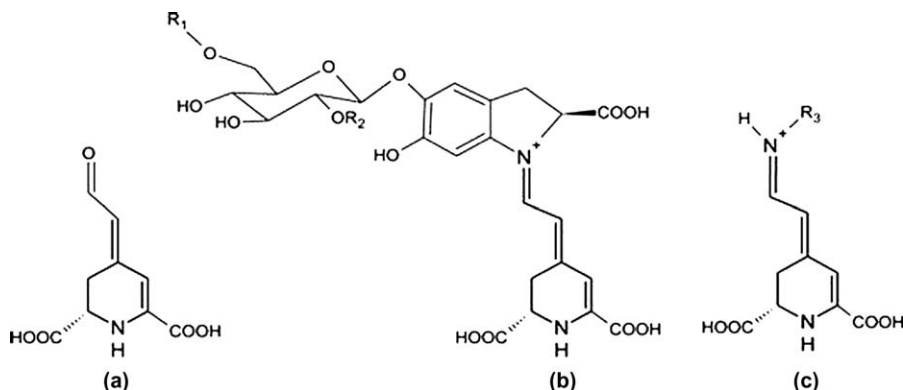


Figure 1.6 Chemical structure of (a) betalamic acid, (b) betacyanins, and (c) betaxanthins (c).⁹⁸

Although structurally related to alkaloids, betalains have no toxic effects in human health as can be deduced from the fact that they are present in high amounts in foodstuffs; therefore they are considered a safe natural colorant source.³⁴ Commercial production of betalains often involves countercurrent solid–liquid extraction with aqueous methanol from plant tissues or cell cultures. A slight acidification of the extraction medium, generally by ascorbic acid addition, may be useful to promote betalain stabilization and to inhibit the possible oxidation by polyphenoloxidase (PPO).^{98–101} Sometimes inactivation of degradative enzymes are achieved by a short heat treatment (70 °C, 2 min).³⁴ The extraction process is followed by aerobic fermentation, generally with *Candida utilis*, to remove the large amount of sugar present. Betanin (E162) is the only betalain approved for use in food and it is almost entirely obtained from red beet (*Beta vulgaris* subsp. *vulgaris*).⁹⁷

Although betalains are well suited for coloring low-acid food due to their stability at pH 3 to 7, they are poorly exploited as coloring agents in food processing, being less commonly used than anthocyanins and carotenoids.¹⁰⁰ Besides betanin from red beet, cactus fruits and Amaranthaceae plants are good alternative sources.¹⁰¹ Cactus fruits from the genera *Opuntia* and *Hylocereus* are edible sources of betalain pigments. The color shade of the juice of *Opuntia ficus-indica* cv. *Rossa* is similar to that of beet preparations, whilst the juice from *Opuntia ficus-indica* cv. *Gialla* displays a yellow tonality and the juice from *Hylocereus ptyrhizus* is characterized by purplish hues. The Amaranthaceae family is a rich source of diverse and unique betacyanins: eight amarantine-type, six gomphrenin-type, and two betanin-type pigments.³²

1.2.1.5 Curcuminoids

Turmeric is an aromatic spice native to Southeastern Asia obtained from the dried ground rhizomes of *Curcuma longa* L., a perennial shrub that belongs to genus *Curcuma* of the Zingiberaceae family. Its dried ground rhizomes provide

a bright yellowish-brown powder also known as yellow ginger or Indian saffron.¹⁰²

The compounds responsible for the yellow color of turmeric are known as curcuminoids. Turmeric also contains essential oils containing monocyclic monoterpenes, sesquiterpenes (bisabolanes and germacranes), arabino-galactans, and *ar*-turmerone, which are responsible for aroma and taste. The three main compounds that comprise the pigmented curcuminoid complex are curcumin (1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), demethoxycurcumin (feruloyl (4-hydroxycinnamoyl) methane), and bisdemethoxycurcumin or bis(4-hydroxycinnamoyl) methane (Figure 1.7).¹⁰²

There are three forms of coloring products based on *Curcuma longa* L. commercially available: turmeric powder, turmeric oleoresin, and purified curcumin. Turmeric powder is obtained from the grinding of dried rhizomes yielding a fine powder. Oleoresin is obtained from turmeric powder by solid-liquid extraction using ethyl acetate, acetone, dichloromethane, methanol, ethanol, or hexane as solvent. After filtration, the solvent is removed by evaporation or distillation resulting in an orange viscous oleoresin. Curcumin powder (E100) is an orange-yellow crystalline powder obtained from turmeric oleoresin by crystallization.¹⁰² Curcumin is an oil-soluble pigment with a melting point of 174 °C. It is stable at acidic pH but readily decomposes at pH above neutral. It is light sensitive, especially in solutions, but highly stable to heat.

Turmeric is a very important herb due to its use in foods, cosmetics, and medicines. Turmeric powder is widely used as culinary ingredient due to its desirable orange-yellow color and spicy flavor having well-established application as coloring agent in mustard paste and curry powder. The oleoresin is generally added to oil-soluble food products such as mayonnaise, fish, meat, soups, and non-alcoholic beverages. On the other hand, curcumin powder is added to products where turmeric is incompatible due to its bitter-peppery taste such as cheese, butter, confectionary, ice cream, and some beverages. Curcumin is also used as an antioxidant to prevent rancidity.¹⁰⁶ Many pharmacological properties have been attributed to curcumin, including cardiovascular protection, antitumor, antioxidant, anti-inflammatory, anti-Alzheimer, anti-hepatotoxic, antibacterial, and antiviral activity.¹⁰⁴ Its unique bioprotective properties have been associated to neutralization of free radicals on the surface of skin, retarding aging and damage due to UV radiation.^{103,105}

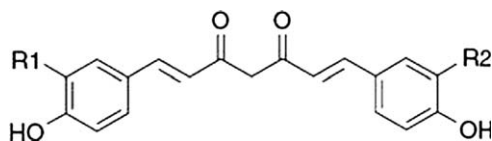


Figure 1.7 Chemical structure of curcumin and its analogs.⁷⁷

1.2.2 Flavors and Fragrances: Essential Oils

Flavors and fragrances are used in a wide variety of cosmetic, pharmaceutical, and edible products. Preference is usually given to natural products, but their shortage, high price, and price fluctuations are often the compelling reasons for partially, if not fully, switching over to synthetic equivalents. The rapid development of the fragrance and flavor industry in the nineteenth century was generally based on essential oils and related natural products. Essential oils produced from aromatic plants are formulated to make flavors and fragrances for a wide range of end uses, such as soaps, cosmetics, perfumes, toiletries, detergents, confectioneries, alcoholic and nonalcoholic beverages, ice creams, baked goods, convenience foods, tobacco products, aerosols, sprays, syrups, and pharmaceutical preparations.^{106,107}

Essential oils have been known to mankind for millenniums. The history of production of essential oils dates back to 3500 years BCE when the oldest-known water distillation apparatus made of burnt clay was employed. In 1480 BCE in Egypt, fragrant plants, oils, and resins were collected and used as ingredients for perfumes, medicines, flavors, and for the mummification of bodies. The use of essential oils as food ingredients has a history dating back to ancient times, with the use of citrus and other pressed (manually or mechanically) oils in sweets and desserts in ancient Egypt, Greece, and the Roman Empire. The fragrance used in the first alcoholic perfume in history was based on rosemary essential oil distillate and was created in the mid-fourteenth century for the Polish-born Queen Elisabeth of Hungary. The beginning of the eighteenth century saw the introduction of 'Eau de Cologne', based on bergamot and other citrus oils, which remains widely used to this day. While knowledge of the science of essential oils did not increase during the seventeenth century, the eighteenth century brought about only small progress in the design of equipment and in refinements of the techniques used. The beginning of the nineteenth century brought progresses in chemistry, including wet analysis and an increased development of hydro-distillation methods. The nineteenth century is generally regarded as the beginning of the modern phase of industrial application of essential oils.^{106,108}

The term 'essential oil' is a contraction of the original 'quintessential oil', a concept dating back to the Aristotelian idea that matter is composed of four elements (fire, air, earth, and water) and the fifth element, or quintessence, was then considered to be the spirit or life force. Distillation and evaporation were thought to be processes of removing the spirit from the plant.¹⁰⁹ Far from being spirit, essential oils are physical in nature, composed of complex mixtures that can contain hundreds of compounds. Nevertheless, they are usually characterized by two or three major components at fairly high concentrations (20–80%), while the rest of the components are present in trace amounts, but are still important in building the aroma.¹¹⁰

Essential oils are secondary metabolites that act in the protection of the plants, having antibacterial, antiviral, antifungal, and insecticide properties; they also act against herbivores by reducing their appetite for such plants. For

these properties, essential oils have been largely employed in pharmaceutical, food, and cosmetic industries.¹¹¹

In the pharmaceutical industry, essential oils are widely employed to prevent and treat human diseases. As examples, essential oil from *Eucalyptus* species produces analgesic and anti-inflammatory effects,¹¹² that from nutmeg (*Myristica fragrans*) has a potent hepatoprotective activity against liver damage caused by certain chemicals,¹¹³ and that from *Origanum onites* L. has anti-angiogenic and anti-tumor activities.¹¹⁴ Essential oil of coriander (*Coriandrum sativum*) is used as carminative or as a flavoring agent to cover the bitter taste of other medicines.¹¹⁵ It has been extensively reported that essential oils can potentially be employed for the prevention and treatment of cancer¹¹⁶ and cardiovascular diseases, including atherosclerosis, by reducing plasma concentrations of cholesterol and triglycerides,¹¹⁷ and thrombosis, by inhibiting platelet aggregation and thromboxane formation.¹¹⁸ They are also used in pharmacy, balneology, massage, and homeopathy. Furthermore, the clinical use of their volatile constituents via inhalation, defined as aromatherapy, have expanded worldwide.¹¹⁹

Essential oils are known to possess potential application as food preservatives due to their antimicrobial properties against a wide range of microorganisms^{120,121} present in a number of food products, such as meat and its products, fish, dairy products, vegetables, rice, and fruits.¹²² Negi¹²³ presents a review about the stability, toxicity, and mechanisms of action of natural antimicrobials, including essential oil and plant extracts, for food application. More recently, many essential oils have been qualified as natural antioxidants,^{124,125} but their use in foods is often limited due to flavor considerations. Anthony *et al.*¹²⁴ analyzed the antioxidant activity of 423 essential oils from 48 plant families and concluded that phenolic terpenes are major constituents of the most effective oils.

Despite their application for their biological properties, the greatest use of essential oils is as flavoring. The coriander oil (*Coriandrum sativum* L.) is used in the liquor, cocoa, and chocolate industries, besides being applied in various food products and in soap.¹²⁹ In the cosmetic industry the majority of essential oils are introduced into fragrance compositions. They are used in perfumes, aftershaves, cosmetics, air fresheners, and deodorizers. In recent years, the importance of essential oils as biocides and insect repellents has also increased.¹⁰⁸ Clove (*Syzygium aromaticum*) oil is traditionally used in dental care as a sealing component and as an antiseptic for mouth hygiene.¹²⁷ The Brazilian cherry tree leaves essential oil has been used by the Brazilian cosmetics industry for its astringent properties, which are associated with its pleasant smell. The main applications are in shampoos, hair conditioners, face and bath soaps, body oils, and perfumes.¹²⁸

Essential oils are volatile, liquid, and clear, are rarely colored, and are characterized by a strong odor. They are highly concentrated substances isolated from aromatic plants by several extraction methods; the most commonly employed are steam distillation and hydro-distillation. The levels of essential oils found in plants can be anywhere from 0.01 to 15 wt % of the total.

Essential oils are soluble in lipids and organic solvents usually with a lower density than that of water. Generally the major components determine the biological properties of the essential oils, but the synergistic effect with minor compounds should not be disregarded.

The first main group that composes essential oil is terpenes and terpenoids, and the other is aromatic and aliphatic constituents, derived from phenylpropane, which comprise aldehyde, alcohol, phenols, methoxy derivatives, and methylene dioxy compounds. Thus, essential oils are classified into terpenoids, shikimates, polyketides, and alkaloids.¹¹¹ There are a number of terpenoids, shikimates, and polyketides of importance in essential oils but very few alkaloids.

1.2.2.1 Polyketides and Lipids

Polyketides and lipids have the simplest biosynthetic pathway. Polyketides are natural products whose biosynthesis can be traced to an intermediate that contains repeating ketide units. The biosynthesis of polyketides is similar to that of fatty acids. They are chemically diverse, but all plant-derived polyketides are produced in the cytosol using enzymes called polyketide synthases, which catalyze the initial steps in polyketide formation via the condensation of a starter (usually acetyl-CoA) and extender molecules (usually malonyl-CoA), resulting in a chain with carbonyl groups.¹²⁹

There are three main paths by which components of essential oils and other natural extracts are formed in this family of metabolites: condensation reactions of polyketides, cyclization of arachidonic acid, and degradation of lipids. Condensation of polyketides leads to phenolic rings. The most important natural products containing polyketide phenols are the extracts of oakmoss and treemoss (*Evernia prunastrii*). The cyclization of arachidonic acid, a polyunsaturated fatty acid, plays a special role as a synthesis intermediate, for compounds such as prostaglandins and methyl jasmonate, in plants and animals.¹⁰⁹

The major metabolic route for fatty acids involves β -oxidation and cleavage resulting in acetate and a fatty acid with two carbon atoms less than the starting acid, that is, the reverse of the biosynthesis reaction. Allylic oxidation followed by lactonization rather than cleavage leads to lactones. A wide variety of aliphatic entities are produced by the reduction of the acid function to the corresponding alcohols or aldehydes.¹⁰⁹ Some examples are shown in Figure 1.8.

1.2.2.2 Shikimic Acid Derivatives

Through photosynthesis, green plants convert carbon dioxide and water into glucose. Cleavage of glucose produces phosphoenolpyruvate, which is a key building block for the shikimate family of natural products. Shikimic acid is synthesized from the condensation of phosphoenolpyruvate and erythrose-4-phosphate, and thus its biosynthesis starts from the carbohydrate pathway.

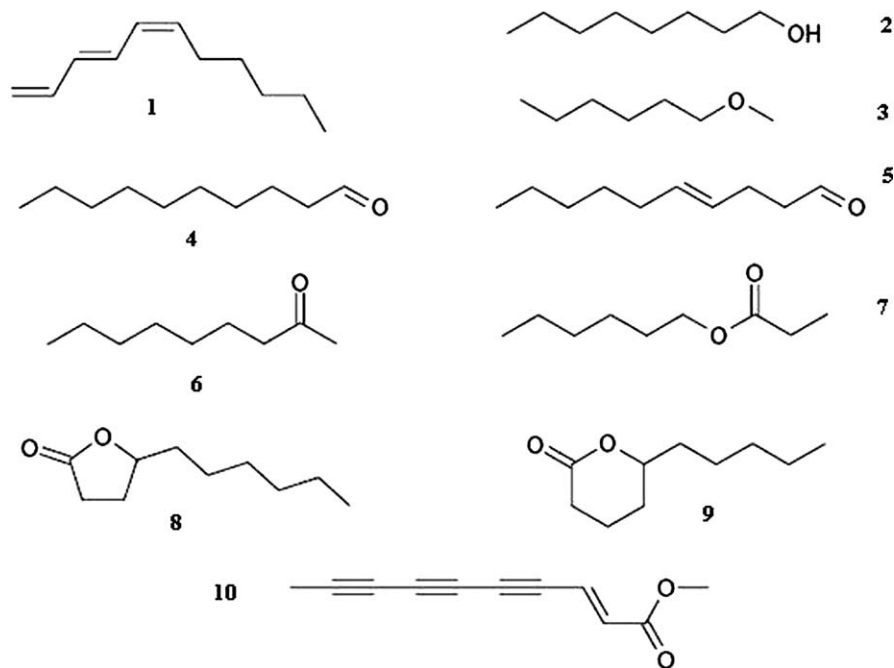


Figure 1.8 Some lipid-derived components of essential oils.

Its derivatives can usually be recognized by the characteristic shikimate pattern of a six-membered ring with either a one- or a three-carbon substituent on position 1 and oxygenation in the third, and/or fourth, and/or fifth positions.¹⁰⁹

Phenylpropanoids originate through the shikimic acid biosynthetic pathway. These compounds are found as the main component of essential oil of certain plants species, such as grass. The main phenylpropanoids and chemotypes are eugenol, methyl eugenols, myristicin, methyl cinnamate, elemicin, chavicol, methyl chavicol, dillapiol, anethole, estragole, and apiole.¹³⁰ Figure 1.9 shows some of them. The shikimate pathway, operational only in microorganisms and plants, is the precursor for amino acids (phenylalanine, tryptophan, and tyrosine), aromatic aldehydes (vanillin), and simple aromatic acids (gallic acid). Plant amino acids phenylalanine and tyrosine also formed via the shikimic acid pathway are deaminated, oxidized, and reduced to yield important aromatic substances such as cinnamaldehyde and eugenol.¹³¹

1.2.2.3 Terpenoids

Terpenoids are the most common compounds in essential oils. They are substances composed of isoprene (2-methylbutadiene) units. Figure 1.10 shows the structures of some terpenoids. They are synthesized from five carbon units of isopentenyl pyrophosphate and its isomer, dimethylallyl pyrophosphate. Mevalonic acid, made from three molecules of acetyl CoA, is the key starting

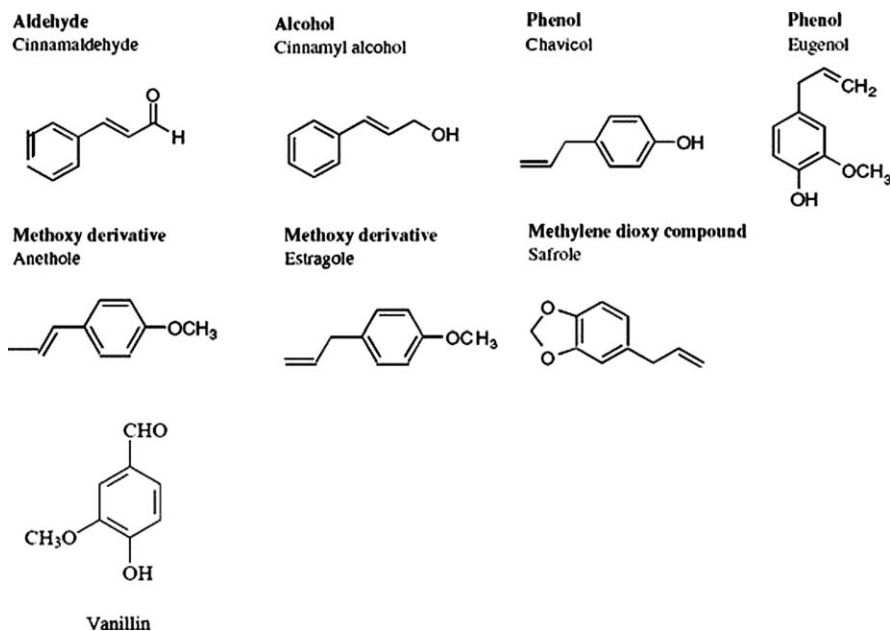


Figure 1.9 Chemical structures of some phenylpropanoids compounds of essential oils.

material for the terpenoids. Phosphorylation of mevalonic acid followed by elimination of the tertiary alcohol and concomitant decarboxylation of the adjacent acid group gives isopentenyl pyrophosphate. Terpenoid structures will always contain a multiple of five carbon atoms when they are first formed, and they are classified depending on the number of these units in their skeleton. The components of essential oils of the majority of plants belong to hemiterpenoids (C_5), monoterpenoids (C_{10}), and sesquiterpenoids (C_{15}) families.^{109,130}

Hemiterpenoids. The hemiterpenoids (C_5) consist of a single isoprene unit. They are the smallest plant terpenoids and can be formed directly from dimethylallyl diphosphate by terpenoid synthase activity.¹³² Many alcohols, aldehydes, and esters with a 2-methylbutane skeleton occur as minor components in essential oils. Esters such as prenyl acetate give fruity top notes to essential oils and the corresponding thioesters contribute to the characteristic odor of galbanum.¹⁰⁹

Monoterpenoids. The monoterpenes are formed from the coupling of two isoprene units (C_{10}). They are the most representative molecules of the essential oils, constituting 90%. They allow a great variety of structures and have been classified according to their functional groups as well as based on their linear or cyclic nature.¹¹¹

Myrcene, geraniol, citronellol, fenchone, limonene, and menthol are widespread in nature. Some sources of myrcene are hops and most of the common

Hemiterpenoids

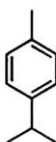
isoprene



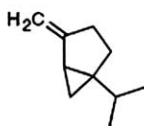
methylbutane



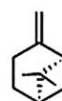
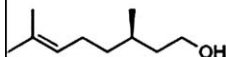
2-methylbut-3-en-2-ol

Terpenes**Carbure monocyclic**
Cymene ("y") or p.cymene

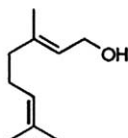
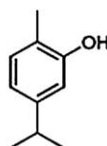
Sabinene

**Carbure bicyclic**
Alpha-pinene

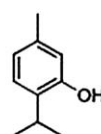
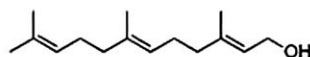
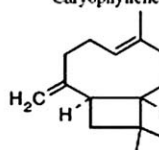
Betapinene

**Alcohol acyclic**
Citronellol

Geraniol

**Phenol**
Carvacrol

Thymol

**Sesquiterpenes****Carbure**
Farnesol**Alcohol**
Caryophyllene**Figure 1.10** Chemical structures of some terpenoids compounds of essential oils.

herbs and spices. The oil of *Monarda fistulosa* contains over 90% geraniol and its level in palmarosa is over 80%; geranium contains about 50% geraniol and citronella and lemongrass each contain about 30%. Citronella and related species are used commercially as sources of geraniol. Rose, geranium, and citronella are the oils with the highest levels of citronellol. Fenchone occurs widely in fennel, cedar leaf, and lavender. Limonene is present in many essential oils but the major occurrence is in the citrus oils, which contain levels up to 90%. *L*-Menthol is found in various mints and is responsible for the cooling effect of essential oils, the two most important sources being cornmint (*Mentha arvensis*) and peppermint (*Mentha piperita*).¹⁰⁹

Sesquiterpenoids. The sesquiterpenes are formed from the assembly of three isoprene units (C₁₅). The extension of the chain increases the number of cyclizations, which allows a wide structural diversity. A few important skeletal types are farnesal, nerolidol, bisabolene, germacrone, vetinone, and caryophyllene.¹¹¹

Farnesol and nerolidol are the only known acyclic sesquiterpenoid alcohols. Farnesol was first isolated from *Abelmoschus moschatus* Moensch, but has also been obtained from numerous other essential oils.¹³³ α -Bisabolol is the simplest of the cyclic sesquiterpenoid alcohols, found in many species as chamomile, lavender, and rosemary. It has a faint floral odor and anti-inflammatory properties. Clove is the best-known source of caryophyllene and α -humulene (the all *trans* isomer). The ring systems of these two compounds are very strained, making them quite reactive chemically. Caryophyllene, extracted from clove oil as a by-product of eugenol production, is used as the starting material in the synthesis of several fragrance ingredients.¹⁰⁹

Vetiver and patchouli are two oils of great importance in perfumery. Both contain complex mixtures of sesquiterpenoids, mostly with complex polycyclic structures. The major components of vetiver oil are α -vetivone, β -vetivone, and khusimol, but the most important components as far as odor is concerned are minor constituents such as khusimone, zizanal, and methyl zizanoate. Nootkatone is an isomer of α -vetivone and is an important odor component of grapefruit. Patchouli alcohol is the major constituent of patchouli oil but, as is the case with vetiver, minor components are more important for the odor profile. These include nor-patchoulol and nor-tetrapatchoulol.¹⁰⁹

1.2.2.4 Essential Oil Sources

Essential oils are present in various parts of the plant including seeds, roots, wood, bark, leaves, flowers, fruits, berries, rhizome, peel, and resin.

Seeds. Anise oil is obtained conventionally by steam distillation from dry ripe seeds of anise (*Pimpinella anisum* L.) or star anise (*Illicium verum* Hook. f.), Apiaceae, but other techniques have been evaluated for better extraction performances, such as solvent extraction and supercritical fluid extraction.^{134,135} The main constituent of anise essential oil is *trans*-anethole, present to about 90–95%, followed by estragole (2.4%). Other constituents present in concentrations higher than 0.06% are (*E*)-methyleugenol, α -cuparene, α -himachalene, β -bisabolene, *p*-anisaldehyde, and *cis*-anethole. Anise oil is an established flavoring agent used in the manufacture of perfumes, toothpaste, and liquors. It is also used as food flavoring in fish, poultry, soups, ice cream, chewing gum, pickles, cake, sweet snacks, and alcoholic drinks.¹³⁶

The essential oil of nutmeg (*Myristica fragrans*), Myristicaceae, an important spice used for the flavoring of numerous food products, is composed of terpenes such as α -, β -, and γ -pinene, sabinene, limonene, and 4-terpineol. Other important components are safrol, elimicin, eugenol, and myristicin; the

last one is responsible for the nutmeg characteristic aroma.¹³⁷ Some of these compounds have insecticidal properties. Nutmeg essential oil is also used as a component of certain types of perfumes and as a flavoring agent for dentifrices.¹³⁸

Seeds and fruits of the families Apiaceae, Piperaceae, and Myristicaceae usually require grinding up prior to steam distillation. In many cases, the seed has to be dried before comminution takes place.

Barks. The well-known bark oils are obtained from birch, cascarilla, cassia, cinnamon, and massoia.¹⁰⁸ Cinnamon and cassia have long been held in high esteem as aromatics as well as ingredients of foods and perfumes. Their bark have an aromatic and sweet taste with a spicy fragrance. Cinnamaldehyde and the phenylpropenoid eugenol are the major constituent of cinnamon and cassia essential oils.¹⁰⁹ These oils are used in food, pharmaceutical, and perfume industries. They find extensive use in flavoring meat and fast food, sauces, pickles, baked foods, candies, confectionery, liqueurs, and soft drinks. In pharmaceutical preparations, bark oil is used to mask the unpleasant taste of medicines. It is also used to impart a woody and musky undertone to perfumes. However, the use of bark oil in the perfume industry is limited due to its skin sensitizing property.¹³⁹ Cinnamon leaf oil has a quite different flavor compared to bark oil. It has spicy cinnamon, clove-like odor and taste, whereas cinnamon bark oil has a bitter flavor, is slightly pungent and burning. Eugenol is the main component of cinnamon leaf oil.¹⁴⁰

Woods. Wood oils are derived mostly from species of *Santalum* (sandalwood), cedar, amyris, cade, rosewood, agarwood, and guaiac. In order to achieve complete recovery of the essential oil, the wood has to be reduced to a very fine powder prior to steam distillation, but in some cases coarse chipping of the wood is adequate for efficient essential oil extraction.¹⁰⁸

Sandalwood oil has very good fixative properties and is very light in color, so it can be added without interfering in the ultimate coloration of products. It also has such a delicate aroma that it can be blended in small quantities without altering the dominant fragrance. It is used in soaps, cosmetics, incense, perfumes, and confectioneries. Conventionally, steam distillation is employed for recovering sandalwood oil, with a 3.8% yield after 24 h; liquid CO₂ extraction yields 4.9% oil in 2 h.¹⁰⁷ Santalols are the main components of sandalwood oil.¹⁰⁹

Rosewood oil is obtained from one of the species of the Lauraceae family, the *Aniba rosaeodora* Ducke. All parts of the tree are fragrant, although only the trunk wood is harvested and distilled. The oil is colorless to pale yellow with a woody-floral fragrance. The main constituent of rosewood oil is the monoterpene alcohol linalool, which is an ingredient used in many fragrance compounds. It may be found in decorative cosmetics, fine fragrances, shampoos, toilet soaps, and other toiletries as well as in non-cosmetic products

such as household cleaners and detergents.¹⁴¹ Linalool is also used to create natural flavors, e.g. as a component of natural apricot flavor.¹⁴⁰

Camphor occurs in many essential oils in both enantiomeric forms. Its richest source is the oil of camphor wood (*C. camphora*, *L. Sieb* and a number of related varieties), but it is also an important contributor to the odor of lavender, sage, and rosemary.¹⁰⁹ Almost all the camphor oil is obtained by steam distillation of the wood (yield 2.2%). About 70% of camphor is removed from crude oil to give camphor oil. This oil is further fractionated to obtain three oils: white camphor oil (13% of the original oil: 46% 1,8-cineole, 22% α -pinene, 21% camphor), which can be rectified to result in an oil with some similarity to eucalyptus oil; brown camphor oil (14% of the original oil: 32% isosafrole, 14% safrole); and blue camphor oil (0.7% of the original oil: azulenes).¹⁴⁰

Rhizomes. Ginger is one of the major spice essences with widespread use in food (sauces, soups, embedded food, bakery, and confectionery products), beverages, and medicines. Brown ginger is produced from unpeeled rhizomes, whereas white ginger comes from skinned rhizomes.¹⁴⁰ The major pungent constituents of ginger oil are the gingerols. The steam distillation cannot recover these pungent components because they are thermally degraded to shogaols, volatile aldehydes, or ketones. Industrially, ethanol, acetone, trichloroethane, and dichloroethane are used to recover gingerols. Another preferred alternative is CO₂ extraction when the ginger extract is intended to be used in high-quality formulations. For use in soft drinks, CO₂ extracts offer both pungency and flavor in the most stable form and can be used for bottled syrup.¹⁰⁷ Furthermore, CO₂ extract is closer to the original raw material in terms of sensory characteristics.¹⁴²

Ginger extract has also been used for over 5000 years in Asia for therapeutic purposes. It is indicated for the treatment of diseases of the gastrointestinal and respiratory systems, arthritis, cramps, dementia, infectious diseases, muscle pains, sprains, migraine, fever, hypertension, impotence, heart palpitations, rheumatism, and even cancer.^{143–147}

Leaves. Numerous leaves are used as source of essential oils. Among them, basil, oregano, rosemary, and pepper oils are the most important to food industries due to their spicy and herbal flavors.¹⁰⁶ Basil is an herbaceous plant cultivated as a culinary herb in Europe. The essential oil of basil is generally obtained by steam distillation or hydro-distillation from the leaves of the plant. About 140 components of basil oil are known, mostly oxygenated monoterpenes and phenylpropane derivatives; the major compounds are methyl chavicol, linalool, 1,8-cineole, and eugenol.¹⁴⁸ The oil is mainly used in seasoning blends but can be useful in small quantities in a wide range of natural flavors.¹⁴⁰

A rather interesting example of diversity is oregano, which counts as the commercially most valued spice worldwide. More than 60 plant species are used under this common name showing similar flavor profiles characterized mainly

by cymyl compounds such as carvacrol and thymol.¹⁴⁹ Oregano oil is particularly rich in *p*-cymene, which has also been identified in thyme oils.¹⁰⁹ The major component of thyme oil is thymol (37–55%). This oil is used in seasoning blends and in traces in many flavors.¹⁴⁰

Peppermint oil is composed primarily of menthol and menthone, but it is not used for the production of menthol due to its high price. The oil is used to give peppermint flavor to confectionery products, liquors, tobacco, cosmetics, toothpastes, other oral hygiene products, and bubble gum. It is also used in mint and herbal blends.¹⁵⁰

Eucalyptus oil is isolated from fresh or partially dried leaves. It has a characteristic camphoraceous odor and has a pungent, spicy, and cooling taste. Eucalyptus essential oil is commonly used in traditional medicine for its expectorant and balsamic activities. Although more generally associated with medicinal use, it is also used in perfumery; the main oil component, 1,8-cineole (sometimes referred to as eucalyptol), contributes to its fragrance. Eucalyptus oil can also be used as a cleaning agent and as an insect repellent.¹⁵¹

The leaves of rosemary (*Rosmarinus officinalis* L.) are best known as a spice and flavoring agent but they are also reported as herbal remedy with antioxidant, anti-inflammatory, anticarcinogenic, antidiuretic, and hepatotoxic protective properties.¹⁵² The major components of its essential oil are α - and β -pinene, camphene, and camphor. The main use of the rosemary oil is in seasoning blends.¹⁴⁰

The essential oils of leaves are removed by steam distillation or selective solvent extraction. Extraction with volatile organic solvents, such as hexane, petroleum ether, benzene, toluene, ethanol, isopropanol, ethyl acetate, acetone, water, etc., is also commonly used, but this process can co-extract some undesirable components, depending on their polarity and on the solvent polarity.¹⁰⁷

Resins. Copaiba oil is an oleoresin obtained by tapping the trunk of the trees from several *Copaifera* L. species (Leguminosae). It is extensively commercialized in Brazil as capsules or crude oil. Copaiba oil is characterized by its terpenic content; the major compounds are volatile sesquiterpenes like β -caryophyllene, α -copaene, and α -humulene.¹⁵³ It is also rich in kaurenoic acid, a diterpene that has been shown to exert anti-inflammatory, hypotensive, and diuretic effects *in vivo* and antimicrobial, smooth muscle relaxant, and cytotoxic actions *in vitro*.¹⁵⁴ The cosmetic industry uses copaiba oil in shampoos, capillary lotions, and bathing foams.¹⁵⁵

Flowers. The characteristic fragrances of flowers are due to the presence of volatile essential oils in their petals. These oils may occur in a free form as in rose, or in a combined form (as glycosides) as in jasmine. The recovery of flavors and fragrances from flowers is crucial because of their short life span. Besides, due to natural enzyme reactions, there is a continuous change in the odor profile. The extraction of essential oils from flowers can be carried out

by a variety of both old and new processes. Maceration and enfleurage are the most primitive methods used, but they are tedious, time-consuming, and inefficient. Therefore, they have been replaced by the solvent extraction method or most efficiently, by supercritical fluid extraction.¹⁰⁷

Jasmine is only successfully extracted by solvent extraction, not by steam distillation. Like many flowers used in perfumery, the hot steam would alter and destroy the floral accords for which jasmine is so prized.¹⁵⁶ In France jasmine is traditionally extracted by enfleurage. The major component of jasmine oil is the benzyl acetate. Indole also makes a very significant odor contribution to it, but it also occurs in many other essential oils.¹⁰⁹ Jasmine enjoys extensive use in perfumery in a large variety of compositions for its intense, tenacious, warm, sweet-floral note. Cosmetic and toiletry products also use its aromatic benefits. In the food flavoring industry jasmine is used in alcoholic and soft drinks and in a wide range of food products.¹⁵⁶

The essential oil extracted from the dried flower buds of clove, *Eugenia caryophyllata* L. (Myrtaceae), is used as a topical application to relieve pain and to promote healing and also finds use in the fragrance and flavoring industries, due to a characteristic clove-like aroma and burning, spicy flavor.^{140,157–159} The main constituents of its essential oil are eugenol (around 75%), eugenyl acetate, and β -caryophyllene (10–15% each). In food industry, clove oil's main use is as flavoring, antimicrobial, and antioxidant agent.^{160–168} It also has fungicidal, antiviral, antitumor, and insecticide properties, besides acting on gastrointestinal disorders and respiratory diseases, which favors its use in pharmaceutical applications.^{127,166–170}

Lavender essential oil has been used for centuries for a variety of therapeutic and cosmetic purposes. It is usually produced by steam distillation, from both the flower heads and foliage, but the chemical composition differs greatly, with the sweeter and most aromatic oil being derived from the flowers. Major components are linalool (40%) and linalyl acetate (25%). The lavender products are mainly used in fragrances, for example, with combination with bergamot oil, in Earl Grey tea flavors, and they are also often used in aromatherapy or incorporated into soaps and other products as a pleasant fragrance or as an antimicrobial agent.^{140,170}

Hop extracts are used by the brewing industry to give bitterness and aroma to beer. The major components of hop essential oil are hydrocarbon terpenes, of which the most abundant are the monoterpene myrcene and the sesquiterpenes α -humulene and β -caryophyllene. Although the terpenes comprise well over 90% of the total oil of a fresh hop, their importance as such to the flavor of beer is generally inconsequential, as they are all virtually water-insoluble and have relatively high flavor thresholds. Amongst the sesquiterpenes, humulene in particular is a precursor to some oxygenated compounds that may positively influence beer flavor.¹⁷¹

Chamomile is a common flowering plant and a member of the daisy family. There are two types: German (*Matricaria recutita*) and Roman chamomile (*Chamaemelum nobile*). Major components of Roman chamomile are isobutyl angelate (30%), isoamyl angelate (12–22%), and other esters. German

chamomile oil contains α -bisabolol oxide (40%), farnesenes (20%), and chamazulene (6%), resulting in its characteristic blue color. German chamomile is much less useful in flavor terms than the Roman chamomile oil. The Roman chamomile oil is used in many natural fruit flavors, particularly apple, pear, peach, apricot, mango, and passion fruit.¹⁴⁰

Ylang-Ylang (*Cananga odorata*) essential oil is derived from the flowers, and it is primarily extracted by water or water-and-steam distillation. This distillation is typically interrupted multiple times based on specific gravity, thereby yielding fractions of varying desirability and value from a perfumery perspective. This oil has a medium to strong initial aroma that is described as fresh, floral, sweet, slightly fruity, fragrant yet delicate. In general terms, it consists of sesquiterpene hydrocarbons, alcohols, esters, ethers, phenols, and aldehydes. Ylang-Ylang oil is used topically as a sedative, antiseptic, hypotensive, and aphrodisiac. In addition, it is used in foods and beverages as a flavoring agent and in cosmetics and soaps as a fragrance.¹⁷²

Peels. Limonene is naturally found in many essential oils, especially citrus fruit peel, such as bergamot, grapefruit, lemon, lime, and orange.¹¹⁹ Although the major component of the grapefruit oil is limonene (88–95%), nootkatone (0.2%) is the most important odor component;¹⁰⁹ other components are α - and β -pinene (<12%), γ -terpinene (<9%), and citral (geranial and neral, <3%). Its main use is in soft drinks and confectionery, to add juicy character.

Bergamot oil is a complex mixture of more than 300 compounds. Major components are limonene (30–45%), linalyl acetate (22–36%), linalool (3–15%), γ -terpinene (6–10%), and β -pinene (6–10%); minor compounds comprise geranial, neral, neryl acetate, geranyl acetate, and bergaptene.¹⁴⁰ The major use of bergamot is to impart citrus flavor to food, beverages, and confectionery. Bergamot oil was also a component of the original Eau de Cologne.

Orange oil is widely used in orange flavors and many other natural flavors. Lemon oil is also widely used in lemon and other natural flavors, such as pineapple, butterscotch, and banana, and can be mixed with other citrus oils, such as lime, orange, and grapefruit.¹⁴⁰

Citrus oils constitute the largest sector of the world essential oil production.¹⁷³ Cold expression is the process usually applied to recover essential oils from lemon, bergamot, and orange peels or when essential oils are highly thermolabile. In this process, oil cells are broken by rolling the peels in hollow vessels fixed with spikes on the inside surface for the abrasion of the peel, allowing the oil to ooze out from the outside surface in the form of an aqueous emulsion, which is subsequently centrifuged. Citrus oils obtained by this process have superior odor characteristics when compared to steam distilled oils, because of the non-thermal processing. However, the unavoidable raise of temperature due to the mechanical friction in the process causes some thermal degradation, the result of which is that cold pressed oil is dark in color.¹⁰⁷

Roots. Roots are source of valerian oil. This perennial herb is indigenous to Europe and Asia. The main components are bornyl acetate (32–44%), camphene (16–25%), and α - and β -pinene (6–12%). However, the most important flavor component is isovaleric acid (1–4%). The oil is used in many fruit flavors but at low levels.¹⁴⁰

1.2.3 Edible Fats and Oils

Edible fats and oils are water-insoluble substances that consist predominantly of glyceryl esters of fatty acids, or triglycerides, with some non-glyceridic materials in small or trace quantities. The choice of the terms ‘fats’ and ‘oils’ is usually based on the physical state of the material at ambient temperature; fats appear solid and oils appear liquid.¹⁷⁴

The processing of edible fats and oils involves a series of stages in which both physical and chemical changes are made to the raw material. Processing is initiated by an extraction or rendering process to remove the fat or oil from the seed, bean, nut, fruit, or fatty tissue. The crude fats and oils recovered contain compounds responsible for the development of undesirable odors, flavors, and colors; therefore, several further steps of processing are carried out to remove the unwanted compounds.¹⁷⁵ After extraction, the processing of vegetable oil almost always includes neutralization or refining, bleaching, and deodorization. Rendered animal fats are normally clarified to remove impurities, bleached, and deodorized. Clarification, neutralization, bleaching, and deodorization are all purification processes which affect the flavor, flavor stability, and appearance of the fat or oil product while removing harmful impurities.¹⁷⁴

1.2.3.1 Sources of Fats and Oils

Fats and oils occur naturally in a wide range of sources, including oil seeds, fruit pulp, animals, and fish. Oil seeds are the major source for the production of edible oils; seeds specifically grown for the production of oil or protein include corn, soybean, canola, rapeseed, sunflower, palm, and olive. Other sources of vegetable oils include by-products of crops grown for fiber, such as cottonseed and flaxseed, crops grown for food and their co-products, such as corn germ, wheat germ, rice bran, coconut, peanuts, sesame, walnuts, and almonds, as well as non-edible crops, such as castor, tung, and jojoba. Animal fats can be obtained from a variety of animal tissues, such as beef, chicken, pork, and fish. Examples of edible animal fats are butter, lard (pig fat), tallow, ghee, and fish oil. They are obtained from fats in the milk, meat, and under the skin of the animal. Fish oil is the lipid extracted from the body, muscle, liver, or other organ of the fish. Oils and fats can still be obtained from microbial products, algae, and seaweed. There are many physical and chemical differences among these diverse biological materials that define the characteristics of the individual fat or oil, which in turn determines the suitability of this ingredient in applications.^{176–178}

1.2.3.2 Commercial Application

Edible fats and oils are the raw materials for oils, shortenings, salad dressing, margarines, and other specialty or tailored products that are functional ingredients in food products prepared by food processors, restaurants, and at home. The major non-food product uses for fats and oils are soaps, detergents, paints, varnish, animal feed, resins, plastics, lubricants, and fatty acids.¹⁷⁴

Shortening Products. Shortening was originally the term used to describe the function performed by naturally occurring solid fats like lard and butter in baked products.¹⁷⁹ The term 'bakery' includes not only the production of bread, but also all food products in which flour is the basic material and to which heat is applied directly by radiation from the walls or top and bottom of an oven or heating device. Therefore, it includes the production of bread, cake, pastry, biscuits, cracker, cookies, pies, toppings, frostings, fillings, etc. Shortenings are very important ingredients for the baking industry because they comprise from 10% to 50% of most baked products. Their functions include: (1) imparting shortness, or richness and tenderness, to improve flavor and sensory characteristics; (2) enhancing aeration for leavening and volume; (3) promoting desirable grain and texture qualities; (4) providing flakiness in pie crusts, Danish, and puff pastries; (5) providing lubrication to prevent the wheat gluten particles from adhering together, which retards staling; (6) enhancing moisture retention for shelf-life improvement; and (7) providing structure for cakes, icings, and fillings.¹⁷⁹ Today, shortening has become virtually synonymous with fat, and it includes many other types of edible fats designed for purposes other than baking.

Spread Products

Cocoa butter. Cocoa butter is the natural vegetable fat obtained through the crushing and grinding of cocoa beans. It contains glycerides of stearic, palmitic, and lauric acids. Cocoa beans are the source of two important ingredients of chocolate: cocoa powder and a solid fat called cocoa butter.¹⁸⁰ Besides, cocoa butter is a traditional emollient employed in several cosmetic products for skin, hair, and lips care; it is considered the most known and most stable butter of natural origin.¹⁸¹

Cupuassu butter. The seeds of cupuassu, a Brazilian Amazonian fruit, contain high amounts of fat (around 60%) with digestibility and chemical and sensory characteristics similar to cocoa butter, although they have a different fatty acid profile. The seeds have a big potential to substitute cocoa in chocolate production.¹⁸² In cosmetic products, cupuassu butter can be used as emollient to soften the skin. The seeds have not been widely explored and in most situations they are still used by farmer as animal feed.¹⁸³

Margarine. Margarine was developed as a butter substitute. It is a flavored food product containing 80% fat and fortified with vitamin A. It is made by blending selected fats and oils with other ingredients such as milk, emulsifiers, preservatives, and coloring agents, to produce a table, cooking, or baking fat product that serves the purpose of dairy butter, but is different in composition and can be used for different applications.¹⁷⁴ Margarine production involves three basic steps: emulsification of the oil and aqueous phases, crystallization of the fat phase, and plasticification of the crystallized emulsion.¹⁸⁴ Over 10 different types of margarines are produced today, including regular, whipped, soft tub, liquid, diet, spread, no fat, restaurant, baker's, and specialty. These margarines are made from a variety of fats and oils, including soybean, cottonseed, palm, corn, canola, safflower, sunflower, lard, tallow, palm kernel, and coconut. Margarine products cater to the requirements of all the consumers: retail, food service, and food processor.¹⁷⁹

Liquid Oils. Liquid oils are usually identified by their physical state at ambient temperature and classified according to their functionality traits: cooking, salad, and high stability. Cooking oils are typically used for pan frying, deep fat frying, sauces, gravies, marninates, and other non-refrigerated food preparations where a clear liquid oil has application. Salad oils are suitable for the production of mayonnaise or salad dressing emulsion and are stable at low temperatures. The high stability oils possess an exceptional oxidative or flavor stability, and are a clear liquid at room temperature.¹⁷⁴ The source of liquid oils available are canola, corn, cottonseed, olive, peanut, safflower, soybean, palm, sunflower, their blends, and some other specialty oils.

Sunflower oil. Sunflower oil is the non-volatile oil extracted from sunflower (*Helianthus annuus*) seeds. It is commonly used in food as frying oil, and in cosmetic formulations as an emollient. Typically up to 90% of the fatty acids in conventional sunflower oil are unsaturated, namely oleic (16–19%) and linoleic (68–72%). Palmitic (6%), stearic (5%), and minor amounts of myristic, myristoleic, palmitoleic, arachidic, behenic, and other fatty acids account for the remaining 10%.¹⁸⁵ Sunflower oil also contains lecithin, tocopherols, carotenoids, and waxes. The three types of sunflower oils produced are high linoleic, high oleic, and mid oleic. High linoleic sunflower oil typically has at least 69% linoleic acid. High oleic sunflower oil has at least 82% oleic acid. Variation in fatty acid profile is strongly influenced by both genetics and climate.¹⁷⁸

Corn germ oil. Corn oil is the oil extracted from the germ of corn (maize) and is almost entirely used for food. Corn oil is regarded as exceptional in flavor and quality, with a healthy image for incorporation into processed foods, and also for snack food frying due to its high smoke point. It is also a key ingredient in mayonnaise and salad dressings.¹⁸⁰ Corn oil contains 9–17% palmitic acid, 20–42% oleic acid, and 39–63% linoleic acid. Refined

corn oil is 99% triglycerides, with proportions of approximately 59% of polyunsaturated fatty acids, 24% of monounsaturated fatty acids, and 13% of saturated fatty acids.¹⁷⁸

Safflower oil. Safflower oil is flavorless and colorless. It is used mainly as a cooking oil and for the production of margarine. It may also be taken as a nutritional supplement.¹⁷⁸ This oil exhibits the highest polyunsaturated level and polyunsaturated/saturated ratio levels commercially available. Its lack of wax, low free fatty acids, and low unsaponifiable levels allow it to be easily refined and deodorized. It contains low levels of phosphatides and unsaponifiables. The phospholipids included are phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl myoinositol, and phosphatidyl serine. The major fatty acid found in the phosphatides is linoleic acid, and the unsaponifiables are mostly sterols and terpenes.¹⁸⁶

Soybean oil. Soybean oil is less expensive than corn, safflower, and sunflower oils, yet it has many of the desirable characteristics of premium vegetable oils: it has a high level of unsaturation compared to some other vegetable oils. Crude soybean oil contains approximately 95–97% triglycerides, formed by both saturated and unsaturated fatty acids. It contains a high concentration of polyunsaturated linoleic and linolenic acids.¹⁸⁷ Soybean oil stands out for its nutritional qualities, permanent supply, considerable economic value, and high functionality. It is an important source of natural lecithin, tocopherols, and phytosterols for pharmaceutical and food uses. This oil can be used as a solvent, a lubricant, and as biodiesel after suitable modification.¹⁷⁸

Olive husk oil. Olive husk is a solid residue derived from olive oil extraction. Its main constituents are water, oil, olive peel, and kernels. This residue contains fat levels in the range of 20–25%, which are recovered by treating olive husk with organic solvents, usually hexane. The crude olive husk oil must be refined to be edible. This oil is similar to olive oil and it is gaining importance in the food industry.^{188,189} Refined olive and husk oils differ little in fatty acid composition; oleic acid is the main component, with minor but nutritionally relevant contributions from palmitic acid and the essential linoleic acid. Among the substances with antioxidant properties, the total phenols content has a strong positive contribution for the high stability (shelf-life) of these oils, but they have a very different phenolic composition.¹⁹⁰

Grape seed oil. Grape seed oil has been applied in various fields from cosmetics to cooking. The oil has a relatively high smoke point, around 216 °C, and it can be safely used for cooking at high temperatures. Grape seeds have 10–20% oil and large amounts of vitamin E. The oil presents several benefits for human health, due to the high content of unsaturated fatty acids and antioxidant compounds like monomeric flavan-3-ols, phenolic

acids, and oligomeric proanthocyanidins.^{191–194} In the conventional extraction process, the seeds are pressed, and then extracted with n-hexane, but the recovery of grape seed oil by supercritical technology as an alternative process, especially because of n-hexane's high flammability and hazardous effects to human health, has recently been studied. Grape seed oil consists mainly of triglycerides, which are rich in unsaturated fatty acids such as oleic and linoleic acids.¹⁹⁵

Palm oil. Palm fruits from the Amazon region, belonging to the Arecaceae family, promise to be an alternative and abundant source of vegetable oils with high nutritional value. Palm oil is obtained by pressing the palm oil (*Elaeis guineensis*) fiber. It is one of the few vegetable oils relatively high in saturated fats. It contains almost equal proportions of saturated (palmitic 48%, stearic 4%, myristic 1%) and unsaturated acids (oleic 37%, linoleic 10%). Valuable by-products obtained from palm oil are carotene, tocopherols and tocotrienols (vitamin E), and palm-fatty acid distillate. Palm oil is reddish because it contains high amounts of α - and β -carotene and it is very nutritious due to the high amounts of vitamin E.¹⁷⁸

Palm oil is used mainly for food purposes but also in cosmetic products, engine lubricants, and biofuel production.¹⁹⁹ Because it is semi-solid at ambient temperature, it is a good natural hardstock for shortenings, margarines, vanaspati, and processed foods such as cream fillings, ice-cream, filled milk, coffee whiteners, whipping creams, infant formula, dry soup mixes, and salad oil.¹⁹⁷

Buriti oil. Buriti oil is extracted from buriti (*Mauritia flexuosa*) fruits, Arecaceae. This oil is composed mainly of fatty acids, tocopherols, and carotenes. The high concentration of monounsaturated fatty acids provides buriti oil with a high nutritional quality and blood cholesterol-lowering properties. In addition, the low concentration of polyunsaturated fatty acids gives buriti oil high oxidative stability. The nutraceutical fraction of buriti oil consists of tocopherols and carotenes, which are natural antioxidants forming vitamin E and pro-vitamin A, respectively.^{198,199} Buriti is the richest known source of carotenoids.²⁰³ The high nutritional value of its oil makes it interesting for the food industry. Besides, it has also been frequently used in cosmetic production.²⁰⁰

Rice bran oil. Rice bran is a by-product of rice milling that contains 15–20% oil by weight. Its oil is edible, it has nutritional value and a nut-like taste; it finds use in cooking and nutritional applications. The major fatty acids in rice bran oil are palmitic, oleic, and linoleic acids, with smaller amounts of stearic and linolenic acids, and traces of other fatty acids. It also contains waxes (2–4%), which are esters of saturated fatty acids with saturated alcohols, monomethyl sterols, dimethyl sterols, and tocotrienols. The latter together with oryzanols impart high oxidative stability to rice bran oil.²⁰¹

Jojoba oil. Jojoba oil is not a typical oil consisting mainly of triacylglycerols; rather, it is a mixture of long-chain esters containing small amounts of triacylglycerols and other materials such as phospholipids and tocopherols. Because of its properties, it has found large application in skin care products and cosmetics.²⁰¹

Wheat germ oil. Wheat germ oil is extracted from the germ of the wheat kernel. It is rich in linoleic acid and also contains α -linolenic, palmitic acid, and oleic acids. The oil shows high vitamin E activity, due to the high content of tocopherols. Wheat germ oil is particularly high in octacosanol as the main active component. Octacosanol is a 28-carbon long-chain saturated primary alcohol found in a number of different vegetable waxes. It can be used as a low density lipoprotein (LDL) control, as a protectant against atherosclerosis and hepatic injury progression, and as antiplatelet, anti-ischemic, and antithrombotic agent, with good tolerance by the human body.^{202–210} Extracts from wheat germ oil have beneficial effects on the physical performance of athletes, due to the octacosanol. As a cooking oil, wheat germ oil is strongly flavored, expensive, and easily perishable.^{178,202}

Biodiesel Feed Stock. Edible oils, non-edible oils, wild oils, used cooking oils, and animal fats have been identified as possible raw materials to produce biodiesel. Soybean, palm, rapeseed, and sunflower oils are used in the industry; the majority of biodiesel produced worldwide is from rapeseed oil, with 84% of total production.¹⁹⁶ The biodiesel production from waste cooking oils is an effective way to reduce the raw material cost and to solve the problem of waste oil disposal.²¹¹ In spite of edible oils being a biodiesel feedstock, its use is significantly affected by the food-versus-fuel issue. Currently, more than 95% of the world biodiesel is produced from edible oil, which is easily available on large scale from the agricultural industry. However, continuous and large-scale production of biodiesel from edible oil without proper planning may cause a negative impact to the world, such as depletion of food supply leading to economic imbalance. A possible solution to overcome this problem is to use non-edible oils or waste edible oils.²¹²

1.2.4 Functional Foods and Nutraceuticals

Essentially all foods and food ingredients play important sensory and nutritional roles providing color, texture, flavor, and nutrients (carbohydrates, proteins, fats, vitamins, and minerals), which are essential to growth, development, maintenance, and other physiological functions of the human body. In addition, some foods and food components may provide extra important functionality, imparting to food health benefits or desirable physiological effects beyond basic nutrition.²¹³ These foods and food components are called functional foods. The ADA (American Dietetic Association)²¹⁴ defines functional foods as ‘any food or modified ingredient that can provide beneficial effect beyond that provided by the nutrients it contains’.²¹⁵ A functional food

must remain food and it must demonstrate its effects in amounts that can normally be expected to be consumed in the diet. It is not a pill or a capsule, but part of the normal food pattern.²¹⁶ In the last decades, nutritional sciences are advancing from the classical concepts of avoiding nutrient deficiencies to nutritional adequacy since there is increasing scientific evidence that consuming some foods and food components may have additional functional effects and may reduce the risk of disease and specifically contribute to maintain the state of health and well-being.

On the other hand, nutraceuticals are health-promoting compounds or products isolated or purified from food sources. The term 'nutraceutical' is often used to refer to a food, dietary supplement or biologically active compound that provides health benefits. A nutraceutical is defined as any substance that may be considered as a food or part of a food and provides medical or health benefits including the prevention and treatment of disease. Nutraceuticals may range from isolated nutrients, dietary supplements, and diets to genetically engineered 'designer' foods and herbal products. Examples are isoflavonoids isolated from soybean, fish oil capsules, herbal extracts, glucosamine, chondroitin sulfate, lutein-containing multivitamin tablets, and antihypertensive pills that contain fish protein-derived peptides.^{217,218}

Functional foods are one of the most promising fields in the nutritional sciences. These foodstuffs are interesting from the consumer point of view with the prospect of maintaining health and preventing diseases by using natural foods as part of the usual diet, and also from the industry point of view, for the added value of the products.²¹⁹ Public health authorities consider prevention and treatment with nutraceuticals a powerful instrument to maintain health and to act against nutritionally induced acute and chronic diseases, thereby promoting optimal health, longevity, and quality of life.^{217,218} Bioactive functional ingredients can come from a variety of sources, including plants, animals, and microorganisms. Some lipid-based materials, such as phosphatidylcholine and sphingolipids, can be recovered from all three of them. Plants provide the greatest variety of bioactive ingredients, especially terpenes and phenolics. The carbohydrates are also primarily found in plant-based products. Amino acids, proteins, and peptides can come from plants, animals, or microbial fermentation. Many interesting bioactive peptides have been recovered from milk.

The major classes of bioactive ingredients found in functional foods and nutraceuticals are: polyunsaturated fatty acids (methylene-interrupted polyenes, conjugated fatty acids, pinolenic acid, etc.); phenolic compounds, which comprise natural monophenols, flavonoids (flavonols, flavanones, flavones, flavan-3-ol, anthocyanins, isoflavones), phenolic acids, hydroxycinnamic acids, lignans (phytoestrogens), and tyrosol esters; terpenes that include carotenoids (carotenes and xanthophylls), monoterpenes, and saponins; phytosterols (β -sitosterol, campesterol, stigmasterol, sitostanol, campestanol, etc.); tocopherols (vitamin E); betalains (betacyanins and betaxanthins); organosulfides (dithiolthiones, polysulfides, sulfides); indoles (glucosinolates/sulfur compounds); protein inhibitors; and other organic acids.^{14,111,220,221}

One important class is terpenoids. Phenolics are also a major group; it is composed of extremely diverse compounds, which exert significant bioactivity. As examples, isoflavones from soybean are used to reduce LDL cholesterol, and anthocyanins and phenolic acids are strong antioxidants used to reduce the expression of proinflammatory genes in *in vitro* systems. Carbohydrates generally deliver fiber, which enhances digestive health. Still in the gastrointestinal system, prebiotics are fermented by the gut flora, resulting in the production of short-chain fatty acids in the colon. Probiotics, on the other hand, contain microorganisms that when ingested may help to establish a healthier gut flora.

Phytochemicals may be present in indigenous plants or crops (food), spices, seaweed, fungi, lichens, mosses, and microorganisms. In plants, they can be found in fruits, berries, seeds, leaves, needles, stems, branches, roots, bulbs, flowers, barks, buds, shoots of wood, etc.^{14,222}

1.2.4.1 Polyunsaturated Fatty Acids

The polyunsaturated fatty acids (PUFAs) contain more than one double bond in their structure. PUFAs can be classified in various groups by their chemical structure: (1) methylene-interrupted polyenes; (2) conjugated fatty acids; and (3) other polyunsaturates. The methylene-interrupted polyenes comprise the ω -3 essential fatty acids (hexadecatrienoic acid, α -linolenic acid, stearidonic acid, etc.), ω -6 fatty acids (linoleic acid, γ -linolenic acid, eicosadienoic acid, etc.), and ω -9 fatty acids (oleic acid, erucic acid, mead acid, etc.). The conjugated fatty acids have two or more conjugated double bonds, like linoleic acids (rumenic acid) and linolenic acids (β -calendic acid). Pinolenic acid and podocarpic acid are examples of other polyunsaturates.

Currently, the consumption of products naturally containing PUFAs, such as fish, has decreased; these changes in food habits of the industrialized countries may be related to the increased rates of many diseases related to inflammatory processes. Some studies show that consuming food products containing ω -3 fatty acids can alleviate symptoms of several psychiatric disorders.^{223,224} The biological effects of ω -6 fatty acids are used to develop pharmaceutical drugs and treatments for atherosclerosis, asthma, arthritis, vascular disease, thrombosis, inflammatory-immune processes, and cancer.^{225,226} Unlike the ω -3 fatty acids, the ω -6 and ω -9 fatty acids are not classified as essential fatty acids because they can be synthesized by the human body from unsaturated fat.²²⁷

With respect to the conjugated fatty acids, linoleic acid is distinguished by its importance in the manufacture of quick-drying oils, which are useful in oil paints and varnishes. Linoleic acid has also become increasingly popular in the industry of beauty products because of its beneficial properties on skin, in the pharmaceutical industry as an anti-inflammatory and acne-reducing agent, and in the food industry for its antioxidant effects on natural phenols.^{228,229}

1.2.4.2 Phytoesters

The phytosterols and phytostanols, the saturated form of phytosterols, are steroidal compounds similar to cholesterol, but from plant origin; they vary only in their carbon side chains and/or presence or absence of a double bond. Vegetable oils and products containing them can be rich sources of phytosterols. Grain products, vegetables, fruits, and berries are not as rich in phytosterols as vegetable oils, but they can also be significant sources of phytosterols due to their high consumption, reaching 150–450 mg/day. Most common phytosterols in the human diet are β -sitosterol (65%), campesterol (30%), and stigmasterol (3%). Over 200 stanols have been identified, and the most common in the human diet are sitostanol and campestanol, which combined constitute about 5% of dietary phytosterols.^{230,231}

Free phytosterols extracted from oils are widely used in fortified foods and dietary supplements. Commercially available products containing plant sterols and/or stanols in their free forms and ester type include margarine, yoghurt, yoghurt drinks, and orange juice. As tablets and capsules, they are particularly attractive because of the ease of incorporating these in a regimen of cholesterol reduction when compared to diet.^{232–234} The plant sterols and stanols are known to reduce low density lipoprotein (LDL) serum cholesterol levels, and foodstuffs containing such compounds of plants are widely used as a dietary therapeutic option to reduce plasma cholesterol and the risk of atherosclerosis. However, recent evidence suggests that phytosterols/phytostanols may regulate proteins involved in cholesterol metabolism in both hepatocytes and enterocytes, although its effects have not been proven to reduce cardiovascular disease risk or overall mortality.^{235,236}

1.2.4.3 Tocopherols and Tocotrienols

Tocos comprise a class of chemical compounds that comprise various methylated phenols and from which many have vitamin E activity. Four tocopherols and four tocotrienols compose vitamin E. Both tocopherols and tocotrienols occur in groups of four (α , β , γ , δ) lipophilic antioxidants synthesized by photosynthetic organisms (Figure 1.11). The tocopherols occur mainly in seeds and leaves of plants. The seed oils (olive, sunflower, corn, and soybean) contain high concentrations of γ -tocopherol and leaf lettuce contains high concentration of α -tocopherol.^{237–239}

The vitamin E form preferentially absorbed and accumulated in the human body is α -tocopherol. This form is available in foods of the every day diet, such as vegetable oils, grains, peanut, corn, poppy seeds, asparagus, oat, chestnut, coconut, tomato, walnut, carrot, and goat milk.²⁴⁰ α -Tocopherol has numerous biological properties; however, it causes indigestion, thus its bioavailability in the intestine is affected. Therefore its consumption as nutritional supplements in the form of tocopherol succinate and tocopherol acetate is indicated.²⁴¹

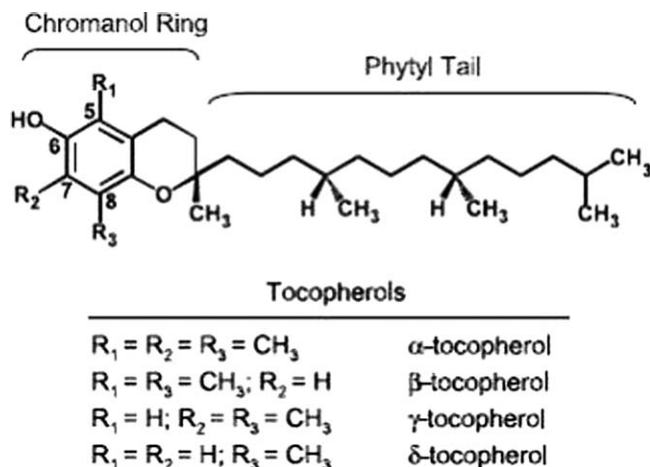


Figure 1.11 Structures of tocopherol variants (alpha, beta, gamma, delta). Modified from Lee *et al.*, 2009.²⁴⁶

In general, tocopherols and tocotrienols are fat-soluble antioxidants that may have many biological functions, such as relieving stress situations and premenstrual tension, preventing cellular damage, improving blood circulation, tissue regeneration, and intermittent claudication, among others. Additionally, the antioxidant activity of tocopherols is associated with inhibition of membrane lipid peroxidation and the elimination of reactive oxygen species.²⁴² Vitamin E (α -tocopherol) is also recognized for preserving fertility in mammals.^{243,244}

1.2.4.4 Ginseng

Ginseng is a slow growing perennial plant with fleshy roots that has two large genera: *Panax* (*Panax quinquefolius* L., *Panax ginseng* CA Meyer), also known as Asian Ginseng, and *Pfaffia* (*Pfaffia iresinoides*, *Pfaffia glomerata*, and *Pfaffia paniculata*), known as Brazilian Ginseng. Ginseng is found mainly in the Northern Hemisphere and eastern Asia, usually in colder climates. Among the countries of South America, Brazil stands out as the most important center for the cultivation of plants of the genus *Pfaffia*.^{245–247}

The dried root of *Panax ginseng* species contain saponins as active ingredients, called ginsenosides. In the case of *Pfaffia* species the main compounds are sitosterol, stigmasterol, allantoin, pfaffic acid, and pfaffosides A, B, C, D, E, and F.²⁴⁸ The chemical structures of ginsenoside and pfaffic acid are shown in Figure 1.12.

Ginseng is marketed in energy drinks, tea, or capsules containing powdered root, mixed or not with ethanol extracts of these plants.²⁴⁹ The use of ginseng as a dietary supplement is related to its medical properties: improving physical and mental performances, especially by relieving symptoms of endocrine, immune, cardiovascular and central nervous systems.^{250,251} The Brazilian

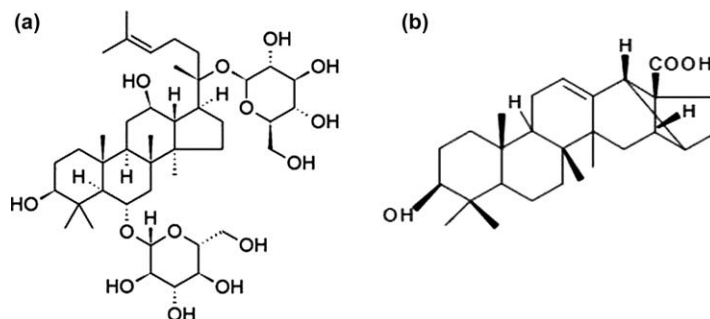


Figure 1.12 Chemical structure of (a) ginsenoside and (b) pfaffic acid.

Ginseng is marketed for the same purposes as Asian Ginseng, except that the bioactive compounds responsible for its invigorating properties belong to a different class, pfaffosides, as opposed to ginsenosides in the case of Asian Ginseng. Brazilian Ginseng acts as a cell regenerator, and it is indicated for physical and mental exhaustion and for treatment of circulatory irregularities, stress, anemia, diabetes, etc.^{252,253}

1.2.4.5 Carotenoids

In the plant kingdom, there are four main groups of bioactive compounds: nitrogenous substances, sulfurous substances, terpenes and phenolics. Carotenoids belong to the terpenes. They have been discussed for their coloring properties (see Section 1.2.1), but they also present extremely important biological properties.²⁵⁴ The carotenoids are the main dietary source of vitamin A precursors, especially in poorer countries. Although β -carotene is the main compound with pro-vitamin A activity, any carotenoid with at least one unsubstituted β ring, such as α -carotene and β -cryptoxanthin, have the added advantage of being able to be converted to vitamin A.⁴⁰ Furthermore, the interest in carotenoids has been increasing due to epidemiological studies that strongly suggest that consuming carotenoid-rich foods reduces the incidence of several diseases such as cancer, cardiovascular diseases, age-related macular degeneration, cataracts, diseases related to low immune function, and other degenerative diseases.^{10,37,40,41} The antioxidant properties of carotenoids have been suggested as being the main mechanism by which they afford their beneficial effects.

Although more than 700 carotenoids have been identified in nature only 20 have been identified in human blood and tissues. At about 90% of the carotenoids in the human diet and body are β - and α -carotene, which are commonly found in yellow-orange vegetables and fruits; α -cryptoxanthin is present in orange fruits; lutein is provided by dark green vegetables; and lycopene is obtained from tomatoes and its products.¹⁰

Even though lycopene is a carotenoid with no pro-vitamin A activity⁷¹ it is an important antioxidant and free radical scavenger.⁷⁰ Due to its 11 conjugated

and 2 non-conjugated double bonds, it was found to be a more efficient antioxidant (singlet oxygen quencher) than β -carotene, α -carotene, and α -tocopherol.⁷⁶ Lycopene plays an important role in human health through the protection against many degenerative diseases such as cancer, atherosclerosis, cataracts, and age-related macular degeneration, as well as to premature aging.⁷⁰ Processed foods are frequently fortified with carotenoids such as lycopene to increase their nutritive value and/or enhance attractiveness.⁷²

The quality of paprika is evaluated according to the red color intensity and to its pungency. Its degree of pungency originates from the group of components called capsaicinoids. They are vanillylamides of branched fatty acids, with 9–11 carbons, of which capsaicin (vanillylamide of 8-methylnona-*trans*-6-enoic acid) and dihydrocapsaicin (vanillylamide of 8-methylnonanoic acid) occur in quantities higher than 80%.⁵³ They play an important role in human health as antibacterials, antioxidants, and immunoenhancers, helping to prevent cancer, cardiovascular diseases, age-related macular diseases, degeneration, cataracts, diseases related to low immune function, arthritis, cystitis, and other degenerative diseases.^{40,53} Furthermore, of the paprika carotenoids, β -carotene and β -cryptoxanthin also have pro-vitamin A activity.²¹

1.2.4.6 Phenolics

Phenolics are a diverse group of aromatic secondary plant metabolites that are widely distributed throughout the plant kingdom. They originate from phenylalanine and, to a lesser extent, from tyrosine.²⁵⁵ They comprise compounds that possess at least one aromatic ring bearing one or more hydroxyl groups.²⁵⁶ Phenolic compounds can be divided into at least 10 different classes depending on their chemical structure, which basically include phenolic acids (simple phenols) and polyphenols (complex phenols), depending on the number of phenol subunits attached to it. Phenolic acids possess just one phenol subunit, comprising thus low molecular weight compounds. Polyphenols possess two or more phenol subunits including intermediate (flavonoids) or high (hydrolysable or condensed tannins, stilbenes, and lignans) molecular weight compounds.²⁵⁶

Phenolic acids are widely represented in plant kingdom. They are mainly located in the cell wall of plants and their main sources are fruits and vegetables.²⁵⁷ Two classes of phenolic acids can be distinguished: the hydroxybenzoic (HBA) and hydroxycinnamic acid (HCA) derivatives. The hydroxycinnamic acid derivatives are aromatic compounds with a three-carbon side chain (C6–C3); *p*-coumaric, caffeic, and ferulic acids are the forms that occur most frequently, usually as simple esters with hydroxy carboxylic acids or D-glucose. On the other hand, the hydroxybenzoic acids have in common the C6–C1 structure, and include *p*-hydroxybenzoic, gallic, and ellagic acids; they are presented mainly in the form of glucosides.²⁵⁸

Polyphenols possess two or more phenol subunits including intermediate (flavonoids: anthocyanins, flavonols and flavones, flavanones, chalcones and dihydrochalcones, isoflavones, and flavanols) or high (hydrolysable or

condensed tannins, stilbenes, and lignans) molecular weight compounds. The polyphenols are also generally divided into hydrolyzable tannins, which are gallic acid esters of glucose and other sugars and phenylpropanoids such as lignin, flavonoids, and condensed tannins. The phenol substructures of polyphenols have various further nomenclatures depending on the number of phenolic hydroxyl groups (Figure 1.13).

The main sources of polyphenols are berries, tea, beer, grapes/wine, olive oil, chocolate/cocoa, nuts, peanuts, pomegranates, yerba mate, and other fruits and vegetables. Obviously, each matrix type has a different polyphenol composition and concentration. As an example, whilst hydroxycinnamic acids are the main polyphenolic compounds in coffee, they also exist in tea, although at lower concentrations.^{259,260} The dominating polyphenolic compounds found in tea are flavonols or flavones.^{261,262} The polyphenol compounds of mate tea can be used as natural antioxidants to increase the shelf-life of various foods, processed and unprocessed, suggesting that the incorporation of polyphenolic extracts of yerba mate in foods can improve their nutritional and sensory quality as well as extending their shelf-life. The seeds of cocoa are known to be rich in flavanol monomers (+)-catechin and (–)-epicatechin and procyanidin oligomers.^{263,264}

Among the bioactive compounds commonly found in foods, phenolic compounds are amongst the most studied due to their antioxidant properties. There are several reasons for this interest, including the increasing knowledge about reactive oxygen and nitrogen species, the definition of predictive markers for oxidative damage, new evidence linking chronic diseases and oxidative stress, and growing data supporting the idea that some of the health benefits associated with fruits, vegetables, and red wine consumption may be linked to the polyphenolic compounds they contain.^{264–271}

The potential of soybeans as a functional food is being currently explored. Indeed, soybeans and soy foods like soymilk, tofu, and miso are widely promoted and consumed based on assumed relationships between their ingestion and beneficial health effects in humans, including chemoprevention of breast and prostate cancer, osteoporosis, cardiovascular diseases and as a reliever of menopausal symptoms. The basis of this relationship includes the evidence provided by both epidemiological studies showing a lower incidence of these health conditions in Asian countries like Japan and China, where soybean and its derivatives are widely consumed, and intervention studies.²¹⁹

Several classes of phytochemicals have been identified in soybeans, including protease inhibitors, phytosterols, saponins, phenolic acids, phytic acid, and isoflavones.^{272–275} The isoflavones are particularly noteworthy because soybeans are the only significant dietary source of these compounds. Isoflavones are a subclass of flavonoids that are also described as phytoestrogen compounds, since they exhibit estrogenic activity (similar effects to estradiol hormones). The basic characteristic isoflavone structure is a flavone nucleus, composed by two benzene rings (A and B) linked to a heterocyclic ring C (Figure 1.14). The benzene ring B position is the basis for the categorization of

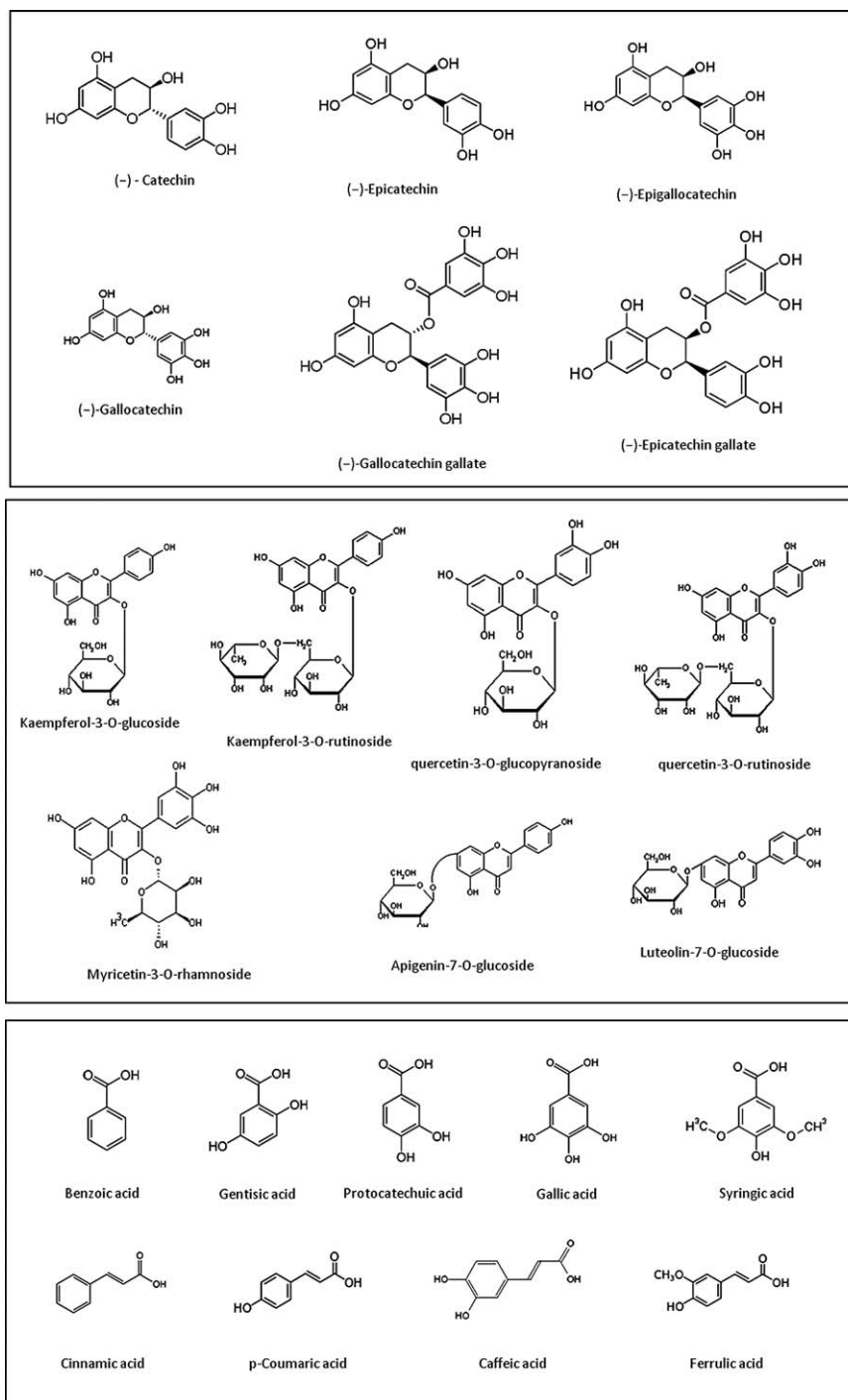
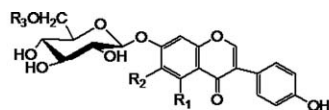
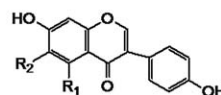


Figure 1.13 Chemical structure of main phenolic compounds.



Glucosides



Aglycones

Isoflavone	Abbrev.	R1	R2	R3
Malonyl Daidzin	MDi	H	H	COCH ₂ COOH
Malonyl Glycitin	MGly	H	OCH ₃	COCH ₂ COOH
Malonyl Genistin	MGi	OH	H	COCH ₂ COOH
Acetyl Daidzin	ADi	H	H	COCH ₃
Acetyl Glycitin	AGly	H	OCH ₃	COCH ₃
Acetyl Genistin	AGe	OH	H	COCH ₃
Daidzin	Di	H	H	H
Glycitin	Gly	H	OCH ₃	H
Genistin	Gi	OH	H	H

Isoflavone	Abbrev.	R1	R2
Daidzein	De	H	H
Glycitein	Gle	H	OCH ₃
Genistein	Ge	OH	H

Figure 1.14 Factors that may be involved in the reduction of cardiovascular disease risk by consumption of soybeans isoflavones.

the flavanoid class (position 2) and the isoflavonoid class (position 3). The main isoflavones found in soybeans are genistein, daidzein, glycitein, and their respective acetyl, malonyl, and aglycone forms.^{276–278}

Isoflavones are being extensively studied because of *in vitro* and *in vivo* biological activity consistent with the potential health effects associated with the consumption of soybeans. There is indication that isoflavones, at least in part, may play a role on the effects of soy foods on improving health.^{279–284}

However, the mechanisms are not yet fully understood and may depend on several factors. The prevention of cardiovascular diseases by soybeans, for example, may depend on the concentration of bioactive components (such as isoflavones), processing and storage conditions of soybeans and foods, the amount, frequency and for how long they are consumed, individuals' genetics and metabolism, among other factors. These factors interact and may directly or indirectly determine an effective reduction of cardiovascular disease risk on a specific subject. At the present time, the scientific data available is solid enough only to point to a possible relationship between soybeans and reduced cardiovascular diseases risk. Isoflavones are also being studied for the relief of menopausal symptoms and as hormone replacement therapy. Although there is no conclusive scientific evidence that isoflavones (or soybeans *in natura*) have positive health effects for the general population, they are increasingly being used as additives in milk and soy beverages and commercialized as nutritional supplements with an important market volume.

1.3 Conclusions

In this chapter we tried to present the most important and useful applications of natural products in order to illustrate their importance. Extraction processes affect the composition and bioactivity of the extracts; that is why it is so important to understand the mechanisms involved in the extraction processes. This and other aspects will be covered in the next chapters. But it is important to highlight that most techniques discussed in the next chapters may undoubtedly be used to extract the phytochemicals presented in this chapter from their natural sources.

Acknowledgements

The authors acknowledge the financial support from CNPq (project 2009/17234-9 and 2010/08684-8) and FAPESP (project 12/10685-8 and 11/19817-1). The authors also acknowledge the contribution of Matheus A. Gigo (FAPESP 2012/11561-0) and Roberta C. C. Celestrino (FAPESP 2012/11459-1) in the revision of the references of this manuscript.

References

1. M. Herrero, A. Cifuentes and E. Ibañez, *Food Chem.*, 2006, **98**, 136.
2. J. Hugenholtz, M. Kleerebezem, M. Starrenburg, J. Delcour, W. M. Vos and P. Hols, *Appl. Environ. Microbiol.*, 2000, **66**, 4112.
3. W. A. Poola, A. R. Nevesb, J. Koka, H. Santos and O. P. Kuipersa, *Met. Eng.*, 2006, **8**, 456.
4. M. Karaaslan, M. Ozden, H. Vardin and H. Turkoglu, *LWT – Food Sci. Tech.*, 2011, **44**, 1065.
5. J. F. Ayala-Zavala, V. Vega-Vega, C. Rosas-Domínguez, H. Palafox-Carlos, J. A. Villa-Rodriguez, M. D. Wasim Siddiqui, J. E. Dávila-Aviña and G. A. González-Aguilar, *Food Res. Int.*, 2011, **44**, 1866.
6. V. Habauzit and M.-N Horcajada, *Phytochem. Rev.*, 2008, **7**, 313.
7. R. H. Liu, *J. Nutr.*, 2004, **134**, 3479S.
8. A. Agarwal, H. Shen, S. Agarwal and A. V. Rao, *J. Med. Food*, 2001, **4**, 9.
9. V. Dewanto, X. Wu, K. K. Adom and R. H. Liu, *J. Agric. Food Chem.*, 2002, **50**, 3010.
10. A. V. Rao and L. G. Rao, *Pharmacol. Res.*, 2007, **55**, 207.
11. S. Bent and R. Ko, *Am. J. Med.*, 2004, **116**, 478.
12. A. C. Williams and B. W. Barry, *Adv. Drug. Deliv. Rev.*, 2004, **56**, 603.
13. K. Cal, K. Kupiec and M. Sznitowska, *J. Dermatol. Sci.*, 2006, **41**, 137.
14. E. Antignac, G. J. Nohynek, J. Clouzeau and H. Toutain, *Food Chem. Toxicol.*, 2011, **49**, 324.
15. J. Delwiche, *J. Food Qual. Pref.*, 2003, **14**, 137.
16. K. K. Park, Y. J. Surh and J. A. Miller, *Cancer Lett.*, 1995, **94**, 33.

17. D. Guo, D. T. Horio, J. S. Grove and R. H. Dashwood, *Cancer Lett.*, 1995, **95**, 161.
18. C. V. Rao, A. Rivenson, B. Simi and B. S. Reddy, *Cancer Res.*, 1995, **55**, 259.
19. S. Izawa, N. Harada, T. Watanabe, N. Kotokawa, A. Yamamoto, H. Hayatsu and S. Arimoto-Kobayashi, *J. Agric. Food Chem.*, 1997, **45**, 3980.
20. O. Hänninen and C. K. Sen, in *Nutraceutical and Functional Food Regulations in the United States and Around the World*, ed. D. Bagchi, Elsevier, Amsterdam, 2008, p. 11.
21. C. Cavaliere, P. Rea, M. E. Lynch and M. Blumenthal, *HerbalGram*, 2009, **82**, 58.
22. B. C. Foster, J. T. Arnason and C. J. Briggs, *Annu. Rev. Pharmacol. Toxicol.*, 2005, **45**, 203.
23. AOAC International, Methods for Dietary Supplements: successful conclusion of a 5-year effort. <http://www.aoac.org/dietsupp6/Dietary-Supplement-web-site/>, 2008.
24. C. Dimitri and C. Greene, *Agric. Info. Bull.*, 2002, **777**, 1.
25. G. R. Gibson and M. B. Roberfroid, *J. Nutr.*, 1995, **125**, 1401.
26. N. Kok, M. Roberfroid, A. Robert and N. Delzenne, *J. Nutr.*, 1996, **76**, 881.
27. M. B. Roberfroid, J. A. E. Van Loo and G. R. Gibson, *J. Nutr.*, 1998, **128**, 11.
28. A. Al-Ahmad, M. W. Al-Ahmad, T. M. Ausschill, M. Follo, G. Braun, E. Hellwig and N. B. Arweiler, *Arch. Oral Biol.*, 2008, **53**, 765.
29. A. L. Delamare, I. T. Moschen-Pistorello, L. Artico, L. Atti-Serafini and S. Echeverrigaray, *Food Chem.*, 2007, **100**, 603.
30. J. K. Kumar and A. K. Sinha, *Nat. Prod. Res.*, 2004, **18**, 59.
31. A. Totosa, in *Handbook of Processed Meats and Poultry Analysis*, ed. L. M. L. Nollet and F. Toldra, CRC Press, 2008, p. 129.
32. J. Wilska-Jeszka, in *Chemical and Functional Properties of Food Components*, 3rd edition, ed. Z. E. Sikorski, CRC Press, 2006, p. 245.
33. C. Socaciu, *Food Colorants*, CRC Press, 2007.
34. F. Delgado-Vargas, A. R. Jimenez and O. Paredes-Lopez, *Crit. Rev. Food Sci. Nutr.*, 2000, **40**, 173.
35. M. B. A. Gloria, in *Handbook of Food Analysis*, 2nd edition, ed. L. M. L. Nollet, CRC Press, 2004, p. 1513.
36. Y.-K. Lee and H.-P. Khng, in *Food Additives*, ed. A. L. Branen, P. M. Davidson, S. Salminen and J. Thorngate, CRC Press, 2001, p. 527.
37. E. S. M. Abdel-Aal and M. H. Akhtar, *Curr. Pharm. Anal.*, 2006, **2**, 195.
38. Q. Su, K. G. Rowley and N. D. H. Balazs, *J. Chromatogr. B*, 2002, **781**, 393.
39. A. V. Rao and L. G. Rao, *Pharmacol. Res.*, 2007, **55**, 207.
40. C. O. Perera and G. M. Yen, *Int. J. Food Prop.*, 2007, **10**, 201.
41. G. E. Bartley and P. A. Scolnik, *Plant Cell*, 1995, **7**, 1027.
42. E. Riggi, *Food Nutr. Agric.*, 2010, **2**, 75.

43. W. B. Van Scheppingen, I. A. L. A. Boogers and A. L. L. Duchateau, *Food Chem.*, 2012, **132**, 1354.
44. C. L. C. Albuquerque and M. A. A. Meireles, *Rec. Pat. Eng.*, 2011, **5**, 94.
45. C. L. C. Albuquerque and M. A. A. Meireles, *J. Supercr. Fluids*, 2012, 86.
46. H. Van Chuyen, N. T. N. Hoi and J. B. Eun, *Int. J. Food Sci. Tech.*, 2012, **7**, 1333.
47. A. Satyanarayana, P. G. P. Rao and D. G. Rao, *J. Food Sci. Tech.*, 2003, **40**, 131.
48. C. R. Cardarelli, M. T. Benassi and A. Z. Mercadante, *LWT – Food Sci. Technol.*, 2008, **41**, 1689.
49. G. F. Silva, F. M. C. Gamarra, A. L. Oliveira and F. A. Cabral, *Braz. J. Chem. Eng.*, 2008, **25**, 419.
50. S. Melo, G. M. N. Costa, A. C. C. Viana and F. L. P. Pessoa, *J. Supercr. Fluids*, 2009, **49**, 1.
51. A. J. Degnan, J. H. Vonelbe and R. W. Hartel, *J. Food Sci.*, 1991, **56**, 1655.
52. R. R. Chao, S. J. Mulvaney, D. R. Sanson, F. H. Hsieh and M. S. Tempesta, *J. Food Sci.*, 1991, **56**, 80.
53. A. Topuz, C. Dincer, K. S. Özdemir, H. Feng and M. Kushad, *Food Chem.*, 2011, **129**, 860.
54. J. P. Fernández-Trujillo, *Grasas y Aceites*, 2007, **58**, 252.
55. S. M. Yusop, M. G. O'Sullivan, M. Preuß, H. Weber, J. F. Kerry and J. P. Kerry, *LWT – Food Sci. Technol.*, 2012, **46**, 349.
56. C. C. Licón, M. Carmona, R. Rubio, A. Molina and M. I. Berruga, *Dyes and Pigments*, 2012, **92**, 1355.
57. B. Montalvo-Hernández, M. Rito-Palomares and J. Benavides, *J. Chromatogr. A*, 2012, **1236**, 7.
58. L. Maggi, M. Carmona, S. D. Kelly, N. Marigheto and G. L. Alonso, *Food Chem.*, 2011, **128**, 543.
59. B. D. Ribeiro, D. W. Barreto and M. A. Z. Coelho, *Food Bioprocess Tech.*, 2011, **4**, 693.
60. D. O. Edem, *Asian J. Clinical Nutr.*, 2009, **1**, 65.
61. D. B. Rodriguez-Amaya, M. Kimura, H. T. Godoy and J. Amaya-Farfan, *J. Food Compos. Anal.*, 2008, **21**, 445.
62. H. van den Berg, R. Faulks, H. F. Granado, J. Hirschberg, B. Olmedilla, G. Sandmann, S. Shouthon and W. Stahl, *J. Sci. Food Agric.*, 2002, **80**, 880.
63. P. Subra, S. Castellani, P. Jestin and A. Aoufi, *J. Supercr. Fluids*, 1998, **12**, 261.
64. J. M. Prado, P. C. Veggi and M. A. A. Meireles, *Curr. Anal. Chem.*, 2012, in press.
65. H. K. Sharma, J. Kaur, B. C. Sarkar, C. Singh and B. Singh, *Int. J. Food Sci. Tech.*, 2009, **44**, 1.
66. A. Ranalli, S. Contento, L. Lucera, G. Pavone, G. Di Giacomo, L. Aloisio, C. Di Gregorio, A. Mucci and I. Kourtikakis, *J. Agric. Food Chem.*, 2004, **52**, 4795.

67. M. Sun and F. Temelli, *J. Supercr. Fluids*, 2006, **37**, 397.
68. E. A. Boss, R. M. Filho, M. R. W. Maciel, L. F. Rios and H. I. Q. Perez, *Mech. Chem. Eng. Trans.*, 2009, **1**, 1.
69. H. L. N. Lau, Y. M. Choo, A. N. Ma and C. H. Chuah, *J. Food Lip.*, 2006, **13**, 210.
70. H. L. N. Lau, Y. M. Choo, A. N. Ma and C. H. Chuah, *J. Food Lip.*, 2007, **14**, 396.
71. L. Ciurlia, M. Bleve and L. Rescio, *J. Supercr. Fluids*, 2009, **49**, 338.
72. B. K. Ishida and M. H. Chapman, *J. Agric. Food Chem.*, 2009, **57**, 1051.
73. A. L. S. Eh and S. G. Teoh, *Ultrason. Sonochem.*, 2012, **19**, 151.
74. Q. Su, K. G. Rowley and N. D. H. Balazs, *J. Chromatogr. B*, 2002, **781**, 393.
75. K.-W. Kong, N. F. Rajab, K. N. Prasad, A. Ismail, M. Markom and C.-P. Tan, *Food Chem.*, 2010, **123**, 1142.
76. C. Yi, J. Shi, S. J. Xue, Y. Jiang and D. Li, *Food Chem.*, 2009, **113**, 1088.
77. T. Bechtold and R. Mussak, *Handbook of Natural Colorants*, Wiley, Chichester, UK, 2009, p. 434.
78. R. Halim, A. Hosikian, S. Lim and M. K. Danquah, *Int. J. Chem. Eng.*, 2010, doi:10.1155/2010/391632.
79. U. M. Lanfer-Marquez, *Braz. J. Pharm. Sci.*, 2003, **39**, 227.
80. R. N. Cavalcanti, D. T. Santos and M. A. A. Meireles, *Food Res. Int.*, 2011, **44**, 499.
81. F. J. Francis, *Crit. Rev. Food Sci. Nutr.*, 1989, **28**, 273.
82. A. Patras, N. P. Brunton, C. O'Donnell and B. K. Tiwari, *Trends Food Sci. Tech.*, 2010, **21**, 3.
83. A. Castaneda-Ovando, M. de Lourdes Pacheco-Hernandez, M. Elena Paez-Hernandez, J. A. Rodriguez and C. Andres Galan-Vidal, *Food Chem.*, 2009, **113**, 859.
84. N. Mateus and V. Freitas, in *Anthocyanins: Biosynthesis, Functions, and Applications*, ed. C. Winefield, K. Davies and K. Gould, Springer, New York, 2009, p. 284.
85. Y. Yang, S. Zhao, Y. Xu and Z. Yu, *Adv. Mat. Res.*, 2012, **361–363**, 691.
86. I. J. Seabra, M. E. M. Braga, M. T. Batista and H. C. De Sousa, *J. Supercr. Fluids*, 2010, **54**, 145.
87. I. J. Seabra, M. E. M. Braga, M. T. P. Batista and H. C. de Sousa, *Food Bioprocess Tech.*, 2010, **3**, 674.
88. K. H. Deubert, *J. Agric. Food Chem.*, 1978, **26**, 1452.
89. W. Luo, R. Zhang, L. Wang, J. Chen, Z. Guan, X. Liao and M. Mo, *Conference on Electrical Insulation and Dielectric Phenomena (CEIDP)*, 2008, 630.
90. A. T. Serra, I. J. Seabra, M. E. M. Braga, M. R. Bronze, H. C. de Sousa and C. M. M. Duarte, *J. Supercr. Fluids*, 2010, **55**, 184.
91. A. T. Serra, A. A. Matias, A. P. C. Almeida, M. R. Bronze, P. M. Alves, H. C. de Sousa and C. M. M. Duarte, *J. Supercr. Fluids*, 2011, **55**, 1007.
92. C. Y. Lien, A. Y. F. Lee, C. F. Chan, Y. C. Lai, C. L. Huang and W. C. Liao, *Applied Eng. Agric.*, 2010, **26**, 441.

93. C. Y. Lien, C. F. Chan, Y. C. Lai, C. L. Huang and W. C. Liao, *Sep. Sci. Tech.*, 2012, **47**, 1241.
94. G. Patil, M. C. Madhusudhan, B. Ravindra Babu and K. S. M. S. Raghavarao, *Chem. Eng. Process.*, 2009, **48**, 364.
95. A. M. Gizir, N. Turker and E. Artuvan, *Eur. Food Res. Tech.*, 2008, **226**, 363.
96. N. Türker and F. Erdoğan, *J. Food Eng.*, 2006, **76**, 579.
97. L. C. P. Gonçalves, M. A. D. S. Trassi, N. B. Lopes, F. A. Dörr, M. T. D. Santos, W. J. Baader, V. X. Oliveira Jr and E. L. Bastos, *Food Chem.*, 2012, **131**, 231.
98. H. M. C. Azeredo, *Int. J. Food Sci. Tech.*, 2009, **44**, 2365.
99. M. I. Khan, P. S. C. Sri Harsha, P. Giridhar and G. A. Ravishankar, *LWT – Food Sci. Tech.*, 2012, **47**, 315.
100. D. Strack, T. Vogt and W. Schliemann, *Phytochemistry*, 2003, **62**, 247.
101. K. M. Herbach, F. C. Stintzing and R. Carle, *J. Food Sci.*, 2006, **71**, R41.
102. P. Adela, in *Food Colorants*, ed. C. Socaciu, CRC Press, 2007, p. 303.
103. D. S. Sogi, S. Sharma, D. P. S. Oberoi and I. A. Wani, *J. Food Sci. Tech.*, 2010, **47**, 300.
104. T. T. Dao, P. H. Nguyen, H. K. Won, E. H. Kim, J. Park, B. Y. Won and W. K. Oh, *Food Chem.*, 2012, **134**, 21.
105. C. Dimakou and V. Oreopoulou, *LWT – Food Sci. Tech.*, 2012, **46**, 393.
106. W. S. Brud, in *Handbook of Essential Oils Science, Technology, and Applications*, ed. H. C. Baser and G. Buchbauer, CRC Press, 2010, p. 843.
107. M. Mukhopadhyay, *Natural Extracts Using Supercritical Carbon Dioxide*, CRC Press, 2000.
108. E. Schmidt, in *Handbook of Essential Oils Science, Technology, and Applications*, ed. K. Hüsni, C. Baser and G. Buchbauer, CRC Press, 2009, p. 83.
109. C. Sell, in *Handbook of Essential Oils Science, Technology, and Applications*, ed. K. Hüsni, C. Baser and G. Buchbauer, CRC Press, 2009, p. 121.
110. M. S. P. Moya and A. Jiménez, in *Encyclopedia of Chromatography*, 3rd edition, ed. J. Cazes, CRC Press, 2009, p. 809.
111. F. Bakkali, S. Averbeck, D. Averbeck and M. Idaomar, *Food Chem. Toxicol.*, 2008, **46**, 446.
112. J. Silva, W. Abebe, S. M. Sousa, V. G. Duarte, M. I. L. Machado and F. J. A. Matos, *J. Ethnopharmacol.*, 2003, **89**, 277.
113. T. Morita, K. Jinno, H. Kawagishi, Y. Arimoto, H. Suganuma, T. Inakuma and K. Sugiyama, *J. Agric. Food Chem.*, 2003, **51**, 1560.
114. R. B. Bostancıoğlu, M. Kürkçüoğlu, K. H. C. Başer and A. T. Koparal, *Food Chem. Toxicol.*, 2012, **50**, 2002.
115. R. Kapoor, B. Giri and K. G. Mukerji, *J. Sci. Food Agric.*, 2002, **82**, 339.
116. P. M. Döll-Boscardin, A. Sartoratto, B. H. L. N. S. Maia, J. P. Paula, T. Nakashima, P. V. Farago and C. C. Kanunfre, *J. Evid. Based Complementary Altern. Med.*, 2012, doi:10.1155/2012/342652.

117. B. H. Ali and G. Blunden, *Phytother Res.*, 2003, **17**, 299.
118. V. Ballabeni, M. Tognolini, M. Chiavarini, M. Impicciatore, R. Bruni, A. Bianchi and E. Barocelli, *Phytomedicine*, 2004, **11**, 596.
119. A. E. Edris, *Phytother Res.*, 2007, **21**, 308.
120. A. Ekanayake, P. H. Zoutendam, R. J. Strife, X. Fu and G. S. Jayatilake, *Food Chem.*, 2012, **133**, 767.
121. M. Turgis, K. D. Vu, C. Dupont and M. Lacroix, *Food Res. Int.*, 2012, **48**, 696.
122. S. Burt, *Int. J. Food Microbiol.*, 2004, **94**, 223.
123. P. S. Negi, *Int. J. Food Microbiol.*, 2012, **156**, 7.
124. K. P. Anthony, S. A. Deolu-Sobogun and M. A. Saleh, *J. Food Sci.*, 2012, doi: 10.1111/j.1750-3841.2012.02795.x.
125. M. B. Hashemi, M. Niakousari, M. J. Saharkhiz and M. H. Eskandari, *Nat. Prod. Res.*, 2012, **26**, 1458.
126. M. H. Eikani, F. Golmohammad and S. Rowshanzamir, *J. Food Eng.*, 2007, **80**, 735.
127. K. Chaieb, H. Hajlaoui, T. Zmantar, A. B. Kahla-Nakbi, M. Rouabhia, K. Mahdouani and A. Bakhrouf, *Phytother Res.*, 2007, **21**, 501.
128. A. C. L. Amorim, C. K. F. Lima, A. M. C. Hovell, A. L. P. Miranda and C. M. Rezende, *Phytomedicine*, 2009, **16**, 923.
129. C. P. Ridley and C. Khosla, in *Encyclopedia of Microbiology*, 3rd edition, ed. M. Schaechter, Academic Press, 2009, p. 472.
130. N. S. Sangwan, A. H. A. Farooqi, F. Shabih and R. S. Sangwan, *J. Plant Growth Regul.*, 2001, **34**, 3.
131. R. L. Smith, S. M. Cohen, J. Doull, V. J. Feron, J. I. Goodman, L. J. Marnett, P. S. Portoghesi, W. J. Waddell, B. M. Wagner, R. L. Hall, N. A. Higley, C. Lucas-Gavin and T. B. Adams, *Food Chem. Toxicol.*, 2005, **43**, 345.
132. B. Miller, C. Oschinski and W. Zimmer, *Planta*, 2001, **213**, 483.
133. R. Bryant, in *Rodd's Chemistry of Carbon Compounds*, 2nd edition, ed. S. Coffey, E. H. Rodd, M. F. Ansell and M. Sainsbury, Elsevier, Amsterdam, 1964, p. 256.
134. P. F. Leal, T. S. Almeida, G. H. C. Prado, J. M. Prado and M. A. A. Meireles, *Sep. Sci. Tech.*, 2011, **46**, 1848.
135. L. M. L. Nollet, in *Handbook of Analysis of Active Compounds in Functional Foods*, ed. L. M. L. Nollet and F. Toldra, CRC Press, 2012, p. 641.
136. M. M. Özcan and J. C. Chalchat, *Ann. Microbiol.*, 2006, **56**, 353.
137. S. Machmudah, A. Sulaswatty, M. Sasaki, M. Goto and T. Hirose, *J. Supercr. Fluids*, 2006, **39**, 30.
138. E. A. Bejnarowicz and E. R. Kirch, *J. Pharm. Sci.*, 1963, **10**, 988.
139. B. Krishnamoorthy and J. Rema, in *Cinnamon and Cassia: The Genus Cinnamomum*, ed. P. N. Ravindran, K. Nirmal Babu and M. Shylaja, CRC Press, 2004, p. 311.
140. D. Kalemba and A. Wajs, in *Food Flavors Chemical, Sensory and Technological Properties*, ed. H. Jelen, CRC Press, 2011, p. 193.

141. C. S. Letizia, J. Cocchiara, J. Lalko and A. M. Api, *Food Chem. Toxicol.*, 2003, **41**, 943.
142. Y. Yonei, H. Ohinata, R. Yoshida, Y. Shimizu and C. Yokohama, *J. Supercr. Fluids*, 1995, **8**, 156.
143. J. Wu and J. S. Yang, *J. Agric. Food Chem.*, 1994, **42**, 2574.
144. X. G. He, M. W. Bernart, L.-Z. Lian and L.-Z. Lin, *J. Chromatogr. A*, 1998, **796**, 327.
145. O. I. Aruoma, J. P. E. Spencer, D. Warren, P. Jenner, J. Bulter and B. Halliwell, *Food Chem.*, 1997, **60**, 149.
146. B. H. Ali, G. Blunden, M. O. Tanira and A. Nemmar, *Food Chem. Toxicol.*, 2008, **46**, 409.
147. D. Li, G. W. Sybert and R. T. Gow. Compositions and methods comprising Zingiber species. Patent WO 2008/010183, 2008.
148. R. Hiltunen and Y. Holm, in *The Genus Ocimum*, ed. R. Hiltunen and Y. Holm, CRC Press, 1999, p. 77.
149. C. Franz and J. Novak, in *Handbook of Essential Oils Science, Technology, and Applications*, ed. H. C. Baser and G. Buchbauer, CRC Press, 2010, pp. 39.
150. R. Eccles, *J. Pharm. Pharmacol.*, 1994, **46**, 618.
151. J. Beerling, S. Meakins and L. Small, in *Eucalyptus: The Genus Eucalyptus*, ed. J. J. W. Coppen, CRC Press, 2002.
152. E. Offord, *Herbal and Traditional Medicine: Biomolecular and Clinical Aspects*, CRC Press, 2004.
153. J. P. B. Sousa, A. P. S. Brancalion, A. B. Souza, I. C. C. Turatti, S. R. Ambrósio, N. A. J. C. Furtado, N. P. Lopes and J. K. Bastos, *J. Pharm. Biomed. Anal.*, 2011, **54**, 653.
154. B. C. Cavalcanti, L. V. Costa-lotufo, M. O. Moraes, R. R. Burbano, E. R. Silveira, K. M. A. Cunha, V. S. N. Rao, D. J. Moura, R. M. Rosa, J. A. P. Henriques and C. Pessoa, *Food Chem. Toxicol.*, 2006, **44**, 388.
155. M. J. Del Nunzio, *Aerosol Cosmetol.*, 1985, **7**, 7.
156. P. Holmes, *Int. J. Aromather.*, 1998, **8**, 8.
157. L. Cai and C. D. Wu, *J. Nat. Prod.*, 1996, **59**, 987.
158. C. Ghelardini, N. Galeotti, L. Di Cesare Mannelli, G. Mazzanti and A. Baartolini, *Il Farmaco*, 2001, **56**, 387.
159. A. Alqareer, A. Alyahya and L. Andersson, *J. Dent.*, 2006, **34**, 747.
160. K. Lee and T. Shibamoto, *Food Chem.*, 2001, **74**, 443.
161. K. V. Menon and S. R. Garg, *Food Microbiol.*, 2001, **18**, 647.
162. I. Gulçin, S. Gungor, S. Beydemir, M. Elmastas and O. I. Kufrevioglu, *Food Chem.*, 2004, **87**, 393.
163. B. M. Naveena, M. Muthukumar, A. R. Sem, Y. Babji and T. R. K. Murthy, *Meat Sci.*, 2006, **74**, 409.
164. K. Chaieb, H. Hajlaoui, T. Zmantar, A. B. Kahla-Nakbi, M. Rouabhia, K. Mahdouani and A. Bakhrouf, *Phytother. Res.*, 2007, **21**, 501.
165. G.-Q. Zheng, P. M. Kenney and L. K. T. Lam, *J. Nat. Prod.*, 1992, **55**, 999.
166. C.-B. Yoo, K.-T. Han, K.-S. Cho, J. Ha, H.-J. Park, J.-H. Nam, U.-H. Kil and K.-T. Lee, *Cancer Lett.*, 2005, **225**, 41.

167. S. Inouye, K. Uchida, Y. Nishiyama, Y. Hasumi, H. Yamaguchi and S. Abe, *Jap. J. Med. Mycol.*, 2007, **48**, 27.
168. A. Fabio, C. Cermelli, G. Fabio, P. Nicoletti and P. Quaglio., *Phytother. Res.*, 2007, **21**, 374.
169. F. Dusan, S. Marián, D. Katarína and B. Dobroslava., *Toxicol. in Vitro*, 2006, **20**, 1435.
170. H. M. A. Cavanagh and J. M. Wilkinson, *Phytother. Res.*, 2002, **16**, 301.
171. T. R. Roberts and R. J. H. Wilson, in *Handbook of Brewing*, 2nd edition, ed. F. G. Priest and G. G. Stewart, CRC Press, 2006, p. 177.
172. G. A. Burdock and I. G. Carabin, *Food Chem. Toxicol.*, 2008, **46**, 433.
173. G. D. Dugo and A. Di Giacomo, *Citrus: The Genus Citrus*, CRC Press, 2002.
174. R. D. O'Brien, *Fats and Oils-Formulating and Processing for Applications*, 2nd edition, CRC Press, 2003.
175. R. D. O'Brien, in *Handbook of Food Science, Technology, and Engineering*, Vol. 4, ed. Y. H. Hui, CRC Press, 2006, pp. 155.1–155.14.
176. V. Tatum and C. K. Chow, in *Fatty Acids in Foods and their Health Implications*, 3rd edition, ed. C. C. K. Chow, CRC Press, 2008, pp. 493–510.
177. P. J. Wakelyn and P. J. Wan, *Extraction Optimization in Food Engineering*, CRC Press, 2003.
178. R. D. O'Brien, *Formulating and Processing for Applications*, 2nd edition, CRC Press, 2004.
179. S. Arvanitoyannis, T. H. Varzakas, S. Kiokias and A. E. Labropoulos, in *Advances in Food Biochemistry*, ed. F. Yildiz, CRC Press, 2010, pp. 131–201.
180. R. D. O'Brien, in *Fats and Oil: Formulating and Processing for Applications*, 3rd edition, CRC Press, 2008.
181. V. K. S. Shukla, in *Handbook of Functional Lipids*, ed. C. C. Akoh, CRC Press, 2006, pp. 279–307.
182. E. C. V. Oliveira, K. P. Boock, M. Maruno and P. A. Rocha-Filho, *J. Dispersion Sci. Tech.*, 2011, **32**, 1135.
183. M. L. Medeiros, A. M. I. B. Ayrosa, R. N. M. Pitombo and S. C. S. Lannes, *J. Food Eng.*, 2006, **73**, 402.
184. B. A. Azevedo, U. Kopcak and R. S. Mohamed, *J. Supercr. Fluids*, 2003, **27**, 223.
185. F. D. Gunstone, *Chemistry, Nutrition, and Biotechnology*, 3rd edition, CRC Press, 2008.
186. S. Jocić, Z. Sakač, N. Lečić and D. Škorić, *Can. J. Appl. Physiol. Pharmacol.*, 2008, **86**, 215.
187. J. R. Smith, in *Safflower*, ed. J. R. Smith, AOCS Publishing, 1996, p. 32.
188. J. John, M. Bhattacharya and R. B. Turner, *J. Appl. Polym. Sci.*, 2002, **86**, 3097.
189. A. Lucas, I. Gracia, J. Rincón and M. T. García, *Ind. Eng. Chem. Res.*, 2007, **46**, 5061.
190. M. M. Esquivel and G. B. Gil, *J. Supercr. Fluids*, 1993, **6**, 91.

191. A. Molero Gómez, C. Pereyra López and E. Martínez de la Ossa, *Chem. Eng. J.*, 1996, **61**, 227.
192. G. K. Jayaprakasha, R. P. Singh and K. K. Sakariah, *Food Chem.*, 2001, **73**, 285.
193. J. K. Prasain, N. Peng, Y. Dai, R. Moore, A. Arabshahi, L. Wilson, S. Barnes, J. M. Wyss, H. Kim and R. L. Watts., *Phytomedicine*, 2009, **16**, 233.
194. Y. Yilmaz and R. T. Toledo, *J. Food Comp. Anal.*, 2006, **19**, 41.
195. M. Bouaziz, I. Fki, H. Jemai, M. Ayadi and S. Sayadi, *Food Chem.*, 2008, **108**, 253.
196. A. Hanganu, M.-C. Todasca, N.-A. Chira, M. Maganu and S. Rosca, *Food Chem.*, 2012, **134**, 2453.
197. K. T. Tan, K. T. Lee, A. R. Mohamed and S. Bhatia, *Renew. Sustain. Energ. Rev.*, 2009, **13**, 420.
198. O.-M. Lai, *Healthful Lipids*, AOCS Publishing, 2005.
199. S. M. Silva, K. A. Sampaio, T. Taham, S. A. Rocco, R. Ceriani and A. J. A. Meirelles, *J. Am. Oil Chem. Soc.*, 2009, **86**, 611.
200. M. L. S. Albuquerque, I. Guedes, P. Alcantara Jr., S. G. C. Moreira, N. M. Barbosa Neto, D. S. Correa and S. C. Zilio, *J. Braz. Chem. Soc.*, 2005, **16**, 1113.
201. C. F. Zanatta, V. Ugartondo, M. Mitjans, P. A. Rocha-Filho and M. P. Vinardell, *Food Chem. Toxicol.*, 2008, **46**, 2776.
202. P. Pons, J. Illnait, R. Más, M. Rodríguez, C. Alemán, J. C. Fernández, L. Fernández and M. Martin, *Curr. Ther. Res.*, 1997, **58**, 26.
203. R. Más, P. Rivas, J. E. Izquierdo, R. Hernández, L. Fernández, J. Fernández, S. D. Orta, J. Illnait and Y. Ricardo, *Curr. Ther. Res.*, 1999, **60**, 458.
204. I. Gouni-Berthold and H. K. Berthold, *Am. Heart J.*, 2002, **143**, 356.
205. J. L. Hargrove, P. Greenspan and D. K. Hartle, *Exp. Biol. Med.*, 2004, **229**, 215.
206. M. Noa and R. Mas, *Arch. Med. Res.*, 2005, **36**, 441.
207. G. Castaño, M. L. Arruzazabala, L. Fernández, R. Mas, D. Carbajal, V. Molina, J. Illnait, S. Mendoza, R. Gámez, M. Mesa and J. Fernández, *Curr. Ther. Res.*, 2006, **67**, 174.
208. S. Lowther and J. Sbordone, Food product containing policosanols, Patent CA 2544227, 2007.
209. Y. Ohta, K. Ohashi, T. Matsura, K. Tokunaga, A. Kitagawa and K. Yamada, *J. Clin. Biochem. Nutr.*, 2008, **42**, 118.
210. G. Knothe, in *Handbook of Bioenergy Crop Plants*, ed. D. R. Shonnard, CRC Press, 2012, p. 793.
211. S. H. Mitmesser, in *Nutritional Ergogenic Aids*, ed. I. Wolinsky and J. A. Driskell, CRC Press, 2004, pp. 247–254.
212. L.-H. Huynh, N. S. Kasim and Y.-H. Ju, in *Biofuels*, ed. A. Pandey, C. Larroche, S. Ricke, C.-G. Dussap and E. Gnansounou, Elsevier, Amsterdam, 2011, p. 375.
213. M. M. Gui, K. T. Lee and S. Bhatia, *Energy*, 2008, **33**, 1646.

214. C. M. Hasler, *J. Food Technol.*, 1998, **52**, 63.
215. ADA Reports Position of the American Dietetic Association, *Functional Foods J. ADA*, 1995, **95**, 493.
216. I. Goldberg, *Functional Foods: Designer Foods, Pharmafoods, and Nutraceuticals*, Chapman & Hall, New York, 1994.
217. R. Tsau and A. M. H. Akthar, *Food Agri. Env.*, 2005, **3**, 18.
218. W. Andlauer and P. Furst, *Food Res. Int.*, 2002, **35**, 171.
219. L. M. Ohr, Nutraceuticals: health foods stand at forefront, *Food Technol.*, 2007.
220. M. A. Rostagno, A. Villares, E. Guillamón, A. García-Lafuente and J. A. Martínez, *J. Chromatogr. A*, 2009, **1216**, 2.
221. I. E. Milder, I. C. Arts, B. Van de Putte, D. P. Venema and P. C. Hollman, *Br. J. Nutr.*, 2005, **93**, 393.
222. G. J. Nohynek, E. Antignac, T. Re and H. Toutain, *Toxicol Appl. Pharmacol.*, 2010, **243**, 239.
223. I. B. Allemann and L. Baumann, *Int. J. Dermatol.*, 2009, **48**, 923.
224. A. Simopoulos, *World Rev. Nutr. Diet*, 2001, **88**, 18.
225. S. C. Cunnane, *Prog. Lipid Res.*, 2003, **42**, 544.
226. A. Simopoulos, *Biomed. Pharmacoth.*, 2002, **56**(8), 365.
227. L. E. M. William, *Ann. NY Acad. Sci.*, 2005, **1055**, 179.
228. S. D. Phinney, R. S. Odin, S. B. Johnson and R. T. Holman, *Am. J. Clin. Nutr.*, 1990, **51**, 385.
229. G. L. Darmstadt, M. Mao-Qiang, E. Chi, S. K. Saha, V. A. Ziboh, R. E. Black, M. Santosham and P. M. Elias, *Acta Paediatr.*, 2002, **91**, 546.
230. M. N. Peyrat-Maillard, M. E. Cuvelier and C. Berset, *J. Am. Oil Chem. Soc.*, 2003, **80**, 1007.
231. S. W. Andersson, J. Skinner, L. Ellegård, A. A. Welch, S. Bingham, A. Mulligan, H. Andersson and K. T. Khaw, *Eur. J. Clin. Nutr.*, 2004, **58**, 1378.
232. L. M. Valsta, A. Lemström, M. L. Ovaskainen, A. M. Lampi, J. Toivo, T. Korhonen and V. Piironen, *Br. J. Nutr.*, 2007, **92**, 671.
233. S. S. Abumweis, R. Barake and P. J. Jones, *Food Nutr. Res.*, 2008, **52**, doi: 10.3402/fnr.v52i0.1811.
234. I. Demonty, R. T. Ras, H. C. Van der Knaap, G. S. Duchateau, L. Meijer and P. L. Zock, *J. Nutr.*, 2009, **139**, 271.
235. K. C. Maki, A. L. Lawless, M. S. Reeves, M. R. Dicklin, B. H. Jenks and E. Shneyvas, *Int. J. Food Sci. Nutr.*, 2012, **63**, 476.
236. L. Calpe-Berdiel and J. C. Escolà-Gil, *Atherosclerosis*, 2009, **203**(1), 18.
237. B. Genser, G. Silbernagel, G. De Backer, E. Bruckert, R. Carmena, M. J. Chapman, J. Deanfield and O. S. Descamps, *Eur. Heart J.*, 2012, **33**(4), 444.
238. D. DellaPenna, *J. Plant Physiol.*, 2005, **162**, 729.
239. J. Kruk and A. Trebst, *Biochim. Biophys. Acta*, 2008, **1777**, 154.
240. R. Szyman and J. Kruk, *Phytochemistry*, 2008, **69**, 2142.

241. USDA – National Nutrient Database for Standard Reference, 2009. www.ars.usda.gov/nutrientdata.
242. B. M. Folmer, D. Barron, E. Hughes, L. Miguet, B. Sanchez, O. Heudi, M. Rouvet, L. Sagalowicz, P. Callier, M. Michel and G. Williamson, *Biochem. Pharmacol.*, 2009, **78**(12), 1464.
243. D. Hofius, M. R. Hajirezaei, M. Geiger, H. Tschiersch, M. Melzer and U. Sonnewald, *Plant Physiol.*, 2004, **135**, 1256.
244. M. H. Shishehbor, R. J. Aviles, M. L. Brenna, X. Fu, M. Goormastic, G. L. Pearce, N. Gokce, J. R. Keaney, J. F. Gokce, M. S. Penn, D. L. Sprecher, J. A. Vita and S. L. Hazen, *JAMA*, 2003, **289**, 1675.
245. K. Hensley, E. J. Benaksas, R. Bolli, P. Comp, P. Grammas, L. Hamdheydari, S. Mou, Q. N. Pye, M. F. Stoddard, G. Wallis, K. S. Williamson, M. West, W. J. Wechter and R. A. Floyd, *Free Radical Biol. Med.*, 2004, **36**, 1.
246. H. J. Lee, J. Ju, S. Paul, J. Y. So, A. DeCastro, A. Smolarek, M. J. Lee, C. S. Yang, H. L. Newmark and N. Suh, *Clin Cancer Res.*, 2009, **15**, 4242.
247. W. Li and J. F. Fitzloff, *Liq. Chrom. Relat. Tech.*, 2002, **25**, 17.
248. C. S. Freitas, C. H. Baggio, J. E. Da Silva-Santos, L. Rieck, C. A. M. Santos, C. C. Junior, L. C. Ming and D. A. G. Cortez, *Life Sci.*, 2004, **74**, 1167.
249. S. Nakai, N. Takagi, H. Miichi, S. Hayashi, N. Nishimoto, T. Takemoto and H. Kizu, *Phytochemistry*, 1984, **23**(8), 1703.
250. P. Matsuzakia, G. Akisueb, S. C. S. Olorisa, S. L. Górniakc and M. L. Z. Dagli, *Life Sci.*, 2003, **74**(5), 573.
251. N. Fuzzati, *Anal. Tech. Biomed. Life Sci.*, 2004, **812**, 119.
252. C. X. Qiao, R. Den, K. Kudo, K. Kamada, K. Takemoto, H. Wati and S. Kamba, *Neurosci. Res.*, 2005, **51**, 31.
253. P. Leal, F. Kfoury, B. Marina, F. Alexandre, F. Fagundes, J. Prado, M. Toyama and M. A. A. Meireles, *J. Supercr. Fluids*, 2010, **54**, 38.
254. P. Leal, N. Maia, B. Nilson, Q. A. C. Quirino, R. R. Catharino, M. N. Eberlin and M. A. A. Meireles, *Food Bioprocess Tech.*, 2008, **1**(4), 326.
255. P. M. Etherton, K. D. Hecker, A. Bonanome, S. M. Coval, A. E. Binkoski and K. F. Hilpert, *Am. J. Med.*, 2002, **113**, 71s.
256. F. Shahidi and M. Nacz, *Phenolics in Food and Nutraceuticals*, CRC Press, Boca Raton, FL, 2004. p. 489.
257. M. Leopoldini, N. Russo and M. Toscano, *Food Chem.*, 2011, **125**, 288.
258. Y. S. Velioglu, in *Advances in Food Biochemistry*, ed. F. Yildiz, CRC Press, Boca Raton, 2010, pp. 313–339.
259. I. Ignat, I. Volf and V. I. Popa, *Food Chem.*, 2011, **126**, 1821.
260. M. N. Clifford, *J. Sci. Food Agric.*, 1999, **79**, 362.
261. J. K. Moon, H. S. Yoo and T. Shibamoto, *J. Agric. Food Chem.*, 2009, **57**, 5365.
262. J. Perez-Jimenez, V. Neveu, F. Vos and A. Scalbert, *J. Agric. Food Chem.*, 2010, **58**, 4959.

263. C. Manach, A. Scalbert, C. Morand, C. Remesy and L. Jimenez, *Am. J. Clin. Nutr.*, 2004, **9**, 727.
264. M. G. Sajilata, P. R. Bajaj and R. S. Singhal, *Compr. Rev. Food Sci. Food Safety*, 2008, **7**, 229.
265. Y. Wang and C.-T. Ho, *J. Agric. Food Chem.*, 2009, **57**, 8109.
266. W. O. Song and O. K. Chun, *J. Nutr.*, 2008, **138**, 1543S.
267. J. Valerga, M. Reta and M. C. Lanari, *Food Sci. Tech.*, 2012, **45**(1), 28.
268. M. Bubonja-Sonje, J. Giacometti and M. Abram, *Food Chem.*, 2011, **127**, 1821.
269. G. Trottier, P. J. Boström, N. Lawrentschuk and N. E. Fleshner, *Nat. Rev. Urol.*, 2010, **7**, 21.
270. M. A. Rostagno, N. Manchón, M. D'Arrigo, E. Guillamón, A. Villares, A. García-Lafuente, A. Ramos and J. A. Martínez, *Anal. Chem. Acta*, 2011, **685**, 204.
271. Y. Birk, *Int. J. Pept. Protein Res.*, 1985, **25**, 113.
272. R. L. Andersson and W. J. Wolf, *J. Nutr.*, 1995, **125**, 581S.
273. K. M. Phillips, D. M. Ruggio, J. I. Toivo, M. A. Swank and A. H. Simpkins, *J. Food Compos. Anal.*, 2002, **15**, 123.
274. A. Romani, M. Minunni, N. Mulinacci, P. Pinelli, F. F. Vincieri, M. Del Carlo and M. Mascini, *J. Agric. Food Chem.*, 2000, **48**(4), 1197.
275. N. Ohta, G. Kuwata, H. Akahori and T. Watanabe, *Agric. Biol. Chem.*, 1980, **44**, 469.
276. E. Farmakalidis and P. A. Murphy, *J. Agric. Food Chem.*, 1985, **33**, 385.
277. S. Kudou, M. Shimoyamada, T. Imura, T. Uchida and K. Okubo, *Agric. Biol. Chem.*, 1991, **55**, 859.
278. R. Beaglehole, *Epidemiol Rev.*, 1990, **12**, 1.
279. H. Adlercreutz and W. Mazur, *Ann. Med.*, 1997, **29**, 95.
280. T. A. Pearson, S. N. Blair, S. R. Daniels, R. H. Eckel, J. M. Fair, S. P. Fortmann, B. A. Franklin, L. B. Goldstein, P. Greenland, S. M. Grundy, Y. Hong, N. H. Miller, R. M. Lauer, I. S. Ockene, R. L. Sacco, J. F. Sallis, Jr, S. C. Smith, Jr, N. J. Stone and K. A. Taubert, *Circulation*, 2002, **106**, 388.
281. J. Slavin, *Nutr. Res. Rev.*, 2004, **17**, 99.
282. R. E. C. Wildman, *Handbook of Nutraceuticals and Functional Foods*, 2nd edition, CRC, 2006.
283. G. Rimbach, C. Boesch-Saadatmandi, J. Frank, D. Fuchs, U. Wenzel, H. Daniel, W. L. Hall and P. D. Weinberg, *Food Chem. Toxicol.*, 2008, **46**, 1308.
284. A. García-Lafuente, E. Guillamón, A. Villares, M. A. Rostagno and J. A. Martínez, *Inflamm. Res.*, 2009, **58**(9), 537.

CHAPTER 2

Extraction of Natural Products: Principles and Fundamental Aspects

M. PALMA,^{*a} G. F. BARBERO,^a Z. PIÑEIRO,^b A. LIAZID,^c C. G. BARROSO,^a M. A. ROSTAGNO,^d J. M. PRADO^d AND M. A. A. MEIRELES^d

^a Department of Analytical Chemistry, University of Cádiz, Campus Universitario de Puerto Real, 11510 Puerto Real, Spain; ^b Centro Ifapa Rancho de la Merced, Jerez, Spain; ^c Department of Chemical Engineering, Université Abdelmalek Essaâdi, Tanger, Morocco; ^d LASEFI/DEA/FEA (School of Food Engineering) / UNICAMP (University of Campinas), R. Monteiro Lobato, 80, Campinas, 13083-862, SP, Brazil

*Email: miguel.palma@uca.es

2.1 Introduction

Bioactive compounds are largely obtained from natural sources. For the determination of phytochemicals from solid samples several consecutive steps are usually necessary, and if one of them is not properly followed, the overall performance of the analysis will be poor, errors will be introduced, and consequently, inconsistency in the results can be expected.

Sample preparation is used to increase the efficiency of an analysis, to eliminate or reduce potential interferences, to enhance the sensitivity of the analysis by increasing the concentration of the analyte in the assay mixture, and sometimes to transform the analytes of interest into a more suitable form that can be easily

RSC Green Chemistry No. 21

Natural Product Extraction: Principles and Applications

Edited by Mauricio A. Rostagno and Juliana M. Prado

© The Royal Society of Chemistry 2013

Published by the Royal Society of Chemistry, www.rsc.org

separated, detected, and/or quantified. The sample produced in this step should have a high concentration of target analytes free of interfering compounds from the matrix. One of the key procedures in this step is the extraction of target analytes.

Extraction is the step of the analytical protocol in which a compound or group of compounds is preferentially transferred from a matrix into a different phase. The final goal of this step is making the sample available for introduction into analytical instruments, i.e. to have the target analytes in a liquid phase ready for use in chromatographic systems at the correct levels.¹ In quantitative analyses, it is important to achieve the complete extraction of the target analytes while preserving their original profile and distribution. In contrast, when dealing with qualitative analyses, the achievement of exhaustive extractions and complete analyte stability are not regarded as important. In both cases, as long as the sample is suitable for the correct analysis of its components, the amount of co-extracted and undesirable components present in the sample usually does not represent an immediate priority.

However, in semi-preparative and preparative-scale separations extraction is a fundamental issue that represents the key to produce extracts highly concentrated on the target compounds. In this case, the main objective is to produce sufficient amounts of high purity extracts composed of only a few compounds or classes of compounds. The importance of the co-extracted compounds depends on their nature and the desired purity of the extracts. For the industrial production of extracts from natural products, for economic reasons it is required to achieve an adequate balance between extraction efficiency, extraction yields of the target and co-extracted compounds, and concentration of target compounds, in order to minimize costs. Another aspect that needs to be considered is that the target compounds may not be completely stable under extraction conditions, but in some cases a certain amount of degradation may be tolerated. For example, higher extraction temperatures may be used if they result in a significant increase in the extraction efficiency while causing a small degradation of the target compounds.

Although these approaches (analytical, semi-preparative, preparative, and industrial production of extracts) have particular characteristics and goals, they are governed by the same processes and mechanisms and are influenced basically by the same process variables. Furthermore, depending on the application, the same techniques and methods may be used at the different scales, and the knowledge available may be explored in order to optimize process conditions to achieve specific goals for each one of them. In this context, the principles and fundamental aspects of the extraction process and the main conventional extraction techniques will be discussed in the next sections.

2.2 Principles and Fundamentals of Extraction

The main goals of the extraction process are related to one or more important properties:

- high yield: the target compounds are exhaustively or approximately exhaustively recovered;

- high selectivity/purity: the resulting extract has a low amount of interfering or undesirable co-extracted compounds;
- high sensitivity: the resulting extract allows for different quantification techniques that produce a high slope in the calibration curves;
- low limit of detection/quantification: components in the extracts can be detected/quantified at low levels because low noise levels are obtained in the analytical system.

These properties differ in terms of importance depending on the process scale. As an example, at the analytical scale, selectivity, sensitivity, and limit of detection are the most important properties, whereas for semi-preparative and preparative separations and at the industrial scale the yield and purity are the key properties.

From the phenomenological point of view, extraction is a mass transfer process of one or more components from one phase to another one. When dealing with natural products, in most cases the sample to be extracted is a solid material, although in some cases liquid samples are used. The extracting solvent is usually a liquid, but it can also be a solid or a supercritical fluid.

In order to understand how any extraction technique works, both the target compounds and the extraction solvent must be considered. Therefore, knowledge of the properties of the solute, mainly its chemical properties, is important in order to understand the extraction process. Moreover, it is important to know the properties of the solvent medium in which the target material is to be dissolved during the extraction process. The interactions between solute and solvent are determined by the vapor pressure of the solute, the solubility of the solute in the solvent, the hydrophobicity, and the acid/base properties of both solute and solvent.

Some of these properties only relate to the compound of interest (solute), while others concern the solvent used for the extraction process. The compatibility between solvent and solute is based on assessing the polarity of the molecular structure to predict their solubility and miscibility. As a general rule, it is assumed that non-polar solutes are dissolved by non-polar solvents while polar solutes are dissolved by polar solvents. For example, water dissolves glucose due to the attraction between the partially positively charged atom of the glucose molecule to the partially negatively charged atom of the water molecule while at the same time the partially negatively charged atom of the glucose molecule is attracted to the partially positively charged atom of the water molecule. If the target component from the raw material is freely available and the polarity of the solvent and of the solute is compatible, the solvent dissolves the solute to form a homogeneous solution.

The thermodynamics of the process allows understanding the formation of a solution. The equation of the Gibbs free energy (ΔG_{soln}) describes the solution formation:

$$\Delta G_{\text{soln}} = \Delta H_{\text{soln}} - T\Delta S_{\text{soln}} \quad (2.1)$$

where ΔH_{soln} is the enthalpy of the solution formation, T is the temperature (in Kelvin), and ΔS_{soln} is the entropy of the solution formation.

When $\Delta G_{\text{soln}} < 0$ the formation of a solution will be spontaneous. Since the entropy always increases with solution formation, ΔS_{soln} is always positive. Therefore, the spontaneity of formation of a solution will be a function of the sign of the enthalpy of solution, ΔH_{soln} . Assuming that ΔH_{soln} is the sum of three individual enthalpies:

$$\Delta H_{\text{soln}} = \Delta H_1 + \Delta H_2 + \Delta H_3 \quad (2.2)$$

where ΔH_1 is the energy added (positive sign) to break intermolecular forces between solvent molecules, ΔH_2 is the energy added (positive sign) to break intermolecular forces between solute molecules, and ΔH_3 is the energy released (negative sign) from the attraction between solvent and solute molecules.

When $\Delta H_3 > \Delta H_1 + \Delta H_2$, $\Delta H_{\text{soln}} < 0$, which means that an exothermic process is taking place and since ΔS_{soln} is always positive, the formation of a solution will be spontaneous. In contrast, when $\Delta H_3 < \Delta H_1 + \Delta H_2$, $\Delta H_{\text{soln}} > 0$, the process is endothermic and the solution formation will occur only if $T\Delta S_{\text{soln}} > \Delta H_{\text{soln}}$. Consequently, higher entropy and/or higher temperature may promote the formation of a solution.

Without any doubt, the solubility of compounds of interest in the solvent is one of the key aspects of the whole extraction process. In fact, it is one of the most important parameters to be optimized during the method development. However, the extraction of a given compound from a complex matrix, as is the case of natural products, is much more complex and several factors simultaneously affect the process. These factors need to be understood so they can be properly controlled to enhance the efficiency of the process. The characteristics of the raw material matrix are important because they provide the possible sites where target compounds (usually small molecules) may be found (Figure 2.1).²

It is conventionally assumed that the compounds present in natural products may be adsorbed on the surface of the matrix (2), dissolved in the pore of the matrix and/or adsorbed on the pore surface (1), dissolved/adsorbed in a micro/nano pore (3), chemically bounded to the matrix (4), or dissolved in the bulk solution (5).² Depending on the solute location in the solid matrix, it is more easily accessed or accessed with more difficulty by the solvent and extracted by it. In seeds, fruits, and roots the solutes usually are uniformly distributed in the solid, whereas in leaves and flowers the solutes are inside fragile glandular trichomes.³

The extraction mechanism is schematically presented in Figure 2.2, and follows these steps:

1. the solvent is transferred from the fluid phase to the solid surface and pervades it;
2. the solvent penetrates into the solid matrix by molecular diffusion;

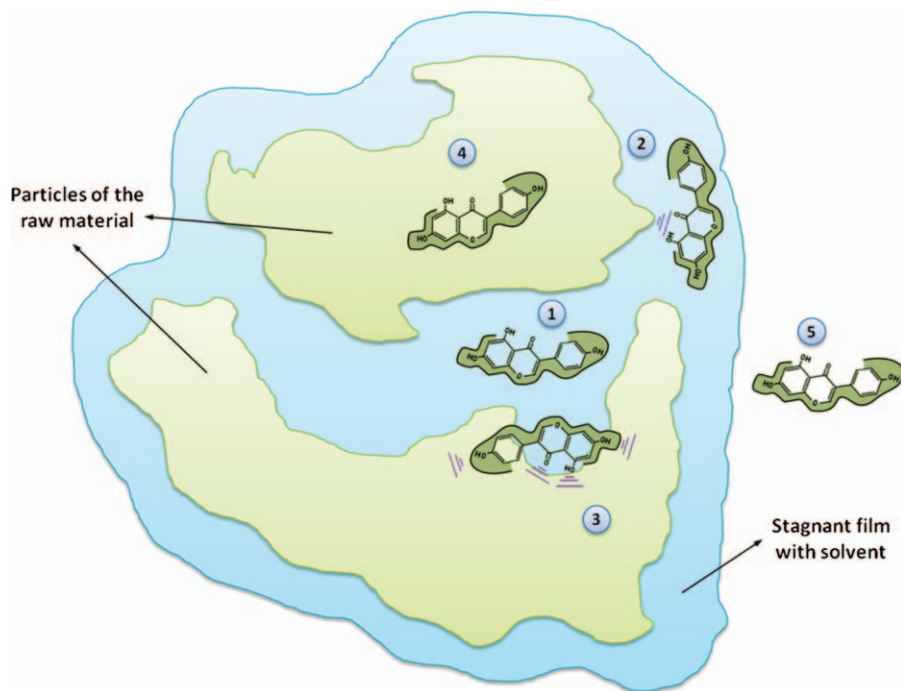


Figure 2.1 Conceptualization of a natural matrix and the possible sites where small molecules may be found.
Adapted from S. H. Rizvi, *Separation, Extraction and Concentration Processes in the Food, Beverage and Nutraceutical Industries*, Woodhead Publishing, Oxford, UK, 2010, p. 665.

3. the soluble material is solubilized by desorption from the matrix and solvation into the extraction solvent – the breakage of chemicals bonds may be required for desorption of target analytes from the solid matrix;
4. the solution containing the solutes returns to the surface of the solid by molecular diffusion;
5. the solution is transferred from the solid surface to the bulk fluid by natural or forced convection.

The extraction of a chemical component X from a phase A to a second phase B begins when the two phases come into contact. The two phases should therefore not be miscible if they are both liquids. Furthermore, phase A can be in the solid or semi-solid state. The distribution of X between the immiscible phases occurs as soon as it can be transferred from phase A to phase B and back from phase B to phase A. The solubilization limit is the equilibrium concentration between the phases. The equilibrium can be represented as:



where X_A is the component X in phase A and X_B is the component X in phase B.

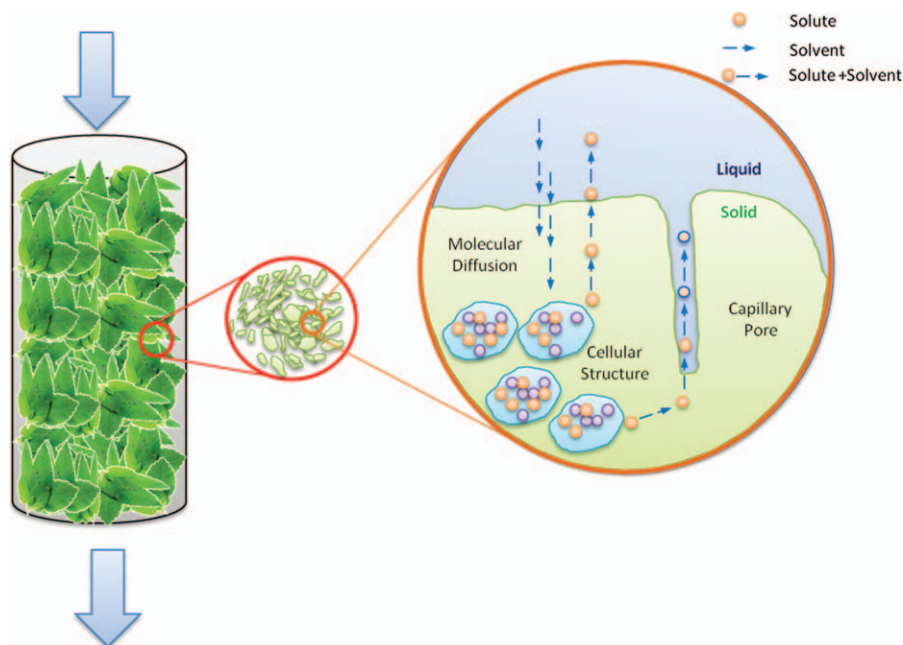


Figure 2.2 Schematic representation of the extraction mechanism.

From Equation (2.3) the partition coefficient K_d can be calculated as:

$$K_d = \frac{[X_B]}{[X_A]} \quad (2.4)$$

where the brackets denote the activities of X in each phase at constant temperature. Usually concentrations are used rather than activities. Therefore, this equation can be used to calculate the amount of component X in phase A after determining component X in phase B.

The concentration of X transferred to phase B appears in the numerator of Equation (2.4), which means that the higher the K_d the higher the recovery of component X during extraction. The equilibrium constant does not depend on the rate to achieve equilibrium, and reaching the equilibrium has some very important consequences: the relative recovery of the extraction process can be used for quantitative purposes; and a more robust extraction method will be developed because lower effects from different working variables will be apparent. However, in practical applications of the extraction process the equilibrium is almost never reached because the amount of soluble compounds (X) is usually small compared to the amount of solvent available, resulting in a diluted solution in phase B. Therefore, instead of waiting for equilibrium to be reached, usually after a certain amount of component X is transferred to phase B, the process is stopped. This makes the mass transfer kinetics important in the extraction process.

The dissolution rate of the solute X into the fluid phase B is controlled by the mass transfer rate of X that moves from the solid matrix (A) into the bulk solvent (B). The solute transfer inside the solid particle occurs due to a concentration gradient in A, which is strictly controlled by molecular diffusion. The equation that describes the diffusion phenomenon is based on Fick's Law:

$$\frac{\dot{M}_X}{A_T} = -D_{XA} \frac{dC_X}{dz} \quad (2.5)$$

where $\dot{M}_X$ is the mass transfer rate of the solute X, A_T is the mass transfer area, represented by the solid–fluid interface, D_{XA} is the diffusion coefficient of the solute X into the solid phase A, C_X is the gradient concentration of X inside the solid particle, and z is the distance measured from the particle interior.

At the surface of the solid particle the solute transfer occurs due to diffusion and convection simultaneously. In this step, the mass transfer rate can be expressed as:

$$\dot{M}_X = k_B A_T (C_{XAI} - C_{XB}) \quad (2.6)$$

where k_B is the individual mass transfer coefficient of the fluid phase (B), C_{XAI} is the concentration of X in the solution located at the solid–fluid interface, and C_{XB} is the concentration of X in the bulk solution. Usually it is assumed that the concentrations of X in both A and B phases at the interface (C_{XAI} and C_{XBI}) are in equilibrium.

While Equations (2.1) and (2.2) describe the thermodynamics of the extraction process, Equations (2.5) and (2.6) describe the mass transfer mechanism. As a result, the extraction processes usually follow a kinetic curve (Figure 2.3) where it can be noticed that the mass transfer rate is not constant. The extraction curves usually consist of three distinct phases: constant extraction rate period (CER), falling extraction rate period (FER), and diffusion controlled period (DC).⁴ In the CER period the easily accessible solute that surrounds the particle (1 and 2 in Figure 2.1) is removed at an approximately constant rate. In this step the mass transfer resistance is mainly in the stagnant film surrounding the particle. The main mechanism responsible for the mass transfer is convection; therefore providing agitation enhances the efficiency of the process. In the FER period there appears gaps in the solute superficial layer that covers the solid particle; therefore, mass transfer resistance in the solid–fluid interface begins. In this step the extraction rate decreases rapidly as a result of the decrease of the effective mass transfer area and diffusion starts being important. In this transition phase the mass transfer resistance is both in the solid and fluid phases, and both convection and diffusion mechanisms are significant. In the DC period the easily accessible solute layer is depleted; therefore, the extraction rate is determined exclusively by the diffusion rate of the solvent into the solid particle and of the solvent and solute from the solid particle to the bulk solvent. This mechanism characterizes a slow stage of the extraction process.

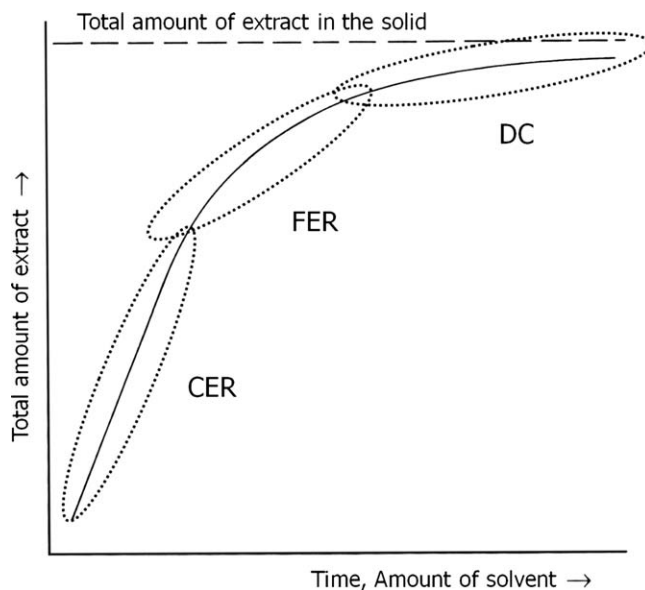


Figure 2.3 Typical kinetic curve observed for the extraction of natural products. Adapted from G. Brunner, *Gas Extraction. An Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Processes*, Springer, New York, NY, 1994, p. 387.

Another difficulty of the extraction process that must be taken into account is that in several cases the target compounds are not freely available and interact with other components from the raw material such as proteins, carbohydrates, and lipids. In this case it is also necessary to break the intermolecular interactions between these molecules before new intermolecular interactions can be established between the solute and the solvent. By providing enough energy to break the linkages it is possible to effectively extract the compounds that interact with the solid matrix, and the amount of energy spent in the process affects the extraction efficiency.

An example of an interaction between target compounds and the solid matrix in the extraction of natural products that may affect the process is the formation of protein–polyphenol complexes. The extraction of isoflavones, a type of polyphenol, is negatively influenced by the protein content of soy products. Using the same conditions, samples with higher protein content produce lower isoflavone yield (41% of the total isoflavone content) when compared to the yield obtained from the sample with lower protein content (58% of the total isoflavone content). This difference was attributed to protein–polyphenol interactions in the sample, which can be due to a variety of interactions including hydrogen bonding, ionic and covalent binding, and hydrophobic interactions, strongly influenced by factors such as temperature, pH, and salt.^{5,6} Thus, the molecular interactions depend on the raw material characteristics and may play a decisive role on the effectiveness of the extraction process of specific classes of compounds.

After the extract containing bioactive compounds is obtained from a natural source, it has further applications. The subsequent processing steps depend on the process scale. For analytical purposes, the next step is usually the qualitative or quantitative determination of the phytochemical profile by spectroscopic or chromatographic techniques. On the other hand, the extract can be the final product in industrial scale processes.

2.3 Exhaustive Versus Non-exhaustive Extraction Methods

In the exhaustive extraction methods virtually all of component X is transferred to phase B either by reaching the partition equilibrium several times or by increasing K_d values, i.e. reaching higher transfer of X from phase A into phase B. K_d is constant at fixed temperature, but other components in the medium can modify the relative values for component X, by modifying K_d or not. For example, pH changes affect the level of free/combined component X in aqueous phases and the addition of salt to an aqueous phase modifies its polarity, thus affecting the distribution of component X.

In non-exhaustive methods, phase B is usually unable to extract a large amount of component X, because the relative amount of phase B is significantly lower than phase A or because the mass transfer is too slow. In this case the knowledge of equilibrium data is mandatory to obtain data about the composition in phases A and B. Non-exhaustive methods are usually faster than exhaustive methods because it is not necessary to reach the partition equilibrium.

Exhaustive methods are most commonly applied in analytical methods of natural products extraction. These approaches include liquid–liquid extraction, Soxhlet extraction, and several sorbent-based extraction methods. They are habitually used in the analytical determination of the phytochemical profile of samples. The process is usually extended as much as required to allow the total recovery of the target components in the sample. The main advantages of exhaustive methods are:

- corrections of the relative recovery data are not required; therefore lower quantification errors are associated with the analytical methods;
- higher total amount of components will be found in the extracts; therefore a higher analytical signal may be obtained.

Exhaustive methods usually require a long process in order to allow the complete removal of the target components from the sample matrix. These methods require that the DC phase of the extraction is reached and extended (Figure 2.3) in order to ensure quantitative recovery. One conservative approach for exhaustive extraction is multiple consecutive extraction steps that can include different extraction methods and/or solvents. Since reaching DC is not a requirement in non-exhaustive methods, in this case the process is shorter,

reaching only FER or the beginning of DC period. In industrial applications, the shorter the process time the higher the number of batches that can be performed; therefore, stopping the process before it reaches the DC period may be economically advantageous.

Another major difference between exhaustive and non-exhaustive extraction methods is the solvent to feed ratio (S/F) used. Higher S/F is used in exhaustive methods (usually 50–100) in order to increase K_d and facilitate the mass transfer between phases A and B. In contrast, lower S/F (5–10) is used in non-exhaustive methods, intending to provide a highly concentrated B phase. This is especially important at industrial-scale processes, where lower S/F implies lower costs associated with solvent removal in the subsequent processing steps. That is why industrial processes are most commonly carried out using non-exhaustive methods for the extraction of natural products.

2.4 Conventional Extraction Techniques

The extraction method to be applied to a particular solid matrix depends on the raw material to be processed and on the product desired. There is no single and standard extraction method for obtaining bioactive compounds from natural products, each one presenting advantages and disadvantages. There are several solid–liquid extraction techniques available. The most commonly used conventional techniques are soaking extraction, Soxhlet extraction, and distillation. Choosing one of them for extracting bioactive compounds from natural products depends on process conditions such as temperature, mechanical action (such as pressure and shaking), and solvent type.⁷ Applying heat and agitation usually accelerates extraction kinetics by making the diffusion of the solute through the interface of the solid matrix with the solvent easier. Next some of the main extraction techniques will be presented.

2.4.1 Soaking

In this process, the untreated or powdered plant material is placed in a container along with the solvent. The plant material stays in contact with the solvent for several hours or even days, during which the soluble material is transferred from the solid sample to the solvent. Usually some kind of agitation is provided to increase the mass transfer rate by increasing the turbulence. Agitation devices are frequently used to process fine particles, since agitation avoids the bed compression and its consequent channeling, which reduces the process efficiency. Furthermore, the dispersion of the particles in the liquid solvent by the agitation facilitates the contact of the solid with the solvent, accelerating the process by favoring the diffusion of the extracted components and avoiding super saturation in the immediate proximity of the surface of the solid to be extracted.⁸ However, care should be taken with excessive agitation, which may cause the disintegration of particle solids.

The most common is to perform the process under room temperature, but heat can be applied to improve the extraction efficiency. However, when

extracting thermosensitive compounds high temperatures should be avoided, thus the tunable process temperature becomes an advantage of soaking extraction method.

When working at room temperature, long extraction times are usually needed to achieve high recoveries. Contini *et al.*⁹ used overnight (20 h) extraction to recover significant amounts of antioxidant compounds from hazelnut – mainly simple phenolics but also some tannic components and some flavonoids. In that case, besides the raw material characteristics, the extraction solvent had a marked effect on the final level of antioxidants in the extracts. The use of ethanol as solvent led to the highest phenolic concentration in the extract from whole hazelnut roasted kernel whilst acetone produced significantly higher values for phenolics than ethanol in the extraction from hazelnut woody shell.

Soaking procedures can be carried out more rapidly when using high extraction temperatures rather than room temperature. For example, Cho *et al.*¹⁰ found a direct relationship between total isoflavone recovery and extraction temperature in the solid–liquid extraction of soybean sprout cotyledon using ethanolic mixtures at different temperatures. The mathematical model obtained after an experimental design approach indicated that extraction temperature influenced the recovery even more than ethanol concentration in the solvent phase. Extraction time also presented lower effect than temperature. A similar result was found by Tsakona *et al.*¹¹ when working with aromatic plants and fruit-bearing tree leaves for phenolics extraction. It was found that temperature was the most important extraction variable after reaching equilibrium for different extraction conditions. Temperature had a greater effect on the yield than the concentration of ethanol in the solvent.

The effect of extraction temperature on the diffusion of target compounds during extraction has been assessed in several studies. In a study on the solid–liquid extraction of resveratrol from grape canes, Karacabey and Mazza¹² established that temperature and ethanol concentration in the extraction solvent were major process variables regarding resveratrol recovery, whereas S/F was found to be insignificant under any conditions. The recovery yields of *trans*-resveratrol, *trans*- ϵ -viniferin, and total phenolics increased with increasing temperature, reaching a maximum at the highest assayed temperature (83.6 °C). These authors determined the diffusivity values of resveratrol in the solid phase by fitting the experimental results to a model derived from Fick's second law (2.5). The effective diffusivity values increased with temperature and the highest predicted level for diffusivity was eight times higher than the value found for the lowest extraction temperature.

The remaining solid material at the end of the process is pressed and it is usually re-extracted. The liquid phases combined from the several extraction steps go through a concentration step to recover the target compounds after the removal of the solvent. There are several concentration methods used to recover the target compounds, including evaporation, solid phase extraction, and freeze-drying.

Despite its advantages, the soaking extraction method also has distinct drawbacks: (1) the time-consuming and labor intensive operation leading to a large volume of hazardous solvents and further clean-up and concentration steps required; (2) the high energy demand for the solvent–solute mixture separation; (3) the product quality loss in solvent evaporation step, in particular for food and pharmaceutical products, due to the retention of undesirable chemical products and the degradation of thermosensitive components; and (4) the mass transfer rate decrease with time because the solvent is continuously enriched with solutes.

2.4.2 Soxhlet

The classical Soxhlet apparatus was designed by Franz von Soxhlet in 1879 and it remains useful until today. It has been used for a long time for the extraction of natural products from plants. It is also useful for soil and sediment analysis as well as for food analysis. Soxhlet is used as a reference extraction method for evaluating the performance of new solid–liquid extraction approaches, even for the most advanced extraction methods, due to its simplicity, low cost per sample, and the inexpensive and robust extraction apparatus. Soxhlet extraction is still used as a reference method in the US EPA official methods, such as 3540B¹³ and others in the AOAC¹⁴ and British Standards.¹⁵ It is a general and well-established technique, which surpasses in performance other conventional extraction techniques except for, in limited field of applications, the extraction of thermolabile compounds.¹⁶ Because of that, Soxhlet is the recommended apparatus for several analytical determinations.

The typical Soxhlet apparatus is shown in Figure 2.4.¹⁷ The ground plant material is placed in a thimble made from thick filter paper or from glass with a porous frit. The thimble is placed in a glass extraction chamber above a flask containing the extracting solvent and below a condenser. The solvent is boiled and the extraction chamber gradually fills with fresh solvent from the distillation flask. When the condensed solvent fills the extraction chamber and reaches a maximum level, it is rinsed back into the distillation flask by a siphon, carrying the extracted solutes into the solvent reservoir below. At this point the extraction thimble does not contain any solvent. The cycle is repeated usually each 10–15 min. In the solvent flask, the solute is separated from the solvent by distillation, i.e. the target components must have lower volatility than the solvent. Therefore, the solute is left in the flask while fresh solvent is evaporated and passes back into the plant solid material. It must be noted that each cycle involves an equilibrium step. It is important to note that fresh solvent reaches the sample in each cycle, and thus saturation of the solvent will not occur, in contrast to classical soaking extraction, even when using hot solvents. After several hours of reflux the extract is concentrated by evaporation of the solvent.

Soxhlet extraction is a general and well-established technique that produces higher yields than other conventional extraction techniques. It is therefore an exhaustive extraction method. It is largely dependent on plant characteristics and particle size, as the internal diffusion may be the limiting step during

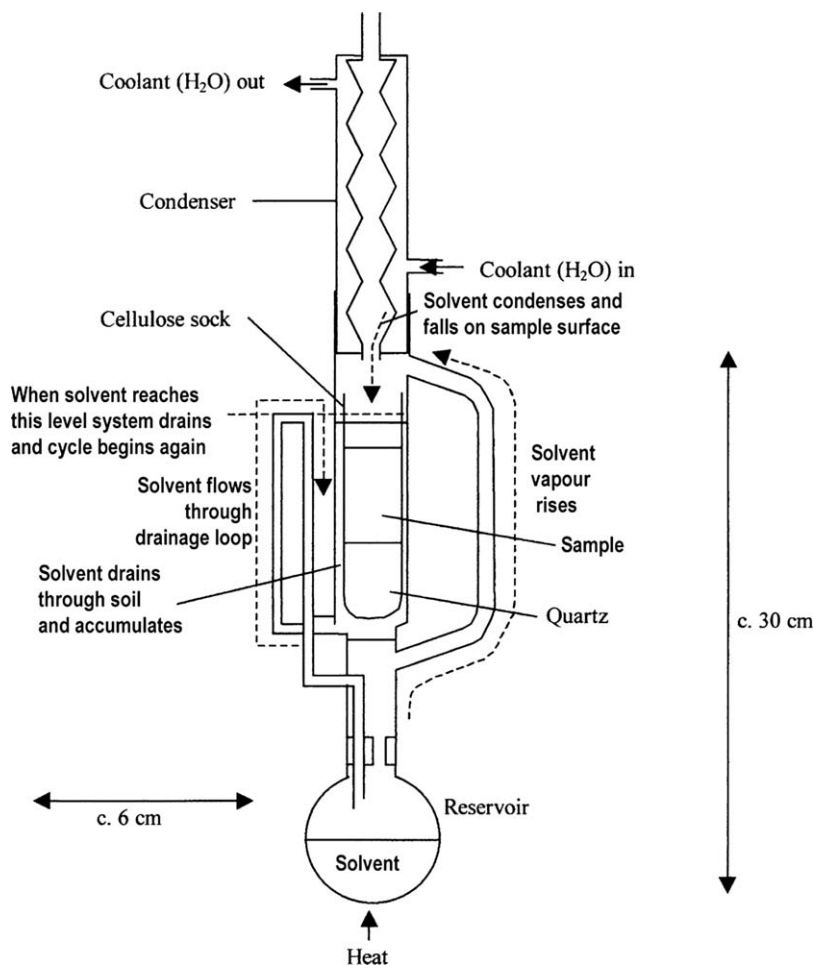


Figure 2.4 A typical Soxhlet extraction system.
Adapted from M. E. Hodson, *Geochim. Cosmochim. Acta*, 2002, **66**, 819.

extraction, and on extraction and evaporation temperatures that affect the quality of the final product.

Several solvents have been used for the extraction of active components from plants.¹⁸ Soxhlet has been specifically applied to extract vegetable oil. In these applications hexane has been the most commonly used solvent. Different types of bioactive compounds have been isolated using this method with hexane as solvent.¹⁹ Hexane has a boiling point of approximately 65 °C, i.e. it should enable the application of Soxhlet extraction to any compound with a boiling point above 65 °C. Hexane is also an excellent solvent for oil because of the high oil solubility in this solvent and also because oil can be easily recovered by distillation. The main drawback for the use of hexane is its high toxicity. As a result, other solvents have been used to substitute hexane in oil recovery,

including some medium polarity alcohols such as isopropanol and ethanol.²⁰ Another option is to use much more polar solvents at a specific pH, e.g. water at pH 12. This solvent produces extracts from plants with lower amounts of free fatty acids.²¹ In most cases the use of solvents other than hexane leads to lower recovery of the lowest polarity components and several applications were therefore developed in which hexane mixtures were used to produce similar recovery to pure hexane, with lower levels of contaminants in the extracts.²²

Finally, some new green solvents have started to come into use in recent years for specific applications. A very good example is the application of terpenes (*d*-limonene, α -pinene, and *p*-cymene) for the recovery of oil from microalgae.²³ In this case, terpenes were obtained from renewable feedstocks, making the whole process greener. Individual levels of fatty acids, including both saturated and unsaturated, were compared after extraction with *n*-hexane and three terpenes. Significant differences in the recovery were found in some cases, although for the main fatty acids in the sample, i.e. oleic acid and palmitic acid, no real variation was found, with slightly higher yield obtained for palmitic acid when using any terpene as extraction solvent and a lower yield of oleic acid when using *d*-limonene. Finally, the total oil yield ranged from 0.88% with *n*-hexane as the extraction solvent to 1.52% with *p*-cymene as solvent.

The main advantages of Soxhlet include: the use of high temperatures, which increase the mass transfer rate; the displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with the solid matrix; and no requirement of a filtration step after leaching. On the other hand, the extraction time is long, a large amount of solvent is used, agitation cannot be provided, and there is the possibility of thermal decomposition of the target compounds because the extraction usually occurs at the boiling point of the solvent for a long time.^{16,24} Reviews of Soxhlet extraction for solid samples were presented by Luque de Castro and Garcia-Ayuso,¹⁶ and by Luque de Castro and Priego-Capote.²⁵

Several modified Soxhlet systems have been designed in an effort to overcome the drawbacks of the classical technique. Most of them focus on speeding up the process in an attempt to reduce the thermal degradation of the target compounds and the solvent consumption. Some alternatives to increase the speed at which the matrix releases components is applying, for example, microwaves^{26,27} or ultrasound.²⁸ A simplified scheme of a focused microwave-assisted Soxhlet extraction (FMASE) system is presented in Figure 2.5.

In FMASE systems the extractor design is the same of a conventional Soxhlet apparatus and the solvent is heated by conventional means (an electrical jacket, for instance). The microwave irradiation is focused only in the part of the extraction vessel containing the sample and is directed to affect both the solvent and the sample. Of course the effect of microwaves on both will depend on their nature and characteristics.

Another modification of the classical Soxhlet extraction is by assistance of ultrasound (ultrasound-assisted Soxhlet extraction – UASE). An experimental UASE system is presented in Figure 2.6. The application of ultrasound directed

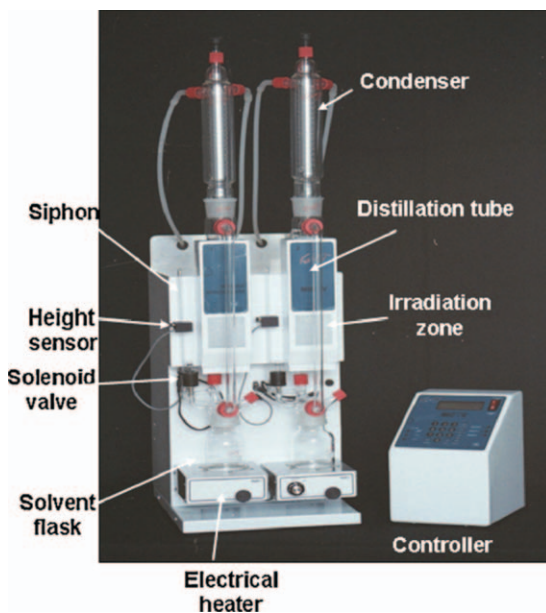


Figure 2.5 Scheme of a focused microwave-assisted Soxhlet extraction FMASE system. Reproduced from Ref. 25 with permission from Elsevier.

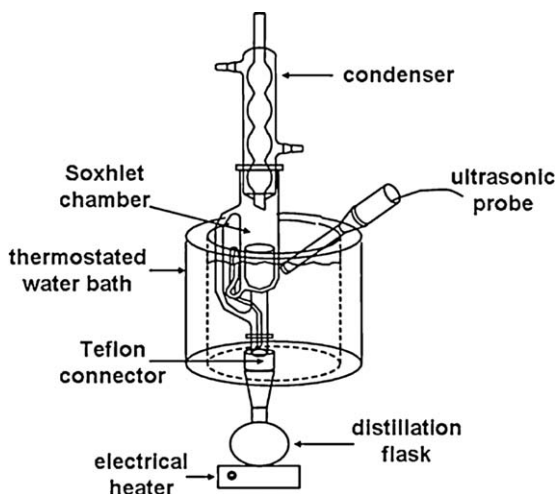


Figure 2.6 Experimental ultrasound-assisted Soxhlet extraction system. Reproduced from M. D. Luque de Castro and F. Priego-Capote, *J. Chromatogr. A*, 2010, **1217**, 2383 with permission from Elsevier.

to the sample and solvent causes the formation of cavitation bubbles that collapse the cell structure and therefore facilitate the extraction process.

The application of ultrasound combined to Soxhlet reduces the number of cycles needed for exhaustive extraction of fats from oleaginous seeds as

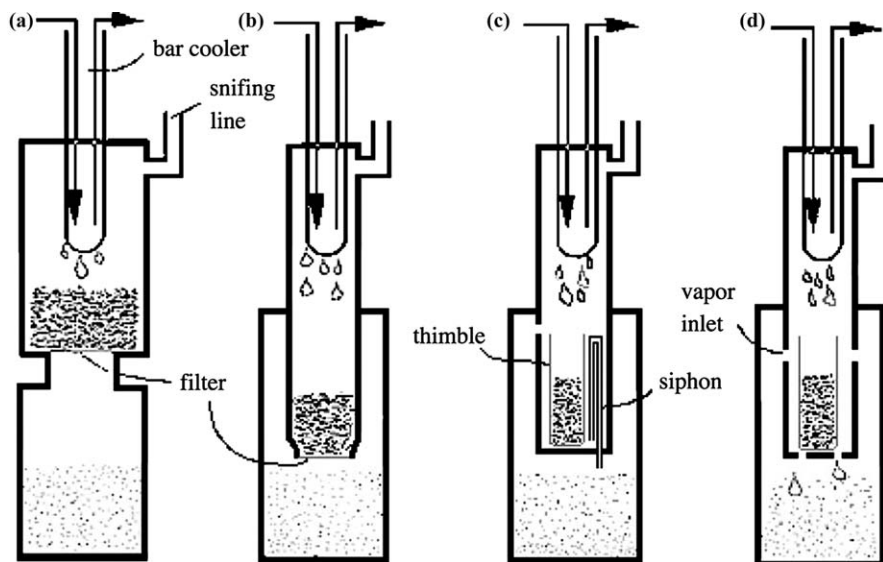


Figure 2.7 Scheme of the different turbulent extraction systems: (a) fluidized-bed extraction (FBE), (b) dive-in FBE, (c) dive-in Soxhlet extraction, (d) dive-in thimble extraction.

Reproduced from D. Bandoniène, M. Gfrerer and E. P. Lankmayr, *J. Biochem. Biophys. Methods*, 2004, **61**, 143 with permission from Elsevier.

sunflower, rapeseed, and soybean. The higher extraction efficiency reduces the process time to at least half of the time needed by the conventional procedure. Furthermore, the application of ultrasound does not affect the composition of the oil.^{20,28}

Another strategy developed to increase the extraction efficiency of Soxhlet (and other techniques) is agitating the extraction bed (Figure 2.7).²⁹ Although only a few applications of this technique are available, the new modified Soxhlet methods have a great potential to be used for the extraction of natural products.

2.4.3 Distillation with Water and/or Steam

Soxhlet is not applicable to highly volatile components, but only to components that have a boiling point below the solvent boiling point. For highly volatile compounds distillation is the preferred alternative. Water and steam distillation are used for the extraction of several volatile bioactive components from plants.³⁰ The product of this process is known as volatile oil or essential oil. It is the most widely used method at industrial scale for the processing of natural products when the target compounds are volatile.

This technique simply involves vaporizing or liberating the volatile compounds from the solid matrix at high temperatures using water and/or steam as extracting agent.³¹ The water/steam heats the solid matrix, which releases the volatile compounds present in it. These are vaporized taking

vaporization heat from the steam, and then are transported to the steam through diffusion. The resulting vapor phase is then cooled and condensed prior to separating water and the organic phase based on their mutual immiscibility. The volatile oil constitutes the upper phase in the decanter, while the bottom phase is constituted of water containing some hydrolyzed compounds, known as hydrosol. The compounds present in hydrosol usually confer to it a pleasant aroma; therefore, it can be used in the formulation of lotions, soaps, ambient aromatizers, etc.

There are three variants of the distillation with water/steam process: direct steam distillation, water distillation (hydro-distillation), and dry steam distillation (Figure 2.8). In direct steam distillation the solid matrix is supported on a perforated grid or screen inserted some distance above the bottom of the still, but it is not in direct contact with water. The boiler can be inside or outside the still. The saturated steam flows up through the solid, collecting the evaporated components. In hydro-distillation the solid matrix is immersed in the boiling water or floating on it, depending on its density. In this case the boiler is inside the still and agitation may be necessary to prevent agglutination. In dry steam distillation the steam flows through the solid matrix bed, as in direct steam distillation, but the steam is generated outside the still and can be superheated at moderate pressures.³³ Because of this feature, it is extensively used to extract terpenes, including mono- and sesquiterpenes, which have boiling points above 150 °C.³² Therefore, superheated steam distillation is more useful than regular hydro-distillation for compounds that have boiling points above 100 °C.

In this process the essential oil must first be extracted from the solid matrix using high temperature and then it must be separated from the water phase. As a result, several points must be considered when applying distillation.

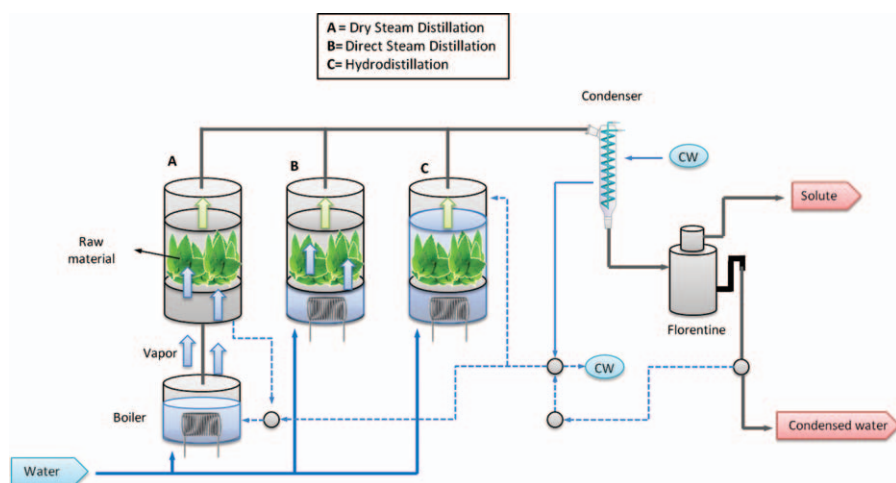


Figure 2.8 Generalized flow sheet of the different types of distillation with water/steam. Adapted from P. Costa, C. Grosso, S. Gonçalves, P. B. Andrade, P. Valentão, G. Bernardo-Gil and A. Romano, *Food Chem.*, 2012, **135**, 112.

Firstly, the bioactive compounds to be isolated cannot be easily degraded by high temperatures. Secondly, as hot water will be in contact with the extracted compounds, stability to hydrolysis should be also confirmed prior to distillation. Finally, an organic–water final mixture will be obtained and complete distribution in the organic phase of the compounds of interest must also be ensured. Only components with boiling points below 100 °C can be easily separated by regular distillation from water. For compounds with boiling points above 100 °C superheated steam distillation is required.

Distillation is still used as a reference method for developing new assisted extraction techniques, mainly in relation to supercritical fluid extraction (SFE). SFE has numerous extraction variables that need to be optimized and, as a result, the recovery can be dramatically modified by using different extraction conditions. In contrast, distillation has few working variables that can be optimized. Therefore, some specific SFE conditions can usually be found that are capable of producing better yields for specific components. On the other hand, there are also results that demonstrate how distillation can produce higher yield than SFE for specific components. It has been found, for example, that the two methods, when applied to *Lavandula viridis* L'Hér,³³ produce different results, with higher yield for most components when using SFE but a higher total number of different compounds obtained by distillation. A similar result was found in a study of the chemical composition of *Lavandula stoechas*.³⁴ The comparison of results obtained by distillation and subcritical water extraction (SWE) showed that the yield of total monoterpene hydrocarbons was higher for distillation than for SWE.

Regarding bioactive properties, similar results were found for extracts obtained by distillation and extracts obtained by microwave-assisted extraction (MAE) in many cases. MAE and distillation applied to *Saccocalyx satureioides* produced very similar results in terms of extract composition, mainly terpenes, and also related to antifungal and antimicrobial properties.³⁵ A study in which the volatile components of clove buds were extracted with both SFE and steam distillation showed that the composition of the clove oil extracted in both cases was very similar. In contrast, the relative concentrations of the compounds were different but both extracts contained approximately the same number of different compounds.³⁶

New methods based on SWE to recover essential oils from *Origanum onites* have also been compared to steam distillation.³⁷ After optimizing the working variables for SWE, clear differences were not obtained in the extracts, although slightly higher yields were obtained than in steam distillation. Pressurized liquid extraction (PLE) has also been compared to steam distillation. PLE can produce a very similar yield of volatile components, but it usually co-extracts various different non-volatile components, for example from thyme herb,³⁸ which makes it a less selective process.

Distillation with water/steam is largely used because it presents a series of advantages compared to other extraction processes: the method generates organic-solvent-free products; there is no need of subsequent separation steps, as the volatile oil is the final product leaving the separator; in industrial scale

this method has a large capacity of processing; equipment is inexpensive; and there is extensive know-how available for this technology.

On the other hand, the process suffers of some serious drawbacks: possible thermal degradation of products; possible hydrolysis, especially for esters, which is an extremely difficult problem to overcome if it occurs; very long extraction times (1–5 hours); and high energy consumption.

Similarly to Soxhlet, several modified distillation systems have been designed in an effort to overcome the drawbacks of the classical technique. Vacuum can be applied to steam distillation to reduce the extraction temperature and time, thus decreasing the thermal degradation of the target compounds (Figure 2.9).³⁹

Hydro-distillation was also combined to microwaves to increase the efficiency of the extraction process of natural products. In some cases, domestic microwave ovens are adapted to assist the process (Figure 2.10).⁴⁰ A more sophisticated approach is the Ohmic-assisted hydro-distillation process (OAHD) (Figure 2.11).⁴¹ OAHD may provide faster extraction kinetics at lower cost while reducing the environmental impact of the process and producing a similar product to those obtained by conventional hydro-distillation.⁴¹

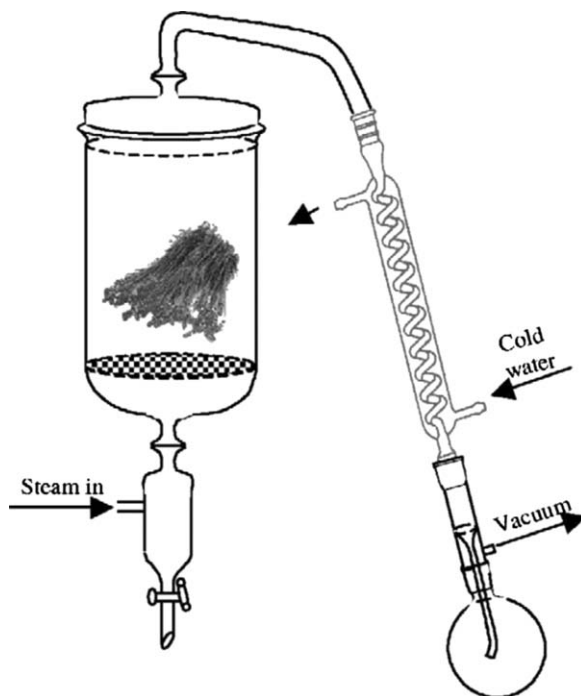


Figure 2.9 Schematic representation of reduced pressure steam distillation apparatus. Reproduced from N.-S. Kim and D.-S. Lee, *J. Chromatogr. A*, 2002, **982**, 31 with permission from Elsevier.

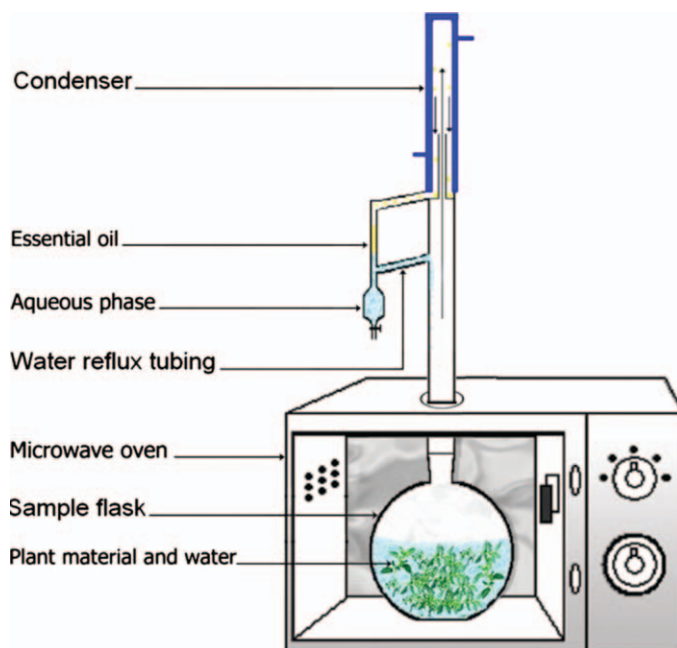


Figure 2.10 Microwave-assisted hydro-distillation set-up with household microwave oven. Reproduced from M.-T. Golmakani and K. Rezaei, *Food Chem.*, 2008, **109**, 925 with permission from Elsevier.

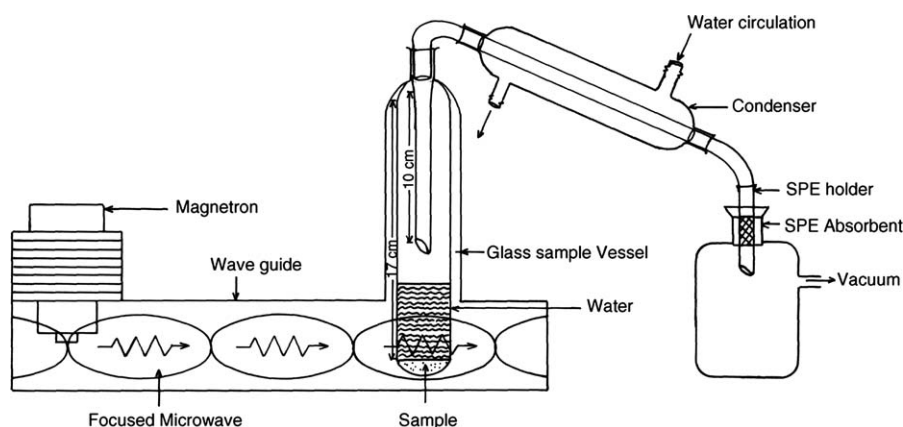


Figure 2.11 Schematic representation of an ohmic-assisted hydro-distillator. Reproduced from M. Gavahian *et al.*, *Innov. Food Sci. Emerg. Tech.*, 2012, **14**, 85 with permission from Elsevier.

One possible combination of microwaves to steam distillation is focused microwave-assisted steam distillation (FMASD) (Figure 2.12).⁴² As the name implies, in FMASD, the microwave irradiation is directed to the sample and the solvent inside the extractor. The extracted material is cooled down and

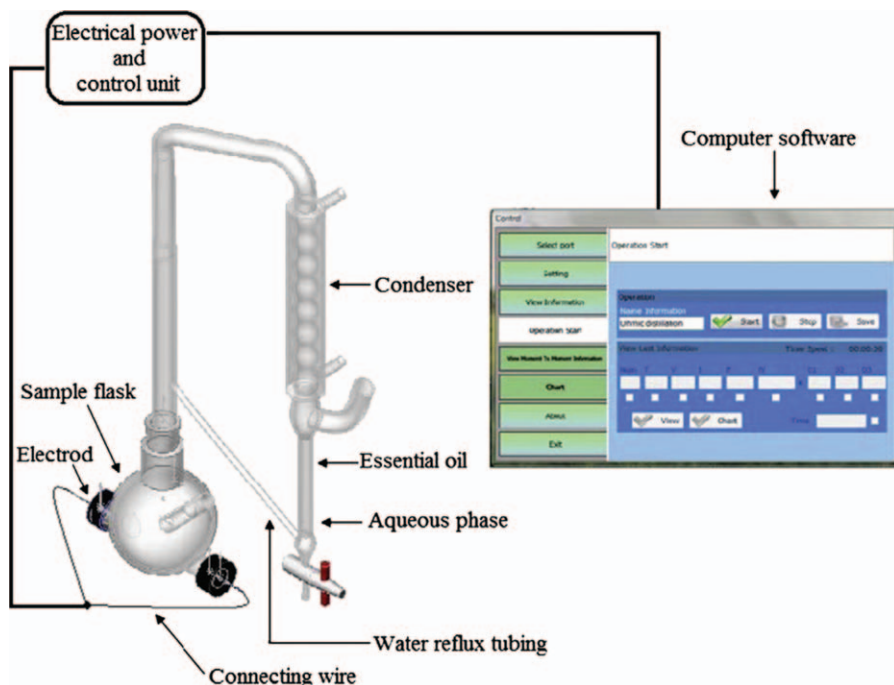


Figure 2.12 Focused microwave-assisted steam distillation set-up with on-line solid phase extraction enrichment arrangement. Reproduced from R. Ganeshjeevan *et al.*, *J. Chromatogr. A*, 2007, **1140**, 168 with permission from Elsevier.

collected in a separation vessel. Furthermore, other techniques can be coupled to the system. In the example shown in Figure 2.12, a solid-phase sorbent was used after the condenser to retain specific compounds, increasing the selectivity of the process.

2.5 Main Extraction Variables

Besides the technique, extraction efficiency is also a function of the process conditions. Several factors affect the concentration of the desired components in the extract, such as solvent type, temperature, solvent to feed ratio, contact time, particle size, etc. Therefore, the best extraction method with optimized conditions should be employed for preparing each particular product. Next the main process variables are presented.

2.5.1 Preparation of the Solid

Extraction processes are largely influenced by the natural characteristics and components of the raw material. The content of the target compounds in the raw material may vary with the degree of plant ripeness, cultivar, and

edaphoclimatic conditions. Moreover, the pre-processing of the plant material can alter its phytochemical profile.

When preparing the solid material for extraction, there are a few aspects that should be taken into account so that the subsequent extraction step is more efficient. Some of the most important are particle size, moisture content, homogeneity, and porosity. The mass transfer rate also depends on the location of the solute inside the solid particle (Figure 2.1), as it dictates the diffusion, which is the main limiting mass transfer mechanism of the process.

The mass transfer rate of the solute from the surface of the solid particle to the solvent depends on the solid superficial area. Reducing the particle size results in higher superficial area, which increases extraction rates. Moreover, it decreases the intraparticle diffusion path, leading to more efficient extraction. However, there is a limit for comminuting the solid; too fine particles can cause bed compression, leading to channeling, which decreases the process efficiency. Therefore, the particle size should be appropriately assessed to guarantee adequate balance between increased mass transfer area while avoiding channeling.

As an example, in the extraction of tea by soaking with agitation in water, while powder tea extraction was completed within 5 min, the same process with leafs took 30 min.⁴³ On the other hand, the extraction of volatile oil from flowers and whole leaves by distillation with water/steam may not require prior comminution because their structure is sufficiently permeable to allow the vaporization of the solutes, and milling the raw material can cause exposure of the solutes to oxidation.³

The water present in the solid matrix may compete with the solvent for dissolving the extract, which would affect the process efficiency. On the other hand, in some cases the moisture is necessary to allow the solute transfer during the extraction. For the extraction of hydrosoluble compounds the presence of water is beneficial, while for liposoluble compounds the raw material should be dried prior to extraction. The drying should be controlled, though, because when high temperature is used in this step, it may affect the profile and concentration of the phytochemicals present in the raw material since some of them can be submitted to thermal degradation. Therefore, when dealing with natural products, low drying temperatures are always preferred.

2.5.2 Solvent

In extraction processes the solvent type is the primary parameter that affects the efficiency of the process, because it determines two important factors: the solubility of the target compounds; and the penetrability into the matrix. The solvent characteristics that should be considered include:⁴⁴

- selectivity: it guarantees the solubilization of target compounds and their purity;
- reactivity: the solvent should not react with the target compounds;

- chemical and thermal stability: they must be assured under the extraction conditions;
- viscosity: low viscosity increases the mass transfer rate by increasing the diffusion coefficient;
- boiling point: low boiling point requires low energy for solvent removal from the extract;
- flammability: flammable solvents should be avoided;
- toxicity and regulatory issues: environmental and health issues should be considered, for both the consumer of the natural products and for the operator of the extraction system;
- economic aspects: solvent cost can be an important part of manufacturing costs at industrial scale (for further details see Chapter 12).

Considering all these aspects, there is no universal solvent for extracting bioactive compounds from natural products, because specific solvents are required for each raw material and target compound.

Because bioactive compounds obtained from natural products can be destined to use in food and pharmaceutical industries, the US Food and Drug Administration (FDA) classifies the solvents that can be used for this purpose in three levels of toxicity. The solvents acceptable for any alimentary purpose belong to Class 3, and include acetone, ethanol, ethyl acetate, 1-propanol, 2-propanol, and propyl acetate. A small percentage of residual Class 3 solvent is allowed in the final product. Class 2 includes solvents that are allowed in specific cases, with a residual allowance of 50–3880 ppm, depending on the solvent. They present a higher level of toxicity when compared to Class 3 solvents. Acetonitrile, chloroform, hexane, methanol, toluene, methyl ethyl ketone, and dichloromethane can be found in Class 2. Class 1 comprises solvents that although they can be acceptable in some analytical applications, are forbidden in industry because of toxic effects to human health and to the environment: benzene, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethane, and 1,1,1-trichloroethane. Because of these restrictions and the search for greener processes, water, ethanol, hexane, and their mixtures are the most used extraction solvents.⁴⁴

A good example that shows the effect of solvent in the recovery of bioactive compounds from cayenne peppers (*Capsicum* spp.) by Soxhlet was conducted using five different solvents: hexane, ethyl acetate, acetone, methanol, and methanol:water (80:20).⁴⁵ Four different types of bioactive components were studied: phenolics, capsaicinoids, carotenoids, and flavonoids. Hexane extracts contained the highest levels of capsaicinoids and carotenoids, but methanol extracts presented the maximum levels of flavonoids. This selectivity is strongly related to the solubility of the target compounds in the solvents, because when using Soxhlet the effects due to the penetrability of the solvent into the matrix are not as important after equilibrium is reached. Antioxidant properties of hexane extracts were the highest, probably due to the strong antioxidant properties of capsaicinoids

and carotenoids and the high levels of those components detected (up to 3.5 mg/g for capsaicinoids in Ixtapa pepper variety and 0.7 mg/g for carotenoids in Tuxtla pepper variety); the hexane extracts did not contain any phenolic compounds. The hexane extracts presented 79.6–95.1% 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition in the DPPH scavenging test versus 25.5–49.4% inhibition of ethyl acetate extracts, which in turn presented the highest phenolic contents (36.4–68.9 mg catechin equivalents/g of extract). Extracts obtained with other solvents resulted in intermediate yield and antioxidant activity.

Another very good example of the effect of solvent on the extraction process was presented by Goulas and Manganaris.⁴⁶ These authors evaluated the recovery of some triterpenic acids (maslinic and oleanolic acids) from olive fruit. Different extraction techniques, including solid–liquid maceration, heated solid–liquid maceration, and automated Soxhlet extraction, were compared. The efficiency of exhaustive solid–liquid extraction for the total triterpenic acids ranged from 2015 to 2372 mg/kg (Fresh weight) for ‘Kalamon’ fruit. The use of methanol:ethanol mixtures in this extraction method led to a significantly higher yield than for any other solvent at room temperature. On the other hand, when the extraction temperature was increased, there were no significant differences between the yields obtained with ethyl acetate, methanol, and methanol:ethanol. This finding indicates that there can be a strong interaction between the different process parameters, which can be influenced by each other. Additionally, ethyl acetate produced the highest yield when using automated Soxhlet.

In summary, the solvent must be optimized not in isolation but in conjunction with the other extraction variables. This factor will be even more important when working with assisted extraction techniques like ultrasound-assisted extraction, microwave-assisted extraction, or pressurized liquid extraction.

For the extraction of carotenoids, carbon disulfide is the best solvent, but volatility, flammability, toxicity, and degradation limit its use, thus, acetone, chloroform, dichloromethane, ethanol, ethyl ether, ethyl lactate, heptane, hexane, isooctane, methanol, petroleum ether, or a mixture of these solvents are being used instead. It is possible to separate xanthophylls from carotenes by using an extraction process with a polar solvent, like methanol, followed by another extraction with non-polar solvent. However, due to toxicity of some of these solvents, ethanol:water mixtures and other green solvents have been preferred, even if the extraction is not exhaustive in this case.⁴⁷

In the recovery of caffeine and polyphenols from tea and coffee, extraction with methanol and acetone usually are the most efficient. Nevertheless, many researchers have been looking for greener solvents, so that now water, ethanol, and their mixture have been used for this purpose.⁴⁸

The conclusion of solvent choice for the extraction of bioactive compounds from natural products is that despite there being no consensus about the

best extraction solvent for each raw material, a general trend is substituting toxic solvents by greener solvents, for both industry and analytical chemists.

2.5.3 Temperature

Temperature usually has a significant effect on the extraction process because changes in it modify the properties of both the solute and the solvent. Temperature affects the solubility and diffusivity of the solutes and also the viscosity and the surface tension of the liquids.⁴⁹ Furthermore, it may provide the necessary energy to disrupt the intermolecular interactions between components of the raw material and make the target components available for the extraction solvent.

On the other hand, in extraction of natural products it may be necessary to use less aggressive conditions, such as moderate temperature and protection from light and oxygen, to prevent the degradation of some thermosensitive compounds that would decrease the product quality. One illustration is the extraction of β -carotene from rose hips; the yield increased with the increase of temperature, but above 45 °C degradation of β -carotene was observed.⁵⁰

2.5.4 Time

Extraction time is a parameter directly related to temperature. Although extending the process increases the yield (Figure 2.3), prolonged exposure of the solid material to high temperatures can lead to the degradation of the compounds of interest.

As an example, some compounds present in tea, such as (–)-epicatechin and (–)-epigallocatechin, depend only on time for extraction efficiency, while others, such as (+)-catechin, (–)-epicatechin gallate, (–)-epigallocatechin gallate, (–)-gallocatechin gallate, proanthocyanidins, and flavonols, depend on time and temperature due to thermal degradation. Thus, it is advisable to use either a combination of high temperature (95 °C) and short extraction time (5–10 min), or low temperature (60 °C or 80 °C) and long extraction time (20 min) in order to avoid major degradation of catechins during the extraction.⁴⁸

2.5.5 Solvent to Feed Ratio

The yield of extraction processes tends to increase with solvent to feed ratio (S/F). A high S/F can be applied in one-step extraction, or lower ratios can be used in a multi-step process. However, high S/F also implies high solvent and energy consumption for solvent removal. Therefore, S/F should be as low as possible, while still ensuring the desired yield for the process.

2.6 Case Study

Leal *et al.*⁵¹ obtained *Foeniculum vulgare* (fennel) and *Pimpinella anisum* (anise) extracts. Fennel and anise both belong to the Umbelliferae family, and are sources of anethole, a compound of interest for food, cosmetics, and pharmaceutical industries. The extracts were obtained from whole and ground raw material, by steam distillation, Soxhlet, cold percolation, ultrasound-assisted extraction, and centrifugal extraction using ethanol as solvent (S/F = 10).

Centrifugal extraction and percolation are techniques largely used to obtain herbal extracts, but literature data on them are scarce. Centrifugation allows phase separation by the centrifuge force. It consists of placing an amount of raw material and solvent in a vessel which is submitted to centrifugation, followed by filtration to separate the exhausted raw material from the solvent + extract. The main disadvantage of this method is the necessity of adding the filtration operation unit.⁵¹

In percolation, the matrix is placed inside a thimble holder, like in Soxhlet, but the solvent + extract mixture is recirculated in the system using a pump.⁴⁴ The main advantages include the possibility to select the process temperature and no necessity of filtration. The major disadvantage is that the solvent recirculated is not fresh, so that the possibility of dissolving more solutes into the liquid phase decreases with time.⁵¹

Fennel and anise yields obtained using different extraction techniques are presented in Figure 2.13. The extraction methods presented approximately the same behavior and relative yields for both raw materials, with a high extraction rate in the first minutes of extraction, with a subsequent rapidly decreasing extraction rate, which is comparable to Figure 2.3. For both raw materials, the highest yields were achieved for Soxhlet extraction, reaching 16.8% and 23.3%, respectively, for fennel and anise. For Soxhlet, the kinetic curves reached a plateau, indicating the exhaustion of extract in raw material. In Figure 2.13a it can also be noticed that milling the raw materials has major impact on extract yield. On the other hand, the extraction techniques that presented lower yield (percolation and centrifugation), presented higher anethole content in the extracts. The highest anethole content among ethanolic extracts was obtained for centrifugal extraction (6.8 mg/g and 143 mg/g for fennel and anise extracts, respectively), which indicates that this extraction method was more selective. The anethole content in anise extracts was 20 to 40 times higher than in fennel extracts. Moreover, the yields obtained for anise were also higher. Therefore, technically, anise is a better source of anethole than fennel, although an economic evaluation should be conducted in order to evaluate the cost of manufacturing these extracts.

Figure 2.14 presents anise extraction by steam distillation. After 300 min the raw material was not exhausted and the total yield was only 0.26%. It can also be noticed the influence of steam temperature on yield during the process. As the steam overcame 150 °C, the extraction rate dropped to almost zero, which can be observed on the constant level reached by the OEC from 35 to 60 min. Comparing extraction with ethanol to steam distillation, it is evident that the

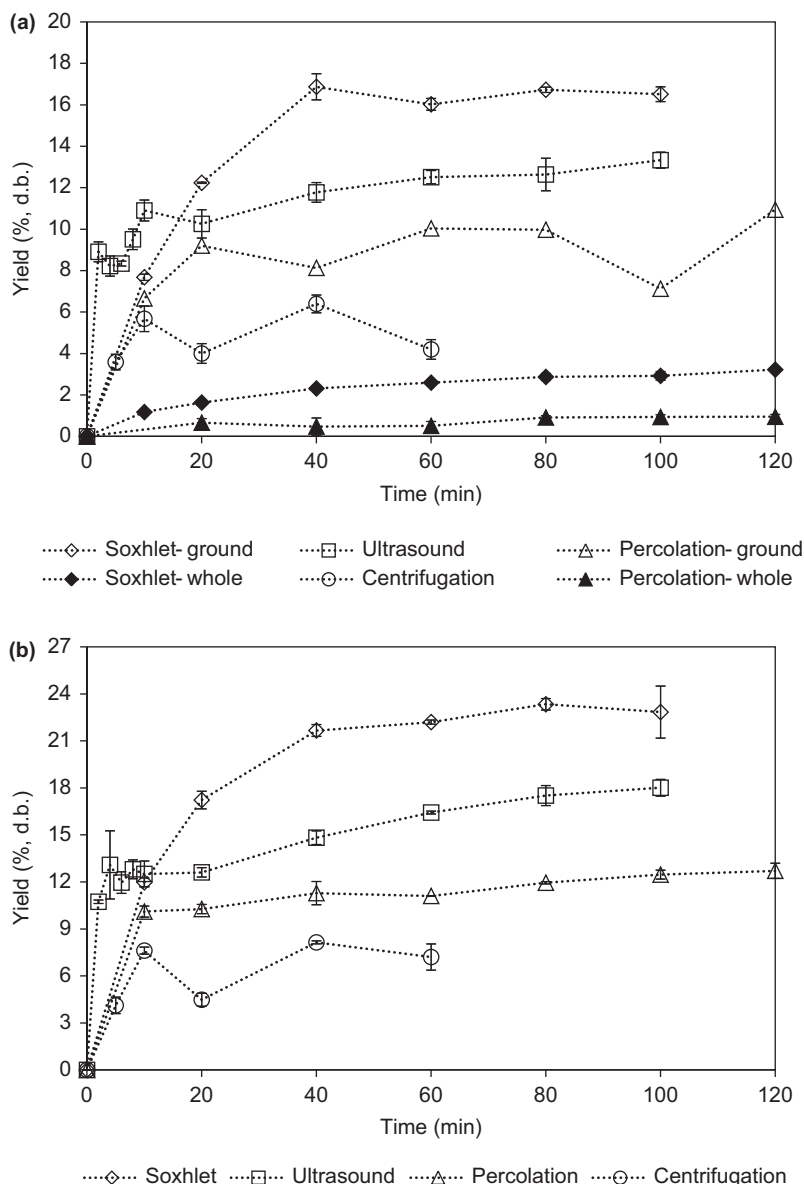


Figure 2.13 Extract yield of (a) fennel and (b) anise obtained by different solvent extraction methods and particle sizes using ethanol as solvent. Reproduced from P. F. Leal *et al.*, *Sep. Sci. Technol.*, 2011, **46**, 1848 with permission from Taylor & Francis Ltd.

former presents higher yields. However, when it comes to application on industry, the quality of the extract, i.e. its chemical composition, is mandatory to determine the best extraction technique. Despite the low yield, high anethole content was found in the volatile oil (68–98%). One option suggested by the

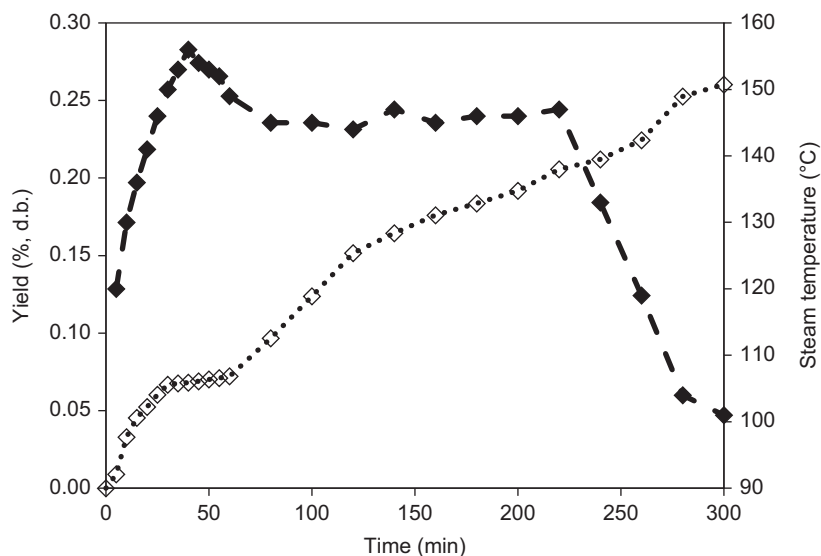


Figure 2.14 Extract yield of whole anise obtained by steam distillation ($\diamond$) and steam temperature ($\blacklozenge$) during the process. Reproduced from P. F. Leal *et al.*, *Sep. Sci. Technol.*, 2011, **46**, 1848 with permission from Taylor & Francis Ltd.

authors is using steam distillation to recover anethole, followed by ethanol extraction to recover an extract containing flavonoids.

2.7 Conclusions

The extraction of natural products is a complex process that is influenced by several factors. Depending on the objective of the extraction (analytical, semi-preparative and preparative separations or industrial production of extracts) different process techniques and operational conditions may be used. In this aspect, it is critical that process conditions are fully optimized with this objective in mind and that these conditions are not universal and may be adjusted for different types of raw materials. Without doubt, the raw material characteristics, the solvent used, the process temperature, and time are the main variables involved in most extractions of natural products and they are irrevocably associated with the success of the process. Another important process parameter is the solvent to feed ratio (S/F). An adequately balanced S/F will ensure that the objective of the extraction process is achieved in the shortest possible process time. There are several techniques that are conventionally used for the extraction of natural products. The most representative techniques are soaking, Soxhlet, and distillation with water and/or steam. Although these are ancient techniques, they are still evolving and their performance is being improved by adopting auxiliary techniques, such as microwave heating and ultrasound application.

Acknowledgements

J. M. Prado is thankful for financial support from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, process 2010/08684-8). The authors acknowledge the financial support from CNPq (project 2009/17234-9) and FAPESP (project 12/10685-8).

References

1. R. Kellner, J. M. Mermet, M. Otto, M. Valcárcel, H. Widmer and H. Michael (eds.), *Analytical Chemistry: A Modern Approach to Analytical Science*, Wiley VCH, Weinheim, 2004, p. 410.
2. S. H. Rizvi, *Separation, Extraction and Concentration Processes in the Food, Beverage and Nutraceutical Industries*, Woodhead Publishing, Oxford, UK, 2010, p. 665.
3. M. G. Cerpa, R. B. Mato, M. J. Cocero, R. Ceriani, A. J. A. Meirelles, J. M. Prado, P. F. Leal, T. M. Takeuchi and M. A. A. Meireles, in *Extracting Bioactive Compounds for Food Products*, ed. M. A. A. Meireles, CRC Press – Taylor & Francis Group, Boca Raton, FL, 2009, p. 9.
4. G. Brunner, *Gas Extraction. An Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Processes*, Springer, New York, NY, 1994, p. 387.
5. A. Papadopoulou and R. A. Frazier, *Trends Food Sci. Technol.*, 2004, **15**, 186.
6. M. A. Rostagno, A. Villares, E. Guillamón, A. García-Lafuente and J. A. Martínez, *J. Chromatogr. A*, 2009, **1216**, 2.
7. T. M. Takeuchi, C. G. Pereira, M. E. M. Braga, M. R. Maróstica Jr., P. F. Leal and M. A. A. Meireles, in *Extracting Bioactive Compounds for Food Products*, ed. M. A. A. Meireles, CRC Press – Taylor & Francis Group, Boca Raton, FL, 2009, p. 137.
8. D. Naviglio, F. Pizzolongo, R. Romano, L. Ferrara, B. Naviglio and A. Santini, *Afr. J. Food Sci.*, 2007, **1**, 42.
9. M. Contini, S. Baccelloni, R. Massantini and G. Anelli, *Food Chem.*, 2008, **110**, 659.
10. S. Y. Cho, Y. N. Lee and H. J. Park, *Food Chem.*, 2009, **117**, 312.
11. S. Tsakona, C. M. Galanakis and V. Gekas, *Food Bioprocess. Technol.*, 2012, **5**, 1384.
12. E. Karacabey and G. Mazza, *J. Agric. Food Chem.*, 2008, **56**, 6318.
13. Federal Register; EPA method 3540B, Soxhlet extraction, Revision 2; EPA: Washington, DC, USA, September, 1994.
14. AOAC Method 963.15, Association of Official Analytical Chemists, USA, 1990.
15. British Standard, BS 4267, 1991.
16. M. D. Luque de Castro and L. E. García-Ayuso, *Anal. Chim. Acta*, 1998, **369**, 1.
17. M. E. Hodson, *Geochim. Cosmochim. Acta*, 2002, **66**, 819.

18. M. Keinanen, *J. Agric. Food Chem.*, 1993, **41**, 1986.
19. A. Shafaghat, *Nat. Prod. Commun.*, 2011, **6**, 1739.
20. P. K. Mamidipally and S. X. Liu, *Eur. J. Lipid Sci. Technol.*, 2004, **106**, 122.
21. P. Hanmoungjai, L. Pyle and K. Niranjani, *J. Chem. Technol. Biotechnol.*, 2000, **75**, 348.
22. S. Sahin, M. Bilgin and M. U. Dramur., *Sep. Sci. Technol.*, 2011, **46**, 1829.
23. C. D. Tanzi, M. A. Vian, C. Ginies, M. Elmaataoui and F. Chemat, *Molecules*, 2012, **17**, 8196.
24. L. Wang and C. Weller, *Trends Food Sci. Tech.*, 2006, **17**, 300.
25. M. D. Luque de Castro and F. Priego-Capote, *J. Chromatogr. A*, 2010, **1217**, 2383.
26. F. Priego-Capote and M. D. Luque de Castro, *Talanta*, 2005, **65**, 98.
27. M. Virost, V. Tomao, G. Colnagui, F. Visinoni and F. Chemat., *J. Chromatogr. A*, 2007, **1174**, 138.
28. J. L. Luque-García and M. D. Luque de Castro, *J. Chromatogr. A*, 2004, **1034**, 237.
29. D. Bandoniene, M. Gfrerer and E. P. Lankmayr, *J. Biochem. Biophys. Methods*, 2004, **61**, 143.
30. F. Augusto, A. L. Lopes and C. A. Zini, *Trends Anal. Chem.*, 2003, **22**, 160.
31. S. Yanniotis, K. Tszilioni, G. Dendrinis and A. Mallouchos, *J. Food Eng.*, 2007, **78**, 82.
32. P. Rubiolo, B. Sgorbini, E. Liberto, C. Cordero and C. Bicchi, *Flavour Frag. J.*, 2010, **25**, 282.
33. P. Costa, C. Grosso, S. Gonçalves, P. B. Andrade, P. Valentão, G. Bernardo-Gil and A. Romano, *Food Chem.*, 2012, **135**, 112.
34. E. S. Giray, S. Kirici, D. A. Kaya, M. T. O. Sonmez and M. Inan, *Talanta*, 2008, **74**, 930.
35. M. Bendahou, M. Benyoucef, A. Muselli, J. M. Desjobert, J. Paolini, A. F. Bernardini and J. Costa, *J. Essent. Oil Res.*, 2008, **20**, 174.
36. W. Q. Guan, S. F. Lin, R. X. Yan, S. K. Tang and C. Quan, *Food Chem.*, 2007, **101**, 1558.
37. M. Z. Ozel and H. Kaymaz, *Anal. Bioanal. Chem.*, 2004, **379**, 1127.
38. A. L. Dawidowicz, E. Rado, D. Wianowska, M. Mardarowicz and J. Gawdzik, *Talanta*, 2008, **76**, 878.
39. N.-S. Kim and D.-S. Lee, *J. Chromatogr. A*, 2002, **982**, 31.
40. M.-T. Golmakani and K. Rezaei, *Food Chem.*, 2008, **109**, 925.
41. M. Gavahian, A. Farahnaky, K. Javidnia and M. Majzoobi, *Innov. Food Sci. Emerg. Tech.*, 2012, **14**, 85.
42. R. Ganeshjeevan, R. Chandrasekar, P. Kadigachalam and G. Radhakrishnan, *J. Chromatogr. A*, 2007, **1140**, 168.
43. D. Komes, D. Horžić and A. Belščak., *Food Res. Int.*, 2010, **43**, 167.
44. T. M. Takeuchi, C. G. Pereira, M. E. M. Braga, M. R. Maróstica Jr., P. F. Leal and M. A. A. Meireles, in *Extracting Bioactive Compounds for Food Products*, ed. M. A. A. Meireles, CRC Press – Taylor & Francis Group, Boca Raton, FL, 2009, p. 137.

45. H. J. Bae, G. K. Jayaprakasha, J. Jifon and B. S. Patil, *Food Chem.*, 2012, **134**, 1912.
46. V. Goulas and G. A. Manganaris, *Phytochem. Anal.*, 2012, **23**, 444.
47. J. M. Prado, P. C. Veggi and M. A. A. Meireles, *Curr. Anal. Chem.*, 2012, in press.
48. C. B. Faria, J. M. Prado, M. A. Rostagno, F. L. Schmidt and M. A. A. Meireles, in *Caffeine: Chemistry, Analysis, Function and Effects*, ed. V. R. Preedy, RSC Publishing, London, UK, 2012, p. 130.
49. S. Myint, W. R. W. Daud, A. B. Mohamad and A. A. H. Kadhum, *J. AOCS*, 1996, **73**, 603.
50. A. Sturzoiu, M. Stroescu, A. S. Guzun and T. Dobre, *Rev. Chim.-Bucharest*, 2011, **62**(3), 344.
51. P. F. Leal, T. S. Almeida, G. H. C. Prado, J. M. Prado and M. A. A. Meireles, *Sep. Sci. Technol*, 2011, **46**, 1848.

CHAPTER 3

Ultrasound-assisted Extraction

DANIELLA PINGRET, ANNE-SYLVIE FABIANO-TIXIER
AND FARID CHEMAT*

Université d'Avignon et des Pays de Vaucluse, INRA, UMR408, Sécurité et
Qualité des Produits d'Origine Végétale, F-84000 Avignon, France

*Email: farid.chemat@univ-avignon.fr

3.1 Introduction

The use of ultrasound in food processing, extraction, and analysis has been widely investigated lately and the number of papers published in the last two decades has increased exponentially.¹ This technology can be used indirectly, to monitor processes minimizing certain drawbacks or enhancing certain benefits in product fabrication or directly, to transform the properties of the final product or the process itself.^{1–4}

Conventional extraction of plant materials comprises solid–liquid techniques depending usually upon organic solvents which present various shortcomings such as toxic residues, chemical transformation of extracts, and toxic wastes.⁵ As a result, an increasing demand from industries for natural molecules produced from a clean extraction with safer solvents is observed. Ultrasound present several advantages in terms of shortening the time of the process, decreasing the volume of the solvent, and increasing the yield of the extract in comparison with conventional methods. In this chapter, some applications of ultrasound in the food domain are presented, as well as an example of a green extraction. The first part is dedicated to the presentation of ultrasound principles, influencing parameters, and instrumentation followed by classical application in the food extraction domain. In the second part, a specific application of ultrasound-assisted extraction of carotenoids is presented,

RSC Green Chemistry No. 21

Natural Product Extraction: Principles and Applications

Edited by Mauricio A. Rostagno and Juliana M. Prado

© The Royal Society of Chemistry 2013

Published by the Royal Society of Chemistry, www.rsc.org

highlighting the advantages of this type of procedure over conventional techniques.

3.2 Ultrasound-assisted Extraction

3.2.1 Ultrasound Principles

The use of ultrasound has been considered an innovative and promising technique of the 21st century, with numerous applications in the pharmaceutical, cosmetic, chemistry, and alimentary fields since the second half of the 20th century. Ultrasound is a mechanical wave that necessitates an elastic medium to spread over and it differs from audible sounds by the wave frequency (Figure 3.1). The audible frequencies to humans are comprised between 16 Hz and 20 kHz, while ultrasound frequencies range from 20 kHz to 10 MHz. From this large range of frequency, two main groups are distinguished and both are used in the food industry: diagnostic and power ultrasounds.⁶ The main physical parameters that characterize ultrasound are the power (in W), the frequency (in Hz), and the wavelength (in cm), from which the ultrasonic intensity (I) is calculated (in $\text{W} \cdot \text{cm}^{-2}$). Diagnostic ultrasound (also called high frequency ultrasound) range from 2 MHz to 10 MHz ($I < 1 \text{ W cm}^{-2}$) and is used in several fields such as medical imaging or even for defect detection in bond inspection for plastics. Conventional power ultrasound (also called low frequency ultrasound) range from 20 kHz to 100 kHz ($I > 1 \text{ W cm}^{-2}$). An extended range is used in sonochemistry (20 kHz to 2 MHz) and in this range, ultrasound is able to produce physical and/or chemical effects into the medium in order to facilitate or accelerate chemical reactions or even for other applications in the industry, such as cutting and plastic welding. Low power and high frequency ultrasound is a non-destructive way of gaining structural and/or chemical information on the used medium.

The major effects of ultrasound in a liquid medium are attributed to the cavitation phenomena, which comes from the physical processes that create, enlarge, and implode micro bubbles of gases dissolved in the liquid. The molecules from which liquid medium is constituted are held together by

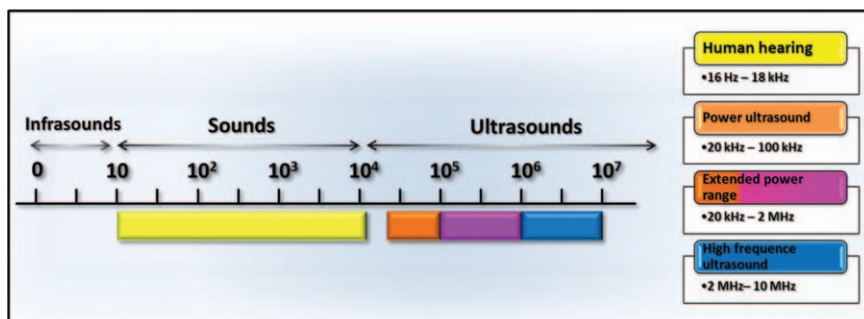


Figure 3.1 Frequency ranges.

attractive forces and as an ultrasound wave passes through an elastic medium, it induces a longitudinal displacement of those molecules, acting as a piston on the surface, resulting from a succession of compression and rarefaction phases (Figure 3.2).⁷ The molecules that form the liquid are temporarily dislodged from their original position and during the compression cycle they can collide with the surrounding molecules. During the rarefaction phase, a negative pressure will be exerted, pulling the molecules apart. The extent of the negative pressure depends on the nature and purity of the liquid. At a sufficiently high power, the attraction forces between them might be exceeded, generating a void in the liquid. The voids created into the medium are the cavitation bubbles which are formed from dissolved gases.^{6,8}

In fact these cavitation bubbles are able to grow by rectified diffusion, since vapors (or gas dissolved in the medium) will enter the bubble during rarefaction phase and will not be fully expelled during the compression cycle.⁶ When the size of these bubbles reach a critical point they collapse during a compression cycle and, since heating is more rapid than thermal transport, a transitory hot spot is created.^{9,10} The temperature and the pressure at the moment of collapse have been estimated to be up to 5000 K and 5000 atm in an ultrasonic bath at room temperature, creating hotspots that are able to accelerate dramatically the chemical reactivity of the medium.^{10–12}

When these bubbles collapse onto the surface of a solid material, the high pressure and temperature released generate microjets and shock waves directed towards the solid surface.¹³ In the food industry, these microjets can be useful

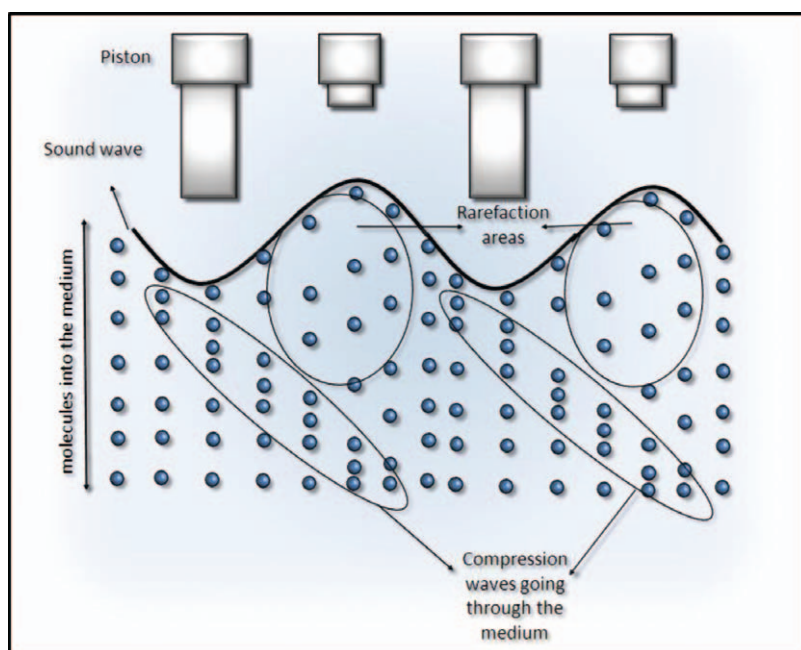


Figure 3.2 Compression and rarefaction cycles induced by a sound wave.

to the extraction of vegetal compounds (Figure 3.3). The cavitation bubble generated close to the plant material surface (a) collapses during a compression cycle (b) and a microjet directed toward the surface is created (b and c). The high pressure and temperature involved in this process will destroy the cell walls of the plant matrix and its contents will be released into the medium (d). For example, in the extraction of basil essential oil, it is possible to notice the intact cells and essential oil glands (Figure 3.4A) in comparison with the empty essential oil gland after conventional maceration (Figure 3.4B). However, due to cavitation, the basil cells are completely destroyed after ultrasound-assisted extraction, allowing the total recovery of the essential oil (Figure 3.4C).

There are actually two forms of cavitation bubbles: stable and transient. Stable cavitation bubbles have an existence of many cycles and oscillate often non-linearly around an equilibrium size, while the transient form exist for one, or at most a few, acoustic cycle, during which time they expand to at least double their initial size before collapsing violently into smaller bubbles.¹⁴ The dynamics of a transient cavitation bubble is expressed by the equation of Rayleigh-Plesset.¹⁵

$$\rho \left[R \frac{d^2 R}{dt^2} + \frac{3}{2} \left(\frac{dR}{dt} \right)^2 \right] = \left(P_h - P_v + \frac{2\theta}{R_0} \right) \left(\frac{R_0}{R} \right)^{3k} - P_k - P_a + P_v - \frac{2\theta}{R} \quad (3.1)$$

where ρ is the solvent density, R is the radius of the bubble, P_h is the hydrostatic pressure, P_a is the acoustic pressure, P_v is the vapor or gas pressure, P_k is the critical pressure of bubble nucleation, $k = C_p/C_v$ is the ratio of specific heats, and θ is a parameter that depends on the viscosity and superficial tension of the liquid.

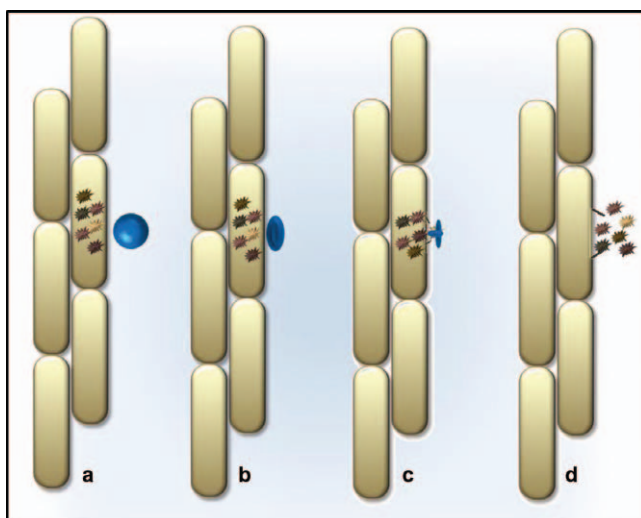


Figure 3.3 Collapse of cavitation bubble and release of plant material.

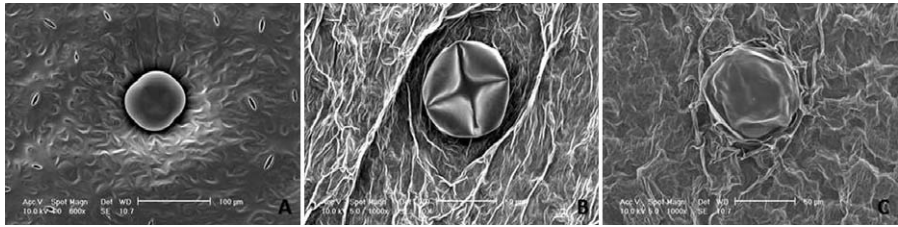


Figure 3.4 Photomicrography of basil leaves in the essential oil extraction (**A**, intact cells and essential oil glands, **B**, essential oil gland after conventional maceration, **C**, basil cells after ultrasound-assisted extraction).

Table 3.1 Comparison of characteristic values at 20 kHz and 500 kHz in the case of water saturated by air at the average intensity of 10 W/cm².

Frequency (kHz)	Amplitude (μm)	Acoustic pressure (atm)	Wavelength (cm)	Collapse duration (μs)	Bubble average diameter, R_0 (μm)
20	2.95	5.4	7.42	10	330
500	1.1	5.4	0.29	0.4	13

The integration of Equation (3.1) allows the calculation of the size of a bubble Equation (3.2), and also the time of implosion Equation (3.3) as a function of ultrasound frequency:

$$R_0 = \frac{4}{3W_a} (P_a - P_h) \left(\frac{2}{\rho P_a} \right)^{1/2} \left[1 + \frac{2(P_a - P_h)}{3P_h} \right]^{1/3} \quad (3.2)$$

where $W_a = 2\pi f_a$ and f is the frequency.

$$t_i \cong 0.915 R_0 \sqrt{\frac{\rho}{(P_h + P_a - P_v)}} \quad (3.3)$$

The Table 3.1 represents the effects of ultrasound frequency on the characteristic parameters of cavitation bubbles calculated by the Equations (3.2 and 3.3).

3.2.2 Instrumentation

All ultrasonic systems are composed of a transducer, which converts electrical energy into sound energy by vibrating mechanically at ultrasonic frequencies, generating ultrasound.¹⁶ Although a wide range of transducer types is available, the purpose is the same. The piezoelectric transducer is based on a crystalline ceramic material that responds to electrical energy. This transducer is the most common type and is used in most ultrasonic processors and reactors, being cited as the most efficient, achieving better than 95% efficiency.¹⁷

The generated ultrasound is irradiated by the emitter (also called the reactor), which can also amplify the waves.¹⁷ Among all the emitters available

in the market, the most used are the bath and the probe systems and this last one is often attached to a horn tip known as sonotrode. The shape of the horn determines the amount of amplification. Hence, the intensity of radiation can be controlled by selecting differently shaped horns. Ultrasounds equipment has been developed for both laboratory and industrial scales. For either application, both bath and probe system are used, although the intrinsic differences between those systems should be taken into account for better adaptation to the desired final purposes. Recently, some continuous-flow apparatuses have been developed for both laboratory and pilot scale. Some advances are still expected on the coupling of ultrasonic equipment to analytical instruments, since it considerably reduces costs by avoiding sample preparation steps such as concentration, filtration and derivatization before analysis.

3.2.2.1 Laboratory Scale

In the laboratory scale, numerous extracts were obtained using ultrasound, such as carotenoids, antioxidants, essential oils, flavors, etc.¹ In order to obtain better results, the choice of the ultrasound equipment is of great importance. The first batch equipment developed was the ultrasonic cleaning bath, which is used for solid dispersion into solvent (solubility of solid particles is increased as the particles size is reduced), for degassing solutions or cleaning small material by immersion (Figure 3.5A). This type of equipment is easy to handle and has very low implementation cost; however, it possesses some important shortcomings such as the declined power over time with attenuation of the intensity (which is dispersed in the water and glassware), decreasing the reproducibility and repeatability of experiments. Recently a new bath system reactor has been developed by REUS (Contes, France) with capacity of 0.5 L to 3 L operating at 25 kHz with an intensity of 1 W cm^{-2} , which is mostly used for extraction

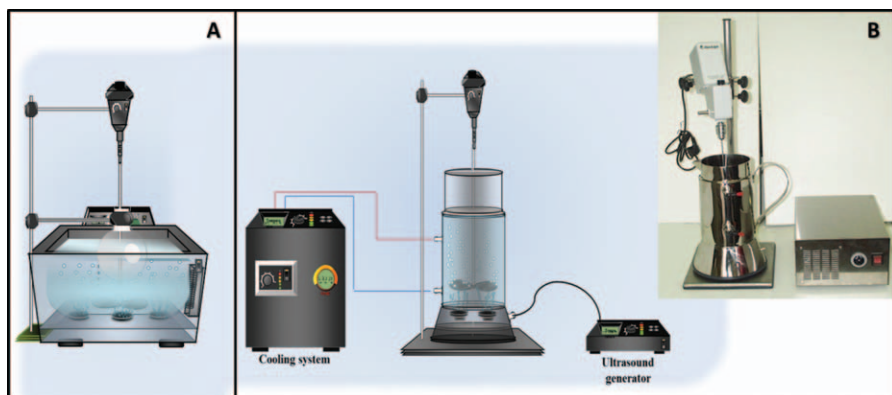


Figure 3.5 Commonly used ultrasonic batch systems: (A) US bath; (B) US reactor scheme and picture.

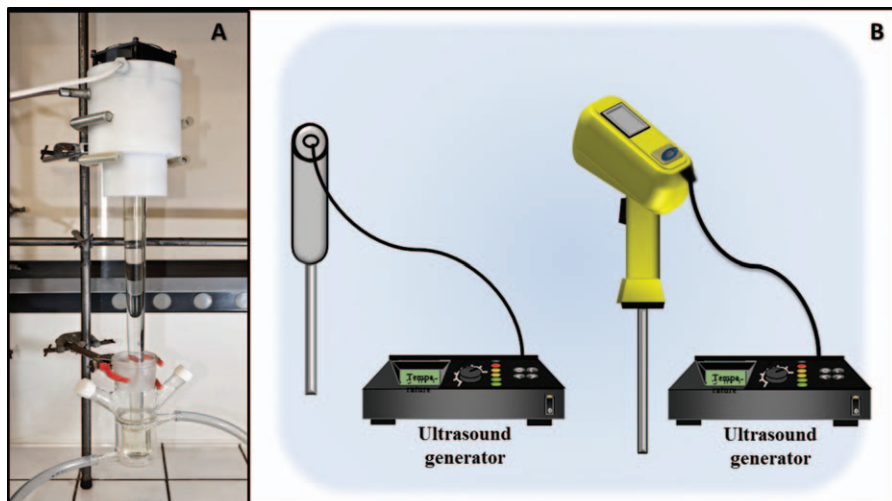


Figure 3.6 Commonly used ultrasonic probe systems: (A) quartz probe; (B) titanium probes.

procedures (Figure 3.5B). The inox jug is equipped with a double mantle to circulate water for controlling the temperature.

For smaller volumes, the probe system (Figure 3.6) is more adapted and is considered to be more powerful, since there is less dispersion of ultrasonic energy. The ultrasonic intensity is delivered by a small surface (the tip of the probe) and immersing the probe directly into the reaction flask avoids attenuation. This system is more frequently used for chemical reactions and has also been used for extraction purposes, but since the cavitation is concentrated in a very small area, the temperature of the sample might rise rapidly; therefore, a temperature control method is often used, e.g. a double mantle reactor.

3.2.2.2 Industrial Scale

Both probe (Figure 3.7A) and bath systems (Figure 3.7B) are used industrially, depending on the application, and several types of ultrasonic devices have been developed for industrial uses or scale-up laboratory experiments by a large number of companies such as Hielscher (Germany), Branson (Switzerland), Vibracell (USA), and REUS (France), among others. The disposition of ultrasound transducers varies upon the device and sometimes an agitation system is also used. Some continuous flow devices have also been developed for both probe and batch systems. REUS has developed reactors from 30 L to 1000 L to which pump systems are coupled in order to fill the ultrasonic bath, to stir the mixture, and to empty the system at the end of the procedure. Hielscher has devices of a wide range of power, from 50 W to 400 W for analytical scales and from 500 W to 16 000 W in industrial scales.



Figure 3.7 Industrial scale ultrasonic devices: (A) probe; (B) bath systems.

Most of the compounds extracted on the industrial scale by ultrasound have immediate use (for instance in liquor production) or can be used as food and cosmetic additives (in the case of essential oil and molecules with special activity). GMC (G. Mariani & C. Spa) is an Italian company specialized in aromatic herb extraction that adapts their extraction system (conventional or innovative) depending on the characteristics of the herbs. GIOTTI is an Italian company that uses ultrasound assistance in extraction of food, pharmaceutical additives, and production of alcoholic drinks. This company works with four continuous batch systems equipped with ultrasound on each side of the tank and an agitation system. Moliserb srl is a company specialized in ultrasound-assisted extraction of thermolabile compounds with alimentary and cosmetic applications.

3.2.3 Important Parameters

The most important parameters that can influence ultrasound-assisted extraction (UAE) are presented in this section. Besides the parameters

intrinsically related to the ultrasonic device (such as the frequency, wavelength, and amplitude of the wave), the ultrasonic power and consequent intensity have also an effect on extraction and can be optimized. The reactor design and also the shape of the probe (if that is the case) can influence the process.

Since the extraction is carried out in a medium, the temperature, time, and solvent-type can affect not only the extraction yield but also the composition of the extract and should thus be taken into consideration. The raw material matrix and the target molecules for UAE should also be carefully considered as a parameter.

The careful study of those influencing parameters is of great importance in order to obtain the best efficacy of extraction and result in the highest yield. However, it is necessary to consider that the highest yield is not always the sole objective of an extraction process, but also the lowest consumption of non-renewable resources and energy. Therefore the optimization of those parameters is necessary to transfer experimental laboratory conditions to industrial scales.

3.2.3.1 Physical Parameters

Since ultrasound is a mechanical wave, the frequency, the wavelength, and the amplitude (Figure 3.8) can influence the cavitation bubbles and, thus, the extraction.

These parameters are described below.

- Frequency (f) is measured in hertz and expresses the number of cycles per seconds. For extraction purposes the most common frequencies are 20–50 kHz.
- Period (P) is the reciprocal of the frequency ($1/f$), and so is the time of one cycle. Both frequency and period are determined by the source of ultrasound only.
- Wavelength (λ) represents the length or distance of one cycle and it is determined by both the source of ultrasound (with a given frequency) and the medium (with a given propagation velocity).

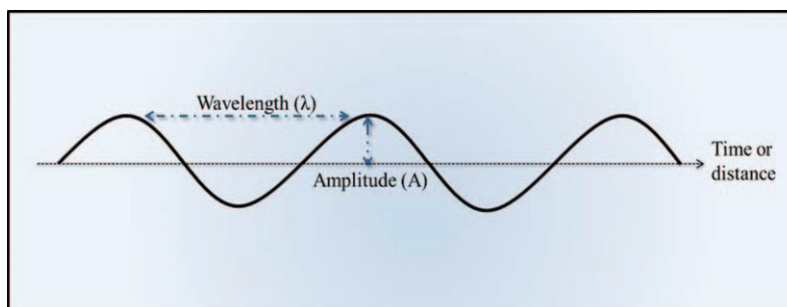


Figure 3.8 Main physical parameters of an ultrasonic wave.

- Amplitude (A) represents the height of the wave and is usually measured in decibels (dB) or pascals (Pa). The amplitude has an effect on the formation and implosion of cavitation bubbles.
- Propagation velocity (C) represents how fast the wave is moving in the specific medium. Propagation velocity is dependent on the medium and is related to the stiffness of the medium and something called the bulk modulus (directly related to the stiffness of the medium), therefore, denser materials also have a faster propagation velocity.

Power and Frequency. The measurement of the actual applied acoustic power in a sonochemical process is not always reported, although numerous physical methods are available that allow the direct or indirect measurement of the applied energy. The available methods estimate the transferred energy by measuring either chemical or physical changes on the medium when ultrasound is applied. The most common physical methods are the measurement of acoustic pressure using hydrophones or optical microscopes, the aluminum foil method, and the calorimetric method.^{18–20} And among the chemical methods, the indirect measurement of $\text{OH}^\bullet$ radicals formed by sonoluminescence or chemical dosimeters are also used.^{12,21} As an example, to calculate the power by calorimetry, it is considered that the actual input power from the device is converted to heat which is dissipated in the medium. In this case, the actual ultrasound power is calculated as shown in Equation (3.4).²²

$$P = m C_p \frac{dT}{dt} \quad (3.4)$$

where C_p is the heat capacity of the solvent at constant pressure ($\text{J g}^{-1} \text{ } ^\circ\text{C}^{-1}$), m is the mass of solvent (g), and dT/dt is temperature rise per second.

Several studies show a great ultrasonic power causes major alterations in materials by inducing greater shear forces (depending on the nature and properties of the medium); however, in the natural product industry this parameter is usually optimized in order to use the minimum power to achieve the best results.¹⁷ Generally, the highest efficiency of UAE, in terms of yield and composition of the extracts, can be achieved by increasing the ultrasound power, reducing the moisture of food matrices to enhance solvent–solid contact, and optimizing the temperature to allow a shorter extraction time. However, some studies showed the power variation can result in a certain selectivity of target molecules, where the ratio of some molecules is a function of the applied power.^{23,24}

The most commonly used frequencies in sonochemistry are between 20 kHz and 50 kHz. With higher frequencies, cavitation would be more difficult to induce, since the cavitation bubbles need a little delay to be initiated during the rarefaction cycle.⁶ The length of the rarefaction phase (during which cavitation bubbles grow) is inversely proportional to ultrasonic frequency; therefore, at high frequencies larger amplitudes are required to generate cavitation. At low frequencies, the transient cavitation bubbles are relatively less numerous,

although with high dimensions, which privileges the physical effects instead of the chemical ones. The effect of the frequency may be linked not only to the cavitation bubble size, but also to its influence on the resistance to mass transfer.¹

Intensity. Intensity can be expressed as energy transmitted per second and per square meter of medium. This parameter is directly correlated with the amplitude of the sound wave; with increase in the amplitude, bubble collapse will be more violent. This last parameter influences directly the acoustic pressure generated when ultrasound is applied to an elastic medium. As described earlier, the actual power can be calculated by numerous different techniques. Nevertheless, the applied ultrasonic intensity (UI) can be calculated using the calculated power (from Equation 3.4) as shown in the Equation (3.5).²⁵

$$UI = \frac{P}{\pi D^2} \quad (3.5)$$

where UI is the ultrasonic intensity (W cm^{-2}), P is the ultrasound power (in W) as calculated by Equation (3.4), and D is the internal diameter (cm) of the ultrasound reactor.

The increase of intensity parameter leads to an increase in the sonochemical effects. It is important to note that high amplitudes can lead to rapid deterioration of the ultrasonic transducer, which results in liquid agitation instead of cavitation and in poor transmission of the ultrasound through the liquid media. However, the amplitude should be increased when working with samples of high viscosity, such as oils.²⁶

Shape and Size of Ultrasonic Reactors. Since ultrasound waves are reflected when a solid surface is reached, in the case of extraction using an ultrasonic bath, the shape of the reaction vessel is critical. The best choice would be a flat bottom vessel such as a conical flask in order to attain a minimum reflection of waves.^{6,14} The thickness of the vessel should also be kept to the minimum to reduce attenuation.²⁶ It is necessary to calculate the optimum reactor dimensions and the position of the emitter in relation to the transducer to attain maximum energy transferred to the medium.²⁷ Further advances have been made by taking into account the lack of homogeneity of the pressure field in the reactor in order to optimize the process efficiency.^{1,28} Also, in the case of ultrasonic probes a rapid decrease of intensity is observed both radially and axially. For this reason a minimal space between the ultrasonic probe and the wall of the container must be respected, while ensuring that the probe does not touch the container to avoid damages on the material.²⁶

In the case of the use of an ultrasonic probe, the shape and diameter of this last one may have an influence on the extraction (Figure 3.9). The stepped probe gives the highest amplitude magnification (i.e. power, amplitude gain $(D/d)^2$) of the shapes shown. Nevertheless, the exponential probe shape offers

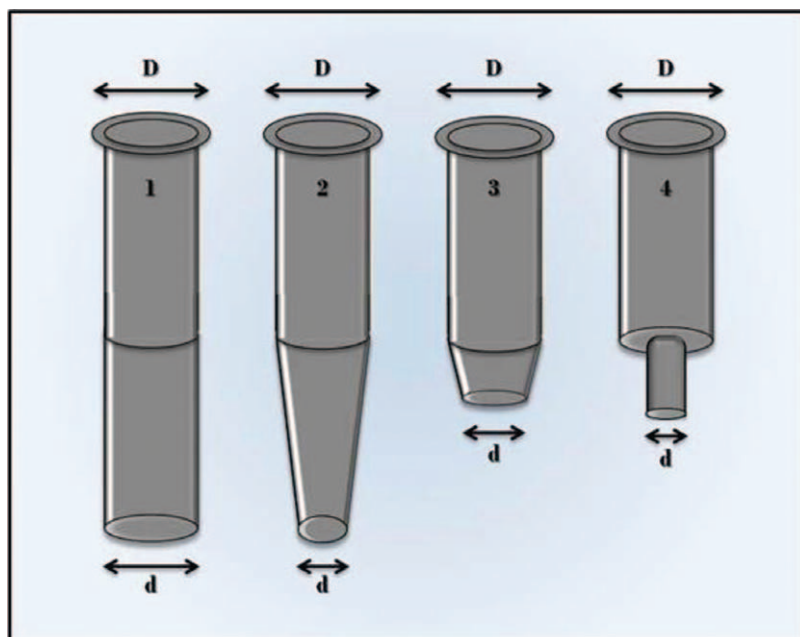


Figure 3.9 Probe shapes: (1) uniform cylinder; (2) exponential taper; (3) linear taper or cone; (4) stepped.

small diameters at its working end, which makes it particularly suited to micro applications.²⁶

Most of the probe emitters are composed of a titanium alloy, since this material is thermoresistant and behaves well under corrosive conditions. However, the erosion of this material is often important, which can provoke the appearance of metal particles in the extraction medium. Some new materials are investigated for ultrasound probe tips, such as quartz and Pyrex, which might solve the problem of metal particles release.²⁹

3.2.3.2 Medium Parameters

The medium to which ultrasound is applied presents intrinsic characteristics that need to be taken into consideration in order to achieve the expected results in the extraction process. Besides the control and optimization of the parameters from the ultrasonic device, those last will achieve the medium where the target compounds are to be extracted. The higher the penetration power and lower the relative strength, smaller the effects on the surface of the medium. To achieve cavitation, as the sonic frequency increases, the intensity of the applied ultrasound must be increased, to ensure that the cohesive forces of the liquid media are overcome and voids are created. Another important parameter that should be taken into consideration is the attenuation phenomenon (which

is inversely proportional to the ultrasound frequency), although it can be reduced by choosing the appropriate shape of the reaction vessel.¹⁴

Solvent Type. Solvent choice is dictated by the solubility of the target analytes in the solvent but also by physical parameters such as viscosity, surface tension, and vapor pressure of the medium. For cavitation bubbles to be effective, the negative pressure during the expansion cycle has to overcome the natural cohesive forces in the medium. The rise of viscosity increases these molecular interactions hence the cavitation threshold rises significantly. In the same way, a high surface tension decreases cavitation phenomena. In this manner, the amplitude should be increased when working with samples of high viscosity. This is because as the viscosity of the sample increases so does the resistance of the sample to the movement of the ultrasonic device, for instance the tip of an ultrasonic probe. Therefore, a high intensity (or high amplitude) is advised in order to obtain the necessary mechanical vibrations that will result in appropriate cavitation.²⁶ Vapor pressure is also directly correlated with the temperature factor, which influences cavitation as well. Therefore, the solvent of choice for UAE should ideally have a very low vapor pressure and the ability to solubilize the molecules of interest.³⁰

Temperature. The temperature increase generates the rise of the vapor pressure and the decrease of the viscosity and surface tension, inducing more solvent vapors to enter the bubble cavity, reducing the pressure difference between the inside and outside of the bubble, which will collapse less violently and reduce sonication effects.²⁶ As a consequence, at higher temperatures, cavitation can be achieved at lower amplitudes. However, the sonochemical effects of such bubbles may be reduced and the use of temperatures above a certain threshold might generate cavitation bubbles that grow very quickly, diminishing its efficacy. For extraction purposes, a higher temperature might result in a higher efficiency due to an increase in the number of cavitation bubbles and a larger solid–solvent contact area, as also an enhancement of solvent diffusivity with consequent enhancement of desorption and solubility of the interest compounds. However, this effect is decreased when the temperature is near the solvent's boiling point, since the bubble's implosion might not induce sufficient energy shear forces to disrupt cell tissues.^{1,31,32} It is important to note also that it is possible to notice a decrease in the extraction yield as the temperature rises, especially in the case of unstable and volatile compounds.^{33,34} The optimization of the temperature parameter can be performed in order to obtain the highest yield of the target compounds without degradation, since this parameter can vary depending on the type of product. Hence, a temperature control is imperative to prevent the degradation of thermolabile compounds.

Presence of Dissolved Gases. Since cavitation bubbles are formed from gas (vapors) dissolved in the liquid, the absence of gases would dramatically

make difficult the creation of those bubbles. Dissolved gases into the solvent act as nuclei for new cavitation bubbles so this would increase the rate of cavitation bubble formation. If the external pressure is increased, then a greater ultrasonic energy is required to induce cavitation, that is, to break the solvent molecular forces. In addition, there is an increment in the intensity of the cavitation bubble collapse and, consequently, an enhancement in sonochemical effects is obtained.²⁶ On another hand, as the creation of the cavitation bubbles is facilitated, they would grow faster and the solvent might undergo boiling: if the bubbles grow too fast, they would not have time to collapse and the liquid would boil without cavitation.

3.2.3.3 Matrix Parameters

Depending on the objective of the UAE and the target molecules, the matrix used can be either fresh or dry. Ultrasound needs an extractive medium to propagate and in order to obtain a correct diffusion of the solvent into the plant cell. Therefore, in the case of dry matrixes, an absorption of the liquid could occur (re-hydration) depending on the porosity of the material and should be studied carefully. Also, the solubility and stability of the target compounds in the chosen solvent and temperature can influence the final yield of the extraction. Likewise, since the extractive system is a heterogeneous and complex porous media, the size of the cavitation bubble has an effect on the efficiency of the extraction. Other parameters related to the solid–liquid extraction such as the solid/liquid ratio and particle size of the material are relevant to the efficacy of the extraction.

3.2.4 Ultrasound-assisted Extraction: Applications in Food

Despite its primary utilization in cleaning of surfaces and instruments, ultrasonic devices have been developed and largely used in the food industry. Because of the wide range of frequencies and power, ultrasound has different effects that allow it to be applied to different and various processes, such as cutting,^{35,36} inactivation of microorganisms and enzymes,^{37,38} homogenization and emulsification,^{39–41} filtration,^{42,43} crystallization and freezing,^{44,45} drying,^{46,47} cooking,^{48,49} degassing,⁵⁰ defoaming,^{51,52} oxidating,^{53,54} and extracting.^{4,55–57}

Each process application has its particularities, but the general principle of ultrasound in these processes is based on mechanical and sonochemical effects that can be observed by the propagation of ultrasonic waves.^{16,17} One should keep in mind that the industry does not always aim for the highest yield, but the objective is to achieve a minimum consumption of non-renewable resources and expense. Ultrasound is generally profitable in large-scale applications, as results include a decrease in energy consumption, a decrease in process time, and an enhancement of quality in the final product, and finally, the initial investments are rapidly paid back.⁵⁸ Table 3.2 summarizes the main applications of ultrasounds in the three types of matrices explored in this section.

Table 3.2 Applications of ultrasound in the food extraction.

<i>Matrix</i>	<i>Processing</i>	<i>Target compounds</i>	<i>References</i>
<i>Fruits and Vegetables</i>			
Citrus peel	UAE bath	Flavonoids	59
<i>Myrciaria cauliflora</i>	UAE bath	Antioxidants	60
Pomegranate peel	Continuous and pulsed UAE	Antioxidants	61
Orange peel	UAE	Polyphenols	62
Green wattle bark, Marigold flowers, Pomegranate rinds, 4'o clock plant flowers and Cocks Comb flowers	UAE probe	Colorants	63
Red raspberry fruits	UAE probe	Anthocyanins	64
Lettuce and cabbage leaves	UAE bath	Ca, Mg, Mn and Zn	65
Tomato	UAE	Lycopene	55, 66
Citrus peel	UAE probe	All-trans- β -carotene	27
<i>Herbs and Spices</i>			
Pepper	UAE bath	Capsaicinoids	67, 68
Caraway seeds	UAE bath	Carvone and limonene	23
Rosemary	UAE bath	Antioxidants	69, 70
<i>Mentha spicata</i>	UAE probe	Flavor compounds	71
Rice and maize wine	UA process	Accelerated aging	72
Red and white wine	UAE bath	Volatile compounds	73, 74
Brandies and oak extracts	UAE bath	Volatile compounds	75
<i>Oleaginous Seeds</i>			
Almond, apricot and rice bran	UAE	Oil	78, 82
Almond	UAE bath	Oil	33
Soybean	UAE bath	Oil	83
Flaxseed	UAE emulsifier	Oil	84
<i>Isatis indigotica</i> Fort	UAE bath	Oil	56
Viz and soybean	UAE/ microwave	Oil	29
Sunflower, rape and soybean seeds	UAE Soxhlet	Oil	85

3.2.4.1 Fruits and Vegetables

In the food domain, the processing of fruits and vegetables is done in multiple steps for simple consumption or even for the extraction of interest molecules intended for direct or indirect applications in food industries or other fields such as pharmaceuticals and cosmetics. To those applications, the vegetable matrices contain a wide range of secondary metabolites that can be extracted and purified for further applications such as lipids, phytochemicals, flavors, fragrances, and pigments.

For instance, antioxidants from plants have numerous applications, being used either for health reasons, as adjuvants in some formulations, or with preservative purposes. In this scenario, antioxidants are able to prevent or slow down the oxidation process by reacting preferably with the oxidizing agent

instead of the target cells or molecules of interest. Ultrasound-assisted extraction (UAE) of antioxidants has been effectively applied to numerous matrices with great recoveries and optimum antioxidant activity.^{59–62}

Other compounds such as colorants^{63,64} and micro- and macronutrients⁶⁵ have also been successfully extracted using ultrasound either coupled to other techniques or alone. The extraction of antioxidants and carotenoids, such as lycopene, from orange, citrus peel, and tomatoes by UAE has been successfully optimized.^{27,55,66}

3.2.4.2 Herbs and Spices

Several interest molecules extracted from herbs and spices are used in the food, cosmetic, and pharmaceutical industries and various processes are used for this end. Among the used techniques, ultrasound has been successfully applied in the recovery of compounds from those matrices. A large range of herbs and spices have been submitted to UAE of compounds using conventional or green solvents.

Different capsaicinoids (noridihydrocapsaicin, capsaicin, dihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin) have been extracted from pepper (*Capsicum frutescens*) and by changing the extraction medium solvent in UAE, a selectivity is observed amongst those compounds.^{67,68} The possibility of selecting the compound of interest by UAE was also observed for caraway seeds, where at low temperatures, a selectivity is observed for carvone extraction instead of limonene.²³ The UAE of rosemary shows carnosic acid is better extracted from dried material in ethanol, while rosmarinic acid is better extracted using methanol as solvent, from which extracts present better antioxidant activity.^{69,70}

Flavors and fragrances are complex mixtures of volatile compounds that are obtained from the secondary metabolism of aromatic plants (including herbs and spices), and usually are present in low concentrations. Those substances generally consist of complex mixtures of mono- and sesquiterpene hydrocarbons, and oxygenated materials biogenically derived from them.⁷¹ The most developed application of ultrasound on the flavor extraction is in the field of alcoholic beverages such as wine and brandy. Aromas are of great importance to those beverages either for quality or appreciation parameters, and ultrasound has been successfully used for aging of rice wine in 1 week or 3 days instead of classical 1 year aging.⁷² On the other hand, some volatiles are markers of quality for wine or brandy and thus, ultrasonic techniques were developed for extraction and analysis of those substances.^{73–75}

3.2.4.3 Oleaginous Seeds

Fats and oils are a main source of energy used by the body. Moreover, they participate in the transmission of nerve impulses, maintain the integrity of cell membranes, have a role in cellular transport, and are precursors of many hormones. From all sources of lipid, oil seeds are complex matrices from which it is possible to extract monoacyl glycerols (MAG), diacyl glycerols (DAG),

triacyl glycerols (TAG), and free fatty acids associated with other minor compounds such as pigments, sterols, alkaloids, etc.⁷⁶

The conventional methods for oil seed extraction are hot or cold pressing, solvent extraction (Soxhlet), and eventually the combination of these. However, the press cake retains considerable amounts of oil and minor compounds and Soxhlet extraction might degrade fatty acids by the high temperature; furthermore, a toxic solvent, hexane, is used.^{77,78}

In the past years, researchers have shown UAE to result in high yields and high-quality oils, allowing faster extraction with great recoveries. Since the oil seeds present a hard shell of the cell wall and its breaking is crucial to oil extraction,^{79,80} cavitation due to ultrasound is able to create more pores in those cells to allow a better contact with the extraction solvent, thus resulting in better yields with a reduced amount of solvent.⁸¹

Numerous oleaginous seeds have been extracted under ultrasound. When used as a pretreatment before extraction, alone or in combination with other techniques such as autoclave, ultrasound has increased the oil yield for almond, apricot, and rice bran, and scanning electron micrographs showed a destructuring of cell walls due to ultrasonic cavitation.^{33,78,82} Flaxseed and soybean have also been extracted by ultrasound resulting in increases of oil yield when compared to conventional and microwave-assisted techniques.^{32,83,84} Ultrasound has also been used in the valorization of by-products such as *Isatis indigotica* Fort. seed oil, providing a commercial importance to seeds from this plant, which is usually commercialized for its leaves.⁵⁶ The combination of extraction methods has also proven to be efficient, as in the case of microwave-assisted extraction of seaweeds and soybeans,²⁹ and also in the case of the innovative ultrasound-assisted Soxhlet extraction, which showed applicability not only for soybeans but also for rape and sunflower seeds.⁸⁵

3.3 Examples of Solvent-free Ultrasound-assisted Extraction of Carotenoids

In this section, an example of application of UAE of carotenoids is presented. A comparison has been made to conventional solvent extraction and the advantages of the proposed extraction method are discussed. For this work, carotenoids were extracted from dried ground carrots and the yield of β -carotene was assessed and compared to the conventional extraction method.

3.3.1 Carotenoids Uses and Conventional Extraction

Besides their use as food additives, cosmetic colorants, and antioxidants in the pharmaceutical industry, carotenoids are precursors of vitamin A and are responsible for numerous benefits in health.⁸⁶⁻⁸⁹ This wide group of compounds can be synthesized by a great number of plants, algae, and bacteria. Carotenoids are formed by polymerization of isoprene units to an aliphatic or alicyclic structure and, by this lipophilic characteristic, their extraction is

usually achieved by organic solvents, generating a great amount of waste of questionable environmental disposal.⁹⁰ Additionally, because of an increase in demand for natural products, alternative extraction methods are under research, such as UAE. The use of vegetable oils for carotenoid extraction has been successfully performed by supercritical fluid extraction using canola, soybean, and olive oil as co-solvents, resulting in a yield 2 to 4 times higher.^{91,92} Therefore, instead of using a non-polar organic solvent, as stated in the current methods, sunflower oil was used as extraction media in an ultrasound-assisted process.

3.3.2 Solvent-free Ultrasound-assisted Extraction of β -Carotene

Ultrasound-assisted extractions (UAE) were performed in an ultrasonic extraction reactor PEX3 (REUS, Contes, France) consisting of an inox jug with 23×13.7 cm internal dimensions and maximal capacity of 3 L, equipped with a transducer at the base of the jug operating at a frequency of 25 kHz with maximum input power (output power of the generator) of 150 W. The double-layered mantle (with water circulation) allowed the control of extraction temperature by cooling/heating systems.

The carrots used for extraction were previously dried and ground, as represented in Figure 3.10. The dry material was submitted to extraction either by solvent in the conventional processor or by oil using ultrasound assistance.

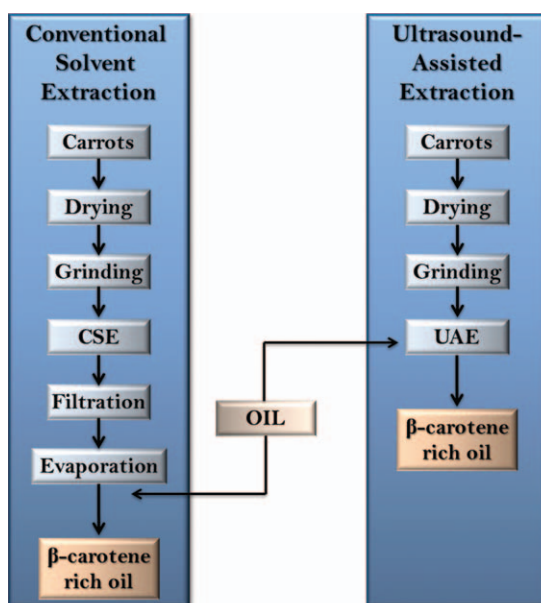


Figure 3.10 Schematic presentation of extraction process steps for β -carotene extraction. CSE: Conventional Solvent Extraction; UAE: Ultrasound-Assisted Extraction.

In the case of solvent extraction, the dried extract was dissolved in sunflower oil before analysis. The yield of β -carotene was measured by absorbance in a UV-spectrophotometer at 450 nm against a standard curve and comparisons between yields from different extraction procedures were made. For comparison purposes, maceration in oil was also performed.

3.3.3 Analysis and Evaluation of UAE Process

A preliminary study was performed in order to establish the better solid/liquid ratio for subsequent extractions and then an optimization of extraction parameters was carried out by a face-centered Central Composite Design (CCD). The ratio of 30% of dry weight was used in all extractions. As presented in Section 3.2.3, the three most important parameters for extraction were studied to obtain the highest yield with the best set of parameters so the quality of the final product is preserved. The parameters chosen for optimization were: ultrasound power (ranging from 30 W to 70 W), temperature of extraction (from 20 °C to 60 °C), and extraction time (from 5 min to 35 min). From the subsequent modeling and statistical analysis, the calculated optimum conditions of β -carotene extraction were 70 W, 40 °C, and 20 min. The yields of extraction under solvent, maceration, and ultrasound were respectively 321.36 mg/L, 294.58 mg/L, and 334.75 mg/L. From the results it is possible to observe that maceration in oil presented the lowest yield of β -carotene, while the traditional solvent extraction method accounted for a yield 9% higher than maceration in oil. However, UAE in oil presented an increase of 13% of the β -carotene yield when compared to maceration and of 4% when compared to solvent extraction (Figure 3.11). In Figure 3.12 it is possible to notice the difference in the color intensity of oil extracts obtained by different extraction methods.

The innovative extraction process presented accounts for numerous advantages, figuring as a green process since the extraction medium is a vegetable oil and reducing considerably the environmental pollution when

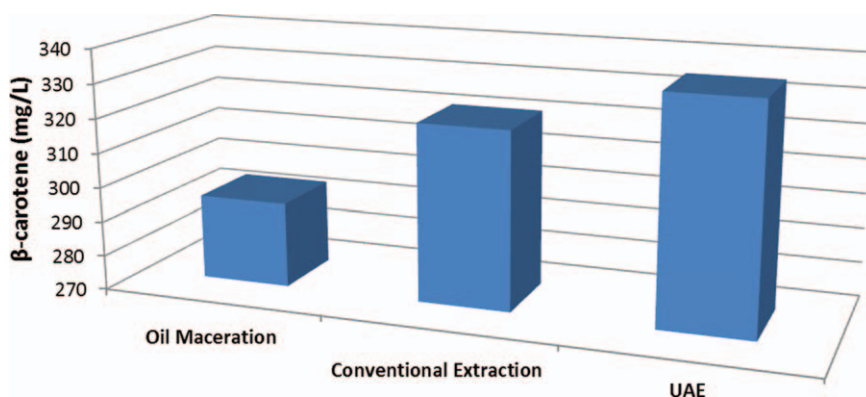


Figure 3.11 Yield of β -carotene obtained from different extraction methods.

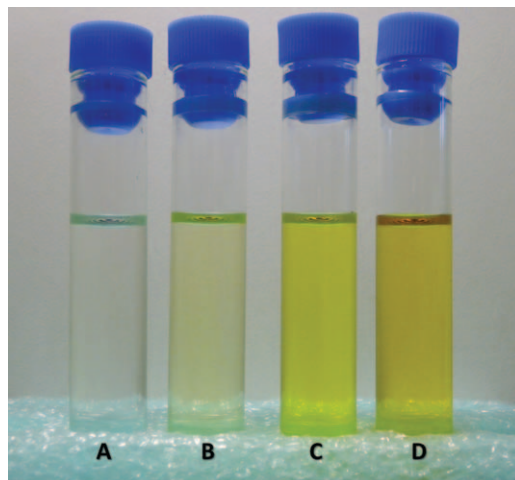


Figure 3.12 β -carotene extracts. (A) Pure sunflower oil; (B) Maceration; (C) Conventional extraction; (D) UAE.

compared to solvent-extraction. The optimization of the extraction also showed a reduction of the process time (20 min instead of 2 h for conventional and solvent extraction) and consequently of the costs, while the mild temperature conditions might prevent possible degradation of the compound, since β -carotene is thermosensible.

In conclusion, the UAE extraction presented a higher β -carotene yield when compared to conventional solvent extraction, showing the potential of the technique with a direct applicability, since the process is free of solvents.

3.4 Costs and Investment in Industrial Ultrasound

The price of industrial ultrasound reactors vary between 10 000 euros (5 L in batch or 5 L/h in continuous mode) and 200 000 euros (1000 L in batch or 1000 L/h in continuous mode). The choice of an ultrasound reactor represents only about 25% more of the initial investment compared to a conventional reactor. However, if we consider that ultrasound use reduces the total time of the procedure by a factor of 10 to 100, together with a decrease of consumed energy and pollution by a factor of 10, the procedures using ultrasound assistance have a production cost and a functioning cost much lower than the costs for conventional procedures.

3.5 Conclusion

Ultrasound use in food extraction has revealed large applicability, minimizing the drawbacks of conventional methods in terms of reducing processing time, solvent used and energy used, being also more effective in mass and energy transfer. This innovative technique results in high reproducibility and simplified

manipulations and leads towards obtaining an economic final product with high purity. Besides the laboratory scale equipment, large-scale industrial ultrasonic devices have been successfully used in the food domain either for the processing of food-related products applied both directly and indirectly to food, cosmetic, and pharmaceutical industries. In this chapter, an application of a solvent-free ultrasound-assisted extraction was also presented, showing the optimization of influencing parameters in order to obtain a quality final product with a better yield and reduced time and costs when compared to conventional techniques.

References

1. M. D. Esclapez, J. V. García-Pérez, A. Mulet and J. A. Cárcel, *Food Eng. Rev.*, 2011, **3**, 108.
2. D. Knorr, M. Zenker, V. Heinz and D.-U. Lee, *Trends Food Sci. Technol.*, 2004, **15**, 261.
3. F. Chemat, Zill-e-Huma and M. K. Khan, *Ultrason. Sonochem.*, 2011, **18**, 813.
4. A. C. Soria and M. Villamiel, *Trends Food Sci. Technol.*, 2010, **21**, 323.
5. M. Ollanketo, A. Peltoketo, K. Hartonen, R. Hiltunen and M.-L. Riekkola, *Eur. Food Res. Technol.*, 2002, **215**, 158.
6. T. J. Mason, E. Riera, A. Vercet and P. Lopez-Buesa, in *Emerging Technologies for Food Processing*, ed. Da-Wen Sun, Academic Press, 2005, p. 323.
7. D. J. McClements, *Trends Food Sci. Technol.*, 1995, **6**, 293.
8. K. S. Suslick, *Sci. Am.*, 1989, **260**, 80.
9. K. S. Suslick, Y. Didenko, M. M. Fang, T. Hyeon, K. J. Kolbeck, W. B. McNamara, M. M. Mdeleleni and M. Wong, *Phil. Trans. R. Soc. Lond. A*, 1999, **357**, 335.
10. E. B. Flint and K. S. Suslick, *Science*, 1991, **253**, 1397.
11. W. B. McNamara, Y. T. Didenko and K. S. Suslick, *Nature*, 1999, **401**, 772.
12. K. S. Suslick, N. C. Eddingsaas, D. J. Flannigan, S. D. Hopkins and H. Xu, *Ultrason. Sonochem.*, 2011, **18**, 842.
13. M. Vinatoru, *Ultrason. Sonochem.*, 2001, **8**, 303.
14. J. P. Lorimer and T. J. Mason, *Chem. Soc. Rev.*, 1987, **16**, 239.
15. W. Lauterborn and T. Kurz, *Rep. Prog. Phys.*, 2010, **73**, 106501.
16. M. J. W. Povey and T. J. Mason, *Ultrasound in Food Processing*, Springer, New York, 1998.
17. H. Feng, G. Barbosa-Canovas and J. Weiss, *Ultrasound Technologies for Food and Bioprocessing*, Springer, 2010.
18. C. J. Martin and A. N. R. Law, *Ultrasonics*, 1983, **21**, 85.
19. M. M. Chivate and A. B. Pandit, *Ultrason. Sonochem.*, 1995, **2**, S19.
20. M. Margulis and I. Margulis, *Ultrason. Sonochem.*, 2003, **10**, 343.
21. K. Makino, M. M. Mossoba and P. Riesz, *J. Phys. Chem.*, 1983, **87**, 1369.

22. M. Toma, S. Fukutomi, Y. Asakura and S. Koda, *Ultrason. Sonochem.*, 2011, **18**, 197.
23. S. Chemat, A. Lagha, H. AitAmar, P. V. Bartels and F. Chemat, *Flavour Fragr. J.*, 2004, **19**, 188.
24. X. Wei, M. Chen, J. Xiao, Y. Liu, L. Yu, H. Zhang and Y. Wang, *Carbohydr. Polym.*, 2010, **79**, 418.
25. B. K. Tiwari, C. P. O'Donnell, A. Patras and P. J. Cullen, *J. Agric. Food Chem.*, 2008, **56**, 10071.
26. H. M. Santos, C. Lodeiro and J.-L. Capelo-Martinez, in *Ultrasound in Chemistry: Analytical Applications*, Wiley, 2008, p. 171.
27. Y. Sun, D. Liu, J. Chen, X. Ye and D. Yu, *Ultrason. Sonochem.*, 2011, **18**, 243.
28. M. D. Esclapez, V. Sáez, D. Milán-Yáñez, I. Tudela, O. Louisnard and J. González-García, *Ultrason. Sonochem.*, 2010, **17**, 1010.
29. G. Cravotto, L. Boffa, S. Mantegna, P. Perego, M. Avogadro and P. Cintas, *Ultrason. Sonochem.*, 2008, **15**, 898.
30. D. J. Flannigan and K. S. Suslick, *Nature Phys.*, 2010, **6**, 598.
31. M. Palma and C. G. Barroso, *Anal. Chim. Acta*, 2002, **458**, 119.
32. Z. S. Zhang, L. J. Wang, D. Li, S. S. Jiao, X. D. Chen and Z. H. Mao, *Sep. Pur. Technol.*, 2008, **62**, 192.
33. Q.-A. Zhang, Z.-Q. Zhang, X.-F. Yue, X.-H. Fan, T. Li and S.-F. Chen, *Food Chem.*, 2009, **116**, 513.
34. H.-F. Zhang, X.-H. Yang, L.-D. Zhao and Y. Wang, *Innov. Food Sci. Emerg. Technol.*, 2009, **10**, 54.
35. F. F. Rawson, in *Ultrasound in Food Processing*, Thomson Science, London, 1998.
36. G. Arnold, S. Zahn, A. Legler and H. Rohm, *J. Food Eng.*, 2011, **103**, 394.
37. P. Piyasena, E. Mohareb and R. C. McKellar, *Int. J. Food Microbiol.*, 2003, **87**, 207.
38. C. P. O'Donnell, B. K. Tiwari, P. Bourke and P. J. Cullen, *Trends Food Sci. Technol.*, 2010, **21**, 358.
39. S. M. Jafari, Y. He and B. Bhandari, *J. Food Eng.*, 2007, **82**, 478.
40. T. S. H. Leong, T. J. Wooster, S. E. Kentish and M. Ashokkumar, *Ultrason. Sonochem.*, 2009, **16**, 721.
41. H. Wu, G. J. Hulbert and J. R. Mount, *Innov. Food Sci. Emerg. Technol.*, 2001, **1**, 211.
42. E. Riera-Franco de Sarabia, J. A. Gallego-Juárez, G. Rodríguez-Corral, L. Elvira-Segura and I. González-Gómez, *Ultrasonics*, 2000, **38**, 642.
43. H. M. Kyllönen, P. Pirkonen and M. Nyström, *Desalination*, 2005, **181**, 319.
44. H. Kiani, Z. Zhang, A. Delgado and D.-W. Sun, *Food Res. Int.*, 2011, **44**, 2915.
45. M. D. Luque de Castro and F. Priego-Capote, *Ultrason. Sonochem.*, 2007, **14**, 717.
46. E. Riera, J. V. Garcia-Perez, J. A. Carcel, V. M. Acosta and J. A. Gallego-Juarez, in *Innovative Food Processing Technologies: Advances in*

- Multiphysics Simulation*, ed. K. Knoerzer, P. Julianio, P. Roupas and C. Versteeg, Wiley and Blackwell, 2011, p. 265.
47. A. Mulet, J. A. Cárcel, N. Sanjuán and J. Bon, *Food Sci. Technol. Int.*, 2003, **9**, 215.
 48. D. Pingret, A.-S. Fabiano-Tixier, E. Petitcolas, J.-P. Canselier and F. Chemat, *J. Food Sci.*, 2011, **76**, C287.
 49. F. Pohlman, M. Dikeman, J. Zayas and J. Unruh, *J. Anim. Sci.*, 1997, **75**, 386.
 50. E. Lagsir and O. Boistier Marquis, *Industries Alimentaires et Agricoles*, 1999, **116**, 23.
 51. G. Rodríguez, E. Riera, J. A. Gallego-Juárez, V. M. Acosta, A. Pinto, I. Martínez and A. Blanco, *Phys. Procedia*, 2010, **3**, 135.
 52. J. A. Gallego Juárez, G. Rodríguez Corral, V. M. Acosta Aparicio, E. Andrés Gallego, A. Blanco Blanco, F. Montoya Vitini and others, 2005, Patent number ES 2212896 B1.
 53. A. C. Chang and F. C. Chen, *Food Chem.*, 2002, **79**, 501.
 54. T. L. Ho and Y. H. Chiu, 2008, Patent number 7334516.
 55. R. Konwarh, S. Pramanik, D. Kalita, C. L. Mahanta and N. Karak, *Ultrason. Sonochem.*, 2012, **19**, 292.
 56. T. Li, X. Qu, Q. Zhang and Z.-Z. Wang, *Ind. Crops Prod.*, 2012, **35**, 98.
 57. T. J. Mason, F. Chemat and M. Vinatoru, *Curr. Org. Chem.*, 2011, **15**, 237.
 58. A. Patist and D. Bates, *Innov. Food Sci. Emerg. Technol.*, 2008, **9**, 147.
 59. J. Londoño-Londoño, V. R. de Lima, O. Lara, A. Gil, T. B. C. Pasa, G. J. Arango and J. R. R. Pineda, *Food Chem.*, 2010, **119**, 81.
 60. D. T. Santos, P. C. Veggi and M. A. A. Meireles, *J. Food Eng.*, 2010, **101**, 23.
 61. Z. Pan, W. Qu, H. Ma, G. G. Atungulu and T. H. McHugh, *Ultrason. Sonochem.*, 2011, **18**, 1249.
 62. M. K. Khan, M. Abert-Vian, A.-S. Fabiano-Tixier, O. Dangles and F. Chemat, *Food Chem.*, 2010, **119**, 851.
 63. V. Sivakumar, J. Vijaeeswarri and J. L. Anna, *Ind. Crops Prod.*, 2011, **33**, 116.
 64. F. Chen, Y. Sun, G. Zhao, X. Liao, X. Hu, J. Wu and Z. Wang, *Ultrason. Sonochem.*, 2007, **14**, 767.
 65. C. C. Nascentes, M. Korn and M. A. Arruda, *Microchem. J.*, 2001, **69**, 37.
 66. A. L.-S. Eh and S.-G. Teoh, *Ultrason. Sonochem.*, 2012, **19**, 151.
 67. G. F. Barbero, A. Liaizid, M. Palma and C. G. Barroso, *Talanta*, 2008, **75**, 1332.
 68. S. Boonkird, C. Phisalaphong and M. Phisalaphong, *Ultrason. Sonochem.*, 2008, **15**, 1075.
 69. S. Albu, E. Joyce, L. Paniwnyk, J. P. Lorimer and T. J. Mason, *Ultrason. Sonochem.*, 2004, **11**, 261.
 70. L. Paniwnyk, H. Cai, S. Albu, T. J. Mason and R. Cole, *Ultrason. Sonochem.*, 2009, **16**, 287.
 71. C. Da Porto and D. Decorti, *Ultrason. Sonochem.*, 2009, **16**, 795.
 72. A. C. Chang and F. C. Chen, *Food Chem.*, 2002, **79**, 501.

73. S. Cabredo-Pinillos, T. Cedrón-Fernández, M. González-Briongos, L. Puente-Pascual and C. Sáenz-Barrio, *Talanta*, 2006, **69**, 1123.
74. D. Hernanz Vila, F. J. Heredia Mira, R. Beltran Lucena and M. Fernández Recamales, *Talanta*, 1999, **50**, 413.
75. I. Caldeira, R. Pereira, M. C. Clímaco, A. Belchior and R. Bruno de Sousa, *Anal. Chim. Acta*, 2004, **513**, 125.
76. M. A. A. Meireles, *Extracting Bioactive Compounds for Food Products*, CRC Press, 2008.
77. E. Moretto and R. Fett, *Tecnologia de Oleos e Gorduras Vegetais na Industria de Alimentos*, Sao Paulo, Brazil, Varela., 1998.
78. A. Sharma and M. N. Gupta, *Eur. J. Lipid Sci. Technol.*, 2004, **106**, 183.
79. R. Eggers, U. Sievers and W. Stein, *J. Am. Oil Chem. Soc.*, 1985, **62**, 1222.
80. B. Shukla, P. Srivastava and R. Gupta, *Oilseeds Processing Technology*, Central Institute of Agricultural Engineering, Bhopal, India, 1992.
81. Z. Lou, H. Wang, M. Zhang and Z. Wang, *J. Food Eng.*, 2010, **98**, 13.
82. A. Sharma and M. N. Gupta, *Ultrason. Sonochem.*, 2006, **13**, 529.
83. H. Li, L. O. Pordesimo, J. Weiss and L. R. Wilhelm, *Transactions of the ASAE*, 2004, **47**, 1187.
84. H. Li, L. Pordesimo and J. Weiss, *Food Res. Int.*, 2004, **37**, 731.
85. J. L. Luque-Garcia and M. D. Luque de Castro, *J. Chromatogr. A*, 2004, **1034**, 237.
86. D. E. Breithaupt, *Food Chem.*, 2004, **86**, 449.
87. M. J. Cocero, S. González, S. Perez and E. Alonso, *J. Supercr. Fluids*, 2000, **19**, 39.
88. L. Jaime, I. Rodríguez-Meizoso, A. Cifuentes, S. Santoyo, S. Suarez, E. Ibáñez and F. J. Señorans, *LWT – Food Sci. Technol.*, 2010, **43**, 105.
89. P. D. Fraser and P. M. Bramley, *Prog. Lipid Res.*, 2004, **43**, 228.
90. R.-A. Delia, *J. Food Compos. Anal.*, 2010, **23**, 726.
91. S. Krichnavaruk, A. Shotipruk, M. Goto and P. Pavasant, *Biores. Technol.*, 2008, **99**, 5556.
92. M. Sun and F. Temelli, *J. Supercr. Fluids*, 2006, **37**, 397.

CHAPTER 4

Microwave-assisted Extraction

EMILIE DESTANDAU,* THOMAS MICHEL AND
CLAIRE ELFAKIR

Institut de Chimie Organique et Analytique, Université d'Orléans-CNRS
UMR 7311, BP 67059, 45067 Orléans cedex 2, France

*Email: emilie.destandau@univ-orleans.fr

4.1 Introduction

The use of microwave energy was mentioned for the first time by Abu-Samra *et al.* in 1975. They used domestic ovens in the laboratory for the treatment of biological samples for metal trace analysis.¹ The extraction of organic compounds by microwave irradiation then appeared with the work of Ganzler *et al.* in 1986.² The first patent for extraction of a natural product using microwaves was filed by Pare in 1995.³

Initially employed as a digestion method for different sample types, such as environmental, biological, and geological matrices, microwave extraction is now widely accepted for extracting natural products from plant materials and has attracted growing interest in recent years. Indeed it allows rapid extraction of solutes from solid matrices, with extraction efficiency comparable to that of the classical techniques, but with the advantages of decreasing solvent quantity, solvent waste, solvent release into the environment, and human exposure. Advances in this process were achieved by eliminating use of solvents in the system and by practicing solvent-free extraction.

With growing interest in green technology, microwave-assisted extraction (MAE) appears among the most promising methods and becomes one of the major techniques for extracting valuable compounds from vegetable materials.

RSC Green Chemistry No. 21

Natural Product Extraction: Principles and Applications

Edited by Mauricio A. Rostagno and Juliana M. Prado

© The Royal Society of Chemistry 2013

Published by the Royal Society of Chemistry, www.rsc.org

Moreover, it is quite adaptable on a small or large volume so technology transfer from laboratory to industry scale can be done.

This chapter will present first the microwave heating theory and the microwave oven design. Then the influence of the main parameters will be discussed. Some of the new developments around microwave-assisted extraction will be described and illustrated by different applications. Finally, a study case showing the development and optimization of a pressurized solvent-free microwave-assisted extraction applied to phenolic compounds of sea buckthorn (*Hippophaë rhamnoides* L.) berries and its comparison to conventional extraction method will be presented to highlight the benefits of microwaves to extract natural compounds from plant matrices.

4.2 Principles of Microwave-assisted Extraction

4.2.1 Microwave Heating Principle

Microwave energy is a non-ionizing radiation that covers a 3-order of magnitude scale from 300 MHz to 300 GHz (wavelength in air or vacuum between 1 m and 1 mm). Figure 4.1 presents the electromagnetic spectrum and the position of microwaves.

Microwaves are electromagnetic waves made up of two oscillating perpendicular fields: electrical field and magnetic field. They can be used as information carriers or as energy vectors. This second application is the direct action of waves on a material which is able to absorb a part of electromagnetic energy and to transform it into heat. In order to avoid interferences with radio communications, domestic and industrial microwaves generally operate at 2450 MHz and occasionally at 0.915 GHz in USA or at 0.896 GHz in Europe.⁴ This frequency corresponds to a wavelength of 12.2 cm and an energy of 0.23 cal/mol (= 0.94 J/mol) and can only cause rotation of molecules.⁵

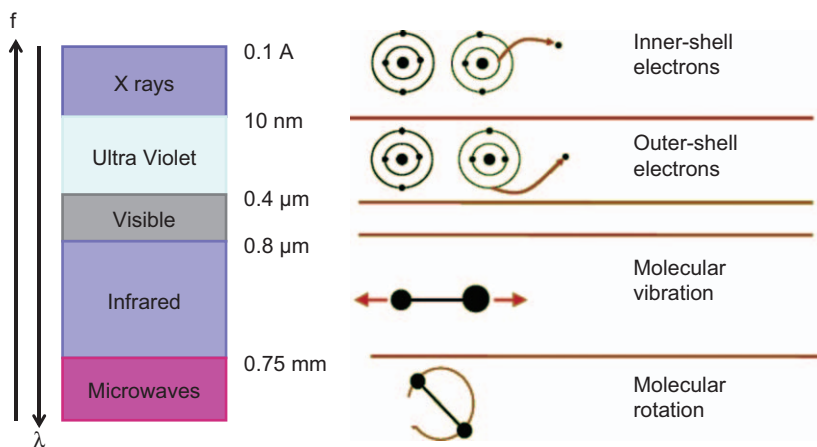


Figure 4.1 Electromagnetic spectrum, adapted from M. Letellier and H. Budzinski, *Analysis*, 1999, **27**, 259.

The principle of heating using microwave energy is based on the direct effects of microwaves on molecules of the material. The transformation of electromagnetic energy in calorific energy occurs by two mechanisms: ionic conduction and dipole rotation in both the solvent and the sample. In many applications these two mechanisms take place simultaneously, which effectively changes microwave energy to thermal energy.

Ionic conduction is due to the electrophoretic migration of ions when an electromagnetic field is applied.⁶ The resistance of the solution to this flow of ions and the collisions between molecules because the direction of ions changes as many times as the field changes sign will result in friction and, thus, heat the solution. Furthermore, the migration of dissolved ions increases solvent penetration into the matrix and thus facilitates the solvation of target compounds.⁷

The dipole rotation is related to alternative movement of polar molecules that have dipole moments (either permanent or induced by the electric field) which try to line up with the electric field. As the field decreases, thermal disorder is restored which results in the release of thermal energy. At 2450 MHz (used in commercial systems), the alignment of the molecules followed by their return to disorder occurs 4.9×10^9 times per second, leading to rapid heating.⁸ Figure 4.2 presents the schematic behavior of dipolar molecules without an electric field and under continuous or high frequency electric fields. The larger the dipole moment of the molecule, the more vigorous is the oscillation in the microwave field. This dipole rotation leads to the disruption of weak hydrogen bonds. A higher viscosity of the medium lowers this mechanism by affecting molecular rotation.⁹

Heat generation in the sample by the microwave field requires the presence of a dielectric compound and release of heat is observed only if the sample has dielectric losses or conducting losses under microwave irradiation. This ability of a solvent to absorb microwave energy and convert it into heat will partly depend on the dissipation factor ($\tan \delta$). The dissipation factor is given by

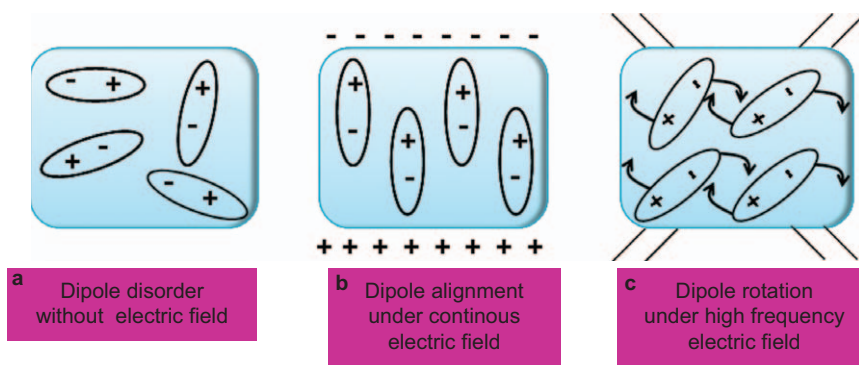


Figure 4.2 Dipolar molecules behavior: (a) without electric field, (b) under continuous electric field, and (c) under high frequency electric field.

Kingston and Jassie.¹⁰ The higher the dissipation factor, the higher will be the thermal energy.

$$\tan \delta = \varepsilon'' / \varepsilon' \quad (4.1)$$

where ε' is the dielectric constant, which expresses the capacity of a molecule to be polarized by an electric field, and ε'' is the dielectric loss factor, which expresses the efficiency of transformation of electromagnetic energy into heat. Polar solvents such as water have high dielectric losses; their permanent dipole moment will be affected by microwaves that were strongly absorbed. However, non-polar solvents such as hexane will not heat up when exposed to microwaves and they are termed as microwave-transparent solvents. Dielectric constants of common solvents are summarized in Table 4.1.

One particularity of microwave heating is, therefore, the selectivity, since only polar molecules can be heated. The second specificity, presented in Figure 4.3, is that unlike classical conductive heating, microwave heating is

Table 4.1 Physical constants of solvents used in MAE.^{6–9}

<i>Solvent</i>	ε'	ε'' (Debye)	$\tan \delta (\times 10^{-4})$
hexane	1.89	<0.1	
heptane	1.92	0	
dichloromethane	8.9	1.14	
2-propanol	19.9	1.66	6700
acetone	20.7	2.69	
ethanol	24.3	1.69	2500
methanol	32.6	2.87	6400
acetonitrile	37.5	3.44	
water	78.3	1.87	1570

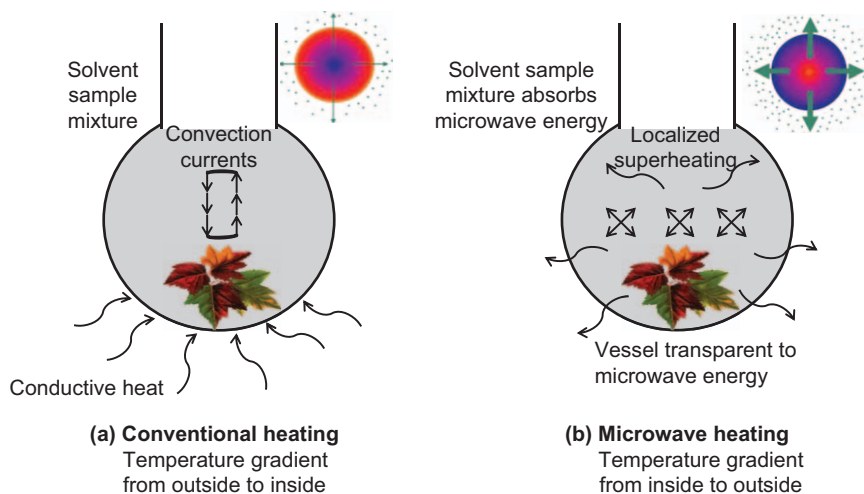


Figure 4.3 Representation of the two heating modes and temperature gradient: (a) by convection and (b) by microwave energy, adapted from M. Letellier and H. Budzinski, *Analysis*, 1999, **27**, 259.

volumetric; the whole sample is heated at the same time. The temperature gradient is reversed compared to conventional heating, because heating takes place in the heart of the solvent–matrix mixture whereas in conventional heating the surface is heated first.¹¹

There is no inertia and the temperature is quickly homogeneous in the medium. The temperature reached by the solvent can be higher than its boiling point. Baghurst and Mingos have shown the superheating effects under microwaves and that solvents such as ethanol or dichloromethane can reach temperatures above their theoretical boiling point (more than 20 °C higher in some cases).¹² They explain the origin of this phenomenon by the lack of nucleation sites.

4.2.2 Microwave Heating Applied to Plant Matrices

In the case of plant sample extraction, the effect of microwave energy is strongly dependent on the nature of both the solvent and the matrix. Most of the time, the solvent selected has a high dielectric constant, so that it strongly absorbs the microwave energy. However, in some cases, only the sample matrix may be heated, so that the solutes are released in a cold solvent; this is particularly useful to prevent the degradation of thermolabile compounds.⁸

The treatment of plant material with microwave irradiation during extraction can result in enhanced recovery of secondary metabolites and aroma compounds.¹³ The forced heating of water in the core of the material may cause liquid vaporization within the cells, which may lead to the rupture of the cell walls and/or plasma membranes.¹⁴ Since a lot of plant secondary metabolites naturally occur in the cell walls or cytoplasm, the cell disruption can shorten the diffusion path and facilitate the mass transfer of the solvent into the plant material and of the secondary metabolites into the solvent, thus allowing the effective extraction. The extracted compounds are dissolved in a suitable surrounding solvent to facilitate the separation from the remaining plant. In this sense, the mechanism of microwave-assisted extraction is different from those of Soxhlet extraction and heat-flux extraction which depend on a series of permeation and solubilization processes to wash the intracellular constituents out of the plant matrix.^{15,16}

In order to elucidate the mechanism of microwave-assisted extraction of natural compounds, structural changes of plant samples after extraction were observed by light microscopy, scanning electron microscopy, transmission electron microscopy, and atomic force microscopy.^{15,17–19} For example, the structure of *Epimedium* leaf sample unprocessed (Figure 4.4A) or processed by microwave irradiation (Figure 4.4B) was examined by light microscopy.¹⁷ Obviously microwave treatment disrupted the tissues of *Epimedium* during this process and a rapid release of chemical substances from the cells into surrounding solvent occurred. Fresh orange peels pre-treated by microwave energy and further observed using scanning electron micrographs also showed a destructive change in the plant tissue.²⁰ These changes in the plant tissue due to microwave result in a considerable increase in the yield of extractable pectin.

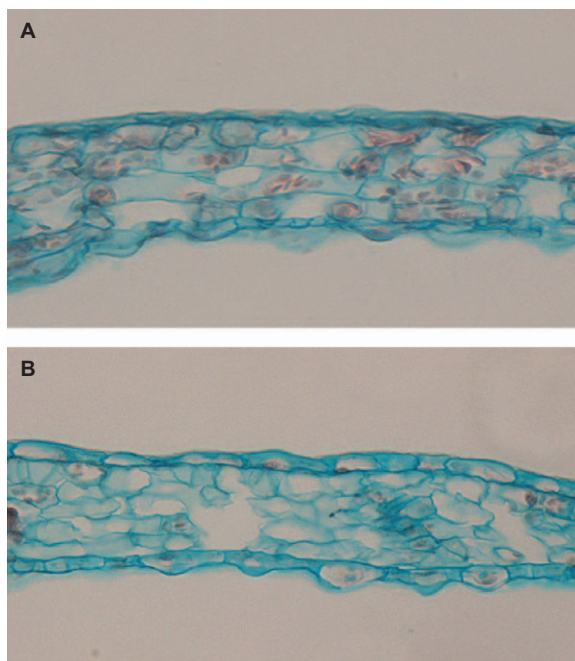


Figure 4.4 Light micrographs of *Epimedium* leaf samples: (A) untreated leaf sample, (B) leaf sample after microwave irradiation, reproduced with permission from H.-F. Zhang *et al.*, *Trends Food Sci. Technol.*, 2011, **22**, 672.

For essential oil extraction, microwaves interact selectively with the free water polar molecules present in glands, trichomes, or vascular tissues. Localized heating near or above the boiling point of water leads to the expansion and rupture of cell walls, which is followed by the liberation of essential oils into the solvent. This process can be applied to fresh plant material or when a dry sample has been re-hydrated before extraction.⁹

The fact that different chemical substances absorb microwave energy to different extents implies that the heating imparted to the surrounding media will vary with the chemical substances used. Hence, for samples with non-homogeneous structural characteristics or that contain various chemical species with different dielectric properties dispersed into a homogeneous environment, it is possible to produce selective heating of some areas or components of the sample.⁶

4.3 Microwave Instrumentation

First experimental setups were mainly laboratory-built systems based on domestic ovens.^{7,21,22} Today, MAE equipment designed for laboratory purposes is safe to work with and offers the user various ways to control the extraction process.⁶ Some manufacturers propose microwave ovens for

digestion or synthesis that can be used for extraction purpose or microwave ovens dedicated to extraction process, such as Milestone's Ethos, CEM's Mars, and Sineo's Master.^{6,23}

4.3.1 Oven Design

Commercial systems are usually constituted of a magnetron tube, a wave guide, a cavity, and circulators (Figure 4.5).

The magnetron tube generates microwaves at a fixed frequency (i.e. 2450 MHz). It consists of a vacuum tube with a central electron-emitting cathode of highly negative potential which is surrounded by a structured anode that forms cavities. They are coupled by the fringing fields and have the intended microwave resonant frequency. The power output of the magnetron can be controlled by the tube current or the magnetic field strength.²⁴

The wave guide transmits the microwave from the source to the cavity. It can itself be used as the applicator for microwave heating when the material is introduced by wall slots and the wave guide is terminated by the matched load. This configuration is called a travelling wave device since the locations of the field maxima change with time.²⁴ The sample introduced in a dedicated extraction vessel (reactor) is then placed in the cavity and the circulator is used for reflection and homogenization of radiation.

Microwave ovens can have monomode or multimode cavities, as presented in Figure 4.5. The monomode cavity (Figure 4.5a) can generate a frequency that excites only one mode of resonance. The sample can be placed in the wave guide where microwaves are focused, on the maximum of the electrical field, as the distribution of the field is known. The multimode cavity is larger (Figure 4.5b) and the incident wave is able to affect several modes of resonance. This superimposition of modes allows the homogenization of the field. Other homogenization systems such as rotating plate can be added.⁵

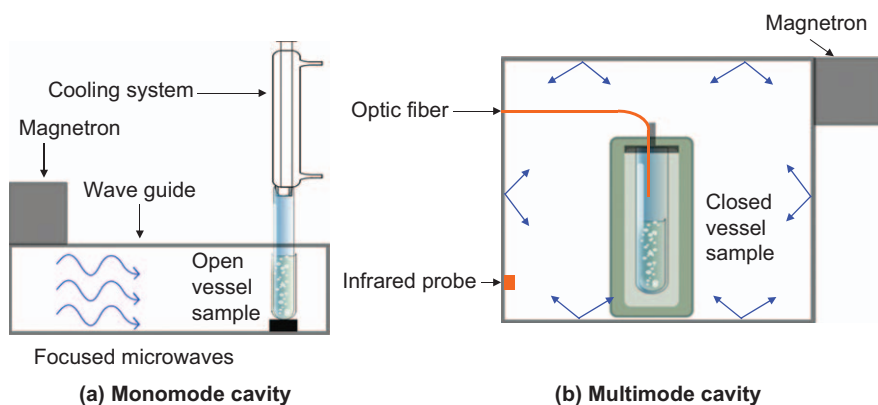


Figure 4.5 Schematic view of (a) open vessel in monomode focused microwave oven and (b) closed vessel in multimode microwave oven, adapted from M. Letellier and H. Budzinski, *Analysis*, 1999, **27**, 259.

The vessels used for extraction are typically made of microwave transparent materials (e.g. glass, polyether imide, or tetrafluoromethoxyl) and are lined with PFA (perfluoroalkoxy) or Teflon liners. Some system also include magnetic stirring inside the extraction vessel that allows continuous contact between the sample surface and the solvent, thus the temperature increases within shorter time and total extraction time is reduced. CEM's Carboflon® and Milestone's Weflon® bars, chemically inert fluoropolymers based on carbon and stable up to 350 °C, which absorb microwave energy and transfer heat to the surrounding medium, can therefore be used to heat non-polar solvents.⁶

4.3.2 Reactor Design

4.3.2.1 Open Systems

With open quartz vessels, extraction is made at atmospheric pressure and is commonly named focused microwave-assisted extraction (FMAE). As a consequence, the maximum possible temperature is determined by the boiling point of the solvent at that pressure. Losses of vapors are prevented by the presence of a cooling system on the top of the extraction vessel that causes condensation of solvent vapors (Figure 4.5a). The power can be modulated and the field is homogenous and reproducible. Hence the heating of the sample is homogeneous and very efficient. A cartridge holder may be used to avoid further filtration. These systems offer increased safety of sample handling compared to extraction in pressurized closed vessels. In addition, larger samples may be extracted in such systems than in closed vessel systems.⁸ Some industrial or pilot installations can offer the possibility to extract up to 100 kg of fresh material.^{25,26} Huayuan technology proposes for example microwave extraction equipment with capacities of 50–500 L.²⁷

4.3.2.2 Closed Systems

With closed vessel extraction processes, pressurized microwave-assisted extraction (PMAE) is performed under pressure (with or without regulation) (Figure 4.5b). Typical pressures reached are below 14 bar, but today's technology can handle up to 100 bar.⁶ The pressure allows temperatures above boiling points of the solvents to be reached enhancing both extraction speed and efficiency (Table 4.2). Commonly power, temperature, and pressure can be controlled to avoid overpressure. Pressure can be measured by a water manometer and the temperature probe is an optic fiber placed inside the reactor or an infra red cell placed in the cavity.

To increase sample throughput many pieces of laboratory equipment incorporate multiple extractions vessels (between 6 and 40 depending on the volume of each vessel) placed in a carousel which rotates through 360° during extraction, which allows simultaneous extractions to be performed. One of the reactors is the reference to control heat and pressure.⁶

Table 4.2 Boiling points and temperatures under pressure of solvents used in MAE.⁵

<i>Solvent</i>	<i>Boiling point (°C)</i>	<i>Temperature at 12 bar (°C)</i>
dichloromethane	39.8	140
acetone	56.2	164
methanol	64.7	151
hexane	68.7	
ethanol	78.3	164
acetonitrile	81.6	194
2-propanol	82.4	145

Today, hazards occasioned by heating highly flammable solvents are overcome through the use of recent security techniques such as high capacity exhaust fans to evacuate air from the cavity, solvent vapor detectors, or pressure-burst safety membranes placed in each vessel.⁹

The main drawback of such systems is that, if the temperature inside the vessel rises rapidly, partitioning of the more volatile solutes into the headspace may occur, leading to losses of these compounds. In addition, once the extraction is finished, the vessels must be cooled to room temperature before opening to avoid losses of volatile solutes, but this step considerably increases the overall extraction time.⁸ Due to this safety aspect, the size of closed vessels is limited to hundreds of mL and carousels are used to increase the raw material amount extracted. For example, the large-volume rotor marketed by Milestone is a medium pressure rotor with 6 vessels of 270 mL each. One Anton Paar's microwave oven can be used up to 1 L and 30 bar.

4.4 Parameter Influence on Microwave-assisted Extraction

The first publications that dealt with the efficiency of microwave heating for organic extraction appeared in 1986. Ganzler *et al.* developed extraction protocols for various types of compounds from soils, seeds, foods, and feeds in a few milliliters of solvent, irradiated for 30 s up to 7 times in a domestic oven (1140 W).² Some optimization of extraction conditions, varying each factor independently,^{28,29} or using factorial,³⁰ central composite,³¹ and Box-Behnken³² experimental designs or using response surface methodology³³ have been reported in the literature. Use of experiment design allows decreasing the number of manipulations needed to know the influence factors on extraction process and the optimal conditions for extraction. The parameters that influence the extraction technique are: choice of solvent composition, solvent to feed ratio, power applied, extraction temperature, extraction time, and size and moisture of the plant material. The selection of parameters and their values depend on solubility, volatility, and stability of target compounds. It will also depend on the interaction of other compounds present in the plant material.³⁴

4.4.1 Solvent

Whatever the extraction technique, the choice of an appropriate solvent is very important for obtaining optimal extraction yields. Thus the solvent composition but also the solvent to feed ratio must be carefully selected to optimize extraction yield and extraction time.

4.4.1.1 Solvent Composition

In the case of plant tissue extraction, the compound or the group of compounds of interest is present in various cells and in different parts of the raw material. To extract these compounds, the solvent has to reach and dissolve them. Solvent usually attacks the cell wall of raw materials and penetrates it to reach the compounds, but it can also dissolve various other impurities. Solvent selection, therefore, should be such that it selectively extracts target compounds.³⁴ Thus, MAE is often performed with the same solvent employed for the traditional extraction. However, the optimal extraction solvents for MAE cannot be always deduced directly from those used in conventional procedures.⁸ Therefore solvent choice for MAE is dictated as for other extraction techniques, by the solubility of the target compounds and by the interaction between solvent and plant matrix, but also by the microwave-absorbing properties of the solvent determined by its dielectric constant.

According to the current developments, three alternative methods for extraction with solvent under microwaves exist.⁶

1. The sample could be immersed in a single solvent or mixture of solvents that strongly absorb microwave energy. These polar solvents could be heated up to their boiling point in an open vessel or above their boiling point in a closed vessel and compounds would be extracted with hot solvent. For example flavonoids from *Hippophaë rhamnoides* were extracted in a focused microwave, and ethanol was chosen because alcohols are most widely used in antioxidant extraction work.³⁵ Oleanolic and ursolic acids were better extracted from fruits of *Chaenomeles sinensis* with methanol compared to absolute ethanol, 95% ethanol, or chloroform.³¹ Even if methanol does not appear to be the most suitable solvent to solubilize triterpenic acidic compounds, its better heating improves the extraction.
2. The sample could be extracted in a combined solvent containing solvents mixture with both high and low dielectric losses. Heating of the extraction solvent is then related to the polar solvent proportion, thus compounds could be extracted in controlled temperature conditions. The MAE of carotenoids from paprika has been optimized.³⁶ Thirty different water:organic solvent mixtures in several proportions were evaluated. Organic solvents tested were acetone, dioxane, ethanol, methanol, and tetrahydrofuran in volume ratio 15, 30, 45, 60, 75, and 90 (percent of organic modifier in water). Both extraction efficiency and selectivity were significantly dependent on the dielectric constant of the extracting solvent mixture. Indeed HPLC profiles of extracts showed different absolute

values of peak areas demonstrating variation of efficiency and different peak ratio demonstrating the different selectivity according to the solvent mixture. The optimal conditions for MAE of pigment from paprika were 120 s extraction time and 50 W energy level. Thanks to these conditions and the use of a solvent mixture containing water and another solvent less able to heat, temperature remains under 60 °C and consequently limits molecule rearrangements that could lead to a decrease of total carotene content.

3. A sample that has a high dielectric loss and is able to absorb microwave could be immersed in solvents with low dielectric losses and transparent to microwave energy. The hot sample releases compounds in cold solvent avoiding any degradation of thermolabile components. If the sample is not able enough to absorb microwave energy, water could be added to the sample matrix increasing its dielectric losses. The addition of absorbing stir bars into the sample-solvent mixture has also been shown to be efficient.⁸ The increased rate of extraction and selectivity were observed when a transparent solvent, petroleum ether, was used to extract piperine from *Piper nigrum* (pepper) compared to dichloromethane and ethanol in MAE.³⁷

Recently, some alternative solvents have been used for MAE of natural compounds. Ionic liquids (ILs) are composed of bulky organic cations and inorganic or organic anions, and they are liquid around room temperature. They have attracted much research interest for a variety of applications, thanks to their excellent properties: negligible vapor pressure, good thermal stability, wide liquid range, tunable viscosity and miscibility with water and organic solvents, as well as good solubility and extractability for various organic compounds. Thus ILs have been used as attractive 'green' alternatives to conventional volatile organic solvents.³⁸ Moreover, ILs can efficiently absorb and transfer microwave energy. ILMAE was used in the extraction of essential oil and biphenyl cyclooctene lignans simultaneously from *Schisandra chinensis* fruits,³⁸ carnosic acid, rosmarinic acid and essential oil from *Rosmarinus officinalis*,³⁹ polyphenolic compounds from *Psidium guajava* leaves and *Smilax china*,⁴⁰ and lichens from *Pertusaria pseudocorallina*.⁴¹ In each case, ILMAE gave better results than conventional methods. Otherwise, the feasibility of employing the non-ionic surfactant triton X100, a biodegradable micellar media, as an alternative and effective solvent for the extraction of glycyrrhizic acid and liquiritin from licorice root was studied. The proposed method offers advantages of fast, simple operation that is free of organic solvents. Compounds can be extracted more selectively with similar or better recoveries in comparison with conventional extraction processes.⁴² The application of polyethylene glycol (PEG) aqueous solution as a green solvent in MAE was also developed for the extraction of flavone and coumarin compounds from medicinal plants.⁴³

Another specific development of MAE, also turned to green chemistry, is solvent-free microwave extraction (SFME). SFME is a combination of

microwave heating and hydro-distillation performed at atmospheric pressure. Fresh or moistened samples with high content of water (at least 70%) are extracted without adding any solvent. Water contained in the matrix is used as the extraction solvent. This technique has been used to obtain essential oil from *Ocimum basilicum* L. (basil), *Mentha crispa* L. (garden mint), *Thymus vulgaris* L. (thyme),⁴⁴ *Elletaria cardamomum* L. (cardamom) seed,¹⁹ *Citrus sinensis* L. (orange) peels,⁴⁵ and *Origanum vulgare* L. (oregano).⁴⁶ In the improved solvent-free microwave extraction, a microwave absorption solid medium, such as carbonyl iron powder, was added and mixed with the sample for the extraction of essential oil from dried plant materials (*Cuminum cyminum* L. and *Zanthoxylum bungeanum* Maxim) without any pretreatment.⁴⁷ Extraction of essential oil by SFME will be discussed later (Section 4.5.3.1).

To conclude, a correct choice of solvent is fundamental for obtaining an optimal extraction process. When selecting solvents, consideration should be given to the microwave absorbing properties of the solvent, the interaction of the solvent with the matrix, and the target compound's solubility in the solvent. Preferably the solvent should have a high selectivity towards compounds of interest excluding unwanted matrix components and also be able to absorb microwave energy. Another important aspect is the compatibility of the extraction solvent with the analytical method used for the further application of the extract.

4.4.1.2 Solvent to Feed Ratio

Generally, a higher solvent to feed ratio (S/F) in extraction techniques can increase the recovery. Indeed, the extraction yield of target compounds is most likely dependent on how the ratio of solid to liquid is regulated (keeping the liquid volume or the solid mass constant).⁴⁸

As a first case, the liquid volume varies and the solid mass remains constant (this case could also be described as only a study of the solvent volume influence). The solvent volume must be sufficient to ensure that the entire sample is immersed, so that the material can swell during extraction. For extraction of triterpenoid saponins from *Ganoderma atrum*, the yield increased up to 75 mL of ethanol for 3 g of plant material which represent a S/F of 25, after which a decreasing trend in yield was observed.⁴⁹ On the other end, no influence on recovery of withanolides was observed with increase in methanol volume from 5 mL to 30 mL for 100 mg of plant material.⁵⁰ However, in the MAE, a higher S/F may give lower recoveries due to inadequate stirring of the solvent by microwaves and excessive swelling of the plant material. Moreover, a higher solvent volume requires higher power and more time to achieve the temperature required. Excessive solvent may also cause the dissolution of other undesirable compounds, lowering the extraction selectivity towards target compounds.⁵¹

In a second case, the solvent volume remains constant while the solid loading changes. The yield of scutellarin from *Erigeron breviscapus* increased with decreasing ratio of solid to liquid from 15.0:100 (g/mL) to 2.5:100 (g/mL).¹⁵ The possible reasons for a lower extraction yield with a higher sample loading could be: (1) increasing the solid mass decreases the surface area available

for solvent to penetrate plant materials and to solubilize the target molecules;³³ (2) microwave energy is absorbed and dispersed by the larger amount of plant materials;¹⁵ (3) the incident microwave radiation per particle decreases with the increase of solid loading at a given power. This should give a relatively low dielectric heating effect, and thus a reduced effect of microwave radiation;³⁴ (4) the absorption of microwave radiation by plant material near the surface of the vessel reduces the penetration depth of microwave radiation into the suspension.⁵² Therefore the raw material in the interior part of vessel will not have the same level of microwave radiation.

The influence of S/F on extraction yield may be associated with the temperature of plant sample–solvent mixture: if the solid mass is kept constant and the liquid volume is increased, the mixture temperature first increases and then decreases, and extraction yield exhibits a similar trend; if the liquid volume is maintained constant and the solid mass decreased, the temperature of the mixture is almost constant and the extraction yield increases gradually.⁴⁸ It may also be associated with properties of the target phytochemicals (e.g. thermostability), microwave-based extractor (with or without stirrer), solvent, and so on. Since the mechanism underlying the impact of S/F on microwave-assisted extraction remains unclear, further investigation is required.¹⁷ Values of S/F frequently employed range from 10:1 to 50:1 (v:w), but this ratio has to be adapted and optimized for each raw material.

4.4.2 Temperature and Pressure

Temperature is an important parameter for all extraction techniques since it contributes to an increase in yield. With a temperature increase, the solvent has higher capacity to solubilize the target compounds, while surface tension and solvent viscosity decrease, which improves sample wetting and matrix penetration. Efficient desorption of compounds from the active site in the matrix takes place, leading to high extraction recoveries. In MAE, temperature depends on the solvent's ability to absorb microwaves and on the microwave energy applied (power). The extraction yield of triterpenoid saponins from *Ganoderma atrum* increased up to 78 °C, after which it decreased because other compounds were co-extracted at elevated temperature.⁴⁹ The influence of temperature in a closed vessel on extraction shows that increasing the temperature of the solvent from 60 °C to 120 °C significantly increases the extraction efficiency. This is because higher temperature causes intermolecular interactions within the solvent to decrease, giving rise to higher molecular motion, and causing the solubility to increase. The increasing temperature may also cause opening of cell matrix, and as a result, anthraquinones availability for extraction increases.⁵³

In closed vessel, the temperature could exceed the boiling temperature of the solvent. Pressure becomes then an important variable; however, pressure is directly dependent on temperature and allows heating above the boiling temperature. At the end of the extraction, the vessel has to be cooled down before opening carefully to avoid loss of volatile solutes which can partition into the head-space, but this step can considerably increase the overall extraction time.

In applications dealing with thermolabile compounds, high temperatures may cause degradation of analytes. Extractions performed between 70 °C and 110 °C presented the same flavonoid percentage extracted from *Radix astragali*, but extraction at 130 °C and 150 °C caused a sharp degradation of some compounds whereas the concentration of others (e.g. formononetin) increased from 70 °C to 150 °C. The degree of degradation at 150 °C increased with the molecular polarity due to the microwave-selective heating of polar molecules.²⁹

Therefore, temperature should be sufficient to ensure a good solubility of compounds and a good penetration of solvent in the plant matrix to enhance extraction yield, but not high enough to degrade the target compounds. In closed vessels, limits on increasing temperature are also given by reactor overpressure.

4.4.3 Extraction Time

One of the main advantages of the microwave-assisted extraction is the very short time (several minutes or seconds) taken compared to conventional techniques. Duration of microwave radiation of 5 min to 30 min was studied for extraction of flavonoids from *Radix astragali*. At the beginning the yield increased with the increase of time and reached its maximum at 25 min, then fell down slightly.²⁹ The yield of triterpenoid saponins from *Ganoderma atrum* increased slightly from 5 min to 15 min, increased more from 15 min to 20 min and then fell down strongly from 20 min to 30 min.⁴⁹ Five minutes were sufficient to extract triterpenoids compounds from olive leaves. Extracts obtained with longer times provided similar results with no degradation detected.⁵⁴ The extraction time is varied to observe the effect of radiation for different durations and in turn to observe an impact on mechanism of interaction between microwaves and various materials like plant cells, target compounds, and impurities.³⁴ With thermolabile compounds, a long extraction time may result in degradation.

Sometimes a sample can be extracted in multiple steps using consecutive extraction cycles. The same solvent can be used for the different cycles with a cooling down step between each irradiation cycle. This practice helps in further improving the extraction yield, preventing prolonged heating using the same S/F. Solvent can also be changed for each extraction cycle to avoid solvent saturation, but it increases S/F. Extraction of flavonoids from *Radix astragali* was performed studying the influence of cycle number up to three cycles. The residue was separated from the solvent and re-extracted three times using fresh solvent each time. Yield of flavonoids increased with the second extraction cycle, but there were almost no more flavonoids extracted in the third cycle.²⁹

4.4.4 Power

The power must be correctly chosen to minimize the time needed to obtain the set temperature without reaching excessive temperature and overpressure in case of closed vessel. However, increased power with longer irradiation time may cause solvent loss by evaporation. Maximum power used ranges between 600 W and 1000 W for closed systems and around 250 W for open systems.⁹

Extraction yield of flavonoids during short irradiation time (5 min and 10 min) was enhanced with microwave power increase from 200 W to 1000 W. But when the sample solutions were heated for a longer time (15 min), the yields under different powers were similar. Thus, the difference of the flavonoid yield between 200 W and 1000 W appears more significant with short irradiation times compared to long irradiation times.²⁹ Extraction yield of ursolic and oleanolic acids from *Chaenomeles sinensis* increased with microwave power rise from 400 W to 600 W but decreased from 800 W to 1000 W.³¹

4.4.5 Nature of the Matrix

4.4.5.1 Matrix Moisture

The nature of matrix in which solutes are present can have an important effect on the extraction yield. The water added or naturally present in the sample is of great importance, as water molecules have a high dipole moment, and thus strongly absorb microwave energy. Therefore water always has an effect on the microwave absorbing ability and hence facilitates the heating process, increasing the polarity of the extracting solvent and/or allowing the sample to be heated. In many cases the matrix moisture improves the extraction recoveries. It may also have a swelling effect on the matrix and influence the solute–matrix interactions, making solutes more available to the extracting solvent.⁶

A dry raw material can be re-hydrated before extraction to facilitate the heating process by changing its dielectric properties and improving extraction efficiency. The sample can be soaked with water for different periods of time. The soaking time varies from 10 min to 24 h and with increase in soaking time an enhanced extraction rate can be observed. Extraction yield of essential oil from cardamom depended if the whole fruit (capsule and seed) or only seed was used as raw material, seeds giving higher yields. Dry seeds were moistened prior to extraction by soaking in water then draining the excess water. This step is essential to raise the initial moisture. Humidity level of the sample was then studied from 30% to 70%, the optimum was found at 67%.¹⁹

The effect of this parameter also depends on the extraction solvent. If the plant material contains enough water that is able to absorb microwave energy, the surrounding solvent can have low dielectric constant and thus remain cold during extraction of thermosensitive compounds. However, it was found that it is impossible to perform good MAE for completely dry as well as for too wet materials when a non-polar solvent such as hexane is used for extraction.⁴

As a consequence, obtaining reproducible results in microwave-assisted extraction requires control of the matrix water content.

4.4.5.2 Matrix Size

Plant particle size and size distribution usually have a significant influence on the efficiency of MAE. The particle sizes of the extracted materials are usually in the range of 100 μm –2 mm. Fine powders can enhance the extraction because the limiting step of the extraction is often the diffusion of chemicals out of the plant matrix, thus smaller size materials have less diffusion depth for molecule

diffusion out of the plant matrix to the surrounding solvent. Moreover, the larger surface area of a fine powder provides contact between the plant matrix and the solvent and smaller particles have less penetration depth that leads to uniform microwave exposure. For example, in MAE of cocaine, finely ground coca leaf powder was more easily extracted than large particles.⁵⁵ The yield of glycyrrhizic acid increased from large pieces and 4–2 mm unrefined powder to 300 μm powder of roots of *Glycyrrhizae radix* (licorice).⁵⁶

In conclusion, MAE can be influenced by many parameters – such as solvent composition, solvent to feed ratio, extraction time, temperature, and irradiation power. These parameters have to be properly chosen to ensure an efficient and selective extraction of target compounds. Moreover, moisture of the vegetable material has to be controlled to obtain reproducible extractions. This large number of parameters that have to be optimized and controlled to performed a good extraction can appear as one of the drawbacks of MAE since a careful optimization of them is needed for each raw material. But in fact they allow the versatility, the selectivity, and the efficiency of the method.

4.5 Trends in Microwave-assisted Extraction and Applications

MAE is today frequently used for extraction of natural compounds from plant matrices since the reduction of the extraction time and solvent consumption and its high efficiency are now well known. Therefore, MAE is considered as a green extraction technique with growing interest. To go further in the versatility of the technique and in the development of green extraction methods, in the past few years some improved extraction methods have been developed using microwaves as heating source; part of them will be described next.

4.5.1 Extraction of Sensitive Compounds

As was shown, high temperature, high irradiation power, and long irradiation time can lead to degradation of some sensitive compounds. To help overcome this drawback MAE can be carried out under an inert atmosphere or under vacuum, so limiting the presence of oxygen in the reactor.

4.5.1.1 Nitrogen-protected Microwave-assisted Extraction (NPMAE)

Oxidation of the active compounds during the extraction process can be prevented by using a pressurized inert gas, such as nitrogen,⁵⁷ in a closed system. In nitrogen-protected microwave assisted extraction (NPMAE), the plant sample is introduced into the vessel with a certain volume of solvent. First, the air in the flask is pumped out by a vacuum pump until a certain degree of vacuum is obtained. Then the vessel is filled with nitrogen from the gas cylinder. This technique has been employed in the extraction of ascorbic acid from guava, yellow pepper, green pepper, and cayenne pepper. Extraction

was performed in aqueous solution containing 0.25% of metaphosphoric acid with a S/F of 10 for 10 min and under microwave power of 400 W. Compared with conventional MAE and solvent extraction methods, the oxidation of ascorbic acid was significantly reduced or prevented in the NPMAE process, providing higher extraction yield of ascorbic acid.

4.5.1.2 Vacuum Microwave-assisted Extraction (VMAE)

Vacuum microwave-assisted extraction (VMAE) is MAE in a vacuum system as presented in Figure 4.6.⁵⁸ The boiling point of the extraction solvent under vacuum is lower than at ambient pressure. Thus, the solvent can be kept boiling

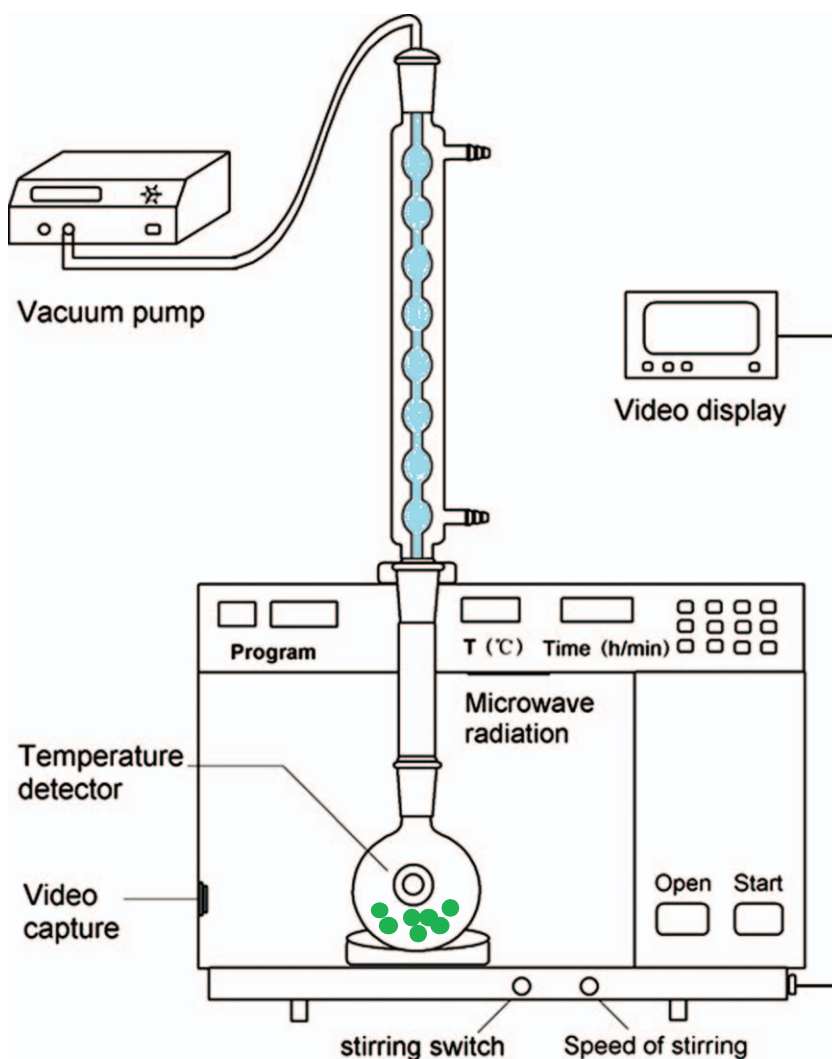


Figure 4.6 Vacuum microwave extraction (VMAE) apparatus, reproduced with permission from J.-X. Wang *et al.*, *J. Chromatogr. A*, 2008, **1198–1199**, 45.

and refluxing at lower temperature, which is good for mixing the sample with the solvent and extracting thermosensitive compounds while preventing their degradation. Furthermore, the air in the extraction system is mostly pumped out, so oxidation of oxygen-sensitive compounds is avoided or reduced. This type of MAE enhances mass transfer mechanism by promoting diffusion of active compounds to the solvent via the suction pressure.⁵⁹ Indeed, the pressure difference between the inside and outside of the cell wall enhances the extraction efficiency of the solutes.⁶⁰ Therefore, VMAE is suitable for extraction of thermosensitive and oxidative compounds at lower temperature and low oxygen content in the extraction process.

All the parameters that influence classical MAE also influence VMAE. One extra parameter in VMAE is the degree of vacuum, which controls the extraction temperature and can have an effect on the extraction yield. For example, vacuum degree and temperature had no clear effect on the extraction yields of resveratrol and emodin from Chinese herbs. In contrast, when the degree of vacuum went from 60 kPa to 40 kPa and the extraction temperature went from 55 °C to 70 °C, the extraction yield of myricetin and quercetin were enhanced. Temperatures above 60 °C at 40 kPa caused the degradation of safflomin A.⁵⁸ Extraction yields of these five compounds with VMAE were higher than those obtained with MAE.

The extraction yields of strong antioxidant compounds ascorbic acid, α -tocopherol, and δ -tocopherol from plant samples in VMAE were higher than those in air-MAE. Since VMAE was performed in vacuum, less oxygen in the system allowed a longer extraction time and a relatively lower extraction temperature, which favoured the extraction. Moreover the sub-pressure *in vacuo* can accelerate the mass transfer rate from the matrix into the extraction solvent.⁵⁹ The poor yields shown by the typical MAE were claimed to be due to both thermal and oxidative degradation.

Extraction of labile compounds such as β -carotene, ascorbic acid, astaxanthin, and aloin A from several plant matrices was optimized under vacuum and low temperature. Optimal temperatures for higher extraction yields were found near room temperature at 25 °C for β -carotene and vitamin C, at 35 °C for aloin A, and at 45 °C for astaxanthin.⁶⁰

Extraction under inert atmosphere or under vacuum prevents the degradation of sensitive compounds, hence better extraction efficiencies can be achieved than in MAE and in conventional solvent extraction.

4.5.2 Extraction Methods Improved by Microwave Heating

Rapid microwave heating of the solvent-sample mixture allows reducing extraction time and energy consumption. So microwave energy was used to speed up the heating and to improve the efficiency of conventional extraction methods.

4.5.2.1 Focused Microwave-assisted Soxhlet Extraction (FMASE)

Focused microwave-assisted Soxhlet extraction (FMASE) is based on the same principles of conventional Soxhlet extraction, but using microwaves as

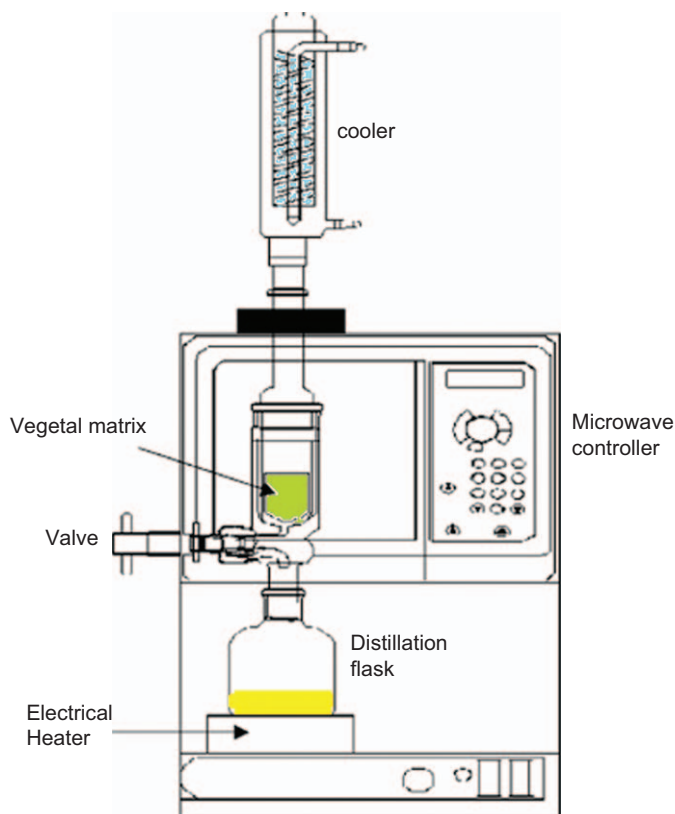


Figure 4.7 Focused microwave Soxhlet extraction (FMASE) apparatus, reproduced with permission from J. L. Luque-Garcia and M. D. Luque de Castro, *Talanta*, 2004, **64**, 571.

auxiliary energy to accelerate the process.⁶¹ Figure 4.7 presents the sample cartridge compartment located in the irradiation zone of a microwave oven.

FMASE preserves the advantages of conventional Soxhlet extraction while overcoming restrictions such as the long extraction time and non-quantitative extraction of strongly retained solutes due to the easier cleavage of solute–matrix bonds by interactions with focused microwave energy. FMASE was first applied to environmental and food samples. The better quality of the extracts was obtained possibly due to the shorter extraction time, but it has been found that the moisture content of samples to be extracted is a critical parameter for a good and reproducible recovery yield.

Thus the method was improved by developing the microwave-integrated Soxhlet (MIS), where the base vessel contains a polytetrafluoroethylene/graphite (Weflon) stir bar capable of absorbing microwaves at the bottom of the vessel.⁶² The use of such a device allows the diffusion of the heat created by the microwaves to the surroundings and is particularly useful when the extraction solvent is transparent to microwave radiations. Thus the sample moisture become less influential and solid material may have a moisture

content ranging from 0 to 90%. Lipids of olive oil were extracted with n-hexane by MIS in 32 min. The extract was compared to that obtained with conventional Soxhlet. The oil profile was equivalent for both extraction methods, except for palmitic acid, which presented a higher percentage in the olive oil obtained by conventional Soxhlet extraction. Microwave irradiation accelerated the extraction process without inducing noticeable changes in the olive oil composition except for the relative amount of palmitic acid.

4.5.2.2 Ultrasonic Microwave-assisted Extraction (UMAE)

Enhancement of mass transfer mechanism in extraction can be achieved by another type of MAE known as ultrasonic microwave-assisted extraction (UMAE). An open microwave and an ultrasonic transducer are used simultaneously (see Figure 4.8). The sample is introduced into the flask with the solvent and then transferred to the microwave cavity and connected with cooling tubes. The transducer is placed outside the microwave cavity under the sample vessel.⁶³

Additional ultrasonic waves emitted by UMAE intensified the mass transfer mechanism as the combined microwave and ultrasonic waves provide high momentum and energy to rupture the plant cell and to elute the bioactive compounds to the extraction solvent.⁶³ As a result, the extraction proceeds with a shorter extraction time and with lower solvent consumption. UMAE has been

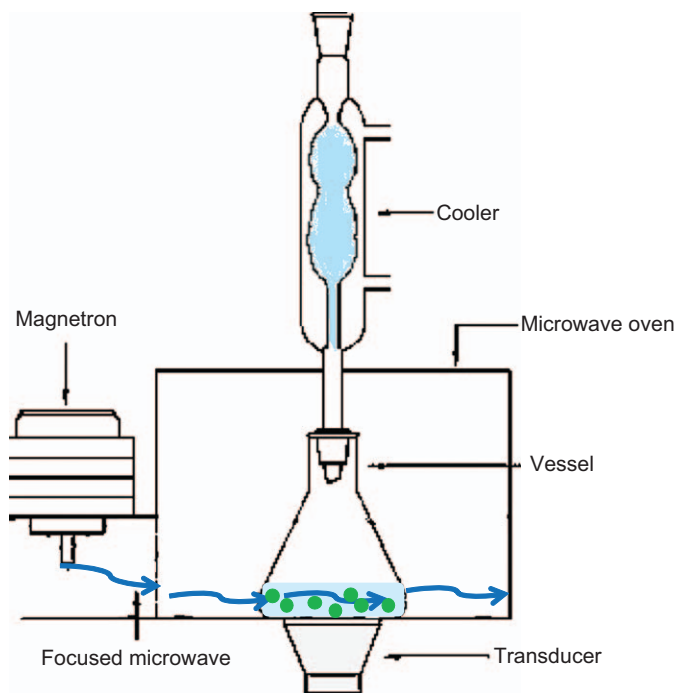


Figure 4.8 Ultrasound microwave-assisted extraction (UMAE) apparatus, reproduced with permission from Y. Chen *et al.*, *Int. J. Biol. Macromol.*, 2010, **46**, 429.

used to extract a variety of active compounds, such as lycopene from tomatoes,⁶⁴ vegetable oil,⁶⁵ and polysaccharides⁶³ from various plants. In a comparison study between UMAE and the conventional methods, the extraction of polysaccharides of *Inonotus obliquus* under optimal conditions of UMAE increased the yield from 2.12% to 3.25% and the purity was 73.16% compared to 64.03% previously recorded by the traditional hot water extraction. In the extraction of lycopene from tomatoes in ethyl acetate, optimal conditions for UMAE were 98 W microwave power together with 40 KHz ultrasonic processing, S/F of solvent to tomato paste was 10.6:1 (v/w). Extraction was carried out at 86.4 °C. The extraction time of UMAE was 6 min with 97.4% yield as compared to 29 min and 89.4% yield by ultrasonic-assisted extraction (UAE).

4.5.2.3 Microwave Hydro-distillation (MWHD or MAHD)

In microwave hydro-distillation (MWHD) presented on Figure 4.9, the hydro-distillation (HD) apparatus is placed inside a microwave oven with a side orifice through which an external cooler joins the vessel containing the plant material and water, inside the oven. The oven is operated at full power, which causes water to boil vigorously and reflux. Essential oil is decanted from the condensate.

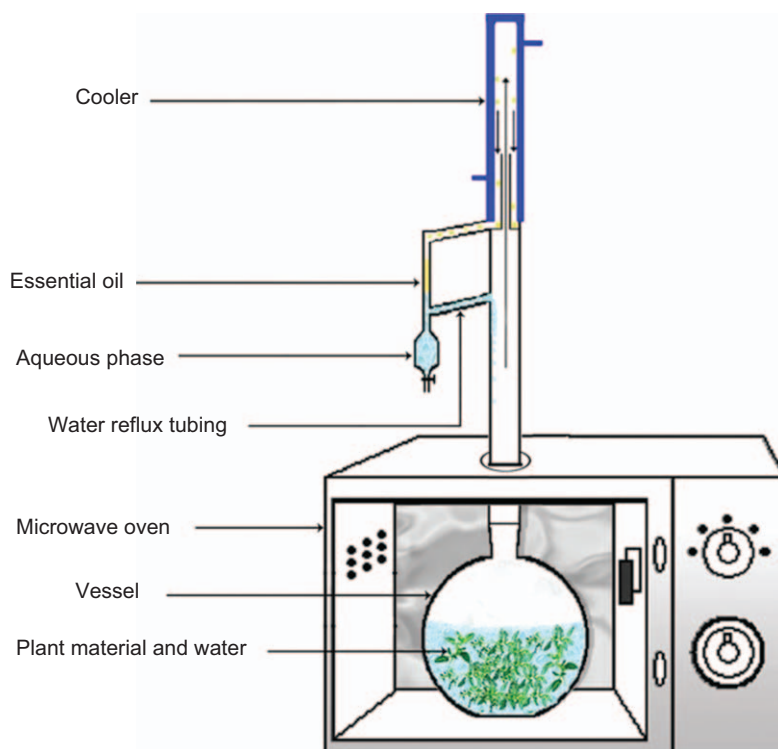


Figure 4.9 Microwave hydro-distillation (MWHD) apparatus, reproduced with permission from M.-T. Golmakani and K. Rezaei, *Food Chem.*, 2008, **109**, 925.

Essential oils extracted from Colombian *Xylopia aromatic* fruits⁶⁶ and from *Lippia alba* leaves⁶⁷ by HD and MWHD were similar in their composition. Almost the same number of components at concentrations above 0.01% was found in the HD essential oils (2 h extraction) and the MWHD essential oils (30 min extraction), with very similar yields.

MWHD of essential oil from the aerial parts (tops) of *Thymus vulgaris* L. was also studied.⁶⁸ The plant material (60 g) was extracted with 1200 mL of water at 990 W for 2 h. Extraction with MWHD started at a much earlier time than with HD (7 min versus 30 min, respectively). This is due to the more efficient heat flow caused by microwaves. The images from the surfaces of thyme leaves obtained by a scanning electron micrograph (SEM) before and after the extraction showed MWHD destroyed the glands in 30 min. This confirms that microwaves penetrate the water inside the plant matrix and cause the glandular walls to rupture more rapidly and more efficiently.

Volatile constituents from the leaves and stems of *Schefflera heptaphylla* L. Frodin were extracted by MWHD and HD.⁶⁹ MWHD was more advantageous than HD in terms of energy saving and extraction time (60 min versus 180 min for MHHD versus HD, respectively). Oil yield was affected by the extraction method and seasonal changes. It ranged from 0.11% to 0.27%, with the maximum amount of oil extracted from the leaves using MWHD in winter and the minimum from the stem oil extraction using HD in spring.

4.5.2.4 Microwave Steam Distillation (MSD)

Microwave steam distillation (MSD)^{70,71} is an improvement of the microwave accelerated steam distillation (MASD).⁷² In the MASD approach the plant material is packed above water separated by a Teflon grid inside the microwave cavity. At the bottom, steam is produced by heating water directly with microwave irradiation. Steam produced in the lower part of the apparatus passes through the vegetal matrix, evaporating and carrying the essential oil towards the cooler on the top of the microwave oven. The problem of this system is that all the microwave energy is absorbed by water to heat and vaporize it and only a small amount is absorbed by the essential oils inside the vegetal sample.

In the MSD approach, depicted in Figure 4.10, an electrical steam generator and a cooler placed outside the microwave oven are connected to a cartridge containing the plant material inside the microwave oven. Therefore, only the plant matrix is submitted to the microwave irradiation, resulting in 'hot spots' by selective heating, since the essential oil inside plants has a significantly higher dielectric loss than the surrounding steam. The plant sample is subjected to microwave heating as soon as the vapor starts to cross it.

Scanning electron microscopy shows that, in the case of microwave heating, the glandular trichomes are subjected to more severe thermal stresses and localized high pressures. This overpressure distends the plant cells causing their rupture. The steam flows through the sample, evaporating and carrying the essential oil. The extraction can be continued until no more essential oil is obtained.

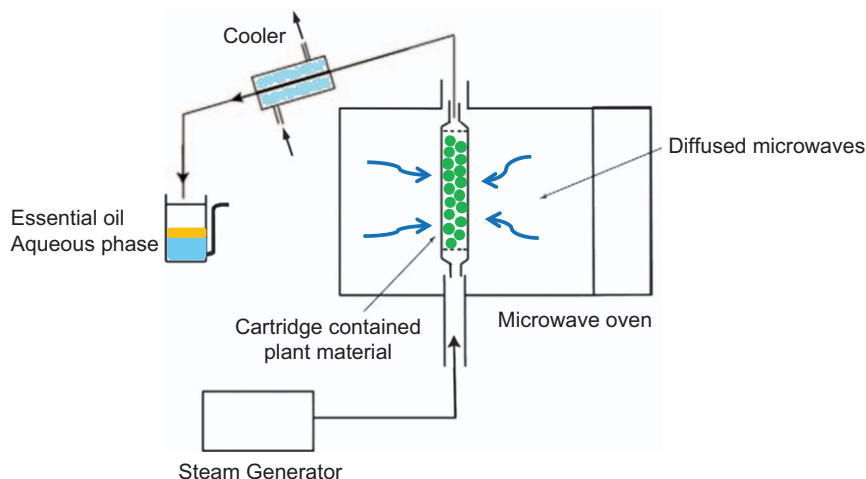


Figure 4.10 Microwave steam (MSD) distillation apparatus, reproduced with permission from N. Sahraoui *et al.*, *J. Chromatogr. A*, 2008, **1210**, 229.

For 20 g of dry lavender flowers, steam flow rate (8 g/min), microwave irradiation (200 W), and time (6 min) were optimized to ensure the complete extraction of essential oil avoiding the loss of volatile compounds with minimum extraction time. Extraction with MSD provided yields and essential oil composition comparable to those obtained after 30 min by conventional steam distillation (SD).⁷⁰ MSD was also applied for the extraction of essential oil from orange peels in 6 min. The temperature measured inside the irradiated sample was 105 °C. Microwave heating of *in situ* water within the orange peels caused a sort of microwave superheating phenomena which facilitated the distension of the plant cells and led to release of the essential oil quickly.²⁵

Microwave steam diffusion (MSDf), where a mixture of hot crude juice and steam moves naturally downwards by Earth's gravity into a spiral condenser outside the microwave cavity, was used for orange essential oil extraction. Compared to conventional steam diffusion (SDf), the MSDf process accelerated the extraction rate (12 min versus 40 min) by a rapid increase of temperature, without changing the volatile composition. This rapid extraction could be due to a synergy combination of the two transfer phenomena, mass and heat, acting in the same way.⁷³

4.5.3 Green Extraction without Solvent

Classical MAE is considered as a green extraction technique because it saves time, solvent, and energy. However, to go further in the development of a green process, MAE is the only technique able to perform extraction of natural compounds from fresh or moistened plant matrices without adding solvent or water. Indeed the extraction can be performed using only the constitutive

(or *in situ*) water of the plant matrix as the extraction solvent. The first developments in this way were designed for essential oil extraction but today other compounds soluble in water can also be extracted without solvent.

4.5.3.1 Solvent-free Microwave Extraction (SFME)

Solvent-free microwave extraction (SFME) is an original combination of microwave heating and dry distillation at atmospheric pressure for obtaining essential oil.^{19,44,74} This method, presented in Figure 4.11, involves placing fresh or rehydrated plant material in a microwave reactor, without any added solvent or water.

The internal heating of the *in situ* water within the plant material distends the plant cells and leads to rupture of the glands and oleiferous receptacles. This process frees essential oil which is evaporated by azeotropic distillation with the *in situ* water of the plant material. A cooling system outside the microwave oven condenses the distillate continuously, which is then collected in a flask. The excess water is refluxed to the extraction vessel in order to restore the *in situ* water to the plant material and to provide uniform conditions of temperature

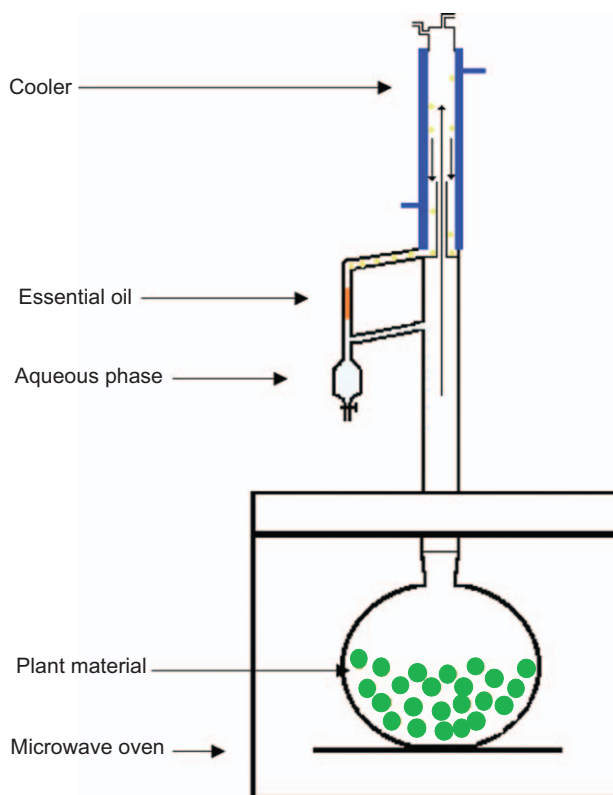


Figure 4.11 Solvent-free microwave extraction (SFME) apparatus, reproduced with permission from M. E. Lucchesi *et al.*, *J. Food Eng.*, 2007, **79**, 1079.

and humidity for extraction. The extraction can be continued at 100 °C until no more essential oil is obtained. The isolation and concentration of essential oil is performed in a single stage. Once the essential oil is extracted it can be analyzed directly without any preliminary clean-up or solvent exchange steps.

SFME was applied to extract essential oil from the aromatic herbs basil (*Ocimum basilicum* L.), garden mint (*Mentha crispa* L.) and thyme (*Thymus vulgaris* L.).⁴⁴ Fresh plant material (250 g) was heated using a fixed power of 500 W for 30 min. Substantially higher amounts of oxygenated compounds and lower amounts of monoterpene hydrocarbons were present in the essential oil extracted by SFME when compared to hydro-distillation (HD), leading to a more valuable essential oil since oxygenated compounds are highly odoriferous. This was probably due to a reduction in the thermal and hydrolytic degradation.

Essential oils of three species, ajowan (*Carum ojowan*), cumin (*Cuminum cyminum*), and star anise (*Illicium anisatum*) were extracted by SFME.⁷⁴ Dry seeds (250 g) were rehydrated prior to extraction by soaking in water for 1 h, then draining off the excess water. The moistened seeds were then placed in the reactor and submitted to microwave heating. SFME allowed a substantial saving of extraction time (1 h versus 8 h for HD) and of evaporation time to dry the extracted essential oil (1 h versus 8 h for HD), thus saving energy. Domination of the oxygenated compounds was also observed.

SFME of the essential oil from oregano (*Origanum vulgare* L.) rehydrated leaf was performed.⁴⁶ Extraction yield increased with irradiation power from 249 W to 622 W and with lower extraction time at higher power (35 min at 622 W, 50 min at 249 W). A general trend was observed of variation of the amount of monoterpene hydrocarbons and sesquiterpenes with extraction time. More volatile monoterpene hydrocarbons were preferentially extracted in the earlier part of the process, whereas higher molecular weight compounds were extracted later. The extraction of oxygenated compounds was very rapid.

In the case of essential oil extraction of *Calamintha nepeta* L., SFME gave a higher content of thermolabile compounds, such as chrysantenone or isomenthone, whereas HD gave higher content of more stable compounds, such as piperitone and menthone. These results suggest that HD favors the conversion or isomerization of compounds whereas SFME limits this type of reaction.⁷⁵

SFME extract from *Rosmarinus officinalis* L. showed stronger antibacterial activity than HD oil against *Staphylococcus aureus* and *Escherichia coli*. The lowest minimum bactericidal concentration (BMC) value was observed from SFME oil against *S. aureus* while the same values were observed for *Bacillus cereus*, *E. coli*, and *Klebsiella pneumoniae*. This enhancing of antibacterial activity in case of SFME could be attributed to the higher content of oxygenated compounds that have been proved to possess strong antibacterial and antifungal activities.⁷⁶

Developed to extract essential oil from fresh or rehydrated plant material, SFME can also be performed directly on a dry sample if a microwave absorption medium (MAM) is mixed with the sample inside the reactor. Three

types of MAM: iron carbonyl powder, graphite powder, and activated carbon powder, were studied. These materials have good microwave absorption capacity and good chemical stability. Thus SFME was applied to the extraction of essential oil from spices *Cuminum cyminum* L., *Zanthoxylum bungeanum* Maxim.,⁴⁷ *Illicium verum* Hook. f. and *Zingiber officinale* Rosc.,⁷⁷ from menthol mint, and from orange peel.⁷⁸ Dry sample (100 g) and 20 g of iron carbonyl powder were put in the reactor. Speed of rotation of 200 rpm enabled the sample and the iron carbonyl powder to mix in the reactor. Extraction was performed during 30 min at 100 °C with an irradiation power of only 85 W.

4.5.3.2 Vacuum Microwave Hydro-distillation (VMHD)

Vacuum microwave hydro-distillation (VMHD) was patented in 1994.^{79–81} In this method fresh or moistened plant matrix placed in the reactor without any solvent or water is subjected to microwave radiation. The microwave radiation is effective to evaporate the water contained in the plant material and to split the cellular structure of the plant sample, which leads to the release of natural compounds. Reduced pressure is intermittently applied within the reactor during the application of microwave radiation to further split up the cellular structure of the plant matrix induced by the application of the microwave radiation. Heating the vessel during a portion of the microwave radiation application compensates for the drop in temperature resulting from evaporation of residual plant material water. Microwave radiation, intermittently applying reduced pressure and heating the reactor causes the hydro-distillation of the natural extract by conveying natural compounds into the steam coming from the biological material as an azeotropic mixture and separating the residual plant matrix from the extract. A part of water resulting from the extract decantation step is injected into the reactor to carry out the hydro-distillation of the natural product remaining in the residue of plant matrix. Thanks to the reduced pressure, extraction can be performed at temperatures lower than 100 °C.

VMHD was applied to extraction of 15% of dry matter peppermint (*Mentha piperita* L.). Extraction was performed at about 70 °C during 15 min with a power put out by the microwave generation at 1150 W. During the extraction, the pump was used to lower the pressure. After only 15 min of extraction and three cycles of pressure reduction, 1.52 mL of essential oil was recovered (giving a yield of 1.01 mL per 100 g of dry matter) whose gas-chromatography profile is similar to that obtained by hydro-distillation for a longer time.

Rather similar conditions were used to extract essential oil from 500 g of garden sage (*Salvia officinalis* L.) having 25% of dry matter at about 70 °C. The power put out by the microwave generation was 1000 W. After 10 min of extraction and two cycles of pressure reduction, 3.06 mL of essential oil was recovered (giving a yield of 2.55 mL per 100 g of dry matter). The theoretical yield by standard hydro-distillation from the same quantity of sage is 2.77 mL/100 g, which shows that VMHD enabled the recovery of more than 90% of the essential oil of the garden sage treated.

4.5.3.3 Microwave Hydro-diffusion and Gravity (MHG)

Microwave hydro-diffusion and gravity (MHG) is a combination of microwaves for hydro-diffusion of essential oils from the inside to the exterior of biological material and Earth's gravity to collect and separate the essential oil.^{82–84} This method, presented in Figure 4.12, involves placing fresh plant material in a microwave reactor at atmospheric pressure, without any added solvent or water.

As mentioned above for SFME, the internal heating of the *in situ* water frees essential oil trapped inside the cells of plant tissues. Thanks to the hydro-diffusion phenomenon, the essential oil diffuses from the inside to the outside of the plant material. The acceleration of extraction rates under microwaves could be due to a synergy combination of the two mass and heat transfer phenomena acting in the same direction (from the hot sample to the colder environment), which are conventionally in opposite of conventional hydro-distillation. Then, the mixture of hot 'crude juice' and steam of *in situ* water moves naturally downwards by Earth's gravity out of the microwave reactor and falls through the perforated Pyrex disc. A cooling system outside the microwave oven cools and condenses the extract continuously. Water and essential oil are collected and separated in the decanter, where essential oil forms a film on the surface of the water, which is skimmed off the top. This green method allows extracting essential oils without distillation and evaporation, which are the most energy-

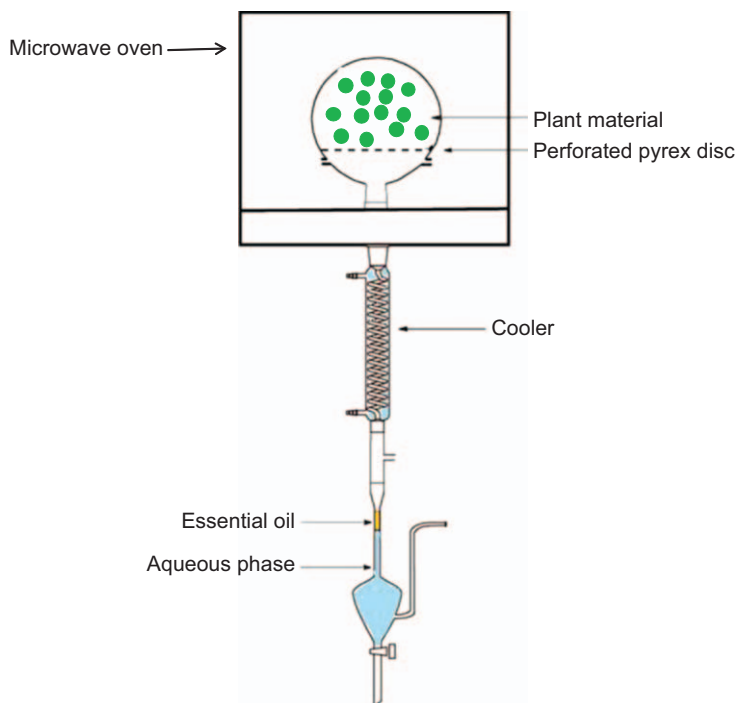


Figure 4.12 Microwave hydro-diffusion gravity (MHG) apparatus, reproduced with permission from N. Bousbia *et al.*, *Food Chem.*, 2009, **114**, 355.

consuming processes among the unit operations. The essential oil produced is in concentrated form, free from any solvent or contaminant, and can be analyzed or used directly without any clean-up, solvent exchange, or centrifugation steps.

MHG was first applied to the extraction of essential oils of *Mentha spicata* L. and *Mentha pulegium* L.⁸² An appropriate microwave irradiation power is important to ensure the essential oil is extracted quickly. However, the power should not be too high; otherwise loss of volatile compounds will result. Extracts obtained by MHG and HD were rather similar in their composition. The same number of volatile secondary metabolites was found in both essential oils with similar yields. The reduced cost of extraction is clearly advantageous for the proposed MHG method in terms of time (15 min versus 90 min), and, therefore, in terms of energy.

MHG extraction of essential oil from fresh citrus peels was performed in 15 min.⁸³ Compared to HD or cold pressing (CP), MHG gave similar extract composition and extraction yield. For MHG and CP, the extracted essential oils presented the same odor of terpene hydrocarbons with fresh, light, flora, woody, and sweet citrusy odor. For HD, the essential oil presented the odor of terpene hydrocarbons – fresh, pungent, but different from fresh fruit, and with a persistent boiled odor. The MHG method offers the possibility for a better reproduction of natural aroma of the fruit essential oil comparable to CP and better than the hydro-distilled essential oil. The same results were observed for extraction of essential oil from rosemary leaves. Moreover, the MHG extract presented higher antioxidant and antimicrobial activities than the HD extract.⁸⁴

MHG was then used for the extraction of non-volatile compounds such as flavonoids (quercetin, kaempferol, myricetin, isorhamnetin, and their glycoside derivatives) from onion bulbs⁸⁵ and from sea buckthorn by-products.⁸⁶ Fruit juices from fresh and frozen plums, grapes, and apricots were also produced by MHG. They were characterized by very bright color, high viscosity, high acidity for plums and apricot, and the flavor of fresh fruit. Yields obtained with MHG were lower than commercial levels but the reduced cost of extraction is clearly advantageous for the MHG method.⁸⁷ Furthermore, the MHG procedure allows one to get a filtrated extract.

The vacuum microwave hydro-diffusion and gravity technique (VMHG) was applied for extraction of flavonoids from onions. Compared to MHG extraction, VMHG can be performed at lower temperatures. During the extraction phase the temperature was 87 °C in the central part of the onion and 81 °C in the reactor for VMHD instead of 100 °C for both onion and reactor for MHG. The extract obtained by VMHG was richer in flavonoid compounds, and thus was more antioxidant. VMHG is an effective method for extraction of heat-sensitive and oxygen-sensitive compounds in the absence of any solvent or water.⁸⁸

Solvent-free microwave extraction and all the techniques developed based on this principle have great advantages. Extractions are rapid, efficient, can produce more valuable essential oil, and can be applied for recovery of both volatile and non-volatile compounds. Moreover, they do not need any solvent and the obtained extract is concentrated and ready to use for further analysis or application.

Table 4.3 gathers the extraction method and the optimum conditions used for the applications cited above, ordered according to the method used.

Table 4.3 Extraction methods, solvent, and optimal conditions used to extract some natural compounds from vegetal matrices
S/F = solvent to feed ratio, M = mass of vegetal sample, P = power, t = time, T = temperature, DV = degree of vacuum,
UF = ultrasonic frequency, UP = ultrasonic power, VF = vapor flow.

Compounds	Plant samples	Extraction techniques	Solvents	Conditions, S/F or M, P, t, T	Ref.
flavonoids	<i>Hippophae Rhamnoides</i>	FMAE	EtOH	10 mL/g, 150 W, 20 min, 60 °C	35
flavonoids	<i>Radix astragali</i>	MAE	90 % EtOH	25 mL/g, 1000 W, 25 min, 2 cycles, 110 °C	29
isoflavone	soybean	MAE	50 % EtOH	50 mL/g, 500 W, 20 min, 50 °C	28
flavones, coumarins	<i>Lysionotus zauciflorus</i> , <i>Cortex fraxini</i>	MAE	PEG	20 mL/g, 10 min, 65 °C	43
anthraquinone	<i>Morinda citrifolia</i> root	MAE	80 % EtOH	100 mL/g, 720 W, 30 min, 60 °C	53
withanolides	<i>Iochroma</i> , <i>gesnerioides</i> leaves	FMAE	MeOH + H ₂ O to moisten dry sample	50 mL/g, 25 W, 40 s	50
scutellarin	<i>Erigeron breviscapus</i>	MAE	25 % EtOH	10 mL/g, 700 W, 40 min, 80 °C	15
oleanolic, ursolic acids	<i>Chaenomeles sinensis</i>	MAE	MeOH	32 mL/g, 600 W, 7 min, 52 °C	31
triterpenoids	olive leaves	MAE	80 % EtOH	8 mL/g, 180 W, 5 min	54
carotenoids	paprika	MAE	50 % acetone	50 mL/g, 50 W, 120 s, 60 °C	36
triterpenoid saponins	<i>Ganoderma atrum</i>	MAE	95 % EtOH	25 mL/g, 800 W, 5 min, 90 °C	49
piperine	<i>Piper nigrum</i>	MAE	petroleum ether	20 mL/g, 450 W, 2 min	37
glycyrrhizic acid	<i>Glycyrrhizae radix</i> root	MAE	50–60 % EtOH, 1–2% ammonia	10 mL/g, 4–5 min, 50–60 °C	56
glycyrrhizic acid, liquiritin	<i>Glycyrrhizae radix</i> root	FMAE	Triton X 100	25 mL/g, 5 min, 100 °C	42
essential oil, lignans	<i>Schisandra chinensis</i> fruits	ILMAE	0.25 M [C ₁₂ mim]Br	12 mL/g, 385 W, 40 min	38
essential oil, carnosic acid, rosmarinic acid	<i>Rosmarinus officinalis</i>	ILMAE	1.0 M [C ₈ mim]Br	12 mL/g, 700 W, 15 min	39
polyphenols	<i>Psidium guajava</i> leaves, <i>Smilax china</i>	ILMAE	2.5 M [bmim]Cl	20 mg/L, 10 min, 60–70 °C	40
lichens	<i>Pertusaria pseudocorallina</i>	ILMAE	[C ₁ mim][MSO ₄], [C ₂ mim][ESO ₄]	20 mL/g, 5 min, 100 °C	41

Table 4.3 (Continued)

Compounds	Plant samples	Extraction techniques	Solvents	Conditions, S/F or M, P, t, T	Ref.
ascorbic acid	guava and peppers	NPMAE	0.25 % metaphosphoric acid	10 mL/g, 400 W, 10 min, DV = 90 kPa	57
resveratrol, emodin	<i>Rhizoma Polygoni Cuspidati</i>	VMAE	MeOH	6 mL/g, 50 °C, 15 min, DV = 50 kPa	58
myricetin, quercetin	<i>Myrica rubra</i> leaves	VMAE	95 % EtOH	6 mL/g, 70 °C, 20 min, DV = 40 kPa	58
safflomin A	<i>Flos Carthami</i>	VMAE	50 % EtOH	6 mL/g, 50 °C, 10 min, DV = 60 kPa	58
ascorbic acid	guava, green pepper	VMAE	1 M acetic acid solution	8 mL/g, 70–80 °C, 2 min, DV = 40 kPa	59
α -tocopherol, δ -tocopherol	soybean, tea leaves	VMAE	EtOH	8 mL/g, 50–80 °C, 10–20 min, DV = 40 kPa	59
ascorbic acid	pepper	VMAE	1 M acetic acid	10 mL/g, 500 W, 10 min, 25 °C, DV = 50 kPa	60
β -carotene	carrot, spinach	VMAE	acetone/EtOH (1/2)	12 mL/g, 500 W, 20 min, 25 °C, DV = 40 kPa	60
astaxanthin	shrimp	VMAE	EtOH	500 W, 15 min, 45 °C, DV = 0 kPa	60
aloin a	aloe	VMAE	H ₂ O	20 mL/g, 500 W, 15 min, 35 °C, DV = 20 kPa	60
lipids	olive seed	MIS	n-hexane, humidity level 55 %	10 mL/g, 720 W, 32 min	62
polysaccharides	<i>Inonotus obliquus</i>	UMAE	H ₂ O	20 mL/g, 90 W, 19 min, UP = 50 W, UF = 40 kHz	63
lycopene	tomatoes	UMAE	ethyl acetate, humidity level 78.86%	10.6 mL/g, 98 W, 6 min, 86.4 °C, UF = 40 KHz	64
vegetable oil	soybean germs	UMAE	hexane	5 mL/g, 100 W, 1 h, 45 °C, UF = 21 kHz	65
essential oils	<i>Xylopiia aromatica</i> fruits	MWHD	H ₂ O	20 mL/g, 800 W, 30 min	68
essential oils	<i>Lippia alba</i> leaves	MWHD	H ₂ O	10 mL/g, 800 W, 30 min	67
essential oils	<i>Thymus vulgaris</i>	MAHD	H ₂ O	20 mL/g, 990 W, 2 h	68
volatile oil	<i>Schefflera heptaphylla</i> , leaves and stems	MWHD	H ₂ O	200 g, 800 W, 60 min	69
essential oils	<i>Lavandula angustifolia</i> flowers	MSD	water steam	20 g, 200 W, 6 min	70
essential oils	<i>Citrus sinensis</i> peels	MSD	water steam, humidity level 75 %	100 g, 500 W, 6 min, VF = 14 g/min	25

essential oil	<i>Citrus sinensis</i> peels	MSDf	water steam, humidity level 90 %	250 g, 200 W, 12 min, 100 °C, VF = 25 g/min	73
essential oil	<i>Carum ojowan</i> , <i>Cuminum cyminum</i> , <i>Illicium anisatum</i>	SFME	H ₂ O to moisten dry sample	250 g, 1 h, 100 °C	74
essential oil	<i>Ocimum basilicum</i> , <i>Mentha crispa</i> , <i>Thymus vulgaris</i>	SFME	humidity level: 90 %, humidity level 95 %, humidity level 80 %	250 g, 500 W, 30 min, 100 °C	44
essential oil	<i>Elletaria cardamomum</i> seed	SFME	humidity level 67 %	100 g, 390 W, 75 min	19
essential oil	<i>Citrus sinensis</i>	SFME	humidity level 90 %	200 g, 200 W, 30 min, 100 °C	45
essential oil	<i>Origanum vulgare</i>	SFME	H ₂ O to moisten dry sample	50 g, 622 W, 35 min	46
essential oil	<i>Calamintha nepeta</i> aerial parts	SFME		60 g, 250 W, 40 min, 100 °C	75
essential oil	<i>Rosmarinus officinalis</i> leaves	SFME		250 g, 40 min	76
essential oil	<i>Cuminum cyminum</i> , <i>Zanthoxylum bungeanum</i>	SFME	humidity level 6.90 %, humidity level 10.21 %	100 g, 30 min, 100 °C, 85 W	47
essential oil	<i>Illicium verum</i> , <i>Zingiber officinale</i>	SFME	humidity level 8.26 %, humidity level 9.94 %	100 g, 85 W, 30 min, 100 °C	77
essential oil	menthol mint, orange peel	SFME	humidity level 10.23 % and 8.17 %	100 g, 85 W, 30 min, 100 °C	78
essential oil	<i>Mentha piperita</i>	VMHD	humidity level 85 %	1150 W ,15 min, 70 °C	79–81
essential oil	<i>Salvia officinalis</i>	VMHD	humidity level 75 %	500 g, 1000 W, 10 min, 70 °C	79–81
essential oil	<i>Mentha spicata</i> , <i>Mentha pulegium</i>	MHG		500 g, 500 W, 15 min	82
essential oil	citrus peels	MHG		500 g, 500 W, 15 min	83
essential oil	<i>Rosmarinus officinalis</i> leaves	MHG	humidity level 60.2 %	500 g, 500 W, 15 min	84
flavonoids	onion bulbs	MHG	humidity level 88.5 %	500 g, 500 W, 27.5 min	85
flavonoids	<i>Hippophaë rhamnoides</i> by-products	MHG	humidity level 57 %	400 g, 400 W, 15 min, 100 °C	86
flavonoids	onions	VMHG	humidity level 84.5 %	500 g, 500 W, 81 °C, DV = 70 kPa	88
flavonoids	<i>Hippophaë rhamnoides</i>	PSFME	humidity level 72 %	4 g, 50 s, 5 cycles, 1000 W, 180 °C	89

This table is not exhaustive and numerous other natural compound extractions from vegetal matrices performed using microwave irradiation can be found in literature.

4.6 Case Study

As solvent-free extraction is a feature of microwave-assisted extraction due to the selective microwave heating of free water inside the plant material, the last part of this chapter details the development and the optimization by experimental design of a solvent-free microwave extraction performed in a closed vessel (PSFME).⁸⁹ This method was applied for the extraction of non-volatile phenolic compounds from sea buckthorn (*Hippophaë rhamnoides* L.) berries. This study shows the influence of the parameters described in Section 4.4, such as irradiation time, cycle number, and power on extraction efficiency. Higher antioxidant activity and richest extraction composition were obtained for PSFME extracts compared to extracts obtained by conventional extraction methods. This example allows highlighting the performance of microwave extraction.

4.6.1 Optimization of the Pressurized Solvent-free Microwave Extraction (PSFME) Procedure

Fresh whole berries (4 g) with humidity level of 72% were introduced without pre-treatment in a 50 mL closed vessel dedicated to microwave extraction without addition of any solvent or water. A schematic extraction procedure is presented in Figure 4.13.

The optimization of the extraction method was achieved by using a two-level full factorial design (−1, 0, +1) to evaluate the most significant parameters on the extraction yield, antioxidant activity, and richness in phenolic compound composition. According to the parameters influencing microwave extraction described above, the three variables chosen were: time of microwave irradiation (t), microwave irradiation power (P), and number of extraction cycles (C). The effect of each factor and of their first-order interaction was tested (tP , tC , CP). As microwave power controls sample heating and thus the extraction temperature, it was decided to cover all the power range of the microwave apparatus (200 W to 1000 W). The number of extraction cycles was limited to 5 cycles maximum in order to get a reasonable total extraction time. Between each cycle the vessel was cooled down in ice to room temperature; this step is quite long (several minutes) and increases the total extraction time. Irradiation time range (10–50 s) was adjusted according to the temperature and pressure increasing inside the reactor. A recorded high temperature (up to 180 °C) and simultaneously an increased pressure inside the reactor occurred at the end of a 50 s extraction cycle with a 1000 W irradiation power. Higher pressure inside the reactor leads to the opening of the safety vessel membrane and consequently to the loss of material. In order to prevent this loss due to either temperature degradation or overpressure, the irradiation time was limited to 50 s.

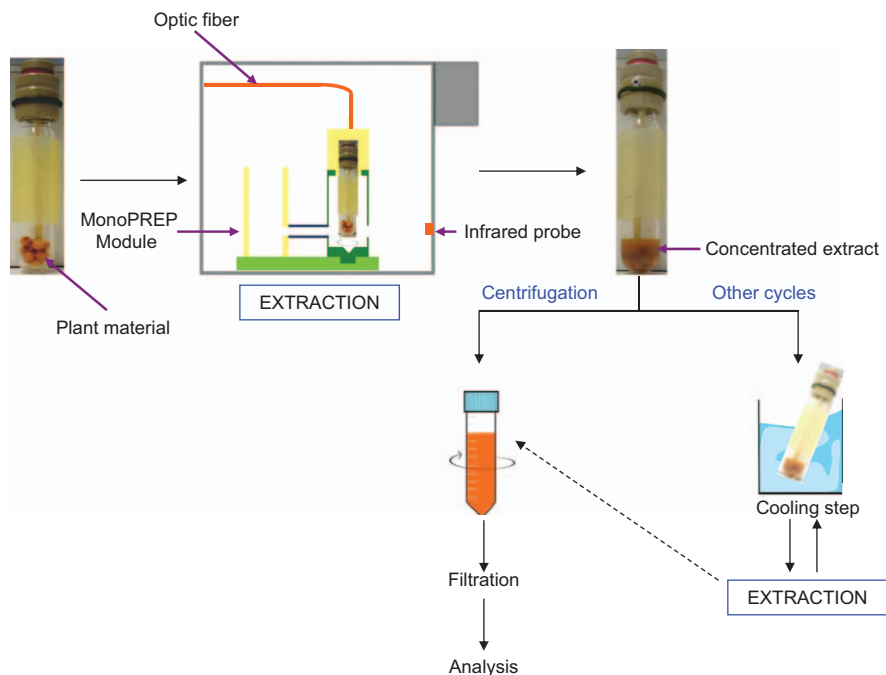


Figure 4.13 Schematic pressurized solvent-free microwave extraction (PSFME) process.

Each extract was tested in terms of bioactivity including *in vitro* antioxidant tests (DPPH and FRAP) and the estimation of phenolic compounds via the test of Folin-Ciocalteu (TPC). The influence of each factor and their first-order interactions were studied. The three parameters studied were significant at 5% and had an influence on the extraction process. Time of microwave irradiation (t) was the most influential parameter and the number of cycles (C) the least influential. The first-order interaction between t and P (tP) was the most significant for the three tests. Optimal conditions were found to be a 1000 W microwave power applied for 50 s and repeated for 5 cycles.

4.6.2 Influence of the Number of Cycles

As the number of cycles is the less influential factor without significant interaction but the most limiting factor for developing a rapid extraction method, this parameter and its influence on extraction was studied more carefully. From cycle 1 to cycle 3, extraction temperature and extraction yield increased strongly from 100 °C to 190 °C and from 4% to 9% respectively. From cycle 3 to cycle 5, temperature and extraction yield no longer changed. Moreover, the antioxidant activity measured with FRAP and DPPH assays followed the same behavior: the responses increased strongly from cycle 1 to cycle 3 and remained constant from cycle 3 to cycle 5. At this stage, three cycles seemed to be enough

to achieve total extraction of phenolic compounds from sea buckthorn berries. But when looking at the TPC results, the TPC increased from cycle 1 to cycle 3 but also increased strongly from cycle 3 to cycle 5, indicating the extraction of more phenolic compounds with five cycles. Moreover, HPLC-UV fingerprint recorded on C18 Alltima column (150×4.6 mm ID, $5 \mu\text{m}$, Alltech, Deerfield, USA) with water and methanol both acidified with 1% acetic acid in elution gradient as mobile phase, showed at 279 nm and 366 nm a good similarity between cycle 1 and cycle 3 chromatograms with just an increase of peak intensity correlated to the increased extraction yield. The chromatogram of cycle 5 was different, with three new compounds appearing at the end of the chromatogram (Figure 4.14). These compounds were identified by HPLC mass spectrometry coupling as quercetin (1), isorhamnetin-7-*O*-rhamnoside (2), and isorhamnetin (3). These chromatographic results are in accordance with the

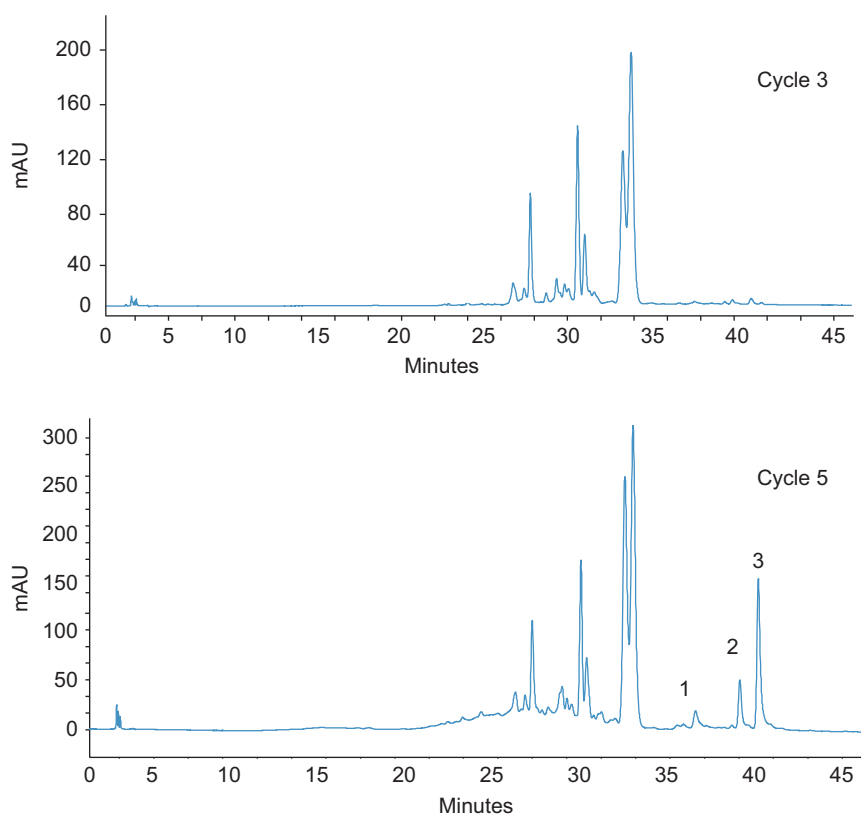


Figure 4.14 Chromatogram fingerprints at 366 nm of PSFME extract (a) after 3 extraction cycles and (b) after 5 extraction cycles. (1) quercetin, (2) isorhamnetin-7-*O*-rhamnoside, (3) isorhamnetin. C18 Alltima column (150×4.6 mm ID, $5 \mu\text{m}$), mobile phase water and methanol both acidified with 1% acetic acid, flow rate = 1 mL/min at room temperature.

TPC results, demonstrating the extraction of three new flavonoid compounds between cycle 3 and cycle 5.

Extraction by PSFME under the optimized conditions leads to high temperature up to 180 °C inside the reactor and up to 200 °C outside the reactor. Even if elevated temperatures enhance the molecule diffusivity resulting in increased extraction kinetics and extraction yield, thermolabile compounds may be degraded during elevated temperature extractions.

To control the stability of flavonoid compounds in these conditions, isorhamnetin-3-*O*-rutinoside and isorhamnetin-3-*O*-glucoside were submitted to 1000 W, 50 s and 5 cycles of irradiation. Concentration of compounds remained stable under irradiation; no degradation and no formation of aglycone compounds was observed.

4.6.3 Proposed Mechanism of PSFME

Figure 4.15 shows the modified structure of the sea buckthorn berries with the number of irradiation cycles.

The first three cycles led to a loss of integrity of berries and then the succession of cycles allowed this destruction to carry on, to reach cell micro-structure and consequently to solubilize compounds of interest. Microwaves are able to penetrate plant matrix and interact with polar molecules such as water. Thus *in situ* polar water of fresh plant material heated immediately above the boiling point, resulted in an internal heating of biomaterial, consequently causing a rapid increase of the pressure inside the plant cells. This pressure increase leads to a breakdown of cell walls and release of solutes (Figure 4.16). Repeated heating led to complete release of water with

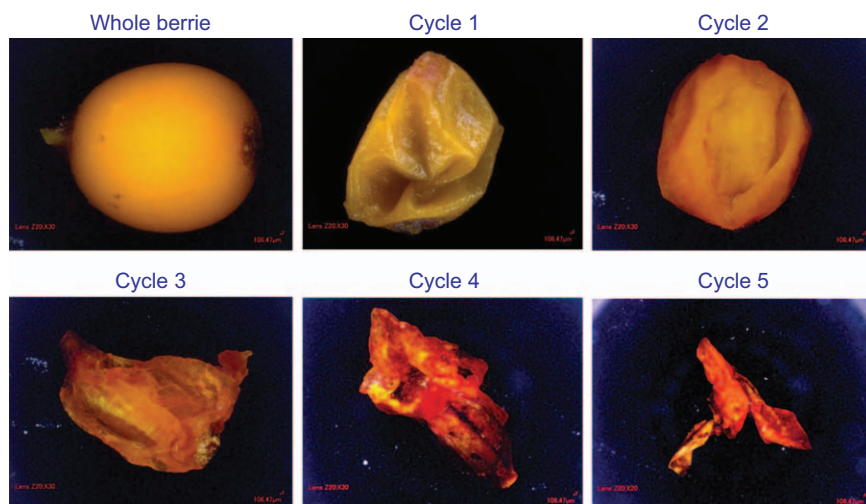


Figure 4.15 Optical microscopy picture of *H. rhamnoides* berries before and after each microwave irradiation cycle.

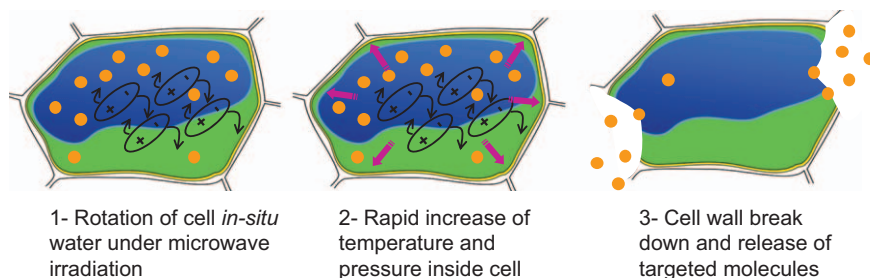


Figure 4.16 Schematic representation of PSFME mechanism.

Table 4.4 Comparison of extraction method for flavonoids extraction from sea buckthorn fresh berries (FV = flush volume)

Method	Vegetal mass	Solvent	Time	Cycle	Temperature	Specific conditions
PSFM	4 g	<i>in situ</i> water	50 s	5	180 °C	1000 W
PLE	4 g	114 mL water	5 min	5	100 °C	FV65%, purge 100 s
PLE	4 g	114 mL water	5 min		40 °C	FV65%, purge 100 s
maceration	4 g	5 mL water	10 min		25 °C	stirring
pressing	4 g	juice			25 °C	

compounds outside the plant matrix. At the end of the extraction procedure berries were destroyed and appeared completely dehydrated.

Moreover, under pressure (closed vessel) and at high temperature (around 180 °C for cycle 3 to cycle 5), polarity and viscosity of water were lower. These conditions allowed water to solubilize and to carry less polar compounds such as aglycone flavonols (generally non-soluble in water at atmospheric pressure and at room temperature) out off the plant matrix. Juice concentrated in compounds was collected, filtered, and was directly usable for further experiments without complementary evaporation step.

4.6.4 Comparison with other Extraction Methods

To evaluate the performances of PSFME, it was compared to pressurized liquid extraction (PLE) at 40 °C and 100 °C and to milder extraction techniques, such as pressing and maceration, free of risk in terms of thermolability. Operating conditions of these different extraction procedures are presented in Table 4.4.

Figure 4.17 depicts the characteristic chromatographic fingerprints obtained from the same amount of berry material extracted either at elevated temperature (PSFME, PLE 100 °C), or at ambient temperature (pressing and maceration).

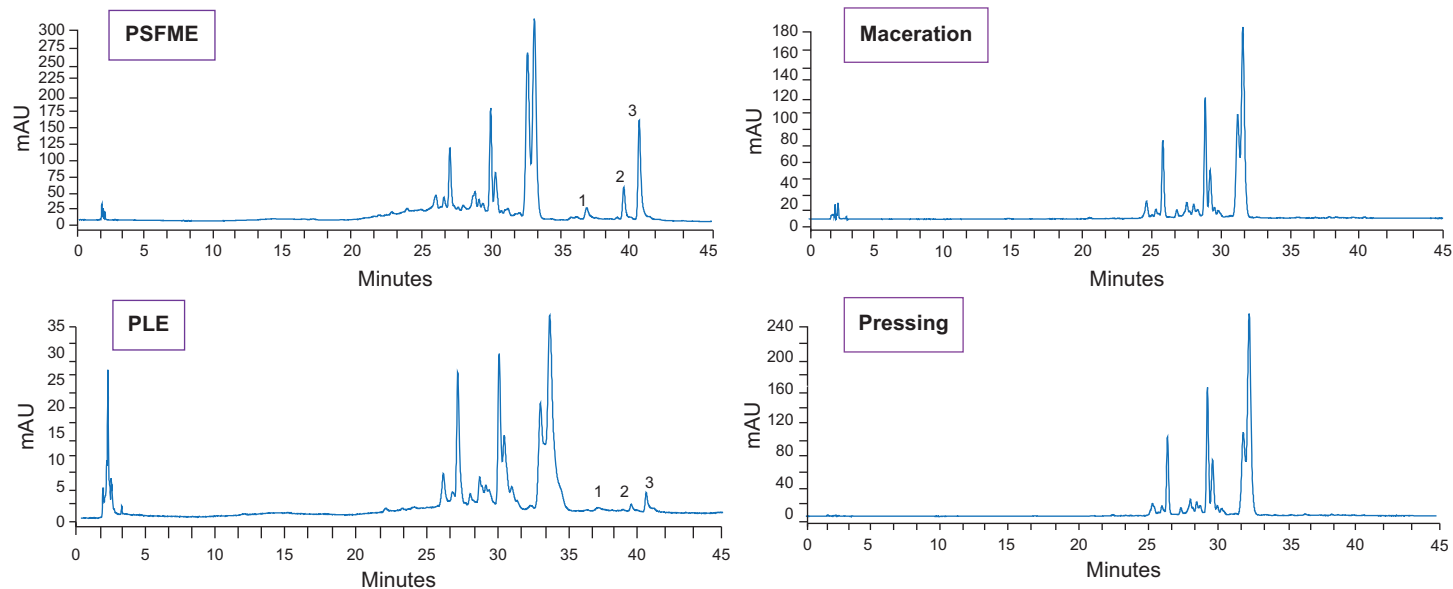


Figure 4.17 PSFME, maceration, PLE and pressing extracts of *H. rhamnoides* L. berries. (1) quercetin, (2) isorhamnetin-7-*O*-rhamnoside, (3) isorhamnetin. C18 Altima column (150 × 4.6 mm ID, 5 μm), mobile phase water and methanol both acidified with 1% acetic acid, flow rate = 1 mL/min at room temperature at 366 nm.

When berries were extracted by PLE, pressing, and maceration, a chromatographic profile was obtained similar to the one obtained by PSFME at least for peaks eluted between 25 min and 35 min. The profile similarity between techniques using low and high temperatures indicated that the heating did not induce evident breakdown of phenolic compounds. Only the chromatogram of PLE extraction at 100 °C for 35 min also showed the presence of the three last peaks corresponding to quercetin, isorhamnetin-7-*O*-rhamnoside, and isorhamnetin in the extract, but with a weaker abundance than in PSFME extract.

PSFME was the procedure which obtained significantly the maximum antioxidant activity according to DPPH and FRAP assays. The higher antioxidant activity observed for PSFME, pressing, and maceration extracts was due to the fact that berries were destroyed and the juice released during these three extraction procedures, in contrast to PLE extracts, where berries were not crushed at the end of the extraction.

4.6.5 Advantages of PSFME

The original PSFME mode, which combines matrix destruction, microwave irradiation, and rise of temperature, promotes molecule transfer from matrix to solvent and consequently a higher release of compounds and a better antioxidant activity. This method is easy to set up, and furthermore is time, energy, and solvent saving since it requires no solvent because the residual water of the berries is in sufficient amounts to be used as the extraction solvent. It can be used favorably in comparison to other more common extraction techniques to obtain berry extracts enriched in phenolic compounds and with a high antioxidant power. Moreover, among the extraction techniques tested it is the only one able to extract quercetin, isorhamnetin-7-*O*-rhamnoside, and isorhamnetin in a low amount of water. High temperature and high pressure of PSFME procedure lower water polarity and viscosity allowing solubilization of quite non-polar compounds such as aglycon flavonoids. Furthermore, the PSFME required no evaporation step after extraction and consequently allowed the simplification of the usually long sample preparation step. After filtration, the PSFME extract is directly usable for further experiments, and leads to obtain a more exhaustive description of the polar compound composition in berries.

4.7 Conclusion

MAE offers main advantages compared to conventional extraction methods such as Soxhlet and maceration. The first advantage comes from the volumetric heating of the sample, leading to a reduction in extraction time (from several seconds to 30 min) and to a reduction in solvent consumption (reduced by 5–10-fold compared to traditional extraction). Moreover, MAE shows evident

advantages with strong penetration force and high selectivity. Thus it is an efficient method giving an extraction yield similar or higher to conventional extraction methods for several compounds and plant matrices.

MAE depends on several parameters, such as power, extraction time, composition, and amount of solvent, plant material loading, etc. Thus, careful method optimization with regulation and control of parameters is essential to effectively recover the compounds of interest. This can appear in a first approach as a limitation of the use of microwave because this optimization process can be quite long, fastidious, besides being sample dependent. However, in fact, it allows the specificity, the selectivity, and the efficiency of MAE to reach levels compared to conventional methods. Hence, MAE can produce extracts of better quality (richer in some family of compounds, more antioxidant, more antibacterial, etc.) than those obtained by conventional methods. MAE allows full control of extraction parameters (time, power, and temperature), enhancing the reproducibility of extraction.

From a current context of green chemistry, MAE is an environmental and human friendly method. It reduces solvent consumption, solvent waste, energy, human exposure to solvent vapors, and hazards. The costs of the process are generally lower than those of conventional techniques. Moreover, MAE is the only technique that can be used without addition of any solvent or water. This is a further step in the green chemistry. Solvent-free microwave extraction provides a more valuable product containing higher amounts of oxygenated compounds. Some medium polar compounds can be solubilized in water under high temperature and pressure conditions.

For heat- or oxygen-sensitive compounds, extraction should be carried out under vacuum or under an inert atmosphere to reduce or prevent degradation.

Nevertheless, in some cases the efficiency of microwaves can be limited when either the target compounds or the solvent are non-polar. Microwave irradiation can sometimes accelerate chemical reactions and thus change the chemical structures of the target compounds; that might result in the reduction of extraction yield. Extraction in closed vessels performed at high temperature can require a cooling step before opening to avoid loss of solvent or volatile compounds. Clean up of extract with a filtration step is often needed.

Initially employed as a digestion method for different sample types such as environmental, biological, and geological matrices, MAE is now widely accepted for extracting natural products from plant materials. Most plant matrix extractions with or without solvent can be performed in a classical laboratory oven in open or closed vessels. Thus a large variety of plant secondary metabolites, volatile or not, can be efficiently extracted. Improved MAE methods (under vacuum, MSD, VMHD, MHG, etc.) need specific apparatus or homemade modified ovens. Presently, microwave-assisted extraction is successfully used in plant extraction at laboratory scale, but the number of industrial-scale utilizations remains too few.

List of Abbreviations

BMC	minimum bactericidal concentration
DPPH	2,2-diphenyl-1-picrylhydrazyl
DV	degree of vacuum
FMAE	focused microwave-assisted extraction
FMASE	focused microwave-assisted soxhlet extraction
FRAP	ferric reducing ability of plasma
FV	flush volume
HD	hydro-distillation
ILMAE	ionic liquid microwave-assisted extraction
ILs	ionic liquids
M	mass of vegetal
MAE	microwave-assisted extraction
MAM	microwave absorption medium
MASD	accelerated steam distillation
MHG	microwave hydro-diffusion and gravity
MIS	microwave-integrated soxhlet
MSD	microwave steam distillation
MSDf	microwave steam diffusion
MWHD or MAHD	microwave hydro-distillation
NPMAE	nitrogen-protected microwave-assisted extraction
P	power
PLE	pressurized liquid extraction
PMAE	pressurized microwave-assisted extraction
PSFME	pressurized solvent-free microwave extraction
S/F	solvent to feed ratio
SDf	steam diffusion
SFME	solvent-free microwave extraction
T	temperature
t	time
TPC	total phenolic content
UF	ultrasonic frequency
UMAE	ultrasonic microwave-assisted extraction
UP	ultrasonic power
VF	vapor flow
VMAE	vacuum microwave-assisted extraction
VMHD	vacuum microwave hydro-distillation
VMHG	vacuum microwave hydro-diffusion and gravity

References

1. A. Abu-Samra, J. S. Morris and S. R. Koirtyohann, *Anal. Chem.*, 1975, **47**, 1475.
2. K. Ganzler, A. Salgó and K. Valkó, *J. Chromatogr. A*, 1986, **371**, 299.

3. J. R. J. Pare, *U.S. Pat.*, Patent 5,458,897, October 17, 1995.
4. L. Wang and C. L. Weller, *Trends Food Sci. Technol.*, 2006, **17**, 300.
5. M. Letellier and H. Budzinski, *Analysis*, 1999, **27**, 259.
6. C. Sparr Eskilsson and E. Björklund, *J. Chromatogr. A*, 2000, **902**, 227.
7. K. Ganzler, I. Szinai and A. Salgó, *J. Chromatogr. A*, 1990, **520**, 257.
8. V. Camel, *Trends Anal. Chem.*, 2000, **19**, 229.
9. B. Kaufmann and P. Christen, *Phytochem. Anal.*, 2002, **13**, 105.
10. H. M. Kingston and L. B. Jassie (eds.), *Introduction to Microwave Sample Preparation*, American Chemical Society, Washington, DC, 1988.
11. V. Camel, *Analyst*, 2001, **126**, 1182.
12. D. R. Baghurst and D. M. P. Mingos, *J. Chem. Soc., Chem. Commun.*, 1992, 674.
13. D. A. J. Starmans and H. H. Nijhuis, *Trends Food Sci. Technol.*, 1996, **7**, 191.
14. M. A. Rostagno, A. Villares, E. Guillamón, A. García-Lafuente and J. A. Martínez, *J. Chromatogr. A*, 2009, **1216**, 2.
15. M. Gao, W. Huang, M. RoyChowdhury and C. Liu, *Anal. Chim. Acta*, 2007, **591**, 161.
16. H.-Y. Zhou and C.-Z. Liu, *J. Chromatogr. A*, 2006, **1129**, 135.
17. H.-F. Zhang, X.-H. Yang and Y. Wang, *Trends Food Sci. Technol.*, 2011, **22**, 672.
18. L. Zhongdong, W. Guohua, G. Yunchang and J. F. Kennedy, *Carbohydr. Polym.*, 2006, **64**, 548.
19. M. E. Lucchesi, J. Smadja, S. Bradshaw, W. Louw and F. Chemat, *J. Food Eng.*, 2007, **79**, 1079.
20. M. Kratchanova, E. Pavlova and I. Panchev, *Carbohydr. Polym.*, 2004, **56**, 181.
21. L. G. Croteau, M. H. Akhtar, J. M. R. Bélanger and J. R. Jocelyn Paré, *J. Liq. Chromatogr.*, 1994, **17**, 2971.
22. J. C. Young, *J. Agric. Food. Chem.*, 1995, **43**, 2904.
23. C.-H. Chan, R. Yusoff, G.-C. Ngoh and F. W.-L. Kung, *J. Chromatogr. A*, 2011, **1218**, 6213.
24. W. Routray and V. Orsat, *Food Bioprocess Technol.*, 2012, **5**, 409.
25. N. Sahraoui, M. A. Vian, M. El Maataoui, C. Boutekedjiret and F. Chemat, *Innovative Food Sci. Emerging Technol.*, 2011, **12**, 163.
26. M. Zill-e-Huma, J. F. Abert Vian, Maingonnat and F. Chemat, *J. Chromatogr. A*, 2009, **1216**, 7700.
27. www.Huayuan-tech.com, consulted in July 2012.
28. M. A. Rostagno, M. Palma and C. G. Barroso, *Anal. Chim. Acta*, 2007, **588**, 274.
29. W. Xiao, L. Han and B. Shi, *Sep. Purif. Technol.*, 2008, **62**, 614.
30. L. N. González-Núñez and M. P. Cañizares-Macías, *Food Chem.*, 2011, **129**, 1819.
31. X. Fang, J. Wang, X. Yu, G. Zhang and J. Zhao, *J. Sep. Sci.*, 2010, **33**, 1147.

32. M. Khajeh and A. Ghanbari, *Nat. Prod. Res.*, 2011, **25**, 1766.
33. T. S. Ballard, P. Mallikarjunan, K. Zhou and S. O'Keefe, *Food Chem.*, 2010, **120**, 1185.
34. M. Desai, J. Parikh and P. A. Parikh, *Sep. Purif. Rev.*, 2010, **39**, 1.
35. U. K. Sharma, K. Sharma, N. Sharma, A. Sharma, H. P. Singh and A. K. Sinha, *J. Agric. Food. Chem.*, 2008, **56**, 374.
36. G. A. Csiktusnádi Kiss, E. Forgács, T. Cserhádi, T. Mota, H. Morais and A. Ramos, *J. Chromatogr. A*, 2000, **889**, 41.
37. G. Raman and V. G. Gaikar, *Ind. Eng. Chem. Res.*, 2002, **41**, 2521.
38. C.-h. Ma, T.-t. Liu, L. Yang, Y.-g. Zu, X. Chen, L. Zhang, Y. Zhang and C. Zhao, *J. Chromatogr. A*, 2011, **1218**, 8573.
39. T. Liu, X. Sui, R. Zhang, L. Yang, Y. Zu, L. Zhang, Y. Zhang and Z. Zhang, *J. Chromatogr. A*, 2011, **1218**, 8480.
40. F.-Y. Du, X.-H. Xiao, X.-J. Luo and G.-K. Li, *Talanta*, 2009, **78**, 1177.
41. S. Bonny, L. Paquin, D. Carrié, J. Boustie and S. Tomasi, *Anal. Chim. Acta*, 2011, **707**, 69.
42. C. Sun, Y. Xie and H. Liu, *Chin. J. Chem. Eng.*, 2007, **15**, 474.
43. T. Zhou, X. Xiao, G. Li and Z.-w. Cai, *J. Chromatogr. A*, 2011, **1218**, 3608.
44. M. E. Lucchesi, F. Chemat and J. Smadja, *J. Chromatogr. A*, 2004, **1043**, 323.
45. M. A. Ferhat, B. Y. Meklati, J. Smadja and F. Chemat, *J. Chromatogr. A*, 2006, **1112**, 121.
46. B. Bayramoglu, S. Sahin and G. Sumnu, *J. Food Eng.*, 2008, **88**, 535.
47. Z. Wang, L. Ding, T. Li, X. Zhou, L. Wang, H. Zhang, L. Liu, Y. Li, Z. Liu, H. Wang, H. Zeng and H. He, *J. Chromatogr. A*, 2006, **1102**, 11.
48. G. Spigno and D. M. De Faveri, *J. Food Eng.*, 2009, **93**, 210.
49. Y. Chen, M.-Y. Xie and X.-F. Gong, *J. Food Eng.*, 2007, **81**, 162.
50. B. Kaufmann, P. Christen and J.-L. Veuthey, *Phytochem. Anal.*, 2001, **12**, 327.
51. W. Xiao, L. Han and B. Shi, *Sep. Sci. Technol.*, 2008, **43**, 671.
52. C. T. Ponne, A. C. Möller, L. M. M. Tijskens, P. V. Bartels and M. M. T. Meijer, *J. Agric. Food. Chem.*, 1996, **44**, 2818.
53. S. Hemwimon, P. Pavasant and A. Shotipruk, *Sep. Purif. Technol.*, 2007, **54**, 44.
54. N. Sánchez-Ávila, F. Priego-Capote, J. Ruiz-Jiménez and M. D. Luque de Castro, *Talanta*, 2009, **78**, 40.
55. A. Brachet, P. Christen and J. L. Veuthey, *Phytochem. Anal.*, 2002, **13**, 162.
56. X. Pan, H. Liu, G. Jia and Y. Y. Shu, *Biochem. Eng. J.*, 2000, **5**, 173.
57. Y. Yu, B. Chen, Y. Chen, M. Xie, H. Duan, Y. Li and G. Duan, *J. Sep. Sci.*, 2009, **32**, 4227.
58. J.-X. Wang, X.-H. Xiao and G.-K. Li, *J. Chromatogr. A*, 2008, **1198–1199**, 45.

59. X. H. Xiao, J. X. Wang, G. Wang, J. Y. Wang and G. K. Li, *J. Chromatogr. A*, 2009, **1216**, 8867.
60. X. Xiao, W. Song, J. Wang and G. Li, *Anal. Chim. Acta*, 2012, **712**, 85.
61. J. L. Luque-Garcia and M. D. Luque de Castro, *Talanta*, 2004, **64**, 571.
62. M. Virost, V. Tomao, G. Colnagui, F. Visinoni and F. Chemat, *J. Chromatogr. A*, 2007, **1174**, 138.
63. Y. Chen, X. Gu, S. Q. Huang, J. Li, X. Wang and J. Tang, *Int. J. Biol. Macromol.*, 2010, **46**, 429.
64. Z. Lianfu and L. Zelong, *Ultrason. Sonochem.*, 2008, **15**, 731.
65. G. Cravotto, L. Boffa, S. Mantegna, P. Perego, M. Avogadro and P. Cintas, *Ultrason. Sonochem.*, 2008, **15**, 898.
66. E. E. Stashenko, B. E. Jaramillo and J. R. Martinez, *J. Chromatogr. A*, 2004, **1025**, 105.
67. E. E. Stashenko, B. E. Jaramillo and J. R. Martinez, *J. Chromatogr. A*, 2004, **1025**, 93.
68. M.-T. Golmakani and K. Rezaei, *Food Chem.*, 2008, **109**, 925.
69. H. Wang, Y. Liu, S. Wei and Z. Yan, *Ind. Crops Prod.*, 2012, **36**, 229.
70. N. Sahraoui, M. A. Vian, I. Bornard, C. Boutekedjiret and F. Chemat, *J. Chromatogr. A*, 2008, **1210**, 229.
71. A. Farhat, C. Ginies, M. Romdhane and F. Chemat, *J. Chromatogr. A*, 2009, **1216**, 5077.
72. F. Chemat, M. E. Lucchesi, J. Smadja, L. Favretto, G. Colnaghi and F. Visinoni, *Anal. Chim. Acta*, 2006, **555**, 157.
73. A. Farhat, A.-S. Fabiano-Tixier, M. E. Maataoui, J.-F. Maingonnat, M. Romdhane and F. Chemat, *Food Chem.*, 2011, **125**, 255.
74. M. E. Lucchesi, F. Chemat and J. Smadja, *Flavour Fragrance J.*, 2004, **19**, 134.
75. S. Riela, M. Bruno, C. Formisano, D. Rigano, S. Rosselli, M. L. Saladino and F. Senatore, *J. Sep. Sci.*, 2008, **31**, 1110.
76. O. O. Okoh, A. P. Sadimenko and A. J. Afolayan, *Food Chem.*, 2010, **120**, 308.
77. Z. Wang, L. Wang, T. Li, X. Zhou, L. Ding, Y. Yu, A. Yu and H. Zhang, *Anal. Bioanal. Chem.*, 2006, **386**, 1863.
78. Z. M. Wang, L. Ding, L. Wang, J. Feng, T. C. Li, X. Zhou and H. Q. Zhang, *Chin. J. Chem.*, 2006, **24**, 649.
79. P. Mengal and B. Mompon, *World Pat.*, WO94026853, 1994.
80. P. Mengal and B. Mompon, *European Pat.*, EP698076, 1996.
81. P. Mengal and B. Mompon, *US Pat.*, US7001629, 2006.
82. M. A. Vian, X. Fernandez, F. Visinoni and F. Chemat, *J. Chromatogr. A*, 2008, **1190**, 14.
83. N. Bousbia, M. A. Vian, M. A. Ferhat, B. Y. Meklati and F. Chemat, *J. Food Eng.*, 2009, **90**, 409.
84. N. Bousbia, M. Abert Vian, M. A. Ferhat, E. Petitcolas, B. Y. Meklati and F. Chemat, *Food Chem.*, 2009, **114**, 355.

85. H. Zill e, M. Abert Vian, J. F. Maingonnat and F. Chemat, *J. Chromatogr. A*, 2009, **1216**, 7700.
86. S. Périno-Issartier, H. Zill e, M. Abert-Vian and F. Chemat, *Food Bioprocess Technol.*, 2011, **4**, 1020.
87. A. Cendres, F. Chemat, J.-F. Maingonnat and C. M. G. C. Renard, *LWT – Food Sci. Technol.*, 2011, **44**, 1035.
88. H. Zill e, M. Abert-Vian, M. Elmaataoui and F. Chemat, *J. Food Eng.*, 2011, **105**, 351.
89. T. Michel, E. Destandau and C. Elfakir, *Food Chem.*, 2011, **126**, 1380.

CHAPTER 5

*Accelerated Liquid Extraction*FELICIANO PRIEGO-CAPOTE^{*a,b} AND
MARÍA DEL PILAR DELGADO DE LA TORRE^{a,b}^a Department of Analytical Chemistry, University of Cordoba, Annex C3 Building, Campus of Rabanales, E-14071, Cordoba, Spain; ^b Institute of Biomedical Research Maimónides (IMIBIC), Reina Sofía Hospital, E-14004, Cordoba, Spain*Email: q72prcaf@uco.es**5.1 Introduction**

Over the last decades, an approach called accelerated solvent extraction (ASE) has competitively emerged for treatment of solid samples by using a liquid phase at high pressure and/or temperature, but below its critical point. This approach is considered an efficient way to increase automation, which is one of the pursued goals in the preparation of solid samples, but it also may shorten process times and reduce the amount of solvent required for sample preparation of solids.

The term ‘accelerated solvent extraction’ was originally coined by Dionex Corporation, which patented the technique and used it as the basis of its commercial devices.¹ In fact, the exclusive use of this term in the earliest years was largely the result of the sole commercially available extractor for this purpose being that manufactured by Dionex. At about the same time, Hawthorne, whose group was studying the applicability of water at high pressure and temperature as solvent, named the process ‘subcritical water extraction’.² With time, however, other alternative names such as ‘pressurised liquid extraction’ (PLE), ‘pressurised hot solvent extraction’ (PHSE), ‘high-pressure solvent extraction’ (HPSE), ‘subcritical solvent extraction’ (SSE) and

RSC Green Chemistry No. 21

Natural Product Extraction: Principles and Applications

Edited by Mauricio A. Rostagno and Juliana M. Prado

© The Royal Society of Chemistry 2013

Published by the Royal Society of Chemistry, www.rsc.org

‘superheated liquid extraction’ (SHLE), among others, have gradually replaced ASE, a commercial designation that is not related to the actual physicochemical foundations of the technique. High pressure is not the most salient feature in the ASE process. In fact, most often the only purpose of raising the pressure is to keep the solvent in the liquid state, and pressure rarely exerts an effect over the extraction process. On the other hand, the term ‘subcritical state’ is a wide term since any solvent at temperature and pressure below the critical point would be in this state, even at ambient conditions, and is therefore inappropriate as well. For these reasons, it is recommended to use the generic term ‘superheated solvent extraction’ despite its scant utilisation by the scientific community, which has widely accepted ASE supported on the distribution of the commercial devices. Both ASE and SHLE are used interchangeably in this chapter.

Referring to the basic principle of this sample preparation technique, it is worth mentioning that extraction with an aqueous or organic solvent at a high pressure and/or temperature can be done in a static regime, a dynamic regime – by continuously circulating the solvent through the sample – or in a combined mode of both operation modes (static–dynamic approaches). The basic principles of each operational SHLE mode as well as the main steps for development of both are discussed. The different devices designed for static and dynamic SHLE are also reviewed. In the final sections, a comparison of SHLE versus other competing extraction techniques and the applicability of SHLE for isolation of natural products are evaluated with special emphasis on the diversity of raw materials (leaves, roots, flowers, wood, fruits, vegetables, etc.) and on the different groups of compounds that can be extracted.

5.2 Static Accelerated Solvent Extraction (Static ASE)

Static ASE is the less versatile of the two extraction modes in terms of flexibility and possibility of coupling to other steps of the analytical process, since it is performed in a closed system. Nevertheless, static ASE is by far the most widely used mainly as a result of the availability of commercial extractors from Dionex (series 100, 200 and 300, and the new versions 150 and 350). Although these systems can be used in the static and dynamic modes, they are preferentially operated under static conditions. The static mode is usually selected to avoid dilution of the extract as the transfer equilibrium governing the extraction process is basically displaced under superheated conditions.

5.2.1 Steps Involved in the Static ASE Process

A common practice in extraction from a solid, applicable prior to static ASE, is to pre-treat it in some way depending on its physicochemical properties. Pre-treatment usually involves grinding to reduce particle size and sieving to isolate a homogeneous fraction of solid particles. In cases of solid–liquid samples centrifugation or filtration, drying (storing samples at moderate

temperature for 24–54 h) or freeze-drying is usually required. The last two operations are quite critical, since moisture present in biological samples may detract from the extraction efficiency, particularly for organic non-polar solvents. As in Soxhlet extraction, the addition of sodium sulfate or an alternative desiccant such as Extrelut particles is recommended in handling large amounts of water.

A Figure 5.1A shows the five steps usually involved in a static ASE process.

1. Loading the sample into the extraction cell. The metal frit of the extraction cell is suggested to be covered with a cellulose filter or a small amount of celite in order to prevent clogging at the outlet of the cell. To avoid dead volumes in the extraction cell the sample can be mixed with an inert matrix (e.g. diatomaceous earth, anhydrous sodium sulfate, glass fibre, high-density glass beads, sand, hydromatrix) to ensure proper sample–solvent contact and to reduce solvent consumption. Active materials such as Al_2O_3 , silica or Florisil can also be employed for specific purposes. These materials allow the extraction cell to be filled up but also to perform *in situ* clean-up by the retention of the target compounds once extracted or, on the contrary side, by the retention of undesired compounds. One other operation that can be carried out *in situ* during extraction of analytes is derivatisation by adding a suitable reagent in the extraction cell. The extraction–derivatisation combination is another interesting possibility as many derivatisation protocols are developed under high temperature, and this can be a way to increase sensitivity and/or selectivity.
2. Filling the cell with solvent, heating and pressurising the cell. Once the cell is loaded with sample and the end caps of the cell tightened, this is filled with a solvent of suited composition. At this point, there are two possibilities: to pre-heat the extraction cell before filling it with solvent or to heat it after filling with solvent. The latter is the preferred option by ASE users. The cell is frequently positioned in a vertical position to ensure that the system is completely filled with liquid solvent without air bubbles. Once the operational temperature is selected, the extraction system is thermostated at a constant pressure and equilibrated. Usually, 5 min is enough to equilibrate the system at the desired temperature and pressure.
3. Static extraction. This step is performed after pressure and temperature equilibration, for a pre-set time during which the analytes are released from the solid matrix and transferred to the solvent by diffusion and solubilisation. The role of pressure is later defined.
4. Collecting the extract. The transfer step begins immediately after static extraction is finished. The pressure valve is opened and the extract flows to the collection vial. In general, cooling the vial is not required as it does not seem to influence the recovery or the precision of the process.
5. Purging residual extract and cleaning for a new extraction. This step entails circulating fresh solvent or an additional inert gas – N_2 , for example – through the cell to remove residual extract in it. The use of an inert gas avoids diluting the extract that, in any case, could be

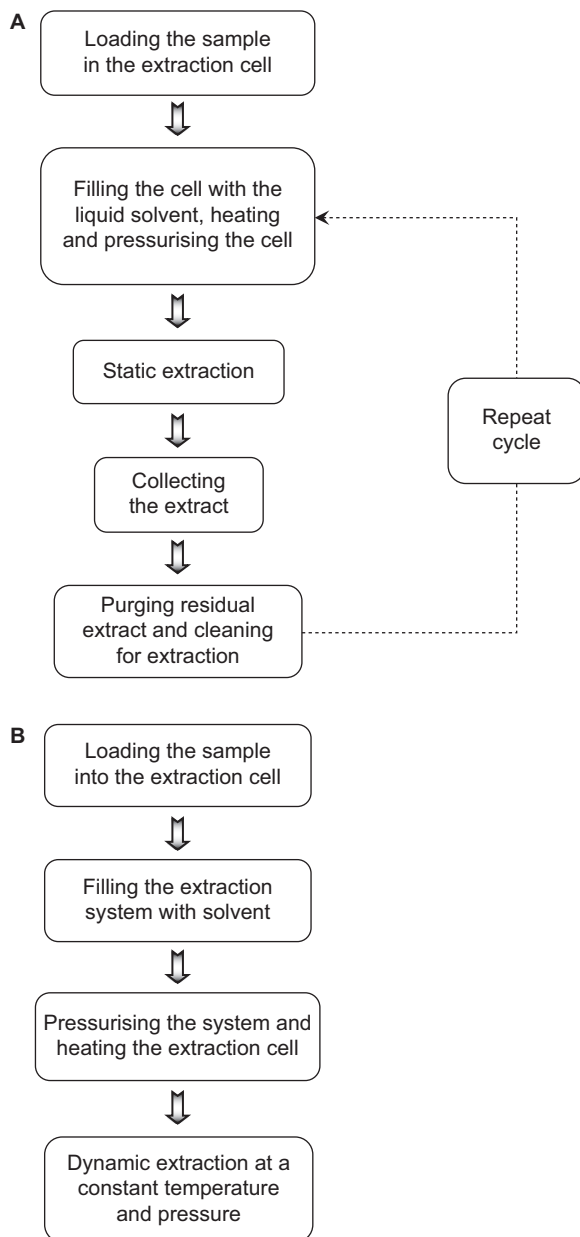


Figure 5.1 Scheme of the main steps involved in: (A) ordinary static and (B) dynamic ASE procedures.

(Reproduced with permission of Elsevier, Luque de Castro, M. D. and Luque-García, J. L. *Acceleration and Automation of Solid Sample Treatment*, Elsevier, Amsterdam, 2002, pp. 244 and 264).

concentrated by evaporation, if required. A 'rinse-solvent' volume corresponding to 60% of the empty extraction cell has proved effective to avoid carry-over between consecutive extractions for most applications.

5.2.2 Static ASE Commercial and Laboratory-designed Devices

The basic equipment required to implement static ASE is quite simple since it consists of the seven basic components depicted in Figure 5.2A, namely: (1) a reservoir for the fresh solvent; (2) a high-pressure pump; (3) a thermostated unit (for instance, an electrically heated oven) with thermal control for placing the extraction cell; (4) a stainless steel extraction cell where the solid-liquid

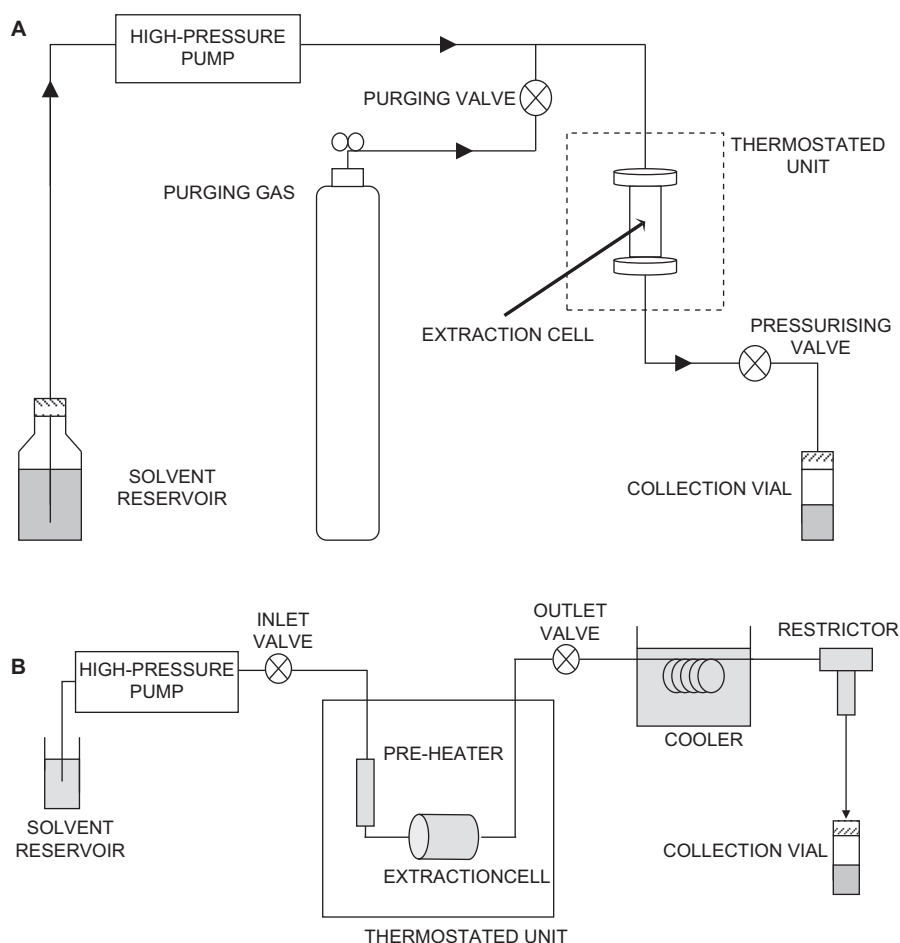


Figure 5.2 Configurations for development of static (A) and a dynamic (B) ASE procedures.

(Reproduced with permission of Elsevier, Luque de Castro, M. D. and Luque-García, J. L. *Acceleration and Automation of Solid Sample Treatment*, Elsevier, Amsterdam, 2002, pp. 245 and 261).

extraction takes place; (5) a back-pressure regulator for controlling the system pressure; (6) a cylinder of inert gas (usually nitrogen) for purging the system after extraction; and (7) a vial to collect the extract.

These components are backbone of an SHLE system, which is not technically complex. Concerning commercial models, only three static ASE systems, all from Dionex, were available until approximately the early 21st century, namely the ASE 100, 200 and 300.³ The main differences between them are, (a) the number of samples that can be simultaneously processed (1, 24 and 12 for ASE 100, 200 and 300, respectively); (b) the capacity of the sample cell (10–100 mL for ASE 100, 1–33 mL for ASE 200 and up to 100 mL for ASE 300); (c) the amount of sample to be processed (between 10 g and 100 g); and (d) the maximum pressure they can withstand (10 MPa for the ASE 100 and 300 models, and 20 MPa for the ASE 200). Two current models (ASE 150 and ASE 350) were developed later as extended versions of the ASE 100 and 300, mainly differing from their older counterparts in specific technological advances to improve their performance. Figure 5.3 shows a picture of the two devices currently commercialised by Dionex. The revised oven designs ensure uniform heating, and precise replicate extractions are possible as a result; Dionium™ cells allow extracting from acid to basic matrices and under extreme pH conditions; new flow-through operational capabilities allow in-line filtration; and faster pumps (up to 70 mL/min) accelerate and allow scaling up the process.

Other custom-made configurations have been the starting point for subsequent industrial development of processes intended to replace their conventional counterparts, which is one of the main benefits of SHLE. One



Figure 5.3 ASE® systems from Dionex.

example is the case of the extraction of non-volatile⁴ and volatile⁵ components of wood, of great interest to the winery industry. The conventional process to obtain extracts from wood, which is based on recirculation of ethanol–water mixtures through a bed of chips of oak wood and vine shoots at ambient temperature and pressure for 8–10 h, can be substituted by superheated extraction at 180 °C and 0.3 MPa for 50 min. The resulting extracts are richer in aroma compounds than the conventional extracts, which could have a favourable impact on the composition of wines and spirits. Also, comparing qualitatively and semi-quantitatively the composition of extracts obtained under certain conditions allows preparing tailor-made extracts.⁶

Taking into account the fundamentals of the static mode, this extraction approach can be technically scaled up to semi-industrial and industrial applications. Semi-industrial devices for extraction with volumes of 250 mL are commercialised by Buchi and FMS (Fluid Management System), among others. Concerning extraction systems for industrial purposes, they can be easily constructed using a pressurised extraction tank with a temperature control unit. These types of extraction devices are designed according to the industrial application and mainly used for the extraction of compounds with applications in fragrances, cosmetics or in the food industry.

5.3 Dynamic Accelerated Solvent Extraction (Dynamic ASE)

Dynamic ASE is employed to take benefit from the continuous contact between the solid matrix and fresh solvent. This favours the displacement of the transfer equilibrium and, therefore, the leaching process. The main limitation of this operational mode is the dilution effect by continuous flow of clean solvent, which makes it mandatory to implement subsequent concentration steps prior to characterisation of natural products, usually at low concentration levels. Unlike static ASE, there is no commercially available equipment for implementing dynamic ASE. This is the reason for the relative paucity of developed applications. In fact, dynamic operation facilitates coupling to other dynamic systems designed for pre-concentration, filtration, chromatographic separation, derivatisation and detection, among the most important.

5.3.1 Steps Involved in the Dynamic ASE Process

Most steps involved in dynamic ASE are similar to those of the static approach (Figure 5.1B), except for a few subtle differences, particularly at the final stages of the process. Thus, the steps involved in the dynamic ASE are as follows:

1. Loading the sample into the extraction cell. Similarly as in the static mode, the sample can be mixed with a dispersant, if required. Dispersant agents in this mode are more important than in the static ASE since continuous circulation of the solvent in the same direction increases

sample compaction. Additional materials such as surfactants, silica or sorption discs can be placed in the cell to facilitate transfer of the compounds from the solid to either the micellar medium or to the sorbent in order to concentrate the target compounds.

2. Filling the extraction system with solvent. The circuit is filled with solvent propelled by a high-pressure pump or a similar device. The extraction cell should be mounted vertically in the oven, with the solvent flowing from top to bottom so the extracted compounds are immediately swept from the cell.
3. Pressurising the system and heating the cell at a pre-set temperature and constant pressure. Before the oven temperature is raised up to the programmed value, the system is pressurised by using an outlet valve. In this way, the flowing solvent – as the valve is closed during this time – produces the overpressure required to maintain the solvent in liquid state at high temperature in the extraction system.
4. Dynamic extraction at constant temperature and pressure. Once the pre-set temperature is reached and stabilised, the outlet valve is partially opened and the liquid phase is continuously circulated through the system for the optimised period (dynamic extraction time). At the same time the extract is cooled by circulation through a coil in a water bath, and then collected at the outlet of the extraction system.

After extraction, the cell is washed with an appropriate solvent at a high flow rate in order to avoid carry-over. No purging of the system with a gas after extraction is required in this mode.

In many cases, the extraction protocols combine dynamic and static operational modes. For this purpose, an inlet valve is installed between the high-pressure pump and the thermostated extractor. The operation mechanism of this combined approach is based on the following steps: (1) the inlet valve is closed once the system is pressurised and the high-pressure pump stopped; (2) the oven temperature is raised up to stable value; (3) the system is maintained under a static regime with both valves closed for a pre-set time and; (4) finally, the valves are opened and the pump works again to keep the solvent flowing during the dynamic extraction period. Several studies, including one by Pérez-Serradilla *et al.*,⁷ have shown a combination of static and dynamic ASE for extraction of natural products. In this particular example, the application of the dual approach resulted in substantially improved sequential extraction of phenol compounds and fatty acids from olive pomace. This lab approach could be scaled up to give place to industrial equipment destined to valorise a semisolid residue generated in the extraction of olive oil.

5.3.2 Dynamic ASE Laboratory-designed Devices

The lack of commercial extractors for dynamic ASE has led to its implementation in laboratory-built designs similar to those for static ASE. The basic elements are shown in Figure 5.2B, namely: (1) a reservoir for storing the liquid

solvent; (2) a high-pressure pump or similar device for propelling the solvent to the extraction cell; (3) a thermostated system such as an electrically heated oven to reach and keep the desired temperature; (4) a pre-heater located prior to the extraction cell for ensuring that the solvent is at the required temperature when reaching the extraction cell; (5) an extraction cell for holding the sample; (6) an inlet valve (if static extraction is combined with the dynamic mode) and an outlet valve to pressurise the system and combine static and dynamic modes; (7) a cooling unit located out of the thermostated system; (8) a restrictor for keeping the pressure within the system at the pre-set level so that the solvent is maintained in the liquid state at the operating temperature; and (9) a vial for collecting the extract.

In dynamic ASE, the propulsion system can be a dual piston pump or a syringe pump, in any case high-pressure devices. The former delivers a continuous supply of solvent (limited by the barrel size in syringe pumps) and allows easy solvent change-over, while syringe pumps deliver a non-pulsating flow. All tubing and elements of a dynamic ASE extractor must be made of stainless steel to avoid corrosion by solvents (particularly acid or alkaline aqueous solutions) used at high temperatures. Special alloys such as hasteloid, which affords working temperatures close to or above 500 °C, can also be used, although the use of these specific materials would increase considerably the cost of the equipment and has limited application to natural products until the present time.

Sequential extraction of polar and non-polar compounds from the same sample with different solvents requires minimal technical changes of the dynamic ASE system. The modifications are aimed at the passage of a gas stream through the sample chamber to remove solvent residues before the next solvent is circulated.⁸ This configuration allows sequential extraction of complex matrices with a predefined order of solvents with different chemical properties (polar, non-polar, acid, alkaline, micellar media, etc.).

In relation to industrial extraction systems, the dynamic approach is not scaled up owing to its technical operational mode. Industrial procedures are mostly based on batch extraction.

5.4 Coupling ASE to Other Steps of the Analytical Process

One of the benefits of ASE when compared to conventional extraction as well as supercritical-fluid extraction (SFE) is the possibility of coupling to other steps of the analytical process such as filtration, pre-concentration, derivatisation, chromatographic separation or detection. Static ASE as implemented in commercial equipment is rarely coupled to other steps of the analytical process. In general, when static ASE is pretended to be coupled to subsequent steps, the extractor has been custom-made due to the compact design of the commercial models which preclude adaptation.⁹ Figure 5.4 shows one of the most complex coupled configurations involving static ASE on-line connected to filtration,

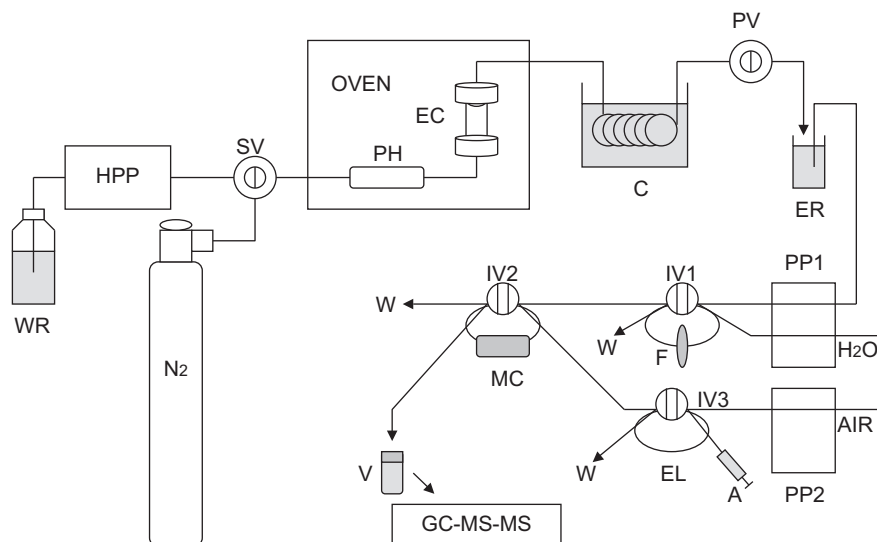


Figure 5.4 Schematic diagram to illustrate the coupling of static ASE to filtration, pre-concentration and chromatographic separation–mass spectrometry detection. WR = water reservoir, HPP = high-pressure pump, SV = switching valve, PH = preheater, EC = extraction chamber, C = cooler, PV = back-pressure valve, ER = extract reservoir, PP1 and PP2 = peristaltic pumps, IV1, IV2 and IV3 = injection valves, F = filter, W = waste, EL = elution loop, A = acetonitrile, MC = minicolumn, V = vial. Reproduced with permission of Elsevier from J. L. Luque-García and M. D. Luque de Castro, *Analyst*, 2003, **128**, 980.

pre-concentration and gas chromatography–mass spectrometry (GC–MS) detection.¹⁰ This configuration, designed for an environmental application, can also be implemented for isolation of natural products. Superheated liquid extraction is carried out as previously described in the static mode. The extract is collected in the reservoir (ER), and an exact volume of the extract is isolated in the loop of the injection valve IV1 and filtrated by action of peristaltic pump PP1. Once the loop is filled, the extract is led to the minicolumn MC using water as carrier. The target compounds are retained in the solid sorbent packed in the minicolumn with removal of interferences. Simultaneously, the loop of IV3 is filled with acetonitrile for subsequent elution of the target compounds. Air is used as carrier in this step to avoid dilution of the eluted compounds, which are collected in a vial (V) for analysis by GC–MS/MS.

Although the preferred choice for characterisation of natural products is the utilisation of off-line protocols, there are some examples in the literature dealing with the coupling of static ASE to other steps of the analytical process. These examples involve mainly coupling of a commercial Dionex extractor to devices such as a commercial liquid handling system (ASPEC) or liquid chromatographs. These couplings have been implemented to determine dianthrone in St. John's wort¹¹ by coupling ASE to solid phase extraction (SPE), and to

characterise proanthocyanidins and other phenolic compounds in brewing processes by coupling ASE, SPE and liquid chromatography coupled to diode array detection (LC–DAD) or liquid chromatography coupled to mass detection (LC–MS).¹² One other characteristic example is that proposed by Zhang *et al.*, who designed an approach coupling a commercial ASE device to a high-performance counter-current chromatograph. This configuration was tested for extraction and determination of caffeoylquinic acids in *Hypericum perforatum* L.¹³

As mentioned, the greatest drawback of dynamic ASE is the dilution of target compounds in the extract, which requires subsequent concentration (usually by batch liquid–liquid extraction, solid-phase extraction or simply evaporation). On the other hand, the versatility of the dynamic mode relative to the static mode can be used to circumvent dilution problems, but also to automate and/or facilitate other steps of the analytical process such as filtration, derivatisation, chromatographic separation and detection. Despite the examples in the literature of on-line configurations based on dynamic ASE coupled to other steps, these have not been applied to characterise natural products.^{9,14}

5.5 Parameters Affecting Performance in ASE

Performance of ASE is influenced by variables that contribute to the transference of compounds from the sample matrix to the bulk solvent, such as temperature, pressure, type of solvent and its characteristics (polarity, volume and – if dynamic mode is used – flow rate), matrix composition, sample size and extraction time. These parameters are briefly discussed in this section.

5.5.1 Temperature

Temperature is the most important parameter influencing the kinetics of mass transfer from the sample matrix to the liquid solvent in ASE and, therefore, it is crucial to succeed in the leaching process. It is well-known that physical properties of solvents are modified at high temperature. One example is found in Figure 5.5 for water at a constant pressure of 24 MPa.¹⁵ The dielectric constant of water decreases with increasing temperature. As a result, the solubility of water in organic solvents increases at high temperature. This is especially interesting in cases where the extraction efficiency of organic solvents at low temperature and pressure is decreased, since they are excluded from water-sealed pores in the sample matrix which contain the target compounds. The increased solubility of water in organic solvents at high temperature favours mass transfer from pores to the organic solvent. Apart from that, the solubility of water is similar to that of methanol at 200 °C and 24 MPa, to that of acetone at 300 °C or even to that of hexane at 500 °C. Therefore, water can be used as solvent for extraction of non-polar compounds at temperature above 200 °C. Modelling the dependence of the solubility on the temperature of an ideal solvent allows estimating the optimum extraction temperature for a given application.

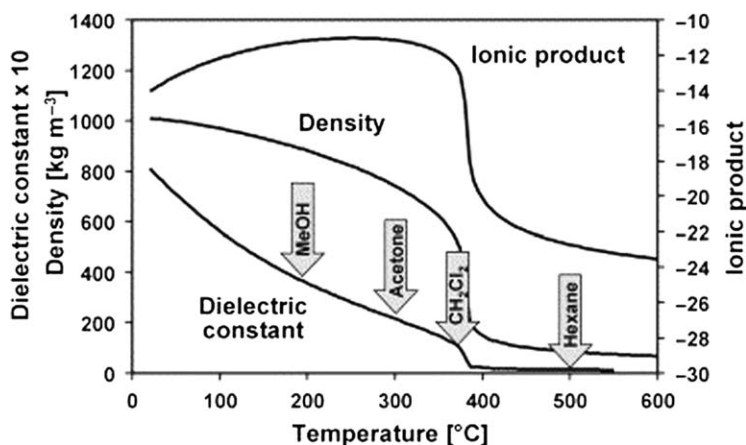


Figure 5.5 Physical properties of water at a pressure of 24 MPa versus temperature. Dielectric constants of typical organic solvents at room temperature are indicated.

Reproduced with permission of Elsevier from P. Kritzer and E. Dinjus, *Chem. Eng. J.*, 2001, **83**, 207.

The use of high temperature during leaching exerts a favourable effect on efficiency through increased diffusion rates. It is difficult to model the effect of temperature on diffusion rate, especially in multi-component systems. In most cases, diffusion rates are estimated to increase by a factor of 2–10 on raising the temperature from 25 °C to 150 °C, which undoubtedly enhances the leaching kinetics.¹⁶ Nevertheless, temperature is a key factor to be optimised in ASE because a high temperature does not always guarantee increased extraction efficiency. In certain cases, increased temperature can promote the formation of adverse effects such as degradation of thermolabile compounds or enhancement of secondary reactions that could influence other steps of the analytical process and/or the quality of the final product. The operation conditions during SHLE can also favour hydrolysis reactions when polymeric matrices are extracted. A particular case taking benefits from this principle is the extraction of lignocellulosic materials such as wood for isolation of lignin monomers (coniferyl and syringyl monomers), which is carried out at 180 °C.¹⁷ The resulting extracts are enriched in monomers when SHLE is performed at high temperature. With these premises, the temperature is only limited when the extraction conditions lead to target compounds degradation by chemical conversion to advanced reaction products. In general, the extraction temperature for isolation of natural products is between 100 °C and 200 °C, being most of the applications developed within the range 160–180 °C.

Temperature affects equilibria occurring at solid surfaces. In fact, it alters strong solute–matrix interactions due to van der Waals forces, hydrogen bonding and dipole attractions. Thermal energy can overcome cohesive (solute–solute) and adhesive (solute–matrix) interactions by decreasing the activation energy required for desorption.

Concerning the effect of temperature on solvents, increased temperatures decrease their viscosity. This effect facilitates solvent penetration through matrix particles and, therefore, enhances extraction. By way of example, the viscosity of 2-propanol decreases 9-fold as the temperature is raised from 25 °C to 200 °C.¹⁶ Apart from that, increased temperature also decreases the surface tension of the solvent, thereby allowing it to better access the sample matrix and to form solvent cavities more easily. Both changes improve contact of the compounds with the solvent and hence the extraction efficiency.

5.5.2 Pressure

A minimum pressure is required in ASE to maintain the solvent in the liquid state and avoid phase transitions. As an example, 2 MPa is sufficient to keep *n*-hexane (atmospheric boiling point of 68.7 °C) in the liquid state at 209 °C. The minimum pressure required to keep solvents in liquid state for a given temperature can be estimated from defined equations.¹⁶ Usually the influence of pressure on the leaching process is null and for this reason overpressure is not necessary. However, in some cases system pressure can be a key variable to displace the system equilibrium. Thus, a high pressure may favour leaching of compounds trapped in matrix pores by forcing the solvent into matrix areas that would normally not be accessible under atmospheric conditions.

Overpressure may also benefit the time required to fill the extraction cell with the solvent, especially with samples of small particle size that increase compactness in the cell.¹⁸ On the other hand, increased pressure can induce changes in the sample by decreasing active surface, which leads to reduced leaching efficiency for compounds in some types of samples.¹⁹

No significant changes in most extraction processes of natural products due to pressure have been detected using laboratory-made ASE systems. For this reason, the pressure is frequently set below 1 MPa. However, protocols developed for commercial ASE devices recommend extraction pressures close to 12 MPa.

5.5.3 Type of Solvent

SHLE can be used with a wide range of solvents, except those with auto-ignition temperatures within 40–200 °C (e.g. carbon disulphide, diethyl ether, 1,4-dioxane) or with low polarity in the eluotropic series (e.g. *n*-hexane).¹⁶ Also, strong bases and acids should be avoided as solvents on account of their corrosiveness, which is enhanced with increased temperature and pressure, causing damage to the fluidic system.

The static extraction mode uses preferentially non-toxic non-residual organic solvents such as ethanol or acetone, but dichloromethane, acetonitrile and hexane as well as mixtures of them have also been used. When the natural products to be extracted are destined for food industry or pharmaceutical applications, non-toxic solvents are selected.

Water is also a quite usual solvent, alone or in mixtures, for isolation of polar and mid-polar compounds in ASE²⁰ for a variety of samples including foods²¹

and plants,²² but also for compounds of widely variable polarity.^{23–26} The use of modifiers occasionally improves leaching. Water can be modified with organic solvents such as methanol, acetone or acetonitrile in low proportions (usually less than 5%) in order to decrease its dielectric constant – and hence its polarity – without drastic increase of temperature.²⁷

Weak acids and bases can be used when the solvent pH plays a decisive role on the leaching efficiency. Micellar media and ionic liquids are also used in certain applications to favour leaching efficiency. One example is the study proposed by Choi *et al.* for extraction of ginsenosides from medicinal plants by using Triton X-100 micellar media.²⁸ Micellar ASE enhanced the extraction efficiency compared to the use of water, leading to results similar to those provided by pure methanol. Therefore, surfactant media can replace organic toxic solvents such as methanol, which is of great interest in the case of natural products. On the other hand, ionic liquids (ILs) also seem to favour leaching kinetics by displacing the system equilibrium. Although few examples are in the literature, the first evidences of these benefits have recently been published, as the method for extraction of organic acid preservatives from glaucous fruits.²⁹ However, cautions must be taken on the use of ILs in dealing with intake of compounds extracted with them as their long-time effects are unknown.

5.5.4 Solvent to Feed Ratio

The amount of solvent required for efficient leaching strongly depends on the extraction mode. Static SHLE usually involves using less than 15 mL of solvent for sample sizes ranging from 1 g to 5 g. Obviously, bigger extraction cells can be used for extraction of higher amount of material. Once the extraction equilibrium is reached, the compounds extracted are rapidly collected by flushing the extraction cell with solvent and an inert gas; as a result, the matrix contains a residual amount of the original solutes, which depends on its partition equilibrium. When new solvent is added, the partition is slightly displaced, and as a consequence more solutes are solubilised. To complete the mass transfer several extraction cycles may be needed in unfavourable cases.

In the dynamic ASE, the solvent is continuously circulated through the extraction cell, so the volume that contacts the sample is a function of the flow rate and extraction time. If the extraction efficiency does not change with the flow rate, then extraction is limited neither by solubility nor by the equilibrium of mass transfer between the matrix and the solvent, so the rate-determining step of the process is diffusion inside the solid particles. In this case, the extraction rate can be increased by raising the extraction temperature. However, if the limiting factor is the solubility of the target compound, doubling the flow rate or the amount of solvent used will double the amount of solutes extracted over the same range; if the solutes undergo several re-adsorption/desorption steps during elution from the extraction chamber, doubling the flow rate will also double the extraction rate.

Flow rates reported for most SHLE methods described in the literature range from 0.5 mL/min to 3.0 mL/min for protocols involving sample size ranging

from 1 g to 5 g. Rates below 0.5 mL/min are not recommended because they can easily cause blockage of the restrictor used to maintain the pressure in the system; also, rates above 3 mL/min provide diluted extracts.

5.5.5 Sample Composition

The sample matrix has a critical influence on extraction of target compounds. Solid samples can differ significantly in physicochemical properties and matrix composition. However, the influence of sample composition is scarcely considered in experimental strategies for optimisation. This parameter can be only evaluated by comparison of extraction protocols for the same group of compounds carried out with different types of samples. As an example, the temperature used for extraction of phenolic compounds depends strongly on the sample composition. Phenolic compounds have been extracted from olive leaves at 140 °C³⁰ while the same compounds required 200 °C for extraction from olive pomace.³¹ It is worth mentioning that both protocols were optimised with the same extraction system.

5.5.6 Particle Size

Particle size is an important parameter that affects extraction efficiency. The influence of particle size depends on the mass transfer mechanism that determines the efficiency in ASE. Obviously, if the ASE efficiency is determined by diffusion, the mass transfer rate can be substantially increased by decreasing particle size up to a limit, below which the efficiency is decreased owing to technical limitations. Other variables with a direct influence on ASE efficiency include sample aging, moisture and presence of dispersant agents. Particle size is not frequently included in optimisation studies. In studies in which this variable is taken into account the common particle size is below 0.4 mm.^{4–6}

5.5.7 Extraction Time

Extraction time in ASE is very short as compared to conventional solid–liquid extraction techniques such as Soxhlet or maceration, and depends on the mass transfer phenomenon that determines the extraction rate. In the static mode, 5–20 min often suffices to ensure quantitative extraction of the target fraction. This is not the case for complex matrices of polymeric structures as wood, in which extraction should be continued over 30–60 min to release monomer compounds with interest for preparation of smoke liquid flavouring.⁶ However, extraction in the static mode is not always quantitative, especially with a single step, since the species to be extracted partition between the solvent and the matrix; as a result, the process is more or less quantitative depending on the partition coefficient for the system in question. In this case, a kinetics study would allow setting the optimum extraction time to isolate the fraction of interest.

Concerning the dynamic mode, where the solvent is continuously circulated through the sample, the leaching process is generally more efficient since the

sample is continuously brought into contact with fresh solvent. Dynamic extraction times usually range from 5 min to 30 min, although special applications could demand longer extraction times in unfavourable cases of complex samples.

5.6 Comparison of ASE with other Extraction Techniques

The exploitation of natural products isolated from different animal and vegetal sources is a growing trend due to the number of fields interested in these products. The bioactivity of natural products is gaining the attention of industries for production of drugs, cosmetics, nutraceuticals or foods, among others. Taking into account the relevance of sample preparation in this scenario it is worth discussing the suitability of ASE versus other solid–liquid extraction techniques presently used to isolate natural products. Prior to entering into discussion, some general aspects are briefly described to introduce the development of modern extraction techniques.

Classical extraction techniques such as maceration, Folch or Soxhlet extraction are typically ascribed to time-consuming protocols and often lead to non-reproducible results, low selectivity and/or low extraction yields. Consequently, repeated extraction cycles are necessary in most cases to obtain high extraction efficiencies. All these aspects constitute crucial reasons to explain why their implementation in the industry or in studies involving the analysis of a great number of samples is not an easy issue. Apart from these aspects, ‘green’ extraction techniques are gaining attention over traditional extraction techniques for isolation of natural products taking into account that they are frequently used for human consumption. Conventional extraction techniques, frequently using high volumes of organic solvents, involve potential danger, not only for the environment but for the laboratory personnel who suffer the consequences of a continuous exposition to a toxic atmosphere.

With the aim of circumventing these negative aspects, exhaustive investigation has been carried out for more than 30 years to develop new techniques that should fulfil the following aims in comparison to classical techniques: higher efficiency, less solvent consumption, ease of automation, more economical and with lower impact on the environment and human health. The result of this exhaustive research has been modern extraction techniques which have widely proved the previously cited benefits. The most important modern extraction techniques are microwave- and ultrasound-assisted extraction (MAE and UAE, respectively) and supercritical fluid extraction (SFE), in addition to ASE. In the light of their characteristics, all of them can give place to ‘green extraction protocols’ and more efficient processes.

The importance of natural products in recent years has promoted numerous studies aimed at comparing the extraction efficiency of classical and modern techniques.^{26,32–39} Taking into account the wide acceptance of ASE for isolation of natural products, it is one of the techniques most frequently

involved in these studies. One notable example in which different extraction techniques were compared is the study of Dawidowicz *et al.* who compared ASE with steam distillation, Soxhlet, SFE and headspace solid-phase micro-extraction (SPME) for isolation of essential oil components from *Thymus Vulgaris* L. ASE extracts were characterised by the highest yield of essential oil components providing similar results to steam distillation, which is recognised as the standard technique for extraction of essential oils in aromatic plants.³⁵ Additionally, the ASE protocol required the shortest extraction time (10 min versus 20 min for SFE and 3 h for steam distillation and Soxhlet).

One other representative study was developed by Luque-Rodríguez *et al.*, in which commercial extracts obtained from skins of red grapes by maceration protocols were qualitatively and quantitatively compared to extracts obtained by ASE.⁴⁰ This study enabled the conclusion that ASE extraction at laboratory level allows obtaining extracts richer in certain families of phenolic compounds than the commercial ones. Indeed, spectrophotometric data demonstrated that the proportion of total flavanols with respect to total anthocyanins and phenols was much higher in ASE extracts, as well as the percentage of monomeric anthocyanins in total anthocyanins (34.4% in ASE versus 17.4% and 20.6% in two different commercial extracts, respectively).

In other cases the extraction efficiency is replaced by other parameters related to the bioactivity of the extracts. Hossain *et al.* reported that extracts from spices such as sage (*Salvia officinalis* L.), basil (*Ocimum basilicum* L.) and thyme (*Thymus vulgaris* L.) obtained by ASE had a higher antioxidant capability than extracts isolated by conventional solid-liquid extraction.⁴¹ SFE by using CO₂ as solvent has been the technique competing with ASE due to the fundamentals of both techniques. CO₂ is a non-toxic, non-flammable and non-corrosive solvent, which is specially suited to obtain natural products for human consumption. One other benefit is that CO₂-SFE is the only technique that allows obtaining a powder without the need of drying.⁴² However, it is mainly limited to non-polar and medium polar substances due to the non-polar character of CO₂.⁴³ One other limitation is the high acquisition and maintenance costs of SFE equipment as compared to ASE, the fundamentals of which support the scaling up of the extraction process for implementation at an industrial level.

Accelerated liquid extraction is generally performed with organic solvents^{32,35} or in aqueous solutions.⁴¹ The current trend is to optimise protocols by using organic solvents compatible with human consumption such as ethanol or acetone. These solvents can be easily removed at very low trace level. Nevertheless, in the last years there is a trend in ASE for the use of water as solvent to convert protocols to completely 'green' methods. This is reflected in the considerable number of studies focused on the use of ASE from an environmental perspective proposed in recent years.^{44,45} In addition, due to ASE fundamentals, it can also be used to extract compounds exhibiting low solubility in the solvent selected. One recent example has been reported by Euterpio *et al.* in the extraction of curcumin from the turmeric rhizome using SHLE by adjusting the pH of water as solvent.⁴⁶

Water is a highly polar solvent with a high dielectric constant ($\epsilon_r = 80.1$) at room temperature and pressure, characterised by the extensive presence of intermolecular hydrogen bonding. However, when the temperature is raised, its permittivity coefficient rapidly decreases as well as its viscosity and surface tension. The associated increase of diffusivity and the lower density under these conditions also makes water specially suited for extraction. From a practical point of view, water can be easily maintained in the liquid state at temperatures up to 250 °C by keeping the pressure above 5 MPa. Under these conditions, its dielectric constant decreases up to 27; this means that its polarity index is between those of methanol and ethanol at 25 °C. Therefore, water under these conditions exhibits similar behaviour to some organic solvents that are widely used to dissolve a broad range of medium and low polarity compounds, and can thus serve as an alternative to traditional organic solvents (see Figure 5.5).

SFE has been successfully used to extract phenolic compounds from grape pomace using CO₂ modified with methanol^{47–49} or ethanol as co-extractant.⁵⁰ Some other studies have compared SFE to ASE as that carried out by Herrero *et al.* in 2010, which revealed that bioactive extracts isolated from rosemary plants using ASE reported not only higher extraction efficiencies (up to 38.6% for ASE working under 200 °C and 10 MPa for 20 min versus 6.5% for SFE under 40 °C, 10 MPa and 300 min with 7% of methanol as a modifier of CO₂), but also higher antioxidant capabilities measured by the DPPH radical scavenging method ($18.2 \pm 0.1 \mu\text{g/mL EC}_{50}$ for ASE versus $12.1 \pm 0.0 \mu\text{g/mL EC}_{50}$ for SFE).³⁴

In addition to the benefits discussed above, ASE has a number of other advantages over other modern techniques. Notably, the use of high pressure may facilitate extraction from samples in which the solutes are trapped in the pores of the sample matrix. The high pressures used may force water into areas of the matrices that would not be accessible to solvents under atmospheric pressure.¹ Moreover, the reduced risk of contamination with exogenous chemicals is an attractive feature, especially when preparing extracts for human consumption (in cosmetics, drugs, foods, etc.). One other complementary factor is that ASE is an ideal choice for the extraction of non-stable compounds such as colorants. The absence of light and air in the extraction chamber reduces significantly degradation and oxidation of these compounds during extraction.⁵¹

One other competing alternative with ASE is the assistance of leaching by microwaves, which have been deeply investigated and applied in analytical chemistry during the last decade to accelerate sample digestion and chemical reactions, and to enhance leaching of solutes from different solid matrices. Microwave energy is a non-ionising radiation that causes molecular motion by migration of ions and rotation of dipoles without changing the molecular structures if the temperature is not too high. Therefore, non-polar solvents such as hexane and toluene are not affected by microwave energy, so it is necessary to add polar additives. MAE is an efficient extraction technique for solid samples and is applicable only to thermally stable compounds, which, in a certain way, is common to ASE. Similarly to ASE, MAE has become a viable

alternative to conventional methodologies due to many substantial improvements over other sample preparation techniques such as reduced extraction time and lower amount of solvent.^{52–54} There are numerous studies comparing the extraction efficiency of ASE and MAE for isolation of natural products. Recently, Taamalli *et al.* have evaluated the leaching efficiency of MAE and ASE together with SFE and conventional solid–liquid extraction for recovering phenolic compounds from Tunisian olive leaves.⁵⁵ The evaluation was supported on the analysis of the extracts by LC–ESI–TOF/MS and LC–ESI–IT–MS.² Higher extraction yields were obtained for ASE followed by MAE using the Folin–Ciocalteu test, while phenolic profiles showed a larger number of phenolic compounds in the extracts obtained using MAE followed by the conventional method. In general, MAE and conventional methods were the best choice for extracting more polar compounds such as oleuropein derivatives, apigenin turinoside and luteolin glucoside. On the other hand, SFE and ASE were more efficient to extract compounds with less polarity such as apigenin, luteolin or diosmetin.

Other comparative studies have revealed the superiority of ASE over MAE, as in that carried out by Delgado de la Torre *et al.*, who have recently compared UAE, MAE and ASE to obtain extracts enriched in bioactive compounds from vine shoots.¹⁷ This study clearly supported ASE as the best extraction technique for isolation of phenols followed by UAE, for which the global determination test based on the Folin–Ciocalteu reagent was used. Figure 5.6 shows the results of extraction efficiency provided by the three extraction techniques in terms of hydroxymethylfurfural and total phenolic concentrations. SHLE led to extracts with higher concentration of total phenols but also with higher concentration of hydroxymethylfurfural formed by degradation of lignocellulosic material.

One other auxiliary energy used to assist solid–liquid extraction is ultrasound. Ultrasound-assisted extraction shares some of the advantages of MAE in terms of enhancing leaching kinetics, reducing solvent volume and possibility of automation. However, UAE is particularly useful for isolation of thermolabile compounds or for sample matrices where disruption favours considerably the contact between solid and liquid phases. In this sense, UAE competes with ASE thanks to the working temperatures reached in UAE, most times at (or close to) ambient conditions. Apart from these aspects, a common aspect to ASE is the low cost of the extraction process thanks to the simplicity of the equipment required.⁵⁶ The main shortcoming of UAE versus ASE is the potential formation of free radicals generated by sonolysis of the solvent, which can produce degradation of some labile compounds by oxidation.⁵⁷ A comparison of the feasibility of ASE versus UAE was carried out by Fojtová *et al.* who applied these extraction methods to walnut-tree leaves prior to GC–MS analysis for quantification of terpenes.⁵⁸ The efficiency of ASE performed with *n*-hexane at 150 °C and 15 MPa in three cycles of 5 min was superior to that of UAE at room temperature using *n*-hexane for 1 h (198.7 µg/g total terpenes versus 59.2 µg/g, respectively). Nevertheless, the relative distribution of particular terpenes changed depending on the extraction

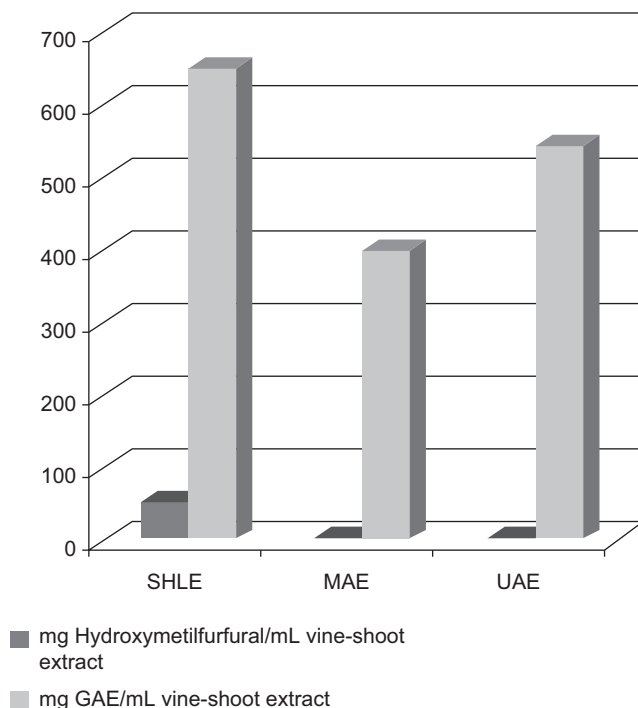


Figure 5.6 Concentration of hydroxymethylfurfural (mg/mL) and total phenolic content expressed as μg equivalent to gallic acid per mL of vine shoot extract obtained by the Folin–Ciocalteu method obtained by SHLE, MAE and UAE approaches.

Reproduced with permission of American Chemical Society from M. P. Delgado-Torre *et al.*, *J. Agric. Food Chem.*, 2012, **60**, 3051.

method, i.e. the relative concentration of β -pirene and limonene was higher for UAE. On the other hand, the final extracts obtained by ASE were found to be clean enough for direct analysis by GC–MS without need for any pre-treatment. This is a great benefit since when working with volatile compounds, every additional handling of samples increases the risk of losses.

5.7 Applications of ASE for the Isolation of Natural Products

Since SHLE was born around 1995, its number of applications has increased exponentially. At the beginning, SHLE arose mainly as a preparation technique to replace conventional techniques based on traditional protocols. In the first period, the trend on leaching applications was primarily marked by a competing technique such as SFE. In fact, US Environmental Protection Agency (EPA) introduced SFE as sample preparation technique in several official methods in the environmental field. However, SFE has not fulfilled the

initial expectations at present while ASE has growing to achieve an unpredicted impact at early years. Nowadays ASE has consolidated its position in some fields, mainly environmental and food analysis, but also it has covered the area of natural products which is characterised by a high number of application fields.

The evolution of ASE in the area of natural products can be charted with bibliographic surveys of publications using 'ASE', 'PLE' or 'SHLE' as key terms. Figure 5.7A shows the number of papers published from 1995 to 2011 found in three databases, namely, Scifinder research tool (which retrieves information contained in databases produced by Chemical Abstracts Service, MEDLINE database and CAPLUS database). The number of ASE applications grew rapidly from 1999 to 2007. Then, the situation was stabilised until 2010 with a new increase. Figure 5.7B shows a distribution of the number of papers according to the name given to the technique. This graph confirms that although ASE is a widely extended name from a commercial point of view, the term 'pressurised liquid extraction' is more accepted in the field of natural products in the last years. Nevertheless, the term 'pressurised liquid extraction' is not well founded.

The wide applicability of ASE in the area of natural products is linked to the diversity of samples extracted with this solid–liquid extraction technique. Vegetal material such as wood, leaves, branches, flowers, fruits, gum, vegetables, agriculture residues, among others, have been treated by ASE. This section has been organised from the perspective of the type of compounds extracted. Thus, three subsections are reviewed to discuss ASE applications for extraction of lipids, volatile compounds and polar compounds. Due to the variability of compounds that could be qualified as polar, the potential application of the isolated natural products will be considered. Table 5.1 summarises

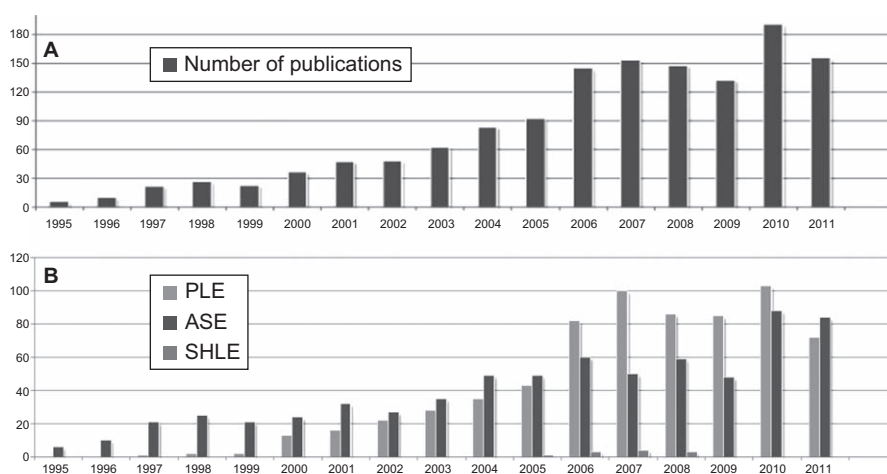


Figure 5.7 (A) Number of publications involving the utilisation of ASE for extraction of natural products in the period 1995–2011. (B) Distribution of the number of publications according to the nomenclature adopted by the authors.

Table 5.1 Characteristics of ASE protocols used for analysis of natural products.

Published on 24 May 2013 on http://www.rsc.org

Sample	Compounds	Pre-treatment	ASE				Post-treatment	Technique	Ref.
			Solvent	T (°C)	Cycles	Extraction time (min)			
Pharmacologically active compounds									
ginseng	gingenosides	dried sample	water + Triton X-100	50–120	1	10	not required	LC–UV	28
<i>Rumex nepalensis</i>	naphtalene and anthraquinone	not required	MeOH	60	1	10	concentration	LC–DAD	33
<i>Spreng. Roots</i>	isoflavones	dried sample	MeOH, MeOH-water (75:25, v/v), acetone and acetone-water (75:25, v/v)	75–125	4	5	concentration	LC–DAD	86
<i>Crifolium L.</i>									
medicinal plants and health supplements	gingenosides	sample between sand layers	MeOH	140	dynamic mode (1 mL/min)	20	not required	LC–DAD	87
ginger	gingerol-related compounds	dried sample	bioethanol/water (70%)	100	1	5	not required	LC–MS	88
medicinal plants	aristolochic acids	sample/sand	MeOH	120	dynamic mode (1.5 mL/min)	20	not required	LC–DAD	90
kava root	kavalactones	not required	water	175	dynamic mode (1 mL/min)	20–40	LLE	GC–FID	92
plant leaves	glycosides	not required	water	100	dynamic mode	30	LLE	MEKC–DAD	93
medicinal plants	aristolochic acids	sample/sand	MeOH	120	dynamic mode (1 mL/min)	20	not required	CZE–UV	94
medicinal plants	alkaloids	sample/sand	MeOH	120	dynamic mode (1 mL/min)	20	not required	CZE–UV	95
coca leaves	cocaine, benzoylecgonine	sample/sand (1:3, w/w)	MeOH	80	dynamic mode (1 mL/min)	10	concentration	GC–MS	96
medicinal plants	alkaloids	sample/sand	water or water/ethanol	95–140	dynamic mode (1 mL/min)	40	concentration	LC–UV	97
natural health products	ephedrine, pseudoephedrine and metabolites	sample/ottawa sand	water/3% MeOH	90	3	5	not required	FI–MS	98
medicinal plants	three glycosides	sample/sand	water + Triton-100	95	dynamic mode (1 mL/min)	40	SPE	LC–MS	99
fruit	furanocoumarins	sample/neutral glass	MeOH	100	1	10	concentration	LC–DAD	100
Chinese herbal medicine	rutin and quercetin	dried sample	1-butyl-3-methylimidazolium (1 M)	125	1	5	not required	LC–CL	101

Phenolic compounds

salt	5 proanthocyanidins	sample/DE	acetone/water (4:1, v/v)	60	2	10	automated SPE	LC-UV, LC-MS	12
<i>Rosmarinus officinalis</i>	bioactive phenolic compounds	air dried	ethanol, water	100/200	1	20	concentration	LC-DAD, LC-MS	32
red grape skins	anthocyanins and other phenols	dried sample	ethanol/water (1:1)	120	1	30	not required	LC-DAD, LC-MS	40
sage (<i>Salvia officinalis</i> L.), basil (<i>Ocimum basilicum</i> L.) and thyme (<i>Thymus vulgaris</i> L.)	total phenolic content	dried sample	32–88% methanol	66–129	1	10	filtration	LC-DAD	41
Tunisian olive leaves	37 phenols	ground under liquid N ₂	ethanol	150	1	20	not required	ESI-TOF/MS	55
vine shoots of <i>Vitis vinifera</i>	14 phenols	dried sample	ethanol/water (80%)	240	1	60	LLE	ESI-IT/MS ² LC-DAD	56
Golden apple	12 phenols	sample/DE (1:1, w/w)	MeOH	40	2	5	concentration	LC-DAD	75
soybeans	isoflavones	freeze-dried sample/sand	ethanol/water (70:30, v/v)	100	3	7	not required	LC-DAD, LC-MS	85
cider apple	16 phenols	freeze-dried sample/DE (1:1, w/w)	MeOH	40	2	5	not required	LC-DAD	102
grape seeds and skins	9 phenols	sample/ sea sand	MeOH	100/150	3	10	not required	LC-DAD	103
grapes	6 phenols	sample + LiChrolut EN sorbent layer	two extractions 1. water 40 °C, 150 atm 2. MeOH 100 °C, 40 atm	100	3	10	not required	LC-DAD	104
aromatic plant (sage)	phenolic diterpenes, phenolic acids	sample/sea sand	water	100	dynamic mode (1 mL/min)	60	SPE	LC-MS	105
rosemary leaves	phenolic diterpenes, flavonoids	not required	water	25–200	dynamic mode (1 mL/min)	30	freeze-drying	LC-MS, LC-DAD	106
carob pods (Mediterranean leguminosae)	41 phenols	sample/DE (1:2, w/w)	acetone/water (1:1, v/v)	60	2	5	SPE	LC-UV, LC-MS	107
tea leaves and grape seeds	flavanols	sample/sea sand	MeOH	130	2	5	-	LC-DAD	108
microalgae	polyphenols	not required	hexane, light petroleum, ethanol, water	115/170	1	9, 15	concentration	MEKC -DAD	109
soybean food	isoflavones	florisil/sand	two extractions: 1. hexane 2. (2)60% MeOH/0.3%FA	100	2	5	concentration	LC-ED	110
Anatolian propolis	13 phenols	not required	ethanol/water/HCl (75:20:5, v/v/v)	40	3	15	not required	LC-DAD	111

Table 5.1 (Continued)

Published

Sample	Compounds	Pre-treatment	ASE				Extraction time (min)	Post-treatment	Technique	Ref.
			Solvent	T (°C)	Cycles					
Essential oils										
<i>Thymus vulgaris</i> L.	essential oils	air-dried	<i>n</i> -hexane, DCM, ethyl acetate and distilled water	20–175	1	5–30	LLE	GC–MS	10	
Chinese medicine	essential oils	not required	water	160	dynamic mode (1 mL/min)	5	(HS)-LPME	GC–MS	32	
mate tea leaves	essential oils	air-dried	<i>n</i> -hexane, toluene, DCM, ethyl acetate, acetone and MeOH.	100	1	10	not required	GC–MS	38	
<i>Origanum onites</i>	essential oils	air-dried	water	100/175	dynamic mode (2 mL/min)	30	SPE	GC–TOF/MS	47	
medicinal plant (fennel)	monoterpenes, oxygenates	not required	water	150	static-dynamic mode (2 mL/min)	30 + 20	LLE	GC–FID, GC–MS	77	
rosemary	terpenes, oxygenates	not required	water	150	dynamic mode (2 mL/min)	30	LLE	GC–FID	112	
peppermint	oxygenates, carbophyllene	air dried sample	water	125–150	dynamic mode (1 mL/min)	20	LLE	GC–MS	113	
majoram leaves	terpenes, pinenes alcohols	not required	water	150	dynamic mode (2 mL/min)	15	LLE	GC–FID, GC–MS	114	
laurel	essential oils	not required	water	150	static-dynamic mode (2 mL/min)	15 + 25	LLE	GC–FID, GC–MS	115	
savory and peppermint	terpenes, oxygenates	air dried sample	water	100–175	dynamic mode (2 mL/min)	12–40	LLE	GC–FID, GC–MS	116	
oregano	11 oregano oil compounds	not required	water	125	dynamic mode (1 mL/min)	24	LLE	GC–FID, GC–MS	117	

lemon grass	essential oils (neral, geranial, geraniol, limonene, citronellal, p-myrcene)	not required	hexane, DCM, acetone, MeOH	40	3	10	not required	GC-FID	118
<i>Thymra spicata</i> L.	essential oils	not required	water	150	dynamic mode (2 mL/min)	30	SPE	GC-TOF/MS	119
lime peel	essential oils (neral, geranial, geraniol, linalool, terpineol)	not required	water/MeOH or ethanol	130	static-dynamic mode (1 mL/min)	5 + 15	LLE	GC-FID, GC-MS	120
Chinese medicine	essential oils	not required	water	150	dynamic mode (1 mL/min)	5	SPME	GC-MS	121
fat matter									
Alperujo	phenols and fatty acids	dried at 70 °C for 24 h	MeOH/water (80 %)	200	static-dynamic	10–13	LLE	GC-MS	7
pepper	terpenes, fatty acids and vitamin e	air dried	ethanol, pentane	85–150	1	10	concentration	GC-MS	37
<i>gaudichaudianum</i> Kunth									
egg yolk, ox liver, calf brain and soybean	total lipids and glycerofosfolipids	freeze drying	chloroform/MeOH (2:1, v/v)	120/150	4	5	LLE	LC-ELSD	59
<i>Ziziphus jujuba</i>	saponins and fatty acids	powdered sample/ diatomaceous earth (1:1)	MeOH/ethyl acetate (95:5)	140	1	15	not required	LC-ELSD	60
egg-containing food	oxysterol	sample/celite	hexane/isopropanol (3:2, v/v)	60	2	8–10	concentration	GC-MS	122
dairy products	fat	sample/celite	hexane, DCM, MeOH, petroleum ether, acetone, ethanol, isopropanol	80–120	2	8–10	concentration	gravimetric	123

CL=chemiluminescence detection, CZE=capillary zone electrophoresis, DAD=diode array detection, DCM=Dichloromethane, ED=electrochemical detection, ELSD=evaporative light scattering detection, ESI-IT/MS²=electrospray ion trap tandem mass spectrometry, ESI-TOF/MS=electrospray time-of-flight mass spectrometry, FID=flame ionization detection, FI-MS=flow injection-mass spectrometry, GC=gas chromatography, (HS)-LPME=headspace liquid-phase microextraction, LC=liquid chromatography, LLE=liquid-liquid extraction, MEKC=micellar electrokinetic capillary chromatography, MeOH=methanol, MS=mass spectrometry, SPE=solid-phase extraction, TOF/MS=time-of-flight mass spectrometry, UV=ultraviolet detection.

the protocols used for extraction of different compounds with special emphasis on operational conditions and preparation of extracts, if required, prior to analysis.

5.7.1 Lipids

Lipidic natural products constitute one of the main compound classes extracted by ASE. Numerous studies have been proposed with common methodological aspects in the extraction protocols. One particular case is that for extraction of total lipids from food for preparation of nutritional supplements. A recent example is the study by Zhou *et al.* who used ASE for extraction of phospholipids from various food matrices, namely: soybeans, egg yolk, calf brain and ox liver.⁵⁹ The protocol was based on the use of 2:1 chloroform–methanol (v/v) at 10 MPa and 140 °C for 5 min. These conditions led to recovery of over 96% phospholipids in each type of food by one single extraction step. The Folch method, a conventional approach for isolation of lipids, required up to four successive extractions to obtain similar leaching efficiency values.

The main application field in the extraction of lipids is the isolation of bioactive compounds such as saponins, liposoluble vitamins, terpenoids, sterols, etc. One example is the research developed by Zhao *et al.* who extracted bioactive compounds as saponins together with fatty acids from a Chinese medicine plant (*Ziziphus jujube*, Suanzaoren).⁶⁰ The extraction protocol in this case was based on a 95:5 methanol–ethyl acetate (v/v) solution and the conditions used were 140 °C and 8.2 MPa for 15 min. In this type of applications, the stability of the fraction of interest is crucial. Thus, in the extraction of vitamin E isomers from seeds and nuts developed by Delgado-Zamarreño *et al.* two extraction cycles were required since the optimum temperature for extraction was set at 50 °C.⁶¹

The main limitation of these applications is the need for organic solvents owing to the lipidic character of these compounds. In this sense, the use of non-toxic organic solvents such as ethanol or acetone should be promoted. Denery *et al.* tested different solvent compositions for isolating carotenoids and kavalactones from green algae.⁶² Among these solvents it is worth mentioning those consisting of acetone, ethanol, 7:3 acetone–ethanol (v/v) and 1:3 methylene chloride–methanol (v/v) mixtures. The methylene chloride–methanol mixture provided the best extraction results by monitoring efficiency for isolation of total pigments, astaxanthin and lutein.

In cases where different families of lipids are co-extracted, the fractionation scheme proposed by Poerschmann *et al.* can be used.⁶³ Essentially, the protocol is based on a two-step extraction process using 9:1 *n*-hexane–acetone (v/v) at 50 °C to obtain neutral lipids followed by 1:4 chloroform–methanol (v/v) at 110 °C to obtain polar lipids. The extraction process was combined with an in-cell fractionation using silica-based sorbents (silicic acid or cyanopropyl silica) placed at the outlet of the extraction cell. Thermally pre-treated sorbents were appropriate to ensure clear-cut boundaries between neutral lipids and

phospholipids. The proposed protocol is superior to the approaches commonly used, which consist of an exhaustive lipid extraction followed by off-line fractionation using SPE, in terms of fractionation efficiency, time and solvent consumption.

Superheated liquid extraction has also been used for specific applications to study or predict biological processes. One example is the use of SHLE for simultaneous isolation of certain families of lipids (straight-chain lipids, plant sterols and terpenoids) from sandy soil profiles under Corsican pine material.⁶⁴ These compounds can be used as vegetation tracers, based on the principle that plant-specific combinations of lipids are preserved in soils and can act as biomarkers to identify past vegetation compositions. The solvent in this case was a 93:7 dichloromethane–methanol (v/v) mixture at 75 °C, 6.9 MPa and 20 min as processing time.

5.7.2 Volatile Compounds

Superheated liquid extraction has frequently been used for isolation of volatile compounds from plants and foods. Among the great number of studies on SHLE of aromatic volatile compounds it is worth mentioning those dealing with compounds contributing to aroma fractions or food flavour. A particular study is that reported by Cincchetti *et al.* in 2009, who proposed a method based on ASE for the authentication of natural vanilla flavours in foods by using detection techniques based on isotopic ratio distributions.⁶⁵

Wood material has also been a vegetal source used for isolation of volatile compounds. Vichi *et al.*⁶⁶ and Natali *et al.*⁶⁷ focused their research on the study of volatile and semivolatile components extracted from oak wood chips. Both studies used ASE to obtain the extracts following the same extraction method: 5 g of sample, 15 mL dichloromethane as solvent, leaching temperature of 150 °C, pressure of 20 MPa, extraction time of 7 min. Similar results were obtained in characterisation by both studies with slight differences as the presence of solerone and two C-13 norisoprenoids identified by Natali *et al.* or the identification for the first time in oak wood of ten lignin dimmer derivatives by Vichi *et al.* A key conclusion from both studies was that the toasting degree was the variable with stronger influence on composition of wood extracts. Also focused on the use of ASE with wood is the study published in 2011 by Dawidowicz *et al.* for isolation of essential oil. This study also monitored the extraction yield as a function of the purge time during the ASE procedure.⁶⁸ Longer purge times led to losses of volatile organic compounds.

Volatile compounds usually correspond to secondary metabolites present at low concentration. The optimisation of the extraction step is focused on them. Numerous examples dealing with optimisation of SHLE to obtain profiles from secondary metabolites can be found in the literature. Two representative examples are those of Cho *et al.*, who worked with *Angelica* roots,⁶⁹ and Liu *et al.*, who used *Nigella sativa* seeds,⁷⁰ both herbaceous plants commonly used for medicinal purposes. Both extraction procedures were

similar with certain differences associated to the sample matrix. Both protocols used *n*-hexane as leaching solvent, but higher temperature and pressure (100 °C and 10 MPa) were applied by Liu *et al.* due to the nature of seeds, a more complex material to be extracted than plant material used by Cho *et al.* (80 °C and 7 MPa).

The volatile fraction from tobacco has been studied using ASE by Vial *et al.*, who used the extracts for discrimination among different varieties of tobacco products. For this purpose the extracts were analysed by GC×GC–MS.⁷¹ Previously, Shen *et al.* applied ASE to different varieties of tobacco for extraction of terpenoids and sterols prior to characterisation of both fractions.⁷² The ASE-based method exhibited better reproducibility and extraction yields than classical methods.

5.7.3 Polar Compounds

Most of applications dealing with ASE and natural products are focused on the extraction of polar compounds. Due to the great variety of these applications in terms of chemical properties of compounds isolated and the diversity of sample matrices, they are distributed depending on the final use of the extracted components as antioxidants, essential oils, nutraceuticals or drugs.

5.7.3.1 Antioxidants

Antioxidant compounds are gaining popularity in the last years thanks to their beneficial properties for human health, but also as food preservatives and dietary supplements. Attending to the potential uses of antioxidants in the clinical and food fields, strong efforts have been made in the last years to obtain antioxidants from a wide range of natural sources, mainly from waste materials from the agrofood industry. Thus, extensive research has been carried out in the Mediterranean countries to isolate antioxidants from residues of the olive oil industry. Taking into account that these extraction protocols are pretended to be implemented at industrial level, ASE has been one of the preferred options for solid–liquid extraction of antioxidants. Different materials from *Olea europaea* have been characterised because of the high content of antioxidant phenolic compounds. Japón-Luján *et al.* optimised an ASE method for isolation of extracts enriched in oleuropein and other bioactive phenols such as verbascoside, apigenin-7-glucoside and luteolin-7-glucoside from olive leaves.³⁰ Under optimal working conditions, complete extraction without degradation of the target compounds was achieved in 13 min. The same authors worked in 2007 with olive pomace, a semisolid residue from the olive oil industry, and used ASE to obtain potent antioxidants such as hydroxytyrosol together with other olive phenols. The extractions were carried out with ethanol–water mixtures, which provided an added value to the extraction protocols due to compatibility of extracts for human consumption.³¹

Residues from the wine industry have also been extensively studied using ASE for isolation of phenolic compounds. Luque-Rodríguez *et al.* proposed a

method for extraction of phenols from grape skins by ASE using ethanol–water mixtures.⁴⁰ Higher extraction efficiencies were obtained by ASE for certain families of phenols such as anthocyanins, total phenols and flavanols than those provided by conventional solid–liquid extraction based on maceration. The same authors also proposed ASE as extraction technique for isolating phenolic compounds and derivatives from vine shoots of *Vitis vinifera*.⁷³ This research proved the significance of the extraction conditions on the qualitative profile of the compounds extracted. The extracts obtained under operation conditions that maximised the concentration of total phenols were especially rich in low molecular mass compounds from lignin degradation (e.g. vanillin, syringaldehyde), while those extracts obtained at low temperature were richer in phenolic acids, particularly those formed by hydrolysis of tannins. This research supports the great chemical variability of antioxidants obtained depending on the ASE extraction protocol. Ju *et al.* have studied the effects of the solvent and temperature on the extraction efficiency of anthocyanins and total phenols from dried red grape skin.⁷⁴ They revealed the significance of both variables on the qualitative profile of the extracts. As example acidified water extracts obtained at 100 °C reported the highest levels of monoglucoside phenols; acidified methanol extracts obtained at 60 °C provided the highest levels of total anthocyanins while at 120 °C the extract contained the highest level of total phenols.

Phenolic extracts have also been obtained from diverse materials such as apple peel and pulp⁷⁵ or rosemary (*Rosmarinus officinalis*).³⁴ Herrero *et al.* have compared the phenolic extracts obtained from rosemary using various extraction techniques and they concluded that SHLE was the most efficient alternative in terms of extraction yield, antioxidant activity and total concentration of phenols.³⁴

5.7.3.2 Essential Oils

Essential oils are very appreciated thanks to their applications in pharmaceutical, cosmetic and food industries. For this reason, it is of great interest to characterise essential oil components isolated from different varieties of aromatic plants. In order to preserve the stability of essential oils, high-efficient, fast, simple and automatable methods are demanded for preparation of extracts. The most common techniques for the isolation of essential oils have been classical steam distillation and maceration. However, the loss of volatile compounds that contribute to the quality of essential oils, low extraction efficiency and degradation effects are common in methods based on classical extraction techniques. That is the reason why other alternatives such as ASE have been implemented for preparation of essential oils.

The number of studies found in the literature supports the superiority of ASE for the extraction of essential oils versus conventional alternatives. In fact, a number of studies compare qualitatively and quantitatively the essential oils obtained using ASE with those obtained using other approaches. One example is the research carried out by Tam *et al.*, who extracted

pharmacological essential oils from *Cyperus rotundus* using three different techniques: hydrodistillation, SFE and ASE.⁷⁶ The last exhibited the highest extraction efficiency for α -copaene, cyperene, β -selinene, β -cyperone and α -cyperone, while SFE reported the best selectivity for extraction of β -cyperone and α -cyperone.

Despite the interest of ASE application for extraction of essential oils began practically since this technique was developed, the operating conditions for isolation of essential oils is still subject of study. In 2000 Gámiz-Gracia *et al.* proposed a continuous extraction method using bidistilled water as solvent for the isolation of essential oil from fennel (*Foeniculum vulgare*), a medicinal plant traditionally used for the treatment of several stomach affections and obesity.⁷⁷ The authors compared their ASE-based method to others based on traditional extraction techniques proving the superiority of ASE in terms of rapidity, efficiency and cleanliness. Water was also used as solvent by Ozel *et al.* for isolation of essential oils from *Thymbra spicata*, where the optimised extraction was run under 150 °C and 3 MPa for 30 min.⁷⁸

On the other hand, Schaneberg *et al.* compared four different procedures for extraction of essential oils from *Cymbopogon citrates* (lemon grass), demonstrating the superiority of ASE in general terms. Apart from that, the composition of the extract was modified as demanded. Dichloromethane extracts contained the highest concentration of marker compounds such as geraniol, limonene, neral and citronellal (13%), while hexane extracts contained the highest concentration of citral (75%).⁷⁹

5.7.3.3 Nutraceuticals and Drugs

The term ‘nutraceutical’ was first coined in 1989 by the Foundation for Innovation in Medicine to define ‘any substance that may be considered a food or part of a food, and provides medical or health benefits, including the prevention and treatment of diseases’. Nutraceuticals do not only maintain, support and normalise any physiologic or metabolic function, but can also potentiate, antagonise, or otherwise modify these functions.^{80,81} In general, nutraceuticals may include dietary fibres, different types of phenolic compounds and antioxidants, polyunsaturated fatty acids, amino acids, proteins and minerals. Therefore, this concept is not referred to a specific family of compounds.

In the last years, ASE is becoming more and more popular for extraction of nutraceuticals. Among them, isoflavones is one of the most studied groups of compounds due to their widely recognised health benefits against menopausal problems as well as their possible preventive role in breast and prostate cancer, osteoporosis and cardiovascular diseases.^{82–84} The critical point in the extraction of isoflavones is to avoid degradation since some isoflavone derivatives are particularly sensitive to hydrolysis. Superheated liquid extraction has faced up this limitation with excellent results. Rostagno *et al.* have proved the absence of degradation in the ASE extracts of isoflavones from freeze-dried soybeans. For this purpose, samples were analysed by LC–DAD

and LC–MS in a well-planned stability study involving both spiked and real samples.⁸⁵ Complementarily, Bajer *et al.* proved the efficiency of ASE for isolation of isoflavones from various plants (*Matricaria recutita*, *Rosmarinus officinalis*, *Foeniculum vulgare* and *Agrimonia eupatoria* L.).³⁶ In another study, Zgórk *et al.* compared ASE to other extraction techniques for leaching of phytoestrogenic active isoflavones from clover species (*Trifolium* L.).⁸⁶

Apart from these compounds, numerous applications based on ASE for extraction of nutraceuticals can be found in the literature. Lee *et al.* employed an ASE dynamic approach for extraction of ginsenosides from *Panax ginseng* and American ginseng as well as from health supplements.⁸⁷ The extraction process was carried out at 10 MPa and 120 °C for 20 min. In a recent research, Hu *et al.* optimised an SHLE procedure for isolation of gingerols from *Zingiber officinale* Roscoe using ethanol as solvent versus water to increase the extraction efficiency.⁸⁸

Superheated liquid extraction has been massively used for isolation of natural products from medicinal plants with pharmacological purposes. Most of these applications are focused on extraction protocols to isolate target compounds with known pharmacological effects such as those by Ong for extraction of glycyrrhizin from *Radix glycyrrhizae*⁸⁹ or that for isolation of berberine and aristolochic acids from different medicinal plants.⁹⁰ Other final purpose of extraction is the identification of bioactive components to explain the pharmacological effects of well-known medicinal plants. One example is the study carried out by Lao *et al.* who optimised an ASE method for isolation of bioactive components (ferulic acid, ligustilide and other phthalides such as butylidenephthalide) from *Angelica sinensis* (Danggui), a well-known Chinese medicine plant.⁹¹ These active components were identified by GC–MS as a first test to elucidate the proved pharmacological effects of the plant.

5.8 Case Study

As a test to evaluate the efficiency of SHLE for isolation of natural products, this approach was applied to characterisation of vine shoots, an agricultural residue obtained in wine-producing countries. The extraction efficiency was assessed by measurement of total phenolic compounds using the Folin–Ciocalteu test and by analysis of phenolic extracts by LC–DAD. The vine shoot variety selected for this study was Pedro-Ximénez due to the geographical importance of this variety in the area where the study was developed. Extraction tests were performed with 1 g of dry material.

5.8.1 Optimisation of the Main Variables Involved in SHLE

The influence of the main variables involved in the leaching process was estimated with a multivariate study. These variables were the percentage of ethanol, temperature and extraction time, while the response variable was the yield of phenolic compounds extracted from vine shoots in as short a time as

Table 5.2 Summary of the experimental designs used for SHL extraction of phenolic compounds from vine shoots.

Design	Variable	Tested range		Selected conditions
		First screening	Second screening	
multivariate	ethanol (%)	20–80	80–100	80
	temperature (°C)	120–180	180–240	240
	time (min)	20–60	60–90	60
univariate	pH	1–13		3

First complete factorial design					
Effect	Sum of squares	DF	Mean square	F ratio	P value
ethanol	50.0	1	50.0	18.72	0.0125
temperature	144.5	1	144.5	54.11	0.0018
time	40.5	1	40.5	15.17	0.0176
total error	10.6818	4	2.67045		
total (corr)	256.182	10			

$R^2 = 95.8304\%$; $R^2(\text{adj for DF}) = 89.5759\%$

Second complete factorial design					
Effect	Sum of squares	DF	Mean square	F ratio	P value
ethanol	50.0	1	50.0	6.05	0.0124
temperature	264.5	1	264.5	32.02	0.0016
time	12.5	1	12.5	1.51	0.0176
total error	33.0455	4	8.26136		
total (corr)	368.545	10			

$R^2 = 91.0335\%$; $R^2(\text{adj for DF}) = 77.5839\%$

possible. The tested ranges and the selected values are shown in Table 5.2 as well as a detailed information about the designs used and the results obtained. The applied pressure (10 MPa) was high enough to guarantee the liquid state of the ethanol–water mixtures in all instances. A complete factorial design was selected for the first approach. The results showed that the three variables had significant positive effects; therefore, the highest value of each variable was chosen as the lowest value in a second complete factorial design. The analysis of the data obtained in the second design showed that the only significant variable was the temperature; the percentage of ethanol and extraction time had negative and positive effects, respectively. According to its effect, 80% ethanol (v/v) was selected. However, in the case of the time, the most reasonable option was to select the shortest (60 min), because the increase of efficiency for longer times was not statistically significant. Under these conditions, higher temperatures (270 °C and 300 °C) were tested, thus increasing the amount of total phenolics extracted. Nevertheless, two trends were also detected, which made inadvisable the use of temperatures above 240 °C: the strong increase of the burnt wood smell of the extract and the decrease in the concentration, or even the disappearance of groups of phenolic compounds such as phenolic aldehydes. Therefore, 240 °C was the temperature selected for further studies.

5.8.2 Influence of Extraction pH

The influence of pH on the yield of the process under the selected conditions was also investigated by a univariate approach. Thus, extractions were carried out adjusting the pH of the extractant at 1, 3, 5, 7, 9, 11 and 13. Figure 5.8 revealed that the yield of phenolic compounds enormously increased as pH decreased. This result can be ascribed to the fact that processing of lignocellulosic materials with acidified water facilitates the breakage of ether linkages in lignin, especially under high temperature conditions, generating a great number of low molecular weight phenols. Nevertheless, fast corrosion of capillary tubes of the system was observed after only a few extraction cycles at pH 1. Consequently, a minimum pH of 3 was used, for which no trace of corrosion was detected after numerous extractions.

5.8.3 Comparison of SHLE with MAE and UAE for Extraction of Vine Shoots

The extraction efficiency of SHLE was compared with that provided by protocols based on MAE and UAE. In both cases 1 g of milled vine shoots was placed into the extraction vessel with 20 mL 80% (v/v) aqueous ethanol at pH 3. In the MAE process, the vessel was positioned at the suited zone for irradiation with focused microwaves. The auxiliary energy was applied at 140 W irradiation power for 5 min, after which the solid residue was removed by centrifugation prior to analysis of the extract. For the UAE process, the

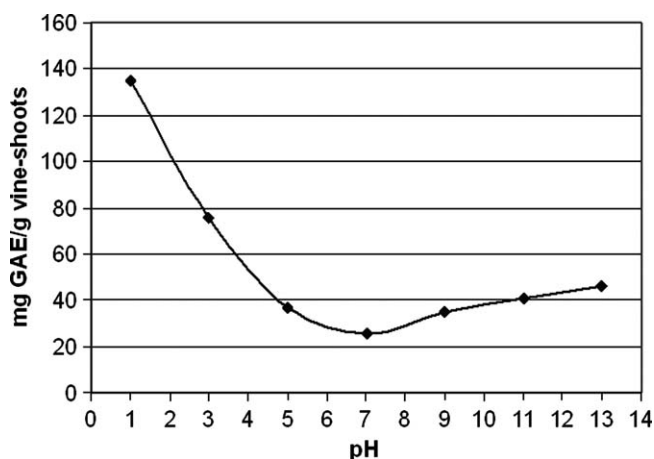


Figure 5.8 Influence of pH on the efficiency of superheated liquid extraction of phenolic compounds from vine-shoots using 80% ethanol (v/v), 240°C and 60 min as extraction parameters. Response variable is the total phenol content measured by the Folin–Ciocalteu test and expressed as mg equivalent of gallic acid/g vine shoots.

Reproduced with permission of American Chemical Society from J. M. Luque-Rodríguez *et al.*, *J. Agric. Food Chem.*, 2006, **54**, 8775.

ultrasonic probe was immersed into the extraction mixture for sonication at 280 W irradiation power for 10 min with a duty cycle of 70% (0.7 s/s irradiation cycles). Similarly, the extract was isolated by centrifugation prior to analysis.

The leaching efficiencies of the three extraction methods were compared in terms of concentration of total phenols estimated by the F–C test. Attending to the results, SHLE provided the highest concentration of phenolic compounds expressed as μg of gallic acid per gram of initial solid vine shoots (3252 $\mu\text{g/g}$ versus 2732 and 2007 $\mu\text{g/g}$ obtained with UAE and MAE, respectively). Therefore, SHLE seems to be the suited strategy for extraction of phenolic compounds from vine shoot, which can be considered a potential vegetal source to obtain this valuable fraction.

5.9 Conclusions: Benefits and Limitations of ASE for Isolation of Natural Products

As a final conclusion of this chapter it can be said that ASE is one of the most competitive solid–liquid extraction techniques for isolation of natural products. The foundations of the technique, its high efficiency for leaching, its ease of scaling-up applications to industrial level, its automatability and its adaptation to the ‘green’ concept contributes to the selection of ASE as one of the main techniques for extraction of natural products. The development of new instrumental configurations with high versatility is demanded to improve the possibilities of this technique. Replacement of toxic organic solvents is a pending goal.

Acknowledgements

The authors would like to thank M. D. Luque de Castro for her suggestions to plan and organise this chapter. The Spanish Ministerio de Ciencia e Innovación (MICINN) and European FEDER program are thanked for financial support through project CTQ2009-07430. F.P.C. is also grateful to the MICINN for a Ramón y Cajal contract (RYC-2009-03921).

References

1. B. E. Richter, B. A. Jones, J. L. Ezzell and N. L. Porter, *Anal. Chem.*, 1996, **68**, 1033.
2. S. B. Hawthorne, Y. Yang and D. J. Miller, *Anal. Chem.*, 1994, **66**, 2912.
3. <http://www.dionex.com/en-us/products/sample-preparation/ase/lp-72855.html/>.
4. J. González-Rodríguez, P. Pérez-Juan and M. D. Luque de Castro, *J. Chromatogr. A*, 2004, **1038**, 3.
5. J. González-Rodríguez, P. Pérez-Juan and M. D. Luque de Castro, *Chromatographia*, 2003, **57**, 363.

6. M. P. Delgado de la Torre, F. Priego-Capote and M. D. Luque de Castro, *J. Agric. Food Chem.*, 2012, **60**, 3409.
7. J. A. Pérez-Serradilla, R. Japón-Luján and M. D. Luque de Castro, *Anal. Bioanal. Chem.*, 2008, **392**, 1241.
8. S. Morales-Muñoz, J. L. Luque-García, M. J. Ramos, A. Fernández-Alba and M. D. Luque de Castro, *Anal. Chim. Acta*, 2005, **552**, 50.
9. J. L. Luque-García and M. D. Luque de Castro, *Trends Anal. Chem.*, 2004, **23**, 102.
10. J. L. Luque-García and M. D. Luque de Castro, *Analyst*, 2003, **128**, 980.
11. *Dionex Application Note 346*. Totally automated sample preparation using accelerated solvent extraction (ASE[®]) coupled with Gilson ASPEC: the determination of dianthrone in St. John's wort.
12. M. Papagiannopoulos, B. Zimmermann, A. Mellenthin, M. Krappe, G. Maio and R. Galensa, *J. Chromatogr. A*, 2002, **958**, 9.
13. Y. Zhang, C. Liu, M. Yu, Z. Zhang, Y. Qi, J. Wang, G. Wu, S. Li, J. Yu and Y. Hu, *J. Chromatogr. A*, 2011, **1218**, 2827.
14. J. L. Luque-García and M. D. Luque de Castro, *J. Chromatogr. A*, 2003, **1010**, 129.
15. P. Kritzer and E. Dinjus, *Chem. Eng. J.*, 2001, **83**, 207.
16. R. H. Perry, D. W. Green and J. O. Maloney, *Perry's Chemical Engineers' Handbook*, McGraw-Hill, Columbus, OH, 1984.
17. M. P. Delgado-Torre, C. Ferreira-Vera, F. Priego-Capote, P. Pérez-Juan and M. D. Luque de Castro, *J. Agric. Food Chem.*, 2012, **60**, 3051.
18. C. G. Arnold, M. Berg, S. Müller, U. Domman and R. P. Schwarzenbach, *Anal. Chem.*, 1998, **70**, 3094.
19. W. H. Ding and J. C. H. Fann, *Anal. Chim. Acta*, 2000, **408**, 291.
20. D. Ragnvaldsson, S. Brochu and H. Wingfors, *J. Hazardous Mat.*, 2007, **142**, 418.
21. T. M. Pawloski and C. F. Poole, *J. Agric. Food Chem.*, 1998, **46**, 3124.
22. M. D. Luque de Castro, M. M. Jiménez-Carmona and V. Fernández-Pérez, *Trends Anal. Chem.*, 1999, **18**, 708.
23. F. Portet-Koltalo, K. Oukebdane, F. Dionnet and P. L. Desbène, *Anal. Bioanal. Chem.*, 2008, **390**, 389.
24. F. Portet-Koltalo, K. Oukebdane, F. Dionnet and P. L. Desbène, *Anal. Chim. Acta*, 2009, **651**, 48.
25. W. Wang, B. Meng, X. Lu, Y. Liu and S. Tao, *Anal. Chim. Acta*, 2007, **602**, 211.
26. P. Wang, Q. Zhang, Y. Wang, T. Wang, X. Li, L. Ding and G. Jiang, *Anal. Chim. Acta*, 2010, **663**, 43.
27. L. Wennrich, P. Popp and M. Möder, *Anal. Chem.*, 2000, **72**, 546.
28. M. P. K. Choi, K. K. C. Chan, H. W. Leung and C. W. Huie, *J. Chromatogr. A*, 2003, **983**, 153.
29. F. Yung-Chang, Z. She-Li, C. Mei-Lan, S. Chao and Z. Yan, *Chin. J. Anal. Chem.*, 2010, **38**, 1785.
30. R. Japón-Luján and M. D. Luque de Castro, *J. Chromatogr. A*, 2006, **1136**, 185.

31. R. Japón-Luján and M. D. Luque de Castro, *J. Agric. Food Chem.*, 2007, **55**, 3629.
32. I. Borrás-Linares, D. Arráez-Román, M. Herrero, E. Ibáñez, A. Segura-Carretero and A. Fernández-Gutiérrez, *J. Chromatogr. A*, 2011, **1218**, 7682.
33. R. Gautam, A. Srivastava and S. M. Jachak, *Phytochem. Anal.*, 2011, **22**, 153.
34. M. Herrero, M. Plaza, A. Cifuentes and E. Ibáñez, *J. Chromatogr. A*, 2010, **1217**, 2512.
35. A. L. Dawidowicz, E. Rada, D. Wianowska, M. Mardarowicz and J. Gawdzik, *Talanta*, 2008, **76**, 878.
36. T. Bajer, M. Adam, L. Galla and K. Ventura, *J. Sep. Sci.*, 2007, **30**, 122.
37. V. Flores-Péres, J. Saffi, M. I. S. Melecchi, F. C. Abad, R. A. Jacques, M. M. Martinez, E. C. Oliveira and E. B. Caramão, *J. Chromatogr. A*, 2006, **1105**, 115.
38. R. A. Jacques, L. D. S. Freitas, V. Flores-Péres, C. Dariva, J. V. Oliveira and E. B. Caramão, *J. Sep. Sci.*, 2006, **29**, 2780.
39. N. Ralota, S. Lacorte, A. Alves and D. Barceló, *J. Chromatogr. A*, 2006, **1114**, 198.
40. J. M. Luque-Rodríguez, M. D. Luque de Castro and P. Pérez-Juan, *Bioresource Technol.*, 2007, **98**, 2705.
41. M. B. Hossain, N. P. Brunton, A. B. Martin-Diana and C. Barry-Ryan, *Food Funct.*, 2010, **1**, 269.
42. J. Shi, H. Nawaz, J. Pohorly, G. Mittal, Y. Kakuda and Y. Jiang, *Food Rev. Int.*, 2005, **21**, 139.
43. E. Reverchon and I. De Marco, *J. Supercrit. Fluids*, 2006, **38**, 146.
44. W. Siri Wong, K. Thirakhupt, D. Sitticharoenchai, J. Rohitrattana, P. Thongkongwong, M. Borjan and M. Robson, *Ecol. Indic.*, 2009, **9**, 878.
45. T. Okuda, N. Yamashita, H. Tanaka, H. Matsukawa and K. Tanabe, *Environ. Int.*, 2009, **35**, 815.
46. M. A. Euterpio, C. Cavaliere, A. L. Capriotti and C. Crescenzi, *Anal. Bioanal. Chem.*, 2011, **401**, 2977.
47. C. Mantell, M. Rodríguez and E. Martínez de la Ossa, *Sep. Sci. Technol.*, 2003, **38**, 3689.
48. C. Mantell, M. Rodríguez and E. Martínez de la Ossa, *Eng. Life Sci.*, 2003, **3**, 38.
49. B. Palenzuela, L. Arce, A. Macho, E. Muñoz, A. Ríos and M. Valcárcel, *Anal. Bioanal. Chem.*, 2004, **378**, 2021.
50. A. Chafer, M. C. Pascual-Martí, A. Salvador and A. Berna, *J. Sep. Sci.*, 2005, **28**, 2050.
51. M. T. Escribano-Bailón and C. Santos-Buelga, Polyphenol Extraction from Foods, in *Methods in Polyphenols Analysis*, ed. C. Santos-Buelga and G. Williamson, RSC, Cambridge, UK, 2003.
52. M. Letellier and H. Budzinski, *Analyst*, 1999, **124**, 5.
53. V. López-Ávila, R. Young and N. Teplitsky, *J. AOAC Int.*, 1996, **79**, 142.

54. M. P. Llompарт, R. A. Lorenzo, R. Cela and J. R. Jocelyn-Paré, *Analyst*, 1997, **122**, 133.
55. A. Taamalli, D. Arráez-Román, E. Barrajón-Catalán, V. Ruiz-Torres, A. Pérez-Sánchez, M. Herrero, E. Ibañez, V. Micol, M. Zarrouk, A. Segura-Carretero and A. Fernández-Gutiérrez, *Food Chem. Toxicol.*, 2012, **50**, 1817.
56. E. Reverchon and F. Senatore, *J. Agric. Food Chem.*, 1994, **92**, 154.
57. M. D. Luque de Castro and F. Priego-Capote, *Analytical Applications of Ultrasound*, Elsevier, Amsterdam, 2007.
58. J. Fojtová, L. Lojková and V. Kubán, *J. Sep. Sci.*, 2008, **31**, 162.
59. L. Zhou, J. Le Grandois, E. Marchioni, M. Zhao, S. Ennahar and F. Bindler, *J. Agric. Food Chem.*, 2010, **58**, 9912.
60. J. Zhao, S. P. Li, F. Q. Yang, P. Li and Y. T. Wang, *J. Chromatogr. A*, 2006, **1108**, 188.
61. M. M. Delgado-Zamarreño, M. Bustamante-Rangel, A. Sánchez-Pérez and R. Carabias-Martínez, *J. Chromatogr. A*, 2004, **1056**, 249.
62. J. R. Denery, K. Dragull, C. S. Tang and Q. X. Li, *Anal. Chim. Acta*, 2004, **501**, 175.
63. J. Poerschmann and R. Carlson, *J. Chromatogr. A*, 2006, **1127**, 18.
64. B. Jansen, K. G. J. Nierop, M. C. Kotte, P. Voogt and J. M. Verstraten, *App. Geochem.*, 2006, **21**, 1006.
65. E. Cincchetti and A. Chaintreau, *J. Sep. Sci.*, 2009, **32**, 1957.
66. S. Vichi, C. Santini, N. Natali, C. Riponi, E. López-Tamames and S. Buxaderas, *Food Chem.*, 2007, **102**, 1260.
67. N. Natali, F. Chinnici and C. Riponi, *J. Agric. Food Chem.*, 2006, **54**, 8190.
68. A. L. Dawidowicz, N. B. Czapczynska and D. Wianowska, *Talanta*, 2012, **94**, 140.
69. S. K. Cho, A. M. Abd El-Aty, J. H. Choi, M. R. Kim and J. H. Shim, *J. Pharm. Biomed. Anal.*, 2007, **44**, 1154.
70. X. Liu, A. M. Abd El-Aty, S. K. Cho, A. Yang, J. H. Park and J. H. Shim, *Biomed. Chromatogr.*, 2012.
71. J. Vial, D. Thiébaud, P. Sassiati, M. S. Beldean-Galea, M. J. Gómez-Ramos, G. Cognon, S. Mallipattu, B. Teillet and M. Bouzige, *J. Chromatogr. Sci.*, 2010, **48**, 267.
72. J. Shen and X. Shao, *Anal. Bioanal. Chem.*, 2005, **383**, 1003.
73. J. M. Luque-Rodríguez, P. Pérez-Juan and M. D. Luque de Castro, *J. Agric. Food Chem.*, 2006, **54**, 8775.
74. Z. Jong Ju and L. R. Howard, *J. Agric. Food Chem.*, 2003, **51**, 5207.
75. R. M. Alonso-Salces, E. Korta, A. Barranco, L. A. Berrueta, B. Gallo and F. Vicente, *J. Chromatogr. A*, 2001, **933**, 37.
76. C. U. Tam, F. Q. Yang, Q. W. Zhang, J. Guan and S. P. Li, *J. Pharm. Biomed. Anal.*, 2007, **44**, 444.
77. L. Gámiz-Gracia and M. D. Luque de Castro, *Talanta*, 2000, **51**, 1179.
78. M. Z. Ozel, F. Gogus and A. C. Lewis, *Food Chem.*, 2003, **82**, 381.
79. B. T. Schaneberg and I. A. Khan, *J. Agric. Food Chem.*, 2002, **50**, 1345.

80. W. Andlauer and P. Furst, *Food Res. Int.*, 2002, **35**, 171.
81. G. Hardy, *Nutrition*, 2000, **16**, 688.
82. V. Beck, U. Rohr and A. Jungbauer, *J. Steroid Biochem.*, 2005, **94**, 499.
83. M. Imjof, A. Gocan, F. Reithmayr, M. Lipovac, C. Schimitzek, P. Chedraui and J. Huber, *Maturitas*, 2006, **55**, 76.
84. F. Occiuto, R. De Pascuale, G. Guglielmo, D. R. Palumbo, G. Zangla, S. Samperi, A. Renzo and C. Circosta, *Phytother. Res.*, 2007, **21**, 130.
85. M. A. Rostagno, M. Palma and C. G. Barroso, *Anal. Chim. Acta*, 2004, **522**, 169.
86. G. Zgórká, *Talanta*, 2009, **79**, 46.
87. H. K. Lee, H. L. Koh, E. S. Ong and S. O. Woo, *J. Sep. Sci.*, 2002, **25**, 160.
88. J. Hu, Z. Guo, M. Glasius, K. Kristensen, L. Xiao and X. Xu, *J. Chromatogr. A*, 2011, **1218**, 5765.
89. E. S. Ong, *J. Sep. Sci.*, 2002, **25**, 825.
90. E. S. Ong, S. O. Woo and Y. L. Yong, *J. Chromatogr. A*, 2000, **904**, 57.
91. S. C. Lao, S. P. Li, K. K. W. Kan, P. Li, J. B. Wang, T. T. X. Dong and K. W. K. Tsim, *Anal. Chim. Acta*, 2004, **526**, 131.
92. A. Kubátová, D. J. Miller and S. B. Hawthorne, *J. Chromatogr. A*, 2001, **187**, 9236.
93. J. Suomi, H. Siren, K. Hartonen and M. L. Riekkola, *J. Chromatogr. A*, 2000, **73**, 868.
94. E. S. Ong and S. O. Woo, *Electrophoresis*, 2001, **22**, 2236.
95. E. S. Ong and S. N. B. Apandi, *Electrophoresis*, 2001, **22**, 2723.
96. A. Brachet, S. Rudaz, L. Mateus, P. Christen and J. L. Veuthey, *J. Sep. Sci.*, 2001, **24**, 865.
97. E. S. Ong and S. M. Len, *Anal. Chim. Acta*, 2003, **81**, 482.
98. M. McCooey, L. Ding, G. J. Gardner, C. A. Fraser, J. Lam, R. E. Sturgeon and Z. Mester, *Anal. Chem.*, 2003, **75**, 2538.
99. E. S. Ong and S. M. Len, *J. Sep. Sci.*, 2003, **26**, 1533.
100. M. Waksmondzka, A. Petruczynik, A. Dragan, D. Wianowska and A. L. Dawidowick, *Phytochem. Anal.*, 2004, **15**, 313.
101. H. Wu, M. Chen, Y. Fan, F. Elsebaei and Y. Zhu, *Talanta*, 2012, **88**, 222.
102. R. M. Alonso-Salces, E. Korta, A. Barranco, L. A. Berrueta, B. Gallo and F. Vicente, *J. Agric. Food. Chem.*, 2001, **49**, 3761.
103. M. Palma, Z. Piñeiro and C. G. Barroso, *J. Chromatogr. A*, 2001, **169**, 921.
104. M. Palma, Z. Piñeiro and C. G. Barroso, *J. Chromatogr. A*, 2002, **1**, 968.
105. M. Ollanketo, A. Peltoketo, K. Hartonen, R. Hiltunen and M. L. Riekkola, *Eur. Food. Res. Technol.*, 2002, **158**, 215.
106. E. Ibañez, A. Kubatova, F. J. Señoráns, S. Cavero, G. Reglero and S. B. Hawthorne, *J. Agric. Food. Chem.*, 2003, **51**, 375.
107. M. Papagiannopoulos, H. R. Wollseifen, A. Mellenthin, B. Haber and R. Galesa, *J. Agric. Food. Chem.*, 2004, **52**, 3784.
108. Z. Piñeiro, M. Palma and C. G. Barroso, *J. Chromatogr. A*, 2004, **19**, 1026.

109. M. Herrero, E. Ibañez, F. J. Señoráns and A. Cifuentes, *J. Chromatogr. A*, 2004, **195**, 1047.
110. B. Klejdus, J. Vacek, V. Adam, J. Zehnalek, R. Kizek, L. Trnkova and V. Kuban, *J. Chromatogr. B*, 2004, **101**, 806.
111. S. Erdogan, B. Ates, G. Durmaz, I. Yilmaz and T. Seckin, *Food Chem. Toxicol.*, 2011, **49**, 1592.
112. A. Basile, M. M. Jiménez-Carmona and A. A. Clifford, *J. Agric. Food Chem.*, 1998, **46**, 5205.
113. A. Ammann, D. C. Hinz, R. S. Addleman, C. M. Way and B. W. Wenclawiak, Fresenius, *J. Anal. Chem.*, 1999, **364**, 650.
114. M. M. Jiménez-Carmona, J. L. Uberta and M. D. Luque de Castro, *J. Chromatogr. A*, 1999, **855**, 625.
115. V. Fernández-Pérez, M. M. Jiménez-Carmona and M. D. Luque de Castro, *Analyst*, 2000, **125**, 481.
116. A. Kubátová, A. J. M. Lagadec, D. J. Miller and S. B. Hawthorne, *Flavour Frag. J.*, 2001, **16**, 64.
117. R. Soto-Ayala and M. D. Luque de Castro, *Food Chem.*, 2001, **75**, 109.
118. O. Chienthavorn and W. Insuan, *Anal. Lett.*, 2004, **37**, 2393.
119. C. H. Deng, N. Li and X. M. Zhang, *J. Chromatogr. A*, 2004, **149**, 1059.
120. C. H. Deng, N. Yao, A. Wang and X. M. Zhang, *Anal. Chim. Acta*, 2005, **536**, 237.
121. M. Z. Ozel and H. Kaymaz, *Anal. Bioanal. Chem.*, 2004, **379**, 1127.
122. E. Boselli, V. Velazco, M. F. Caboni and G. Lercker, *J. Chromatogr. A*, 2001, **239**, 917.
123. R. K. Richardson, *J. AOAC Int.*, 2001, **84**, 1522.

CHAPTER 6

Supercritical Fluid Extraction

JOSE A. MENDIOLA, MIGUEL HERRERO,
MARÍA CASTRO-PUYANA AND ELENA IBÁÑEZ*

Instituto de Investigación en Ciencias de la Alimentación/Institute of Food Science Research CIAL (CSIC-UAM), C/Nicolás Cabrera, 9 (Campus de Cantoblanco), 28049 Madrid, Spain

*Email: elena@ifi.csic.es

6.1 Introduction

There is a wide range of classical extraction techniques used to extract bioactive compounds from natural matrices. Although these techniques are routinely used, they have several recognized drawbacks; besides low selectivity and/or low extraction yields, they are labor intensive, it is difficult to implement automation and therefore they are more prone to present low reproducibility. These shortcomings can be partially or completely overcome by using newly developed advanced extraction techniques. These new extraction techniques are usually faster, more selective towards the compounds to be extracted, and, also very important nowadays, more environmentally friendly. In fact, by using the considered advanced extraction techniques, the use of toxic solvents is highly limited or greatly reduced.

This is especially true for supercritical fluid extraction (SFE), a technique based on the use of solvents at temperatures and pressures above their critical points. SFE can be a fast, efficient, and clean method for the extraction of natural products from several matrices. The ease of tuning the operating conditions in order to increase the solvation power makes this technology a good option for the selective recovery of several types of substances.^{1,2}

RSC Green Chemistry No. 21

Natural Product Extraction: Principles and Applications

Edited by Mauricio A. Rostagno and Juliana M. Prado

© The Royal Society of Chemistry 2013

Published by the Royal Society of Chemistry, www.rsc.org

6.2 Fundamentals of Supercritical Fluid Extraction

Supercritical fluid extraction was first introduced in 1879 by Hannay and Hogarth. Despite the advantages associated to the use of supercritical fluids as extracting agents, it was not until around 1960 that this technique started to be thoroughly investigated as an alternative to conventional extraction methods such as solid–liquid extraction (SLE) and liquid–liquid extraction (LLE), both requiring large amounts of hazardous chemicals such as chlorinated solvents.

The discovery of the critical phenomena is attributed to Charles Cagniard de la Tour in 1822.³ Experiments on steam engines in the late 17th and early 18th centuries motivated interest in the behavior of fluids at high temperatures and pressures. The discovery of what we now call ‘the critical point’ came about with Cagniard de la Tour’s experiments in acoustics; he placed a ball in a digester barrel partially filled with liquid. Upon rolling the device, a splashing sound was generated as the solid ball penetrated the liquid–vapor interface. But heating the system far beyond the boiling point the splashing sound ceased above a certain temperature. This marks the discovery of the supercritical fluid phase. He measured the critical temperature at which the interface tension vanished, as determined by the disappearance of the meniscus, for different substances such as water, alcohol, ether, and carbon bisulfide. In 1869, the term ‘critical point’ was coined by Thomas Andrews, who further elucidated the meaning of Cagniard de la Tour’s *état particulier*.³ The important concept of universality of critical phenomena was introduced by Pierre Curie, who discovered that ferromagnetic materials become demagnetized above the critical temperature.⁴ The field of critical phenomena has blossomed and now forms a keystone of modern science, both experimental and theoretical, and its development exemplifies how a topic of purely fundamental research can diversify into initially unforeseeable directions.

6.2.1 Physical Properties of Supercritical Fluids

As the substance approaches its critical temperature, the properties of its gas and liquid phases converge, resulting in only one phase at the critical point: a homogeneous supercritical fluid. The heat of vaporization is zero at and beyond this critical point, and so no distinction exists between the two phases. On the pressure–temperature diagram (Figure 6.1A), the point at which critical temperature and critical pressure meet is called the critical point of the substance. Above the critical temperature, a liquid cannot be obtained by increasing the pressure, even though a solid may be formed under sufficient pressure. The critical pressure is the vapor pressure at the critical temperature. In the vicinity of the critical point, a small increase in pressure causes large increases in the density of the supercritical phase (Figure 6.1B).

Physical properties of supercritical fluids are between those of a gas and those of a liquid, as can be observed in Table 6.1, in which some data taken from Pereda, Bottini, and Brignole⁵ has been included. For instance, the density of a supercritical fluid is similar to a liquid while its viscosity is similar

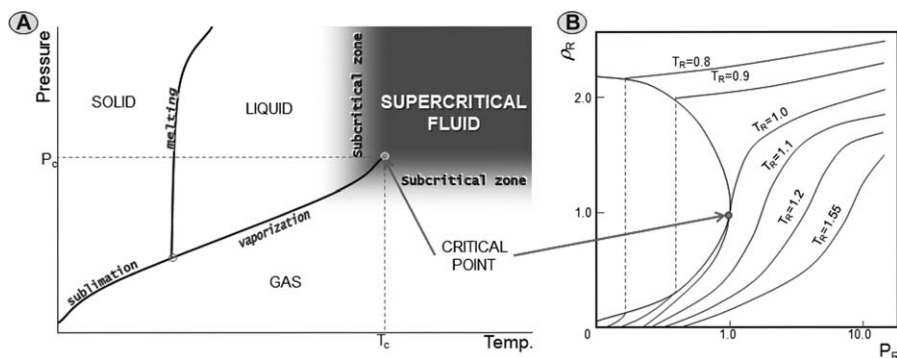


Figure 6.1 Carbon dioxide pressure–temperature phase diagram (A) and density–pressure phase diagram at different temperatures (B) considering reduced variables ($T_R = T/T_C$, $P_R = P/P_C$ and $\rho_R = \rho/\rho_C$).

Table 6.1 Comparison of the physical properties of gas, liquid and supercritical fluids.

Physical property	Gas ^a	Supercritical	Liquid ^a
density (kg m ⁻³)	0.6–2	200–900	600–1000
dynamic viscosity (mPa s)	0.01–0.3	0.1–0.3	0.2–3
thermal conductivity (W/mK)	0.01–0.25	Max. ^b	0.1–0.2
diffusion coef. (10 ⁶ m ² s ⁻¹)	10–40	0.07	0.0002–0.002
surface tension (dyn cm ⁻²)	–	–	20–40

^a At room temperature.

^b Thermal conductivity presents maximum values in the near-critical region, highly dependent on temperature.

to a gas and its diffusivity is placed between gas and liquid. Thermal conductivities are relatively high in supercritical fluids and have large values near the critical point. Surface tension is close to zero in the critical point, being similar to gases and much smaller than for liquids. Many other physical properties such as relative permittivity, solvent strength, etc., highly related to density, show large gradients with pressure above the critical point. Changes in those properties are crucial when dealing with extraction since they are related to changes in solubility and mass transfer ratios, and, therefore, related to changes in the selectivity of the solvent.

The solvent strength of a supercritical fluid can be characterized, among others, by the Hildebrand solubility parameter, δ , which relates to the density of the solvent as follows:

$$\delta = 1.25P_c^{1/2} \left[\rho / \rho_{\text{liq}} \right] \quad (6.1)$$

where P_c is the critical pressure, ρ is the gas density, and ρ_{liq} is the liquid density. At low pressures, the density of a gas is low, so the solvating power is rather low; at near critical conditions, the density increases rapidly approaching

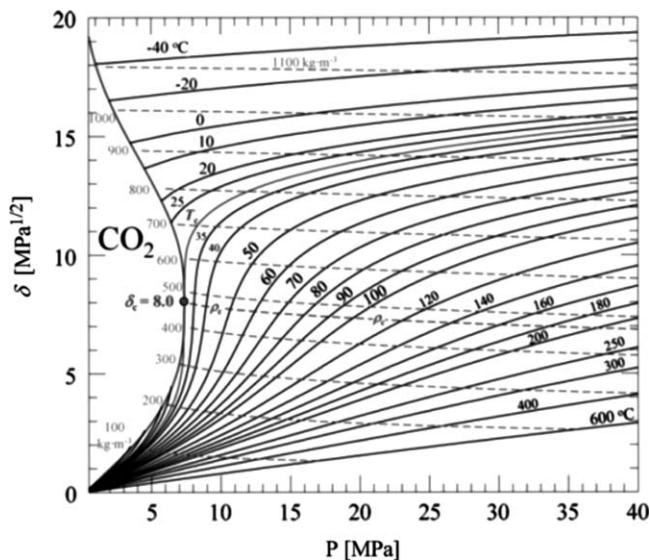


Figure 6.2 Solubility parameter of carbon dioxide.

Reprinted from Machida *et al.*,⁶ *The Journal of Supercritical Fluids*, Vol. 60, December 2011, pp. 2–15, copyright 2011, with permission from Elsevier.

that of a liquid and thus the solubility parameter increases as the critical pressure is approached. This effect can be seen graphically in Figure 6.2 in which the Hildebrand solubility parameter for CO₂ is represented as a function of the pressure for different temperatures.⁶ This is one of the key features of SFE since the solvating power of the fluid can be strongly influenced by small changes in pressure and temperature either favoring the extraction of the target compounds or the precipitation of the solutes dissolved in the supercritical fluid.

6.2.2 Supercritical Solvents

Although there is a wide range of compounds that can be used as supercritical fluids (see Table 6.2 in which the critical properties of several solvents used in SFE are given), it is true that after the Montreal Protocol, introduced in 1987 to restrict or eliminate the manufacture and use of particularly damaging ozone depleting solvents (at present signed by 170 nations), there is a worldwide pressure for the industry to adopt new sustainable processes that do not require the use of environmentally damaging organic solvents.⁷ In this sense, SFE using green solvents has been suggested as a clean alternative to hazardous processes and thus, SFE has found its growing niche.

Among the green solvents used in SFE, carbon dioxide (critical conditions = 31.2 °C and 7.38 MPa) is undoubtedly the most commonly employed. CO₂ is inexpensive, environmentally friendly and generally

Table 6.2 Critical properties of some solvents used in SFE.

<i>Solvent</i>	<i>Critical property</i>			
	<i>Temperature</i> (°C)	<i>Pressure</i> (MPa)	<i>Density</i> (kg/m ³)	<i>Solubility parameter</i> δ_{SCF} (MPa ^{1/2})
ethene	10.1	5.11	200	11.86
water	101.1	22.05	322	27.61
methanol	−34.4	8.09	272	18.20
carbon dioxide	31.2	7.38	470	15.34
ethane	32.4	4.88	200	11.86
nitrous oxide	36.7	7.26	460	14.72
sulfur hexafluoride	45.8	3.82	730	11.25
<i>n</i> -butene	−139.9	3.65	221	10.64
<i>n</i> -pentane	−76.5	3.37	237	10.43

recognized as safe (GRAS). Supercritical CO₂ (SC-CO₂) is also attractive because of its high diffusivity combined with its easily tunable solvent strength. Another advantage is that CO₂ is gaseous at room temperature and pressure, which makes extract recovery very simple and provides solvent-free extracts. Also important for food and natural products is the ability of SFE using CO₂ to be operated at low temperatures using a non-oxidant medium, which allows the extraction of thermally labile or easily oxidized compounds.⁸ As can also be seen in Table 6.2, supercritical CO₂ has a low polarity (with a low solubility parameter, around 15 MPa^{1/2}), and therefore, its efficiency to extract polar compounds from natural matrices is quite limited. To overcome this problem, polar co-solvents (methanol, ethanol, water) are commonly used in small amounts to increase the solubility of polar compounds in the supercritical mixture.

The widest application of supercritical fluids is extraction, especially with carbon dioxide. The first patent dealing with supercritical fluid extraction was filed by Messmore in 1943,⁹ although the first industrial application was developed by Zosel in 1978.¹⁰ Since then, supercritical fluids have been used to isolate natural products, but for a long time applications relied only on a few of them. The development of processes and equipment is beginning to pay off and industries are getting more and more interested in supercritical techniques. This interest is also observed in the high amount of scientific papers dealing with supercritical fluid extraction (SFE) published in recent years. Moreover, industrial applications of SFE have experienced a strong development since the 1990s in terms of patents.^{8,11}

SFE has been used in different fields such as the food, pharmaceutical, chemical, and fuel industries. Due to the absence of toxic residue in the final product, among other advantages, supercritical fluids are especially useful for extraction in two situations: (a) extracting valuable bioactive compounds such as flavors, colorants, and other biomolecules or (b) removing undesirable compounds such as organic pollutants, toxins, and pesticides.² In this chapter we will focus on the use of supercritical fluids to extract valuable compounds from vegetal and marine sources and by-products from the food industry.

6.3 Instrumentation

Supercritical fluid extraction is commonly carried out considering two basic steps: (1) extraction of soluble substances from the matrix by the supercritical fluid and (2) separation or fractionation of the extracted compounds from the supercritical solvent after the expansion.

The basic instrumentation to carry out supercritical fluid extractions should be composed of materials that are capable to withstand high pressures, typically as high as 50 MPa (although systems requiring extractions pressures as high as 70 MPa have also been used). The equipment needed is different depending if the application deals with solid or liquid samples. Figure 6.3 shows the two schemes corresponding to a SFE extractor for solid and liquid samples. As can be observed, the main differences are related to the extraction cell itself. While the solid samples equipment has an extraction vessel of a given internal volume (see, Figure 6.3.A), the liquid samples extraction plant uses an extraction column in which the extraction is performed in countercurrent mode (Figure 6.3B). Countercurrent extraction (CC-SFE) is performed introducing the sample in the system from the top of the column and the pressurized solvent from the bottom; in this process, the components distribute between the solvent and the liquid sample which flows countercurrent through the separation column. Depending on the separation factor between components to be extracted, the desired contact time between the solvent and the sample can be reached by adjusting the height of the sample introduction into the extraction column. It can also be adjusted by modifying the performance of the separation column, in terms of height and diameter, or of the packing material (structured/random, packing dimensions, surface area, etc.). Different methods have been published in the literature concerning the modeling of a countercurrent supercritical fluid extraction system. For an in-depth understanding of CC-SFE, readers are referred to previous papers published by Brunner^{12,13} and Reverchon.¹⁴ Factors such as solvent-to-feed ratio are of crucial importance in this type of extraction, as will be discussed in the following section.

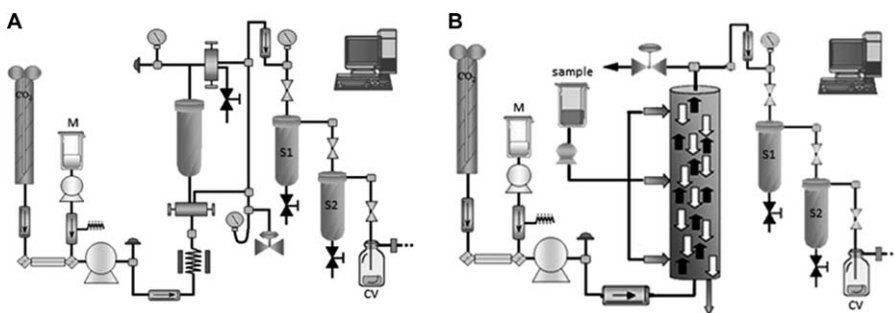


Figure 6.3 (A) Scheme of a typical SFE instrument for the extraction of solid samples. (B) Scheme of a typical SFE plant for the extraction of liquid samples. M = modifier reservoir, S1 = separator 1, S2 = separator 2, CV = collection vessel.

As can be seen in Figure 6.3, both systems are composed by a tank for the extracting solvent, usually CO₂, a pump to pressurize the gas to the desired extraction pressure, a restrictor or valve to maintain the high pressure inside the system, and a trapping vessel (or separation cell, also called fractionation cell) for the recovery of the extracts. Different factors should be optimized in order to avoid losses of extracted compounds. One of these factors is the trapping method, selection of which should be done considering the extract volatility and polarity, the volatility of the extracting agent, the volatility of the modifier (if used), and the solvent flow rate, among other parameters. Also, different trapping methods are available, such as solid trapping, liquid trapping, cool trapping, etc. In pilot or industrial systems, collection of the extracted solutes is done by rapidly reducing the pressure, increasing the temperature, or both. In this case, depressurization after the extraction can be performed in cascade considering that each separation vessel can have a particular temperature and pressure in order to have some of the extracted compounds precipitated and separated.

Additionally, the system may include another pump to introduce an organic modifier (co-solvent) that is sometimes needed to extend the solvent capabilities of, for instance, supercritical CO₂, allowing the extraction and recovery of more polar compounds.

Regarding the extraction mode, at small scale, solid samples can be extracted in dynamic or static modes or even in a combination of both. Under static conditions, the supercritical fluid is introduced in the extraction vessel and is kept in contact with the sample for a given extraction time. Once the desired time is achieved, the extract is released through the pressure restrictor to the trapping vessel. On the other hand, in a dynamic process, the supercritical fluid continuously enters the extraction vessel and flows through the sample to the separators for a cascade fractionation. In the combined mode, a static extraction is performed for a period of time, and subsequently a dynamic extraction is carried out. Medium and large scale SFE are generally carried out in dynamic conditions: the supercritical solvent flows through the solid material extracting the target compounds until the substrate is depleted. On the other hand, liquid samples, according to the design of the extractors, are commonly extracted in a continuous mode.

Figure 6.4 displays an example of a process flow diagram of a SFE process at industrial scale showing the different components needed for large-scale operations;¹⁵ in this particular case, prebiotic carbohydrates were extracted from a complex mixture using a mixture of carbon dioxide and ethanol:water. In this process, carbon dioxide (stream 1) and a co-solvent mixture (stream 2) are pumped (pumps 1 and 2) and mixed into a heat exchanger (HE1) used to guarantee that the solvent reaches the extraction cell at the target temperature. After the extraction cell, a valve is placed to control the extraction cell pressure. Carbon dioxide depressurizes through this valve and is removed as a gas (at low pressures) in stream 9; after that, carbon dioxide is recompressed up to 4–5 MPa, condensed in the heat exchanger 3 (HE3) to be pumped again as a liquid and recirculated into the system. Extracted solutes remain dissolved in the co-solvent and are withdrawn from the collection vessel in stream 11. Other

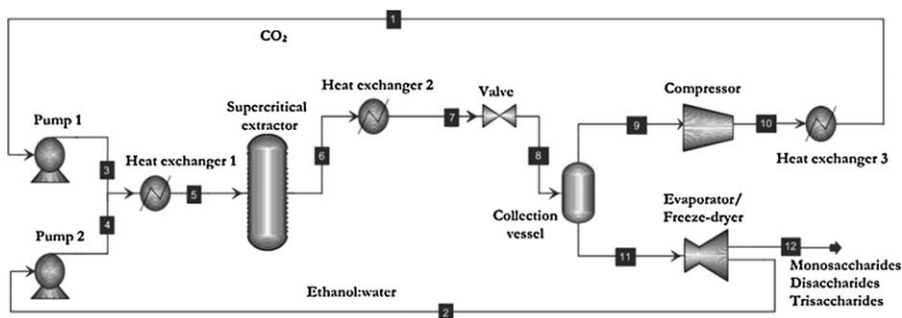


Figure 6.4 Flow diagram of SFE process at industrial scale.

Reprinted from Montañés *et al.*,¹⁵ *Journal of Chromatography A*, Vol. 1250, 10 August 2012, pp. 92–98, copyright 2012, with permission from Elsevier.

operations needed to collect and purify the extracts can be performed afterwards.

6.4 Parameters Affecting the Extraction Process

The extraction of the soluble substances from the matrix can be described by considering several steps, each one influenced by several factors that should be optimized. When dealing with solid samples, there is, at the beginning of the extraction process, diffusion of the solvent into the matrix leading to absorption of the supercritical solvent and therefore to decrease of the mass transfer resistance; after this step, soluble compounds are dissolved into the supercritical fluid and are further transferred by diffusion first into the surface of the solid and later to the bulk of the fluid phase. The extraction process ends with the transport of the solute and the bulk fluid phase and their removal from the extractor. The kinetics of the extraction process can be followed by determining the amount of extract (mass of extract or yield) as a function of process time (or solvent consumption), providing an overall extraction curve (OEC), such as the one shown in Figure 6.5. Although this figure refers to the extraction rate of artemisin,¹⁶ it shows the typical behavior of SFE. A typical extraction curve can be divided into three periods.^{2,17}

1. A constant extraction rate period (CER), characterized by the extraction of the solute contained in the surface of the particles, that is, easily accessible. The mass transfer in this step is controlled by convection.
2. A falling extraction rate period (FER), in which most of the easily accessible solute has been extracted and mass transfer starts to be controlled by diffusion.
3. A diffusion controlled rate period (DCR), in which the easily extractable solute has been completely removed and the extraction process is controlled by the diffusion of the solvent inside the particles and the diffusion of solute + solvent to the surface.

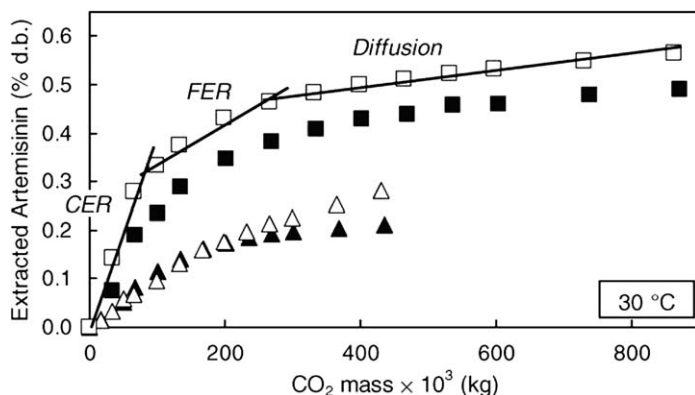


Figure 6.5 Overall extraction curves for artemisinin obtained in different conditions of SFE: ($\blacktriangle$, $\triangle$) 5.5×10^{-5} kg CO_2/s ; ($\blacksquare$, $\square$) 11.1×10^{-5} kg CO_2/s (full symbols: 40 MPa and empty symbols: 20 MPa), reprinted from Quispe-Condori *et al.*,¹⁶ *Journal of Supercritical Fluids*, Vol. 36, Issue 1, 2005, pp. 40–48, copyright 2005, with permission from Elsevier.

In some cases, the slope of the straight line corresponding to CER can be used as an indication of solubility, depending on the flow rate considered (for a more in depth discussion, readers are referred to Rodrigues *et al.*¹⁸).

For liquid samples, the steps are similar although further complexity is introduced by including the dimensions of the column and the size and structure of the packing material in the countercurrent column. Moreover, theoretical calculations of the efficiency of the separation, based on experimental measurements, are sometimes necessary to adjust the experimental conditions for challenging separations.

In the following section, an explanation of the main factors influencing the Supercritical Fluid Extraction process is presented.

6.4.1 Raw Material (Particle Size, Porosity, Location of the Solute, Moisture Content)

Despite the raw material normally being imposed on the process, there are several factors to take into account. The influence of the physical state of the sample (solid, liquid) on the outcome of the extraction is well known. When dealing with solid samples, other factors such as particle size, shape, and porosity of the solid material are of crucial importance since they have direct effects on the mass transfer rate of the process. In order to increase the extraction rate, the solid matrix must be comminuted to increase the mass transfer area. On the other hand, too small particles must be avoided. Their use can compact the bed, increasing the internal mass transfer resistance and causing channeling inside the extraction bed. As a result, the extraction rate decreases due to a non-homogeneous extraction.²

As an illustration, the degree of crushing was a very significant factor in the extraction of carotenoids from *Haematococcus pluvialis* microalgae.¹⁹ It was demonstrated how an increase in the crushing procedure produced an enhancement in the carotenoid extraction yield. This effect can be attributed to an increase of the mass transfer rate as a consequence of the lower particle size as well as to the increase of carotenoids in the medium as a result of the disruption of cells in the heavier crushing procedure.¹⁹ Although supercritical solvents have higher diffusivity in the raw material matrix than liquids, a decrease in the sample particle size generally produces an increase in the extraction yield obtained due to the increase in the contact surface between sample and solvent, mainly when diffusion is limited by internal mass transfer resistance. Nevertheless, in some applications, for example, when dealing with samples of high water content, the use of dispersing agents (e.g. diatomaceous earth) to avoid sample clogging together with hydromatrix to absorb the liquid portion from the sample can be useful. In general, drying the raw material is recommended; however, in some cases the presence of water is necessary to favor the interaction of the solvent with the solute, as in the extraction of caffeine from green coffee beans, or due to its role in the swelling of the cell, which facilitates the flow of the solvent into the cell.²

In the case of liquid samples two main strategies are used: (a) to trap the liquid on a solid support (e.g. sepiolite) and to treat it like a solid or (b) to perform column countercurrent extractions (see Figure 6.3B). The first strategy is mainly used at a small scale since the employment of solid supports can increase the extraction costs. As mentioned, during countercurrent extraction, the liquid sample is continuously added on a column by the top or the middle point, while the supercritical phase is supplied by the bottom point. This strategy has been very useful for oil refining. Hurtado-Benavides *et al.* studied the effect of the type, size, and structure of the column packing on the efficiency and performance of the countercurrent system for the SFE of olive oil; results demonstrated the influence of these factors on the mass transfer ratio.²⁰ For instance, authors showed that the use of a column packing with high surface area provides similar results to decreasing the mean particle size of a solid raw material.

6.4.2 Solubility (Pressure and Temperature)

As previously mentioned, there are several physical parameters of the supercritical fluid that are highly dependent on the pair pressure–temperature. The design of processes using supercritical solvents is strongly dependent on the phase equilibrium scenario, which is highly sensitive to changes in operating conditions. Therefore, phase equilibrium engineering, that is, the systematic application of phase equilibrium knowledge to process development, plays a key role in the development and design of these processes.⁵

In general, both the yield of a solute and the separation selectivity, which are highly dependent on solubility properties, are determined by the operating pressure and temperature. At SFE conditions, the solvent solubilization

capacity increases with pressure at constant temperature, therefore increasing the amount extracted from the raw material. In general terms, increasing the pressure leads to an exponential increase of the solubility close to the critical point (higher densities) (Figure 6.1B).

As a general rule, a component with high vapor pressure has higher solubility in a supercritical medium. Solubility of most components in supercritical fluids (SCFs) increases with the increase of the SCF density, which can be accomplished by increasing the extraction pressure. Other important aspects influencing solubility of components in the SCF are their polarity and molecular weight as well as extraction temperature. In SFE processes using CO_2 , the component solubility is lowered as the polarity and/or the molecular weights of the solutes are increased.

Increasing the temperature, at constant pressure, promotes two opposite effects: it reduces the solvent power of CO_2 by a decrease of the density, and, on the other hand, it increases the vapor pressure of solutes which can be more easily transferred to the supercritical phase. The balanced effect on solubility of the solute in the supercritical solvent will, in fact, depend on the operating pressure. Near the critical pressure, the effect of fluid density is predominant, thus, a moderate increase in temperature leads to a large decrease in the fluid density, and therefore, to a decrease in solute solubility. However, at high pressures, the increase in the vapor pressure prevails, thus the solubility increases with the temperature. This is called a retrograde behavior of the solid solubility, as can be seen in Figure 6.6. At pressures above the P_c , the isotherms exhibit a maximum in solubility.

When dealing with really complex matrices or extracting different components (like most natural product extraction processes), thermodynamic calculation can be very complicated. An alternative to performing highly complicated thermodynamic calculations consists on performing experimental designs to set up a robust extraction process.⁸ They offer a framework

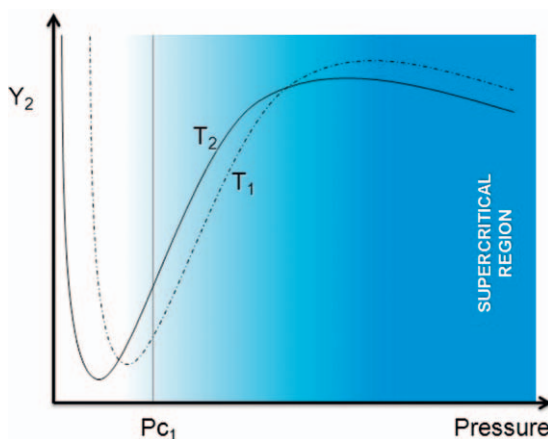


Figure 6.6 Typical isotherms of solid solubility in SFE ($T_1 > T_2 > T_{c1}$).

where researchers create an experiment, controlling experiment factors so as to predict or establish a result based on dependent and independent variables. Experimental designs give the possibility to evaluate interactions between factors, in this case pressure and temperature, while limiting the number of experiments.

Different types of experimental designs have been used to optimize SFE extraction conditions; among them response surface methodology (RSM). RSM was first introduced by Box and Wilson in 1951.²¹ The goal of RSM is to estimate a second-degree polynomial model that describes the response surface obtained in the experimental design. The higher R^2 of the model, the better it can predict future results and optimize the process. Although the extraction yield can be selected as the response variable, the particular composition of the extracts can also be optimized. For example, the use of RSM allowed the simultaneous graphical optimization of the extraction temperature, pressure, and time of different natural products such as passiflora seed oil²² and algal fatty acids.²³ In the extraction of passiflora seed oil,²² 14 experiments plus 6 replicates in the centerpoint were carried out to test 3 variables at 5 levels. Oil extraction yield was the variable to optimize. Results showed a second-order polynomial model with good coefficients of determination ($R^2 = 0.94$) in which the linear and quadratic coefficients of independent variables, temperature, pressure, and extraction time, the interactions between temperature and extraction time and pressure and extraction time had a significant effect on the oil yield. The model predicted that the optimum extraction yield would be obtained using the following conditions: extraction temperature, 56 °C; extraction pressure, 26 MPa; extraction time, 4 h. Under these conditions, the oil yield was 25.83%.

Simplex centroid design (SCD) is another popular choice since it was described by Scheefe in 1963.²⁴ It is an experimental design used to optimize mixture compositions. Mixtures are different from other types of experimental design because the proportions of the constituents must add up to 100%. Increasing the level of one constituent necessarily reduces the level of the others. SCD are used to analyze the relationship involved in a process that contains several variables. SCD are constructed to form a triangle with data points located at each corner (100% of each component), the three midpoints on each side (50%:50% of each), as well as the center (33.3%:33.3%:33.3% of each) and is highly effective at demonstrating the significance related to the three primary components. SCD has been used, for example, to determine the optimum temperature, pressure, dynamic extraction time, and modifier volume that maximize the extraction of essential oil from valerian (*Valeriana officinalis* L.).²⁵ With this strategy four independent variables were tested at five levels by using only 18 experiments.

An interesting chemometric approach suggested by Prof. M. A. A. Meireles' group²⁶ is based on the construction of the extraction yield isotherms (at different T) considering different responses, and the subsequent variable reduction using principal component analysis (PCA). This way, principal components (PCs) are defined able to explain most of the total variance of the results. By plotting the

respective loadings of the PCA variables as a function of pressure and temperature it is possible to draw interesting conclusions about the features of the extraction process, as, for example, the most effective operational conditions for the supercritical CO₂ extraction of phenolic compounds from pomegranate leaves that were suggested equal to 50 °C and 30 MPa.

Therefore, experimental designs show important advantages in terms of reducing the number of experiments and favoring the optimization of several responses at the same time. On the other hand, they do not provide a thermodynamic framework and, therefore, every different raw material should be optimized by itself since no general rules can be extracted (and therefore, extrapolated) to other samples.

6.4.3 Use of Modifiers

CO₂ is largely the most used solvent to perform SFE. From the point of view of natural products extraction, its main drawback is its low polarity, which seriously limits its ability to extract polar components from the raw material. As for many other substances, its dielectric constant may change with density, but even at high densities, CO₂ has a limited ability to dissolve high-polarity compounds. To address this problem, small amounts of co-solvents (modifiers) are added to the CO₂ stream. The addition of modifiers to CO₂ can improve the extraction efficiency by raising the solubility of the solutes. Two mechanisms have been proposed by Pereira and Meireles² to explain the effects:

1. solute–co-solvent interactions, caused by increase in solvent polarity;
2. matrix swelling that facilitates the contact of the solute by the solvent.

The effect is not only dependent on the nature of the modifier used, but also on the type of matrix, and the target solutes.

As a general rule, the amount of modifier used is lower than 10–15%. The most used modifiers are methanol, ethanol, and water. A key point when working with modifiers is to consider that the critical point of the mixture CO₂:modifier is different than the one of pure compounds and it also depends on the proportions of each.²⁷ In fact, two or three different phases may coexist at the same conditions. It must be taken into account that modifiers are not gases at room conditions and, therefore, liquid residues are obtained in extracts and remaining matrix after SFE. This is the main reason for not recommending the use of methanol in the extraction of natural products since the presence of this toxic solvent can preclude the further use of the extracts, for instance, in food applications. Ethanol is a GRAS solvent widely employed as a modifier for natural products extraction, although its final use will be determined by its ability to increase the solubility of the target compound in the CO₂:modifier mixture. Considering only toxicity and polarity, water can be suggested as an interesting modifier, but it presents several drawbacks such as the increase in the formation of ice blockages due to the Joule–Thompson effect in the separator vessel; the possible ionization and hydrolysis of compounds; and foam formation, attributed to the co-extraction of saponins² that thus leads to a loss of selectivity.

Sometimes modifiers are not only used to increase the polarity of the solvent phase, but also to improve the extraction rate of non-polar solvents. The use of oil as modifier came from the observations of Bamberger *et al.*²⁸ that the solubility of a less volatile lipid component was significantly enhanced by the presence of a more volatile triglyceride species in the system. Sun and Temelli demonstrated the ability of vegetable oils used as modifiers to enhance the yield of carotenoids (non-polar and with low volatility) from carrot; without a co-solvent, the extraction yield had a very small variation with changes in pressure and temperature, but when canola oil was employed, extraction yields increased by 3–4 times.²⁹ The extraction yield of total carotenoids with SC-CO₂ using 5% canola oil addition (w/w) was substantially higher than that obtained by traditional solvent extraction.

6.4.4 Solvent Flow Rate (Solvent-to-Feed Ratio)

Solvent to feed ratio (S/F) is the most important parameter for supercritical fluid extraction, once the extraction pressure and temperature have been selected. Solvent flow rate must be high enough to provide a good extraction yield in short time, but it should also grant enough contact time among solvent and solutes. Moreover, it must be considered that higher solvent flow rate promotes an elevation of the operational and capital costs, which should be carefully studied for industrial applications.² In general it is common to use S/F ratios around 25–100:1 for analytical and 5–15:1 for large-scale processes. In this sense it is important to consider the amount of CO₂ spent as a cost when dealing with industrial processes. In fact, when dealing with industrial scale only the first parts of the extraction curve (Figure 6.5) are considered; this is in contrast to analytical operations where quantitation is the main goal. Therefore higher amounts of CO₂ can be consumed in analytical scale and higher S/F ratios are used.

S/F is also very important when dealing with countercurrent column extractions. Generally, the efficiency of the column decreases as the CO₂ flow rate increases, since the HTU (height of a transfer unit) increases with increasing CO₂ loading, as demonstrated by Hurtado-Benavides *et al.*²⁰ and Brunner *et al.*³⁰

6.5 Applications

6.5.1 Plants

SFE has been widely employed to extract interesting compounds from natural matrices, such as plants. In fact, there is a great number of published works in which the use of this extraction technique is described for recovering bioactive compounds from those raw materials. As examples, Table 6.3 summarizes the most remarkable and recent works published dealing with the use of SFE to extract bioactive components from plants. Besides, the reader is referred to other review papers that can be found in the literature in order to gain a deeper insight on the less recent applications.^{2,11,31–34}

Table 6.3 Most recent published works (2010–2012) dealing with the use of SFE for the extraction of bioactive components from plants.

<i>Plant material</i>	<i>Compounds of interest</i>	<i>Related bioactivities</i>	<i>Extraction conditions</i>	<i>Ref.</i>
<i>Boletus edulis</i>	fatty acids		CO ₂ , 35 MPa, 40 °C, 214 min (dynamic, 1.6 L/min)	40
<i>Borago officinalis</i>	fatty acids		CO ₂ + methanol, 35 MPa, 65 °C, 10 min static + 10 min dynamic	41
<i>Camellia sinensis</i>	fatty acids and antioxidants	antioxidant activity	CO ₂ , 32 MPa, 45 °C, 90 min (static)	42
<i>Chamaecyparis obtusa</i>	essential oil		CO ₂ , 12 MPa, 50 °C, 90 min (dynamic, 0.04 L/min)	43
<i>Evodia rutaecarpa</i>	evodiamine, rutaecarpine	different actions	CO ₂ + methanol, 28 MPa, 62 °C, 78 min (dynamic 2 L/min CO ₂ + 0.4 mL/min cosolvent)	44
<i>Hemerocallis disticha</i>	lutein, zeaxanthin	antioxidant activity	CO ₂ , 60 MPa, 80 °C, 30 + 30 min (static + dynamic, 0.01 L/min)	36
kale, spinach	polyphenols, flavonoids	antioxidant activity	CO ₂ + 5% methanol, 25.8 MPa, 50 °C, 30 min (static)	35
<i>Lamiaceae</i> plants	essential oils	antimicrobial activity	CO ₂ , 30 MPa, 40 °C, 90 min (dynamic, 2.4 kg/h)	45
<i>Lippia dulcis</i>	hernandulcin and other sesquiterpenes		CO ₂ , 12 MPa, 35 °C, 60 min (static) + dynamic period	46
<i>Magnolia officinalis</i>	honokiol and magnolol	antioxidant, anti-inflammatory activities	CO ₂ , 40 MPa, 80 °C, 60 + 40 min (static + dynamic, 0.02 L/min)	47
<i>Mitragyna speciosa</i>	alkaloids		CO ₂ + 28.8% ethanol, 30 MPa, 65 °C, 45 min (dynamic)	48
<i>Nelumbo nucifera</i>	alkaloids	several bioactivities	CO ₂ + 10% diethylamine and 1% water, 30 MPa, 70 °C, 120 min (dynamic, 1.1 kg/h)	49

olive leaves	phenolic compounds	cytotoxic activity	CO ₂ + 6.6% ethanol, 15 MPa, 40 °C, 120 min (static)	50
olive leaves	oleuropein	antioxidant activity	CO ₂ + 20% methanol, 30 MPa, 100 °C	51
peach kernels	fatty acids		CO ₂ , 30 MPa, 50 °C, 150 min (static)	52
<i>Psidium guajava</i>	total phenols	antioxidant activity	CO ₂ + 10% ethanol, 30 MPa, 50 °C, 30 min (static) × 4 cycles	53
rosemary (<i>Rosmarinus officinalis</i>)	phenolic compounds	antioxidant activity	CO ₂ , 30 MPa, 40 °C, 300 min (dynamic, 60 g/min)	54
rosemary (<i>Rosmarinus officinalis</i>)	phenolic compounds	antioxidant activity	CO ₂ + 6.6% ethanol, 15 MPa, 40 °C, 120 min (static)	55
rosemary (<i>Rosmarinus officinalis</i>)	phenolic compounds	anti-inflammatory activity	CO ₂ , 35MPa, 80 °C, 30 + 90 min (static + dynamic)	56
<i>Salvia desoleana</i>	sclareol	antioxidant and cytotoxic activity	CO ₂ , 25 MPa, 40 °C, 240 min (dynamic, 16 L/min)	57
<i>Salvia officinalis</i>	essential oil		CO ₂ , 30 MPa, 40 °C (dynamic, 3.23 g/min)	58
sea buckthorn (<i>Hippophae rhamnoids</i>)	tocopherols, lycopene and β-carotene	radical scavenging activity	CO ₂ , 40 MPa, 35 °C, 60 min (static)	59
spearmint (<i>Mentha spicata</i> L.)	essential oil		CO ₂ , 9. MPa, 35 °C, 30 min (static)	37
spearmint (<i>Mentha spicata</i>)	essential oil	antioxidant activity	CO ₂ , 30 MPa, 50 °C, 180 min (dynamic, 5 g/min)	60
spinach	lutein	antioxidant activity	CO ₂ + ethanol, 30. MPa, 50 °C, 90 min (static)	61
strawberry (<i>Arbutus unedo</i>)	total phenolics	antioxidant activity	CO ₂ + 19.7% ethanol, 6 MPa, 48 °C, 60 min (dynamic, 15 g/min)	62
thyme (<i>Thymus vulgaris</i>)	thymol	antiseptic, anti-inflammatory activities	CO ₂ + 3% ethanol, 15 MPa, 50 °C	63
thyme (<i>Thymus vulgaris</i>)	volatiles	antibacterial	CO ₂ , 9 MPa, 40 °C, 240 min (dynamic, 1.1 kg/h)	64

As can be observed from the information presented in Table 6.3, all the applications deal with extraction of solid materials, and most of the applications are directed towards the extraction of compounds possessing a particular bioactivity. In this regard, antioxidant compounds have been the most studied. The bioactives extracted belong to a wide range of compound classes, from more polar phenolic compounds to alkaloids, carotenoids, and other pigments and essential oils. Considering that CO₂ is the supercritical fluid frequently selected, and bearing in mind that bioactive compounds present in natural samples often possess a relatively high polarity, the use of organic modifiers to extract these components is very common.

Ethanol and methanol are the cosolvents most frequently used, although the use of others such as diethylamine and water has been also explored. Normally, proportions of up to 20% have been employed for the modifiers, although proportions as low as 5% have been shown to be useful to extract, for instance, polyphenols.³⁵ In contrast, in the case of the extraction of essential oils neat CO₂ is employed as the polarity of supercritical carbon dioxide is low enough to extract the less polar compounds that are part of the essential oils. Other less polar bioactives could be potentially recovered by using small proportions of modifiers or even using pure CO₂ at higher pressures. In this regard, carotenoids are natural pigments with very low polarity, which have a great potential to be explored by the characteristics and advantages of SFE, especially considering food applications. These components are basically interesting by their antioxidant activities and coloring properties. In general, very high pressures are needed to dissolve carotenoids when using neat CO₂ because their solubility is low. Despite their relatively non-polar structure, the molecular weight is large (536.85 Da for β -carotene), a factor known to reduce solubility in SC-CO₂ due to its low volatility.²⁹ In fact, 60 MPa of pressure were employed for the extraction of lutein and zeaxanthin from *Hemerocallis disticha*.³⁶ The addition of a co-solvent to SC-CO₂ was proven to improve the extraction efficiency, as seen in Section 6.4.3.

In any case, what it is interesting during process optimization is the employment of chemometric tools in order to determine the optimum extraction conditions for the different parameters involved. In this regard, the application of an experimental design is of great help in order to have enough experimental data to subsequently determine the optimum conditions for each studied parameter according to the response variables selected. Taguchi,³⁷ Box-Behnken,³⁸ or central composite experimental designs³⁹ have been used, among others, for the optimization of variables involved in the SFE extraction of bioactives from plants. Extraction pressure and dynamic extraction time as well as modifier volume were the factors studied to maximize the recovery of essential oils from *Myrtus communis*,³⁹ whereas extraction pressure, temperature, and time were the parameters selected in the extraction of *Garcinia mangostana*.³⁸ In this latter case, total extraction yield and radical scavenging activity of the extracts were chosen as response variables and the composition and proportion of co-solvent was kept constant.

Response surface methodology has also been employed. This method allows not only the visualization of the best conditions obtained for the studied factors, but also the graphical observation of the influence of the different factors studied on the response variables observed.^{29,39}

6.5.2 Marine Products

The discovery and development of marine bioactives is a relatively new area compared to those derived from terrestrial sources. Although some plants have demonstrated to be interesting sources of bioactive compounds, the potential of other sources from marine nature have also been pointed out. The high diversity observed in the marine environments from a chemical and biological point of view makes the ocean an extraordinary source of high-value compounds. In this regard, SFE has been widely employed for extracting bioactive compounds from algae, microalgae, and other marine-related organisms such as crustaceans, fish, and their by-products.^{2,11,34,65–69} Table 6.4 summarizes the most relevant literature recently published (from 2010 to 2012) dealing with the recovery of valuable compounds from marine sources using SFE. As can be observed in this table, the main application of SFE developed in the last two years deals with the extraction of ω -3 polyunsaturated fatty acids (PUFAs) and carotenoids.

The possibility of obtaining ω -3 PUFAs from marine sources has been intensively studied in recent years considering their important potential biological properties, such as anti-inflammatory, antithrombotic, anti-arrhythmic, etc.^{70–73} Marine sources, especially fish oil and fish by-products, provide the major natural dietary source of ω -3 PUFAs, mainly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). SFE using non-polar CO₂ is especially well suited to extract this kind of compounds. Regarding fish oil, Lopes *et al.*⁷⁴ studied the possibility, under different temperatures and pressures, of fractionating the triacylglycerols with respect to EPA and DHA from fish oil with low ω -3 fatty acids content (10%) in order to demonstrate that the probability of fractionating the oil with respect to these fatty acids is improved by using fish oil with lower ω -3 fatty acids content as the basis.⁷⁴

The applicability of SFE technology to add value to waste products of the fish industry is also demonstrated by the use of different fish by-products and some marine invertebrate (sea urchin) as raw materials to obtain ω -3 PUFAs. For instance, an interesting work developed by Sánchez-Carmargo *et al.*⁷⁵ demonstrated that the addition of ethanol significantly improves the extraction yields of lipids and astaxanthin from redspotted shrimp waste compared to the extraction without ethanol as co-solvent;⁷⁶ data obtained in this study showed that the extraction yields increase considerably with the increase in the proportion of ethanol in the solvent mixture, reaching maximum recoveries of 93.8% and 65.2% for lipids and astaxanthin, respectively, when employing 15% ethanol. Besides, increasing the proportion of ethanol resulted in increase in the concentration of the ω -3 fatty acids in the lipids of the extract.⁷⁵

Table 6.4 Summary of the most relevant works published in the period 2010–2012 on the recovery of valuable compounds from marine sources using SFE.

<i>Marine source</i>	<i>Compounds of interest</i>	<i>Related bioactivities</i>	<i>Extraction conditions</i>	<i>Ref.</i>
Brazilian red-spotted shrimp waste (shell and tail)	ω -3 PUFAs, astaxanthin	antioxidant activity	CO ₂ + 15% ethanol, 30 MPa, 50 °C, 100 min (dynamic, CO ₂ at 3 L/min and ethanol at 0.001 L/min)	75
Brazilian red-spotted shrimp waste (heads, shell and tail)	ω -3 PUFAs, astaxanthin	anti-inflammatory, reduce risk of certain heart diseases, antioxidant activity	CO ₂ , 30 MPa, 50 °C, (static 20 min + dynamic 200 min, 1.5 L/min)	76
<i>Chlorella vulgaris</i>	lutein	antioxidant activity	CO ₂ + ethanol, 40 MPa, 40 °C, 45 min (dynamic, CO ₂ at 0.003 L/min and ethanol at 3×10 ⁻⁴ L/min)	85
<i>Chlorella vulgaris</i>	canthaxanthin and astaxanthin	antioxidant activity	CO ₂ , 30 MPa, 40 °C, 30 min (dynamic, 0.04 kg/h)	80
<i>Chlorella vulgaris</i> C-C	polyphenols and flavonoids	antioxidant activity	CO ₂ + 50% ethanol, 31 MPa, 50 °C, 20 min (static)	84
fish by-product (Indian mackerel skin)	ω -3 PUFAs	anti-inflammatory, reduce risk of certain heart diseases	CO ₂ , 35 MPa, 75 °C, 180 min (10 static cycles of 18 min)	86
fish by-products (off cuts from hake, orange roughy and salmon, and livers from jumbo squid)	ω -3 PUFAs	anti-inflammatory, reduce risk of certain heart diseases	CO ₂ , 25 MPa, 40 °C, 90 min (dynamic, 13.75 kg/h)	65
fish by-products (trout heads, spines and viscera)	ω -3 PUFAs	anti-inflammatory, reduce risk of certain heart diseases	CO ₂ , 50 MPa, 60 °C (dynamic, 0.6 kg/h)	87

fish oil (<i>Pseudoplatystoma corruscans</i>)	ω -3 PUFAs*	anti-inflammatory, reduce risk of certain heart diseases	CO ₂ , 20 MPa, 33 or 40 °C, (static 30 min + dynamic, 0.8 L/min)	74
<i>Haematococcus pluvialis</i>	astaxanthin	antioxidant activity	CO ₂ + ethanol (2.3 mL/g sample), 43.5 MPa, 65 °C, (static 60 min + dynamic 180 min, CO ₂ at 10 L/min and ethanol at 0.03 L/min)	88
<i>Monoraphidium sp. GK12</i>	astaxanthin	antioxidant activity	CO ₂ + ethanol (biomass/ethanol ratio, 1/20), 20 MPa, 30 °C, 15 min (static)	89
<i>Nannochloropsis oculata</i>	lipids, zeaxanthin	anti-inflammatory, reduce risk of certain heart diseases, antioxidant activity	CO ₂ + 16.7 w/w% ethanol, 35 MPa, 50 °C (dynamic, CO ₂ at 0.01 L/min)	77
Northern shrimp by- products (heads, shell and tail)	ω -3 PUFAs	anti-inflammatory, reduce risk of certain heart diseases	CO ₂ , 35 MPa, 40 °C, 90 min (dynamic, 3–5 L/min)	90
<i>Scenedesmus almeriensis</i>	lutein and β -carotene	antioxidant activity	CO ₂ , 40 MPa, 60 °C, 300 min (dynamic, 0.06 kg/h)	79
<i>Schizochytrium limacinum</i>	lipids	anti-inflammatory, reduce risk of certain heart diseases	CO ₂ + 95% ethanol, 35 MPa, 40 °C, 120 min (static)	78
sea and freshwater algae and cyanobacteria	isoflavones	antioxidant activity	CO ₂ + 3% methanol/H ₂ O (9:1 v/v), 35 MPa, 40 °C, 60 min (dynamic, 0.75–0.85 L/min)	83
sea urchin gonad	ω -3 PUFAs	anti-inflammatory, reduce risk of certain heart diseases	CO ₂ , 28 MPa, 50 °C, 80 min (dynamic, 0.33 L/min)	91
shellfish by-products (Abalone gonad)	ω -3 PUFAs	anti-inflammatory, reduce risk of certain heart diseases	CO ₂ , 28 MPa, 50 °C, 80 min (dynamic, 0.42 L/min)	92

*PUFAs, polyunsaturated fatty acids.

Although SFE has been also applied to carry out the lipid extraction from microalgae, such as *Nannochloropsis oculata*⁷⁷ and *Schizochytrium limacinum*,⁷⁸ the main application of this technology using algae and microalgae as natural sources has been the extraction of antioxidant compounds, namely carotenoids, isoflavones, polyphenols, and flavonoids.

Traditionally, carotenoids have been extracted using organic solvents; however, different studies have discussed the use of SFE for their recovery. They have demonstrated the extraction of carotenoids, such as lutein and β -carotene^{43,79} or canthaxanthin and astaxanthin,⁸⁰ using neat SC-CO₂. However, most of the applications presented in Table 6.4 employed certain amount of a co-solvent (ethanol or methanol) to modify the polarity of the SC-CO₂. Using the mixture co-solvent/SC-CO₂, the extraction efficiency of carotenoids is improved; since volatility of carotenoids is very low, as seen in previous sections, the use of modifiers is generally recommended instead of increasing pressure above 50 MPa. Besides extraction, other applications found in the recent literature include their purification by supercritical anti-solvent precipitation (SAS). For instance, this methodology has been employed to the purification of zeaxanthin from the ultrasonic⁸¹ or Soxhlet⁸² extract of the microalgae *Nannochloropsis oculata*. In addition, Liao *et al.* developed an interesting process considering SFE of lipids and carotenoids from *Nannochloropsis oculata* and SAS of carotenoid-rich solution.⁷⁷ Although in this approach both processes were considered independently, their combination may favor the simultaneous extraction and purification of carotenoids.

Other antioxidant compounds different from carotenoids, such as isoflavones, polyphenols, and flavonoids have also been extracted by SFE using methanol or ethanol as co-solvent from algae, microalgae, and cyanobacteria.^{83,84} Phenolic compounds are slightly polar; for this reason a certain amount of polar co-solvent and pressures above 30 MPa have been used in both papers. These works open a new field for SFE. The microalga *Chlorella vulgaris* extract obtained using SC-CO₂ presents dual inhibitions to lung cancer cell growth and migration ability, which is the index of cancer metastasis. The flavonoid content obtained from SC-CO₂ (3.18 mg quercetin/g lyophilized extract) was also significantly higher than from ultrasonic extraction (0.86 mg quercetin/g lyophilized extract). Accordingly, *C. vulgaris* might be a potential candidate for cancer chemoprevention.⁸⁴

6.5.3 Agricultural and Food By-products

Another potential source of natural products that can be explored using the benefits of SFE is agricultural and food-by-products. Industrial activities generate a large variety of by-products that normally do not have any commercial value. Their conversion into valuable material by, for instance, the extraction of high-value compounds can provide enormous benefits from an environmental and economic point of view. In this sense, SFE has been widely used to add value to agricultural and food by-products.^{1,11,34,93,94} A high variety of agricultural and food by-products have been employed as source of

bioactive compounds (Table 6.5). Several of the studies presented use sophisticated chemometric tools in order to select the most appropriate extraction conditions as well as to study the influence of each experimental parameter (temperature, pressure, or percentage of co-solvent) in the extraction procedure. Among them, factorial experimental design,⁹⁵ response surface methodology,^{88,96–98} central composite non-factorial design,⁹⁹ and mathematical modeling¹⁰⁰ have been employed.

The main bioactive compounds extracted by SFE from agricultural and food by-products are polyphenols and carotenoids with antioxidant properties, but also fatty acids, essential oils, and tocopherols. This fact demonstrates, as mentioned before, the versatility of SFE towards the extraction of lipophilic and hydrophilic compounds when a co-solvent is added to CO₂. For instance, for extracting polyphenols the addition of a moderately polar modifier is critical, so that ethanol is usually added at relatively low levels (10–20%) although extraction using up to 60% has been reported.⁹⁷ Ethanol is the most used co-solvent, but other modifiers can be employed; for instance, Castro-Vargas *et al.*⁵³ compared the extraction yield of phenolic compounds from guava seeds by SFE with CO₂ and with ethyl acetate and ethanol as co-solvents. The phenolic fraction yield increased directly with solvent polarity (CO₂, CO₂/ethyl acetate, and CO₂/ethanol).

Most of the works presented in Table 6.5 dealing with the SFE of polyphenols measure the extraction efficiency by total phenolic content; however, some other studies measure the levels of specific compounds such as resveratrol¹⁰¹ or kaempferol glycosides.⁹⁷

Regarding carotenoids, different SFE methodologies have been developed to extract lycopene, which has the highest antioxidant activity among all dietary antioxidants and plays an important role in the prevention of oxidative and age-related diseases.^{102,103} It represents the most abundant carotenoid in tomatoes, accounting for more than 80% of the pigments present in fully red-ripe fruits. The SFE extraction of lycopene has been mainly carried out from tomato by-products;^{95,104,105} however, it has been also extracted from pink guava, a tropical fruit rich in lycopene.¹⁰⁶ Usually, lycopene recovery does not exceed 20% of the total amount of carotenoids in the absence of a co-solvent. This percentage is considerably increased when a vegetable oil is added as co-solvent. As examples, Lenucci *et al.*¹⁰⁴ demonstrated that the addition of an oleaginous co-matrix consisting of roughly crushed hazelnuts to the lyophilized tomato matrix made it possible to increase the lycopene recovery from 35% to approximately 80% in the oleoresin, whereas Machmudah *et al.*¹⁰⁵ showed how the presence of tomato seed oil helped to improve the recovery of lycopene by SFE from dried tomato peel by-products from 18% to 56%.

In most of the papers dealing with SFE of lycopene from seeds, pulp, and tomato skin, the extraction is preceded by the removal of the humidity from the raw material by using some drying process to further increase the extraction yield of lycopene. However, Egydio *et al.*⁹⁵ developed a SFE methodology to extract lycopene from tomato juice without the need to dry the raw material. The recovery from the pulp of centrifuged tomato juice increased significantly after substituting the water for ethanol before SFE extraction.

Table 6.5 Summary of the works published on the recovery of valuable compounds from agricultural and food by-products using SFE in the period 2010-2012.

<i>Food by-product</i>	<i>Compounds of interest</i>	<i>Related bioactivities</i>	<i>Extraction conditions</i>	<i>Ref.</i>
broccoli leaves	fatty acids	prevention of different diseases	CO ₂ , 30 MPa, 60 °C, (static 10 min + dynamic 90 min, 0.003 L/min)	107
carob pulp kibbles	phenolic compounds	antioxidant activity	CO ₂ + 12.4% ethanol:water (80:20 v/v), 22 MPa, 40 °C, 210 min (static 15 min + dynamic 450 min, CO ₂ at 0.29 kg/h)	99
coffee husks	caffeine		CO ₂ , 30 MPa, 100 °C, 300 min (dynamic, 197 kg CO ₂ /kg husk)	108
grape by-products (seeds, stems, skin and pomace)	resveratrol	antioxidant activity	CO ₂ + 5% ethanol, 40 MPa, 35 °C, (dynamic 180 min, 0.048 kg/h)	101
grape seed	proanthocyanidins	antioxidant activity	CO ₂ + 15–20% ethanol, 25–30 MPa, 30–50 °C, 60 min (dynamic 0.3 kg/h)	109
guava (<i>Psidium guajava</i> L.) seeds	phenolic compounds	antioxidant activity	CO ₂ + 10% ethanol, 30 MPa, 50 °C, 120 min (static 4 cycles of 30 min)	53
guava by-products (decanter)	lycopene	antioxidant activity	CO ₂ + 10% ethanol, 30 MPa, 55 °C, (static 30 min + dynamic 150 min, 0.0025 L/min)	106
hemp seeds	fatty acids		CO ₂ , 30 MPa at 40 °C or 40 MPa at 80 °C, (dynamic, 10 kg/h)	110
Kalahari melon seed	phytosterol-enriched oil		CO ₂ , 30 MPa, 40 °C, (static 30 min + dynamic 180 min, 0.012 L/min)	111
olive oil mill waste	phenolic compounds	antioxidant activity	CO ₂ , 35 MPa, 40 °C, 60 min (dynamic 0.12 kg/h)	112
orange pomace	polyphenols	antioxidant and antimicrobial activities	CO ₂ + 8% ethanol, 25 MPa, 50 °C, (dynamic 300 min, 1.02 kg/h)	100

Piquillo red pepper by-products	vitamin E and provitamin A	different protective effects	CO ₂ , 24 MPa, 60 °C, 120 min (dynamic, 0.03 L/min)	113
pomegranate seed oil	fatty acids, tocopherols	antioxidant activity	CO ₂ , 15–30 MPa, 35–65 °C, 120 min (static)	114
rapeseed	essential oil		CO ₂ , 30 MPa, 50 °C, 180 min (dynamic)	96
red-ripe tomato cultivars	lycopene	antioxidant activity	CO ₂ , 45 MPa, 65–70 °C, variable ratio tomato matrix/roughly crushed hazelnuts (dynamic 180 min, 18–20 kg/h)	104
rice germ	tocols	different protective effects	CO ₂ , 13.8 MPa, 60 °C (dynamic, 5 L/min)	115
rizhomes of <i>Cyperus rotundus</i> Linn.	essential oil		CO ₂ , 30 MPa, 35 °C, 120 min (dynamic, 0.42 L/min)	88
sesame	fatty acids	antioxidant activity	CO ₂ + ethanol, 20 MPa, 35 °C, 210 min (dynamic, 0.15 kg/h)	116
tea seed cake	kaempferol glycoside	antioxidant activity	CO ₂ + 60% ethanol, 20 MPa, 80 °C, 150 min (dynamic, CO ₂ at 2 L/min, ethanol at 5 × 10 ⁻⁴ L/min)	97
tomato juice	lycopene	antioxidant activity	CO ₂ , 35 MPa, 40 °C, (static 5 min + dynamic 180 or 360 min, at 0.1 or 0.05 kg/h respectively)	95
tomato peel by-products	lycopene	antioxidant activity	CO ₂ , 40 MPa, 90 °C, 180 min, ratio tomato peel to seed of 37/63 (dynamic, 0.003 L/min)	105
vinification residues	fatty acids, α -tocopherol, phenolic compounds	different actions	CO ₂ , 25 MPa, 80 °C, 60 min (dynamic, 4.14 kg/h)	117
wheat bran	phenolics compounds and tocopherols	antioxidant activity	CO ₂ , 30 MPa, 60 °C, 120 min (dynamic 1.60 kg/h)	118

Therefore, SFE has demonstrated its ability to extract/isolate/concentrate several high-value compounds from different natural sources, including food by-products. This is an important aspect regarding valorization of low value by-products and is a key factor in the development of green extraction processes complying with the Green Chemistry rules.

6.6 Case Study

In this case study, the main parameters affecting the supercritical fluid extraction of bioactive compounds from cyanobacteria *Spirulina* (*Arthospyra platensis*) will be described. Among the compounds with antioxidant activity from *Spirulina*, vitamin E (α -tocopherol) has been selected for its importance as a lipid-soluble antioxidant compound and because SFE has shown several advantages compared to the extraction with organic solvents (use of non-toxic solvents, high enrichment factors, selectivity, etc.).¹¹⁹ Optimization has been carried out using a response surface methodology (RSM) considering several factors such as extraction pressure and temperature and modifier content. The response selected in this work was the concentration of vitamin E achieved in the process at pilot scale. In a first step, the extraction time was determined by studying the kinetics of the extraction. After that, the experimental design was run considering the two solvents, pure CO₂ and CO₂ plus 10% ethanol as co-solvent, and the response was optimized in order to select those conditions in which there is a high enrichment of vitamin E. Moreover, a new application of carbon dioxide expanded liquids (CXLs) will be discussed at the end of this section, as a new way to increase the efficiency of the extraction process while improving the selectivity towards the compound/s of interest.¹²⁰

6.6.1 Effect of Extraction Time

The extractions were carried out in a pilot-scale plant for supercritical fluid extraction (Iberfluid, Spain) with a 285 mL extraction cell, such as the one shown in Figure 6.3A. The extraction cell was filled with 75 g of microalgae (30–70 μ m trichome length) and 120 g of washed sea sand. All extractions were done using a flow of 3 L/h. The extraction conditions (extraction pressure and temperature) were selected from the experimental design. Cascade fractionation was achieved by setting pressures in separators 1 and 2 equal to 50% of the extraction pressure in the column and 50% of the pressure in separator 1 (with a maximum of 5.5 MPa), respectively. Temperatures in separators 1 and 2 were fixed equal to the extraction temperature and 20 °C, respectively.

Extraction time was fixed previously through sequential extractions of the same sample at selected conditions (central point of the design, that is, 22 MPa and 55 °C without modifier). The extraction time was set at the beginning of the asymptotic curve yield (%)–time (min), considering, as described previously, that at this time the diffusion-controlled rate period started. Thus, the extraction time was fixed equal to 75 min.

6.6.2 Effect of Pressure, Temperature and Modifier

The effects of two factors, pressure (P) and extraction temperature (T), on the concentration of vitamin E, for each solvent tested, were studied using a central composite circumscribed design (CCCD). A total of 10 experiments were carried out, considering the following: four points of a full factorial design, four star points ($\alpha = 1.414$ star distance), and two center points to estimate the experimental errors. By using this design, the two variables were tested at five different experimental levels: pressure at 7.8, 12, 22, 32, and 36.1 MPa, and extraction temperature at 26.7, 35, 55, 75, and 83.3 °C (implying using densities from 0.195 to 0.94 g/mL), in correspondence with the coded levels: -1.414 , -1 , 0 , $+1$, $+1.414$, respectively. The experimental design was done twice, using pure CO_2 and CO_2 plus ethanol as co-solvent. For the extractions using ethanol, the addition started after having reached the selected pressure during 75% of the extraction time. Ethanol was added in an amount corresponding to 10% of CO_2 (v/v).

Figure 6.7 illustrates the importance of the factors through the standardized Pareto charts for the vitamin E concentration when using CO_2 or CO_2 and ethanol as extraction solvent. As can be seen, the terms that have the strongest influence in the response variable, for CO_2 , are the extraction temperature (T) and its quadratic term (T^*T), both having a positive effect. Considering as extracting solvent CO_2 plus ethanol, only the quadratic term (T^*T) was significantly different from zero.

Results obtained are in agreement with previous studies in which different authors also determined that the temperature was the factor that mostly influenced the recovery of vitamin E. Since the temperature affects positively the extraction of vitamin E, it is reasonable to think that its extraction will be

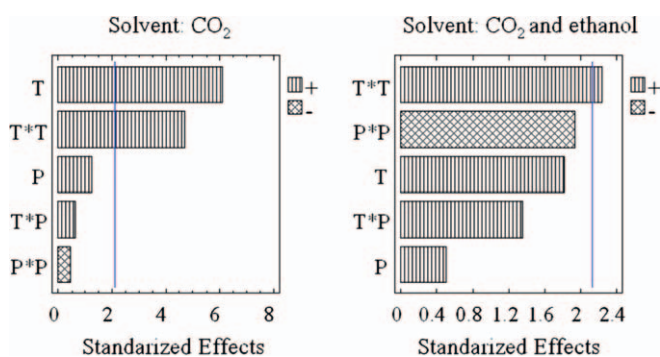


Figure 6.7 Standardized Pareto chart plot with the effect of each term in the model divided by its standard error, for the two response variables. The vertical line in the chart tests the significance of the effects at 90% confidence level. Legend for the bars corresponding to the terms in the model (T =extraction temperature, P =extraction pressure), reprinted from Mendiola *et al.*,¹¹⁹ *Journal of Supercritical Fluids*, Vol. 43, 2008, pp. 484–489, copyright 2008, with permission from Elsevier.

avored by high-extraction temperatures. In this sense, several references can be found in the literature using temperatures up to 80 or 90 °C for extraction of vitamin E from, for instance, soybean flakes¹²¹ or palm leaves.¹²² Moreover, Skerget *et al.*¹²³ provided data for phase equilibria of the binary system vitamin E–CO₂ at different pressures and temperatures up to 80 °C, showing that the solubility of the vitamin E in the light phase increased with pressure and temperature being maximum at 80 °C.

The optimum conditions of the extraction process, provided by the statistical program, are equal to the maximum pressure and temperature tested, that is, 83 °C and 36.2 MPa. The predicted value for vitamin E concentration (mg/g extract) at the optimum is equal to 29.4 mg/g extract. Considering at these conditions, an extraction yield equal to 0.53% (that corresponds to the maximum extraction yield obtained at 36.2 MPa and 55 °C, which are the closest experimental conditions), the concentration of vitamin E per gram of *Spirulina* is equal to 0.155, meaning an enrichment factor of 12 compared to the initial concentration in raw *Spirulina* (that ranged from 0.011 to 0.014 mg vitamin E/g dried microalgae).

6.6.3 Effect of Solvent

Working from the good results found with pure CO₂ and CO₂ modified with ethanol towards the extraction of bioactive compounds from different natural sources such *Spirulina*, it seemed interesting to test the efficacy and selectivity of a new type of solvent such as ethanol expanded with CO₂. This solvent is included in the so-called gas-expanded liquids (GXLs) (specifically, carbon dioxide expanded liquids, CXLs) that consist of a mixed solvent made from a compressible gas dissolved in an organic solvent (see Figure 6.8). The possibilities offered by this new type of solvent are huge since its properties largely vary depending on the CO₂ composition (which can be modified through the tuning of the operating pressure); for example, a large amount of CO₂ may favor mass transfer and, in many cases, gas solubility, while the presence of polar organic solvents enhances the solubility of solid and liquid solutes.

In the present example, this approach was considered to obtain fractions enriched in γ -linolenic acid from *Spirulina*. To test the usefulness of GXL

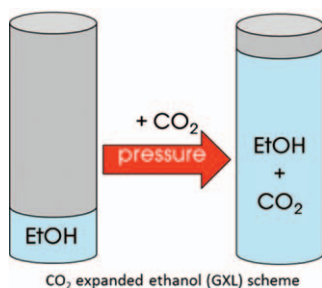


Figure 6.8 Scheme of gas expanded liquid (ethanol) formation.

composed by ethanol and supercritical- CO_2 , a Taguchi L_9 (3^4) orthogonal array experimental design was used. Factors considered were those related to typical SC- CO_2 extractions including extraction temperature, extraction pressure, extraction time, and the fraction of organic solvent that, in this case, ranged from 10 to 50% (v/v) to work as GXLs. All of these conditions have in common the formation of a liquid phase saturated with carbon dioxide, that is, an expanded liquid phase.

As mentioned, the goal of the present work was the optimization of total extraction yield and γ -linolenic acid (GLnA) recovery. Temperature was found to be the less significant factor. On the other hand, the fraction of ethanol and also the extraction time were the main parameters. Total extraction yield for GXL increased by increasing both extraction time and ethanol fraction in the solvent. Such behavior in the extraction with GXL was quite different from that of the traditional SFE, in which temperature and pressure play major roles in changing the solubility of the components and therefore the mass transfer and total extraction yield of the operation. In a GXL, the physical behavior resembles that of a pressurized liquid. Considering the good results achieved in this work, in which extraction yields up to 7% and GLnA recoveries close to 30% were obtained, extraction using GXLs can be considered a valuable alternative to both pressurized liquid extraction (PLE) and supercritical fluids for the extraction of medium-polar compounds.

6.7 Future Trends and Conclusions

In the present chapter we have tried to demonstrate that SFE is nowadays one of the most popular alternative methods for extracting valuable compounds from different natural raw materials such as plants, marine products, and agricultural by-products. Advantages of the use of such technology have been underlined as well as the parameters that can be modified to optimize the process in terms of yields and/or purity of the target compounds. Recent applications have been summarized, allowing us to identify both the target compounds and the key raw materials that have been studied lately. In this sense, it seems that compounds or extracts with associated antioxidant activity are the most popular, mainly because of their suggested relationship with the improvement of health status. Other bioactivities such as anti-inflammatory and antimicrobial have also become of interest. As for the target compounds, carotenoids, phenolic compounds, ω -3 PUFAs, and essential oils are among the most widely studied.

Although SFE has been recognized as an advantageous process from an environmental point of view, sustainability and eco-friendliness of a particular process is a goal that has to be approached through the application of, among other tools, life-cycle analysis (LCA). LCA should be employed to efficiently calculate the impact on the environment of the different available procedures. Future research in this interesting area is expected. Moreover, more focus is needed in terms of economic considerations of SFE processes at large scale. Pioneer works of Meireles have set the basis for a better understanding of

process economics; interested readers are referred to an interesting review on this topic.² Chapter 12 will cover this subject in more depth.

As more advantages are associated with the use of SFE as a viable process for natural product extraction, a wider range of experimental conditions are tested, including sub- and supercritical conditions, and a higher number of solvents are included, trying to cover a wide range of polarities. In this sense, new developments using solvents other than carbon dioxide are every time more common, including, for example, the employment of supercritical ethane to extract all-*trans*-lycopene from tomato industrial wastes,¹⁹ or the extraction of lipids from fermentation biomass using near-critical dimethyl ether (DME).¹²⁴ DME has shown, for instance, important advantages associated to the extraction of wet biomass because of its high solubility in water. This mutual solubility of water and DME enables the co-extraction of water and lipids that can be easily separated afterwards but that allows the processing of the material without a previous drying step.

As already mentioned in the case study, other solvents with great possibilities to be used in SFE are the so-called gas-expanded liquids (GXLs), understanding a GXL as a mixed solvent composed of a compressible gas (such as CO₂ or ethane) dissolved in an organic solvent. CO₂-expanded liquids (CXLs) are the most commonly used class of GXLs. By just modifying the CO₂ composition, a continuum of liquid media ranging from the neat organic solvent to SC-CO₂ is generated, the properties of which can be adjusted by tuning the operating pressure. Moreover, CXLs can be created at relatively mild pressures with a substantial replacement of the organic solvent with CO₂. Therefore, GXLs combine the beneficial properties of compressed gases (such as the improved mass transport) and of traditional solvents (large solvating power), leading to a new class of tunable solvents that are often the ideal type of solvents for a given application. Although these novel solvents have been applied to some processing applications, including gas antisolvent (GAS) processes, particle deposition, etc., just few examples demonstrated the ability of such solvents in extracting valuable compounds from natural matrices.¹²⁰ Other solvents such as ionic liquids (ILs) have started to be explored combined with supercritical fluids. The most obvious benefit of coupling ILs and SFCs is in the integration of reaction and extraction processes into the same system; that is, linking the possibility of carrying out a reaction in the most favorable phase (the ionic liquid) while the reaction products are extracted into the supercritical phase for easy recovery.¹²⁵

In this sense, it is foreseen an important development of green processing platforms, using green solvents such as supercritical carbon dioxide and water, multi-unit operations consisting of raw material pre-treatment, reactions (biocatalysis, transesterification), extraction, and biofuel conversion, etc. New technologies involving the combined use of enzymes, disruption methods such as ultrasounds,¹²⁶ or membrane separation¹²⁷ with supercritical fluids can undoubtedly revolutionize the concept of process sustainability, approaching it to a more promising green biorefinery platform able to give new answers to the demands posted nowadays.

References

1. J. A. Mendiola, M. Herrero, A. Cifuentes and E. Ibáñez, *J. Chromatogr. A*, 2007, **1152**, 234.
2. C. Pereira and M. Meireles, *Food Bioproc. Technol.*, 2010, **3**, 340.
3. B. Berche, M. Henkel and R. Kenna, *J. Phys. Stud.*, 2009, **13**, 3001.
4. P. Curie, *Archiv. Sci. Phys. Nat.* (reprinted in *Oeuvres De Pierre Curie*, p. 214), 1891, **26**, 13.
5. S. Pereda, S. Bottini and E. Brignole, Fundamentals of Supercritical Fluid Technology, in *Supercritical Fluid Extraction of Nutraceuticals and Bioactive Compounds*, ed. J. L. Martinez, CRC Press, Boca Raton, FL, USA, pp. 1, 2007. ISBN: 978-0-8493-7089-2.
6. H. Machida, M. Takesue and R. L. Smith Jr., *J. Supercrit. Fluids*, 2011, **60**, 2.
7. E. Ramsey, Q. Sun, Z. Zhang, C. Zhang and W. Gou, *J. Environ. Sci.*, 2009, **21**, 720.
8. E. Schütz, *Chem. Eng. Technol.*, 2007, **30**, 685.
9. H. E. Messmore, United States Patent 2420185 (1943).
10. K. Zosel, *Angew. Chem., Int. Ed. Engl.*, 1978, **71**, 702.
11. M. Herrero, J. A. Mendiola, A. Cifuentes and E. Ibáñez, *J. Chromatogr. A*, 2010, **1217**, 2495.
12. G. Brunner, *Gas Extraction - An Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Processes*, Springer, Darmstadt, Germany, 1994. ISBN: 3798509441.
13. G. Brunner, *J. Food Eng.*, 2005, **67**, 21.
14. E. Reverchon, *J. Supercrit. Fluids*, 1997, **10**, 1.
15. F. Montañés, T. Fornari, A. Olano and E. Ibáñez, *J. Chromatogr. A*, 2012, **1250**, 92.
16. S. Quispe-Condori, D. Sánchez, M. A. Foglio, P. T. V. Rosa, C. Zetzl, G. Brunner and M. A. A. Meireles, *J. Supercrit. Fluids*, 2005, **36**, 40.
17. M. A. Meireles, Extraction of bioactive compounds from Latin American Plants, in *Supercritical Fluid Extraction of Nutraceuticals and Bioactive Compounds*, ed. by J. L. Martinez, CRC Press, Boca Raton, FL, USA, pp. 243, 2007. ISBN: 978-0-8493-7089-2.
18. V. M. Rodrigues, E. M. B. D. Sousa, A. R. Monteiro, O. Chiavone-Filho, M. O. M. Marques and M. A. A. Meireles, *J. Supercrit. Fluids*, 2002, **22**, 21.
19. B. Nobre, F. Marcelo, R. Passos, L. Beirão, A. Palavra, L. Gouveia and R. Mendes, *Eur. Food Res. Technol.*, 2006, **223**, 787.
20. A. M. Hurtado-Benavides, F. J. Señoráns, E. Ibáñez and G. Reglero, *J. Supercrit. Fluids*, 2004, **28**, 29.
21. G. E. P. Box and K. B. Wilson, *J. Roy. Stat. Soc. Ser. B. (Stat. Method.)*, 1951, **13**, 1.
22. S. Liu, F. Yang, C. Zhang, H. Ji, P. Hong and C. Deng, *J. Supercrit. Fluids*, 2009, **48**, 9.

23. M. G. Sajilata, R. S. Singhal and M. Y. Kamat, *J. Food Eng.*, 2008, **84**, 321.
24. H. Scheffe, *J. Roy. Stat. Soc. Ser. B. (Stat. Method.)*, 1963, **25**, 235.
25. A. Safaralie, S. Fatemi and A. Salimi, *Food Bioprod. Process.*, 2010, **88**, 312.
26. R. N. Cavalcanti, H. J. Navarro-Díaz, D. T. Santos, M. A. Rostagno and M. A. A. Meireles, *J. Food Res.*, 2012, **1**, 282.
27. S. N. Joung, C. W. Yoo, H. Y. Shin, S. Y. Kim, K. P. Yoo, C. S. Lee and W. S. Huh, *Fluid Phase Equilib.*, 2001, **185**, 219.
28. T. Bamberger, J. C. Erickson, C. L. Cooney and S. K. Kumar, *J. Chem. Eng. Data*, 1988, **33**, 327.
29. M. Sun and F. Temelli, *J. Supercrit. Fluids*, 2006, **37**, 397.
30. G. Brunner, *J. Supercrit. Fluids*, 1998, **13**, 283.
31. H. Sovová, *J. Supercrit. Fluids*, 2012, **66**, 73.
32. S. M. Pourmortazavi and S. S. Hajimirsadeghi, *J. Chromatogr. A*, 2007, **1163**, 2.
33. L. Wang and C. L. Weller, *Trends Food Sci. Technol.*, 2006, **17**, 300.
34. M. Herrero, A. Cifuentes and E. Ibañez, *Food Chem.*, 2006, **98**, 136.
35. M. Ligor, T. Trziszka and B. Buszewski, *Food Anal. Meth.*, 2012, doi: 10.1007/s12161-012-9367-9.
36. Y. W. Hsu, C. F. Tsai, W. K. Chen, Y. C. Ho and F. J. Lu, *Food Chem.*, 2011, **129**, 1813.
37. K. Ansari and I. Goodarznia, *J. Supercrit. Fluids*, 2012, **67**, 123.
38. A. S. Zarena, N. M. Sachindra and K. Udaya Sankar, *Food Chem.*, 2012, **130**, 203.
39. E. Ghasemi, F. Raofie and N. M. Najafi, *Food Chem.*, 2011, **126**, 1449.
40. S. Vidović, I. Mujić, Z. Zeković, Z. Lepojević, S. Milošević and S. Jokić, *J. Am. Oil Chem. Soc.*, 2011, **88**, 1189.
41. N. F. Ramandi, N. M. Najafi, F. Raofie and E. Ghasemi, *J. Food Sci.*, 2011, **76**, C1262.
42. Y. Wang, D. Sun, H. Chen, L. Qian and P. Xu, *Int. J. Mol. Sci.*, 2011, **12**, 7708.
43. Y. Jin, D. Han, M. Tian and K. H. Row, *Nat. Prod. Commun.*, 2010, **5**, 461.
44. B. Liu, F. Guo, Y. Chang, H. Jiang and Q. Wang, *J. Chromatogr. A*, 2010, **1217**, 7833.
45. T. Fornari, A. Ruiz-Rodriguez, G. Vicente, E. Vázquez, M. R. García-Risco and G. Reglero, *J. Supercrit. Fluids*, 2012, **64**, 1.
46. P. F. De Oliveira, R. A. F. Machado, A. Bolzan and D. Barth, *J. Supercrit. Fluids*, 2012, **63**, 161.
47. E. L. C. Cheah, P. W. S. Heng and L. W. Chan, *Sep. Purif. Technol.*, 2010, **71**, 293.
48. L. Orio, L. Alexandru, G. Cravotto, S. Mantegna and A. Barge, *Ultrason. Sonochem.*, 2012, **19**, 591.
49. J. Xiao, B. Tian, B. Xie, E. Yang, J. Shi and Z. Sun, *Eur. Food Res. Technol.*, 2010, **231**, 407.

50. A. Taamalli, D. Arráez-Román, E. Barrajón-Catalán, V. Ruiz-Torres, A. Pérez-Sánchez, M. Herrero, E. Ibáñez, V. Micol, M. Zarrouk, A. Segura-Carretero and A. Fernández-Gutiérrez, *Food Chem. Toxicol.*, 2012, **50**, 1817.
51. S. Şahin, M. Bilgin and M. U. Dramur, *Sep. Sci. Technol.*, 2011, **46**, 1829.
52. N. Mezzomo, B. R. Mileo, M. T. Friedrich, J. Martínez and S. R. S. Ferreira, *Bioresour. Technol.*, 2010, **101**, 5622.
53. H. I. Castro-Vargas, L. I. Rodríguez-Varela, S. R. S. Ferreira and F. Parada-Alfonso, *J. Supercrit. Fluids*, 2010, **51**, 319.
54. G. Vicente, M. R. García-Risco, T. Fornari and G. Reglero, *Chem. Eng. Technol.*, 2012, **35**, 176.
55. I. Borrás Linares, D. Arráez-Román, M. Herrero, E. Ibáñez, A. Segura-Carretero and A. Fernández-Gutiérrez, *J. Chromatogr. A*, 2011, **1218**, 7682.
56. C. F. Kuo, J. D. Su, C. H. Chiu, C. C. Peng, C. H. Chang, T. Y. Sung, S. H. Huang, W. C. Lee and C. C. Chyau, *J. Agric. Food. Chem.*, 2011, **59**, 3674.
57. A. M. Posadino, M. C. Porcu, B. Marongiu, A. Cossu, A. Piras, S. Porcedda, D. Falconieri, R. Cappuccinelli, G. Biossa, G. Pintus and L. Pretti, *Food Res. Int.*, 2012, **46**, 354.
58. V. Mičić, Ž. Lepojević, M. Jotanoviaæ, G. Tadić and B. Pejović, *J. Appl. Sci.*, 2011, **11**, 3630.
59. L. D. Kagliwal, S. C. Patil, A. S. Pol, R. S. Singhal and V. B. Patravale, *Sep. Purif. Technol.*, 2011, **80**, 533.
60. P. P. Almeida, N. Mezzomo and S. R. S. Ferreira, *Food Bioproc. Technol.*, 2012, **5**, 548.
61. Z. Chen, J. Chao, B. Wang, H. Cao, S. Wang and C. Lin, *Adv. Mat. Res.*, 2012, **518–523**, 3931.
62. S. Akay, I. Alpak and O. Yesil-Celiktas, *J. Sep. Sci.*, 2011, **34**, 1925.
63. M. R. García-Risco, G. Vicente, G. Reglero and T. Fornari, *J. Supercrit. Fluids*, 2011, **55**, 949.
64. C. Grosso, A. C. Figueiredo, J. Burillo, A. M. Mainar, J. S. Urieta, J. G. Barroso, J. A. Coelho and A. M. F. Palavra, *J. Sep. Sci.*, 2010, **33**, 2211.
65. N. Rubio-Rodríguez, S. Beltrán, I. Jaime, S. M. de Diego, M. T. Sanz and J. R. Carballido, *Innov. Food Sci. Emerg. Technol.*, 2010, **11**, 1.
66. C. Crampon, O. Boutin and E. Badens, *Ind. Eng. Chem. Res.*, 2011, **50**, 8941.
67. M. Plaza, M. Herrero, A. Alejandro Cifuentes and E. Ibáñez, *J. Agric. Food. Chem.*, 2009, **57**, 7159.
68. V. Ferraro, I. B. Cruz, R. F. Jorge, F. X. Malcata, M. E. Pintado and P. M. L. Castro, *Food Res. Int.*, 2010, **43**, 2221.
69. E. Ibáñez, M. Herrero, J. A. Mendiola and M. Castro-Puyana, in *Marine Bioactive Compounds: Sources, Characterization and Applications*, ed. by M. Hayes, Springer Verlag, New York, pp. 55, 2012.
70. A. P. Simopoulos, *Curr. Sports Med. Rep.*, 2007, **6**, 230.

71. F. Shahidi and H. Miraliakbari, *J. Med. Food*, 2004, **7**, 387.
72. F. Shahidi and H. Miraliakbari, *J. Med. Food*, 2005, **8**, 133.
73. I. Calzolari, S. Fumagalli, N. Marchionni and M. Di Bari, *Curr. Pharm. Des.*, 2009, **15**, 4094.
74. B. L. F. Lopes, A. P. Sánchez-Camargo, A. L. K. Ferreira, R. Grimaldi, L. C. Paviani and F. A. Cabral, *J. Supercrit. Fluids*, 2012, **61**, 78.
75. A. P. Sánchez-Camargo, M. Â. A. Meireles, A. L. K. Ferreira, E. Saito and F. A. Cabral, *J. Supercrit. Fluids*, 2012, **61**, 71.
76. A. P. Sánchez-Camargo, H. A. Martinez-Correa, L. C. Paviani and F. A. Cabral, *J. Supercrit. Fluids*, 2011, **56**, 164.
77. B. C. Liau, C. T. Shen, F. P. Liang, S. E. Hong, S. L. Hsu, T. T. Jong and C. M. J. Chang, *J. Supercrit. Fluids*, 2010, **55**, 169.
78. S. Tang, C. Qin, H. Wang, S. Li and S. Tian, *J. Supercrit. Fluids*, 2011, **57**, 44.
79. M. D. Macías-Sánchez, J. M. Fernandez-Sevilla, F. G. A. Fernández, M. C. C. García and E. M. Grima, *Food Chem.*, 2010, **123**, 928.
80. A. M. F. Palavra, J. P. Coelho, J. G. Barroso, A. P. Rauter, J. M. N. A. Fareleira, A. Mainar, J. S. Urieta, B. P. Nobre, L. Gouveia, R. L. Mendes, J. M. S. Cabral and J. M. Novais, *J. Supercrit. Fluids*, 2011, **60**, 21.
81. J. J. Wu, S. E. Hong, Y. C. Wang, S. L. Hsu and C. M. J. Chang, *J. Supercrit. Fluids*, 2012, **66**, 333.
82. C. T. Shen, P. Y. Chen, J. J. Wu, T. M. Lee, S. L. Hsu, C. M. J. Chang, C. C. Young and C. J. Shieh, *J. Supercrit. Fluids*, 2011, **55**, 955.
83. B. Klejdus, L. Lojková, M. Plaza, M. Šnóblová and D. Štěrbová, *J. Chromatogr. A*, 2010, **1217**, 7956.
84. H. M. Wang, J. L. Pan, C. Y. Chen, C. C. Chiu, M. H. Yang, H. W. Chang and J. S. Chang, *Process Biochem.*, 2010, **45**, 1865.
85. D. Ruen-Ngam, A. Shotipruk, P. Pavasant, S. Machmudah and M. Goto, *Chem. Eng. Technol.*, 2012, **35**, 255.
86. F. Sahena, I. S. M. Zaidul, S. Jinap, M. H. A. Jahurul, A. Khatib and N. A. N. Norulaini, *J. Food Eng.*, 2010, **99**, 63.
87. L. Fiori, M. Solana, P. Tosi, M. Manfrini, C. Strim and G. Guella, *Food Chem.*, 2012, **134**, 1088.
88. H. Wang, Y. Liu, S. Wei and Z. Yan, *Food Chem.*, 2012, **132**, 582.
89. K. Fujii, *Food Bioprod. Process.*, 2012, **90**, 762.
90. V. Treyvaud Amiguet, K. L. Kramp, J. Mao, C. McRae, A. Goulah, L. E. Kimpe, J. M. Blais and J. T. Arnason, *Food Chem.*, 2012, **130**, 853.
91. B. W. Zhu, L. Qin, D. Y. Zhou, H. T. Wu, J. Wu, J. F. Yang, D. M. Li, X. P. Dong and Y. Murata, *Eur. Food Res. Technol.*, 2010, **230**, 737.
92. D. Y. Zhou, L. Tong, B. W. Zhu, H. T. Wu, L. Qin, H. Tan and Y. Murata, *J. Food Process. Preserv.*, 2012, **36**, 126.
93. H. Wijngaard, M. B. Hossain, D. K. Rai and N. Brunton, *Food Res. Int.*, 2012, **46**, 505.
94. M. Durante, M. S. Lenucci, L. Rescio, G. Mita and S. Caretto, *Phytochem. Rev.*, 2012, **1**.

95. J. A. Egydio, A. M. Moraes and P. T. V. Rosa, *J. Supercrit. Fluids*, 2010, **54**, 159.
96. J. Yu, J. Wang, C. Liu, Z. Liu and Q. Wang, *Int. J. Food Sci. Technol.*, 2012, **47**, 1115.
97. B. Li, Y. Xu, Y. X. Jin, Y. Y. Wu and Y. Y. Tu, *Ind. Crops Prod.*, 2010, **32**, 123.
98. K. L. Nyam, C. P. Tan, O. M. Lai, K. Long and Y. B. C. Man, *Food Bioproc. Technol.*, 2011, **4**, 1432.
99. M. G. Bernardo-Gil, R. Roque, L. B. Roseiro, L. C. Duarte, F. Gírio and P. Esteves, *J. Supercrit. Fluids*, 2011, **59**, 36.
100. P. Benelli, C. A. S. Riehl, A. Smânia Jr., E. F. A. Smânia and S. R. S. Ferreira, *J. Supercrit. Fluids*, 2010, **55**, 132.
101. L. Casas, C. Mantell, M. Rodríguez, E. J. M. d. l. Ossa, A. Roldán, I. D. Ory, I. Caro and A. Blandino, *J. Food Eng.*, 2010, **96**, 304.
102. A. V. Rao, *Tomatoes, Lycopene and Human Health: Preventing Chronic Diseases*, Caledonian Science Press, UK, 2006. ISBN: 0955356504.
103. A. V. Rao, *J. Nutr.*, 1999, **129**, 1442S.
104. M. S. Lenucci, A. Caccioppola, M. Durante, L. Serrone, R. Leonardo, G. Piro and G. Dalessandro, *J. Sci. Food Agric.*, 2010, **90**, 1709.
105. S. MacHmudah, S. Zakaria, M. Winardi, M. Sasaki, N. Goto, Kusumoto and K. Hayakawa, *J. Food Eng.*, 2012, **108**, 290.
106. K. W. Kong, N. F. Rajab, K. Nagendra Prasad, A. Ismail, M. Markom and C. P. Tan, *Food Chem.*, 2010, **123**, 1142.
107. E. Arnáiz, J. Bernal, M. T. Martín, C. García-Viguera, J. L. Bernal and L. Toribio, *Eur. J. Lipid Sci. Technol.*, 2011, **113**, 479.
108. J. Tello, M. Viguera and L. Calvo, *J. Supercrit. Fluids*, 2011, **59**, 53.
109. E. E. Yilmaz, E. B. Özvural and H. Vural, *J. Supercrit. Fluids*, 2011, **55**, 924.
110. C. Da Porto, D. Decorti and F. Tubaro, *Ind. Crops Prod.*, 2012, **36**, 401.
111. K. L. Nyam, C. P. Tan, O. M. Lai, K. Long and Y. B. C. Man, *Food Bioproc. Technol.*, 2011, **4**, 1432.
112. T. I. Lafka, A. E. Lazou, V. J. Sinanoglou and E. S. Lazos, *Food Chem.*, 2011, **125**, 92.
113. A. Romo-Hualde, A. I. Yetano-Cunchillos, C. González-Ferrero, M. J. Sáiz-Abajo and C. J. González-Navarro, *Food Chem.*, 2012, **133**, 1045.
114. G. Liu, X. Xu, Y. Gong, L. He and Y. Gao, *Food Bioprod. Process.*, 2012, **90**, 573.
115. S. N. Ko, T. Y. Ha, S. In Hong, S. W. Yoon, J. Lee, Y. Kim and I. H. Kim, *Int. J. Food Sci. Technol.*, 2012, **47**, 761.
116. R. H. R. Carvalho, E. L. Galvão, J. A. C. Barros, M. M. Conceição and E. M. B. D. Sousa, *Braz. J. Chem. Eng.*, 2012, **29**, 409.
117. F. Agostini, R. A. Bertussi, G. Agostini, A. C. Atti Dos Santos, M. Rossato and R. Vanderlinde, *Sci. World J.*, 2012, **2012**, art. no. 790486.
118. K.-T. Kwon, M. S. Uddin, G.-W. Jung, J.-E. Sim and B.-S. Chun, *Int. J. Biol. Life Sci.*, 2010, **6**, 117.

119. J. A. Mendiola, D. García-Martínez, F. J. Rupérez, P. J. Martín-Álvarez, G. Reglero, A. Cifuentes, C. Barbas, E. Ibañez and F. J. Señoráns, *J. Supercrit. Fluids*, 2008, **43**, 484.
120. M.-T. Golmakani, J. A. Mendiola, K. Rezaei and E. Ibañez, *J. Supercrit. Fluids*, 2012, **62**, 109.
121. J. W. King, F. Favati and S. L. Taylor, *Sep. Sci. Technol.*, 1996, **31**, 1843.
122. A. Birtigh, M. Johannsen, G. Brunner and N. Nair, *J. Supercrit. Fluids*, 1995, **8**, 46.
123. M. Škerget, P. Kotnik and Ž. Knez, *J. Supercrit. Fluids*, 2003, **26**, 181.
124. O. Catchpole, J. Ryan, Y. Zhu, K. Fenton, J. Grey, M. Vyssotski, A. MacKenzie, E. Nekrasov and K. Mitchell, *J. Supercrit. Fluids*, 2010, **53**, 34.
125. J. Planeta and M. Roth, *J. Phys. Chem. B*, 2005, **109**, 15165.
126. E. Riera, M. Blasco, A. Tornero, E. Casas, C. Roselló, S. Simal, V. M. Acosta and J. A. Gallego-Juárez, *AIP Conference Proceedings*, 2012, **1433**, 358.
127. O. Akin, F. Temelli and S. Köseoğlu, *Crit. Rev. Food Sci. Nutr.*, 2012, **52**, 347.

CHAPTER 7

Recent Trends and Perspectives for the Extraction of Natural Products

M. E. M. BRAGA,^{*a} I. J. SEABRA,^b A. M. A. DIAS^a AND H. C. DE SOUSA^{*a}

^a CIEPQPF, Chemical Engineering Department, FCTUC, University of Coimbra, Rua Sílvia Lima, Pólo II – Pinhal de Marrocos, 3030-790 Coimbra, Portugal; ^b ESAC – Politechnic Institute of Coimbra, Bencanta, 3040-316 Coimbra, Portugal

*Email: marabraga@eq.uc.pt, hsousa@eq.uc.pt

7.1 Introduction

In this chapter it is intended to present the most recent trends and perspectives related to the extraction of natural products. However, and to better comprehend the reasons for such current and future tendencies, it is also necessary to try to understand the most important driving forces, constraints and achievements that led to the present situation. Therefore other relevant issues for these subjects will also be briefly covered and discussed, despite this work being mostly focused on the new developments and future perspectives in terms of the target extracts/compounds, of vegetable raw materials and of the engineering/technical topics concerning the involved extraction, separation and purification methodologies.

Extraction of natural products is as old as humankind and the use of natural extracts and of purified natural compounds in food, medical, cosmetic and

RSC Green Chemistry No. 21

Natural Product Extraction: Principles and Applications

Edited by Mauricio A. Rostagno and Juliana M. Prado

© The Royal Society of Chemistry 2013

Published by the Royal Society of Chemistry, www.rsc.org

agricultural applications dates back thousands of years to the Mesopotamian, Egyptian and Chinese civilizations. Nevertheless, some of these natural products still find common uses in modern societies as, for example and according to the World Health Organization (WHO), around 75% of people worldwide still depend on natural-based traditional medicines for their primary health care.¹

Over the last century a large number of natural-based drugs and medicines have been developed and, in more recent years, a significant interest revival in natural-origin products as potential sources for new drugs was observed. Finally, due to several pertinent reasons and to recent consumer and environmental trends, the use of natural products is also becoming more and more popular as food supplements, phytomedicines, nutraceuticals, cosmetics, natural pesticides, as well as in other industrial, energy and environmental applications.

Since it is estimated that only a small part of world biodiversity (5–10%) has been explored for bioactivity so far and that, for example, further research on previously studied plants continues to lead to some new and useful bioactive substances, it is clear that, together with the availability of natural products and their renewable character, there is still a huge potential to explore in the field of natural products extraction and on their use for new and improved applications.

Due to the typical inherent complexity of these systems and to the great number of potentially useful natural products that can be obtained from so many natural sources, it is also essential to develop 'greener', more specific and more efficient extraction/separation/purification methodologies to be applied in the above referred purposes and applications. However, many other relevant factors should be considered before choosing these adequate methods.

Natural extracts may contain quite different substances that present distinct physicochemical properties. In general terms, these extracts contain carbon-based compounds (e.g. sugars, fatty acids, terpenoids and phenolic compounds), nitrogen-based compounds (e.g. alkaloids, amino acids and protein-based compounds) as well as mineral or inorganic elements (e.g. iron, cobalt, phosphorus, sulfur and potassium).

Therefore, besides the necessary selection of the target extracts/compounds, their identification and of their potential natural origins, it is also of paramount importance to define and to take in consideration several other important topics such as: required extraction yield and selectivity; extracts thermal and chemical stabilities; potential final products/applications; economic and market issues (e.g. raw materials availability and corresponding costs, direct and indirect processing/manufacturing costs, energy demand, final product pricing, market demand and consumer trends); involved legislation (environmental, trade and industrial legislation) and other specific regulatory concerns (namely for the food, cosmetic and pharmaceutical industries), as well as scale-up and other production issues (such as other additional separation/purification processes that may be necessary).

All these topics will help to define the appropriate raw material to be extracted and its pre-treatment procedures, the selection of the most suitable solvents and solvent mixtures (in terms of physicochemical properties, purity/composition and potential risks and toxicity) and/or the most efficient extraction methodologies and operational conditions to be employed. Figure 7.1 summarizes some of the most important and interdependent topics that should always be considered when choosing an efficient extraction methodology that allows obtaining a specific extract and/or target compound for a pre-determined application.

Once the target extracts/compounds and all the other relevant issues concerning their extraction are well known and completely defined, it will also be necessary to identify other physical, chemical and engineering problems (e.g. thermodynamics – phase equilibria and solubility – and mass transfer issues) that should be considered in order to optimize the employed extraction methodology (in terms of extraction yields and target compound selectivity) and to avoid (or to reduce) the number of additional separation and purification steps that may still be required for these purposes.²

Generally, large numbers of chemical substances are simultaneously recovered during an extraction process from a natural raw material and it is quite rare to find a specific extraction method and a solvent mixture that presents a high and specific selectivity for the main target compounds and that will lead to high purity extracts. Despite most of the physicochemical properties of extracts being closely related and strongly dependent on the corresponding properties of their pure compounds, they are usually different from these due to

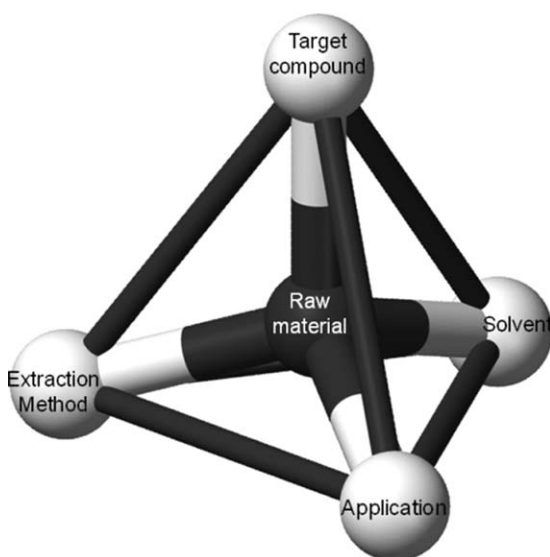


Figure 7.1 Relevant and interdependent topics to be considered when choosing and defining an efficient extraction methodology.

the specific interactions that may be established between all the substances present in these extracts (including solvents or other additives) and to their relative compositions. However, some natural extracts contain substances that are so chemically and physically similar and that are so closely combined that they may present similar properties to those of a specific pure compound and thus they may be easily and mistakenly considered as pure compounds. Furthermore, these chemical and physical similarities will strongly affect and limit the separation and purification of specific target compounds.

This is very important since, after employing the chosen extraction methodology, the obtained extracts must be further and adequately separated, fractionated and purified by several physical and chemical methods such as filtering, sedimentation, centrifugation, evaporation, crystallization, liquid–liquid extraction, adsorption, membrane separation and pervaporation, application of magnetic/electrical fields, or chromatographic methods. However, all these separation/purification methods must also fulfill most of the previously established requirements for the extraction methodologies, namely in terms of purification yields, compounds thermal and chemical stabilities, solvents and solvent mixtures (physicochemical properties and potential risks/toxicity), operational conditions, direct and indirect costs, energy demand, scale-up and processing issues, as well as other important and mandatory environmental, industrial and specific legislation issues.

Harjo *et al.*³ suggested a five-step systematic preliminary evaluation procedure that can be applied for the potential production of phytochemical and other natural-origin products: (i) specification and characterization of the target compound(s) and natural raw material(s) (according to their physicochemical properties); (ii) selection of the adequate extraction/separation techniques and solvents/solvent mixtures to obtain the target compound(s); (iii) flow sheet design and selection of required unit operations and equipment; (iv) selection of the operational conditions for all employed unit operations and equipment; and (v) flow sheet evaluation modeling, cost information analysis and consideration of other specific criteria which may be relevant for the production and commercialization of the envisaged phytochemical or natural compound.

As an example, a typical flow diagram for the extraction of bioactive extracts/compounds intended for potential pharmaceutical applications (but also compliant for food and cosmetic ones) is presented in Figure 7.2. From an engineering/process point of view, the main concerns should be the optimization of the extraction, separation, fractionation, purification and formulation processes. However, other extremely important and obligatory subjects to be considered are those regarding patent and final product approval procedures, non-clinical trials (*in vitro* and *in vivo* experiments), clinical trials, GLP (Good Laboratory Practice), GCP (Good Clinical Practice) and GMP (Good Manufacturing Practice) issues, as well as other scale-up, production, regulatory, economic and marketing topics.

Considering this example and that the envisaged target compound is going to be extracted from a solid vegetable raw material, some of the important

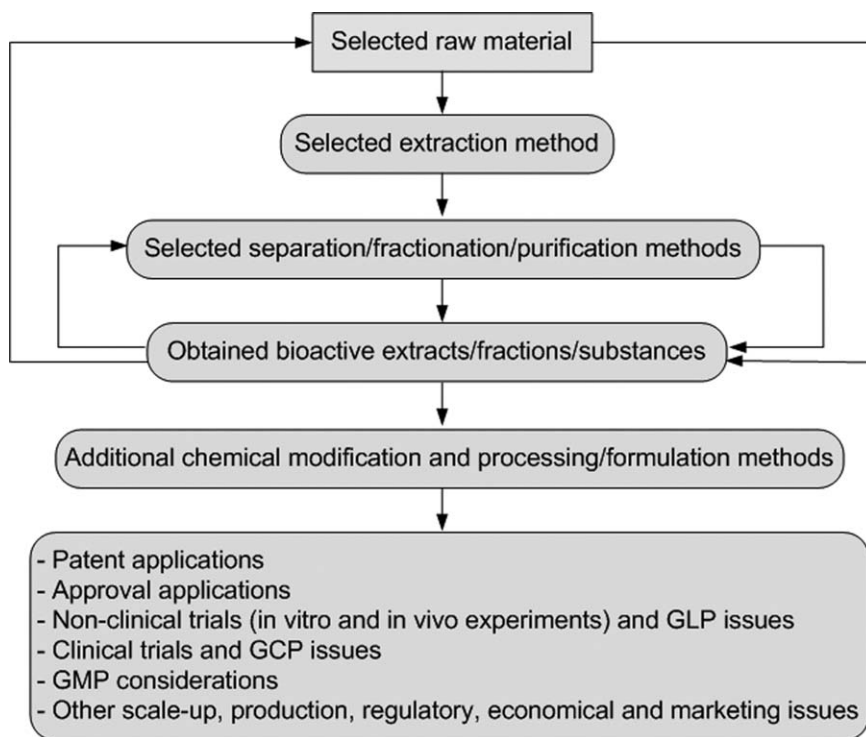


Figure 7.2 Typical process flow diagram for the extraction of bioactive compounds intended for pharmaceutical applications. GLP=Good Laboratory Practice; GCP=Good Clinical Practice; GMP=Good Manufacturing Practice.

engineering/process topics to be considered and optimized can be further summarized (Figure 7.3): raw material collection/selection and pre-treatment; solvent/solvent mixtures; extraction methodologies to be employed; and the operational conditions to be used. Most of these subjects require the knowledge of important raw material, solvent mixtures and target compound physico-chemical properties as well as of other thermophysical and mass transfer issues.

It is important to keep in mind that even for the same and specific target compound, different vegetable raw materials, solvents mixtures and extraction methodologies can be employed. Therefore, although it is possible to transpose some useful information from already studied/employed extraction systems, each new system should always be considered as unique and thus studied, defined and developed according to this premise.

After this brief introduction, the following sections present and detail some of the most recent trends and perspectives in the extraction of natural products, focusing on: the new natural-origin extracts/target compounds that are currently being studied and extracted as well as their potential applications; the most recently studied vegetable raw materials (or the recent tendencies

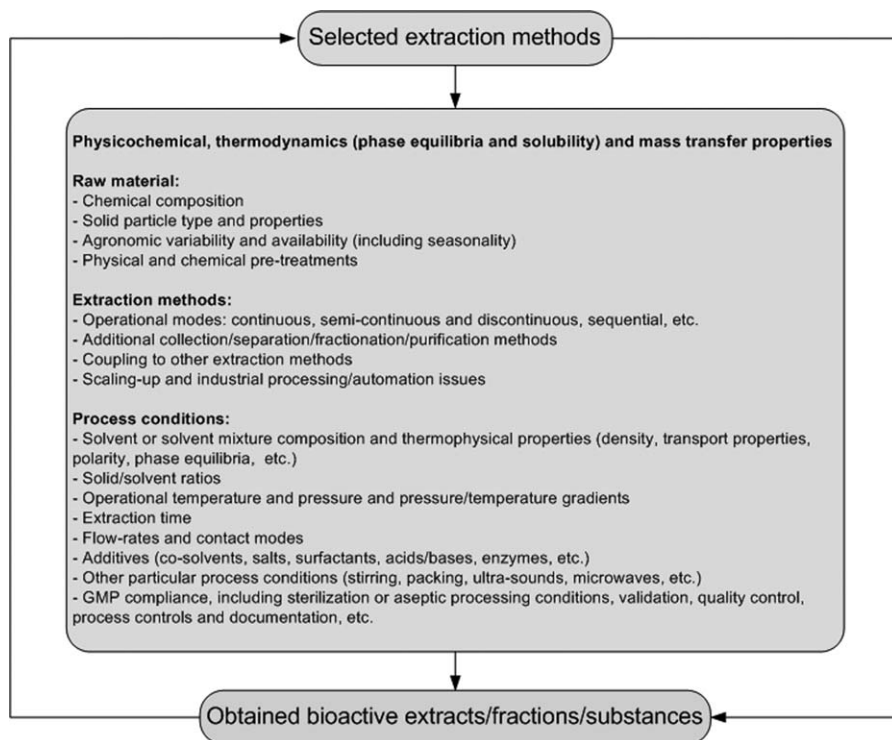


Figure 7.3 Important engineering/process topics that must be considered and optimized for the extraction of bioactive extracts/compounds from vegetable solid raw materials.

regarding previously studied vegetable raw materials) that can be explored as sources for the envisaged target compounds; the current and future strategies regarding the extraction methodologies that are expected to be further developed and employed (including those already being explored but that can be improved and/or combined); and finally, on the novel/alternative solvents, solvent mixtures and extraction additives that could be beneficially used in the above referred extraction methodologies.

7.2 Target Extracts/Compounds

Despite the fact that all compounds from natural origins can be called 'natural products', this term usually refers to secondary metabolites that are extracted from entire (or from parts) of terrestrial and marine organisms (plants, animals or microorganisms). These substances are usually small molecules (with typical molecular weights lower than 2000 g/mol) that are not strictly necessary for the organism's survival. Other high molecular weight substances such as natural polypeptides, proteins and polysaccharides can also be obtained from organisms and be employed in a wide range of applications. However, this type

of substances, their properties and applications, are out of the scope of this work. In addition, plant-origin natural compounds will be the main focus of this review.

A wide range of plant secondary metabolites have been already employed as natural-based drugs and, recently, there has been a significant and renewed interest in these compounds as potential drugs or, alternatively, as sources for new drugs. This happened mostly because some of these compounds have potential to be used as drugs by themselves or, and due to their structural diversity, as chemical precursors/building blocks to synthesize new chemical entities. Moreover, these compounds present intrinsic drug-like ADME properties, i.e. they can be Absorbed, Distributed, Metabolized and Excreted. Finally, mostly due to consumer trends and to market and safety/environmental issues, their use is also becoming very important as food supplements, phytomedicines, nutraceuticals, cosmetics, natural pesticides, as well as in other industrial, energy and environmental applications (where these substances can be employed to replace toxic/harmful compounds, or when degradability is required).

Older and traditional strategies to identify natural target extracts/compounds were essentially based on chemotaxonomic research and on ethnical and traditional use information. These strategies were mainly focused on the extraction, isolation, identification and chemistry of target compounds but not as much on their possible biological activities. However, in recent years other strategies were also implemented, namely those that are more focused on bioactivity such as *in vitro* bioassay-guided isolation and identification, metabolomics, genetic manipulation, as well as on combinatorial chemistry, new and advanced chromatographic spectroscopic methods, automation via high-throughput screening (HTS), natural products libraries and on production of target compounds in microorganisms or cell cultures.

In order to evaluate which are the current trends and future perspectives regarding natural product extraction, namely in terms of the most studied target extracts/compounds, a literature search was performed, covering the years between 2000 and 2011. However, due to the great number of different extracts and valuable target compounds that can be obtained from so many natural sources and to their distinct chemical functionalities and corresponding chemical, physical and biological properties, it is usually a very difficult task to identify and index/classify these substances. Furthermore, several different types of classifications and names for these compounds can be found in literature. These are usually based on natural products chemical classes or even on some of their biological and physicochemical characteristics (such as 'antioxidants' or 'volatile oils') which may make literature searches and reviews quite confusing or even lead to wrong or mismatched results.

A possible classification for all the categories/classes of natural products commonly found in organisms is the one compiled in the *Dictionary of Natural Products*, online edition, and presented in Table 7.1.⁴

The literature search used these specific index terms (descriptors) with some exceptions, since, from our experience, some of them are not commonly

Table 7.1 Categories/classes of natural products commonly found in organisms (Source: *Dictionary of Natural Products*).⁴

<i>Natural Products categories</i>	<i>Examples</i>
Aliphatic natural products	Semiochemicals Lipids, including fatty acids, waxes, etc.
Polyketides	Antibiotics: uvaricin, erythromycin, rifamycin, nystatin, tetracyclines, aflatoxin, etc.
Carbohydrates	Sugars, disaccharides, oligosaccharides and polysaccharides, etc.
Oxygen heterocycles	β -lactones, furans, butanolides, pyrans, pentanolides, 2-pyrones, 4-pyrones, etc.
Simple aromatic natural products	Simple benzene derivatives, phenols, benzyl alcohols, benzaldehydes, aryl ketones, benzoic acids, phenylacetic acid derivatives and phenylpropanoids, benzoquinones, etc.
Benzofuranoids	Benzofurans, benzodifurans and isobenzofurans, phthalides, etc.
Benzopyranoids	1-benzopyran derivatives such as coumarins, etc.
Flavonoids	Simple phenolics, chalcones, aurones, dihydrochalcones, flavanones, flavones, flavanon-3-ols, flavonols, flavan-3,4-diols, anthocyanins, anthocyanidins, flavan-3-ols, proanthocyanidins, flavans, etc.
Tannins	Gallic acid, catechin, gallotannins, ellagitannins, etc.
Lignans	Megaceratonic acid, lappaols, flavonolignan, coumarinolignan, stilbenolignan, xantholignan, etc.
Polycyclic aromatic natural products	Naphthalenes, quinones, naphthoquinones, indenenes, anthracenes and phenanthrenes, phenalenes and fluorenes, etc.
Terpenoids	Monoterpenoids, sesquiterpenoids, diterpenoids, sesterterpenoids, triterpenoids, tetraterpenoids, miscellaneous terpenoids, meroterpenoids, steroids (including sterols), etc.
Steroids	Estrane steroids, androstane steroids, C20 steroids, pregnane steroids, cardanolide steroids, bufanolide steroids, cholestane steroids, ergostane steroids, stigmastane steroids, spirostan and furostan steroids, Vitamin D and related compounds, etc.
Amino acids and peptides	Protein and non-protein amino acids, cyclic- and oligopeptides, β -lactams, glycopeptides, etc.
Alkaloids	Ornithine derivatives, lysine derivatives, nicotinic acid derivatives, polyketide origin, anthranilic acid derivatives, tyrosine and phenylalanine derivatives, isoquinoline alkaloids, tryptophan derivatives, monoterpene indole alkaloids, terpenoid alkaloids, steroidal alkaloids, imidazole alkaloids, oxazole alkaloids, thiazole alkaloids, pyrazine and quinoxaline alkaloids, pyrrole alkaloids, putrescine alkaloids, spermine and spermidine alkaloids, peptide alkaloids, purines, pteridines and analogues, etc.
Polypyrrroles	Haems, bilins, chlorophylls and porphyrins, etc.

employed in literature. Thus, the descriptors used were 'lipids' (instead of 'aliphatic natural products'), 'phenolics' (instead of 'flavonoids'), 'naphthalenes and quinones' instead of 'polycyclic aromatic natural products', 'oligopeptides and amino acids' instead of 'amino acids and peptides', and 'essential oils' (instead of 'terpenoids'). All searches were carried out using the selected natural products categories/classes specific descriptors (in the singular form) in conjunction with the additional descriptor 'extraction'. Literature search was performed using the Scopus search engine⁵ and covered only review and research articles for the 2000–2011 period. Search for descriptors was carried in article title, abstract and keywords.

Literature search results are presented in Figure 7.4. Some categories/classes are not presented since obtained search results were insignificant (if compared to the other categories/classes).

As can be verified, from 2000 up to 2011, the publications dealing with the extraction of lipids are the clear majority (with more than 5200 results) followed by those involving the extraction of phenolics (more than 3200 results). These are followed by those works involving the extraction of carbohydrates, essential oils and steroids (around 2100 results), alkaloids (around 1400 results), naphthalenes and quinones (around 1100 results) and tannins (around 800 results). Finally, with a much lower number of publications (between 200 and 50 results), appear the works searched for lignans, polypyrroles, oligopeptides and amino acids, and polyketides.

The obtained numerical results may contain some inaccuracies and may be slightly over- or under-estimated. This can be due to the employed search

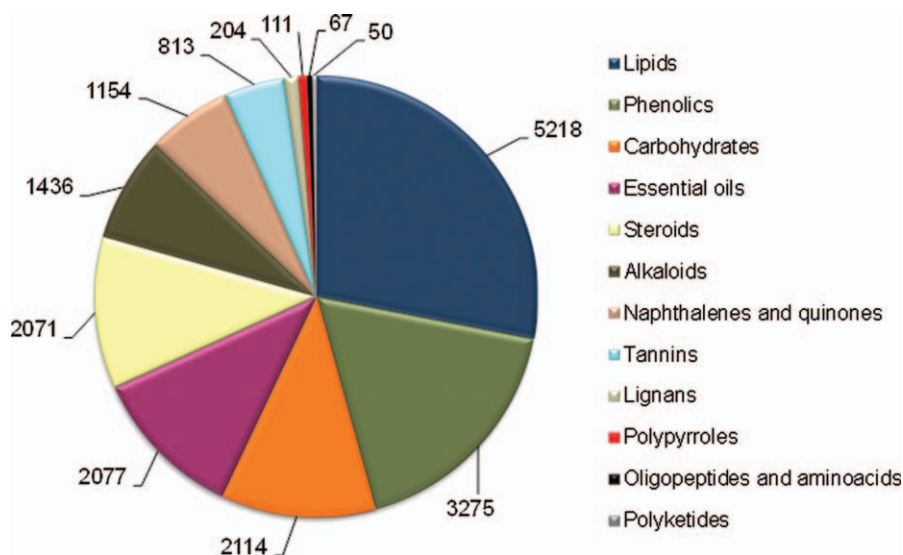


Figure 7.4 Number of publications (review and research articles) dealing with natural product extraction. Literature search was performed in Scopus for the 2000–2011 period.⁵

descriptors (that may be too specific or too broad for the performed search), to the indexed articles titles/abstracts/keywords (that may not contain the most adequate terms or keywords), as well as to other indexing/searching services limitations. However, it would be a gigantic task to verify such a large number of results, article by article, in order to improve their exactness. In fact, the chapter focus was finding/comparing general trends on the extraction of natural products with an acceptable level of confidence (and not in obtaining/comparing very accurate data).

The observed tendencies were somehow expected. Nevertheless, additional literature searches were performed for the compound classes with higher publication numbers and/or for those to which some reservations apply in terms of the results obtained for the selected descriptors. Therefore, literature was further searched for the descriptors 'fatty acids' and 'waxes' (for the 'lipids' class of compounds); for 'flavonoids', 'anthocyanins', 'proanthocyanidins' and 'anthocyanidins' (for the 'phenolics' class of compounds); for 'sugars' and 'oligosaccharides' (for the 'carbohydrates' class of compounds); and for 'volatile oils' and 'terpenoids' (for the 'essential oils' class of compounds). Once again, searches were performed in conjunction with the additional descriptor 'extraction'. The results are presented in Figure 7.5.

From these results, it can be verified that the previously chosen search descriptors were those that led to the larger number of publications with the exception of the term 'carbohydrates', which led to slight lower values than

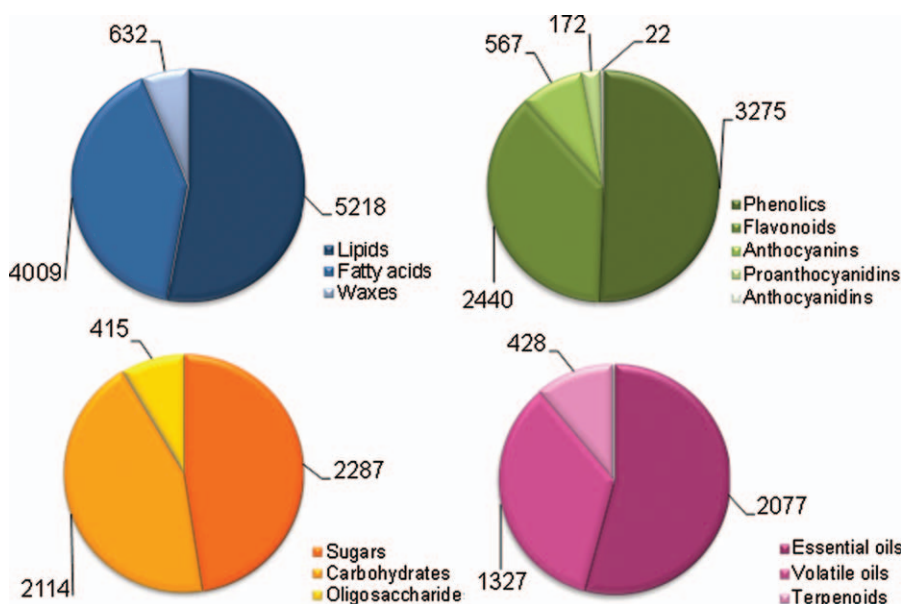


Figure 7.5 Number of publications (review and research articles) dealing with natural product extraction for some specific classes of compounds. Literature search was performed in Scopus, for the 2000–2011 period.⁵

those obtained using the descriptor 'sugars'. Another possible conclusion that can be drawn from these results is that, even considering other alternative descriptors for the same compounds class, the final results in terms of number of publications for each compounds class followed the same tendencies.

Figure 7.6 presents the annual number of publications (review and research articles) for the covered search period. It is clear that, in general terms, the number of publications dealing with the extraction of natural products has been increasing in the last years for all classes of compounds. Moreover, the observed increases were much more pronounced in the last 7 years (2005–2011).

In terms of compound classes, this growing interest was higher for the extraction of tannins (750% increase for the 2000–2011 period and 480% increase for the 2005–2011 period), phenolic compounds (490% increase for the 2000–2011 period and 230% increase for the 2005–2011 period), essential oils (280% increase for the 2000–2011 period and 150% increase for the 2005–2011 period), alkaloids (240% increase for the 2000–2011 period and 210% increase for the 2005–2011 period) and lipids (140% increase for the 2000–2011 period and 80% increase for the 2005–2011 period). Despite the number of publications dealing with the extraction of all other compound classes having also increased in recent years, the corresponding increments were not as high as those observed for the above referred substances.

All these results reflect the recent growing interest and tendencies in this research field as the potential of natural products for new applications have also been further explored and intensified, namely for food, nutraceutical, cosmetic, pharmaceutical, agricultural and environmental applications.

These applications are evidently the result of their advantageous properties as natural colorants, pigments or dyes, flavors, aromas, antioxidants, anti-inflammatories, antiproliferative, antimicrobials, antifungals, analgesics, pesticides, or simply as alternative precursor molecules for the synthesis of

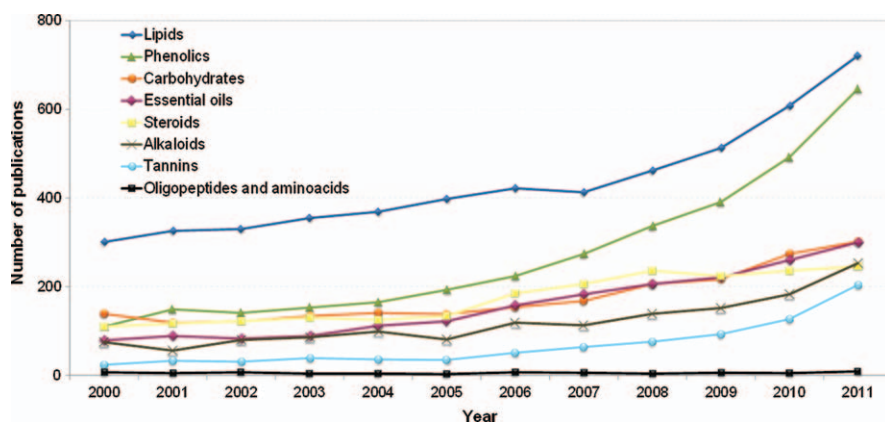


Figure 7.6 Annual number of publications (review and research articles) dealing with natural product extraction. Literature search was performed in Scopus, for the 2000–2011 period.⁵

other substances for these applications. The literature was also searched for the extraction of compounds presenting some specific properties or that can be used for some particular applications. These results are presented in Figures 7.7 and 7.8.

The descriptor 'extraction' was used together with the descriptors 'colorants', 'pigments', 'dyes', 'aromas', 'flavors', 'antioxidants', 'anti-inflammatories', 'antiproliferatives', 'anticancer', 'antimicrobials', 'antifungals' and 'analgesics'.

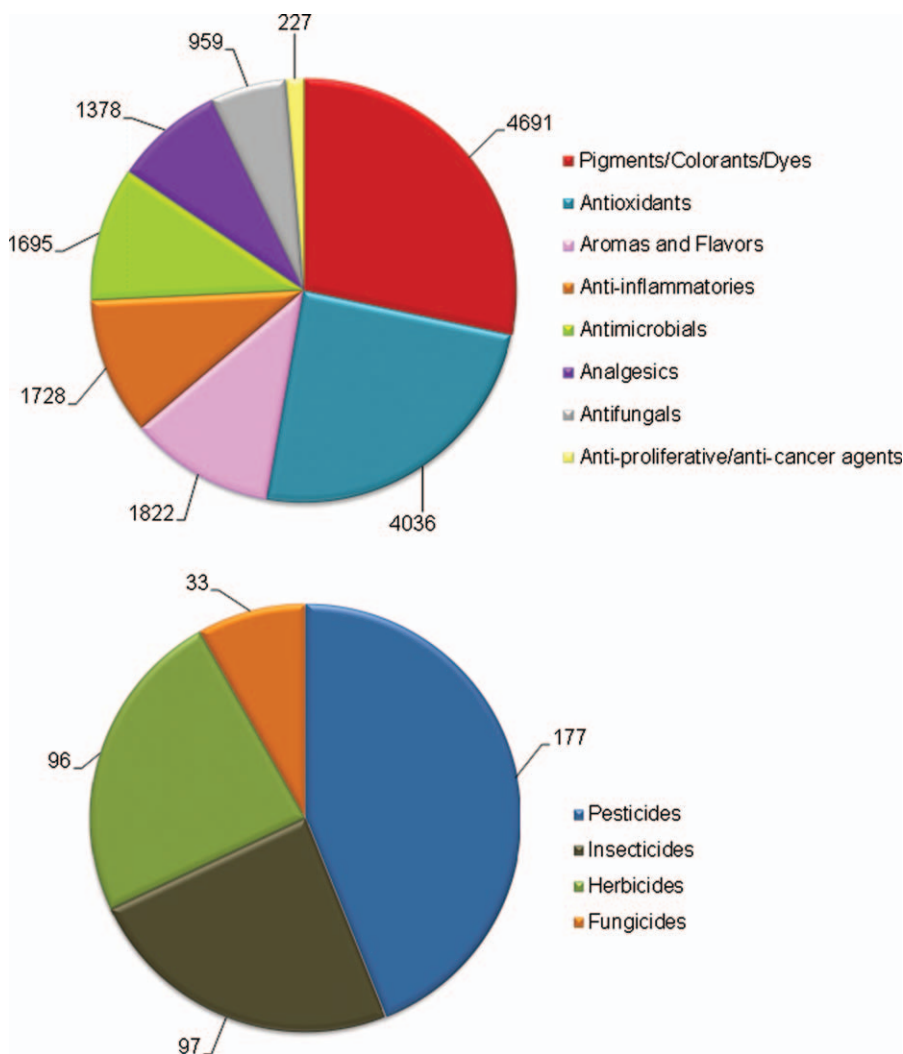


Figure 7.7 Number of publications (review and research articles) dealing with natural product extraction: compounds presenting some specific properties or that can be used for some particular applications. Literature search was performed in Scopus, for the 2000–2011 period.⁵

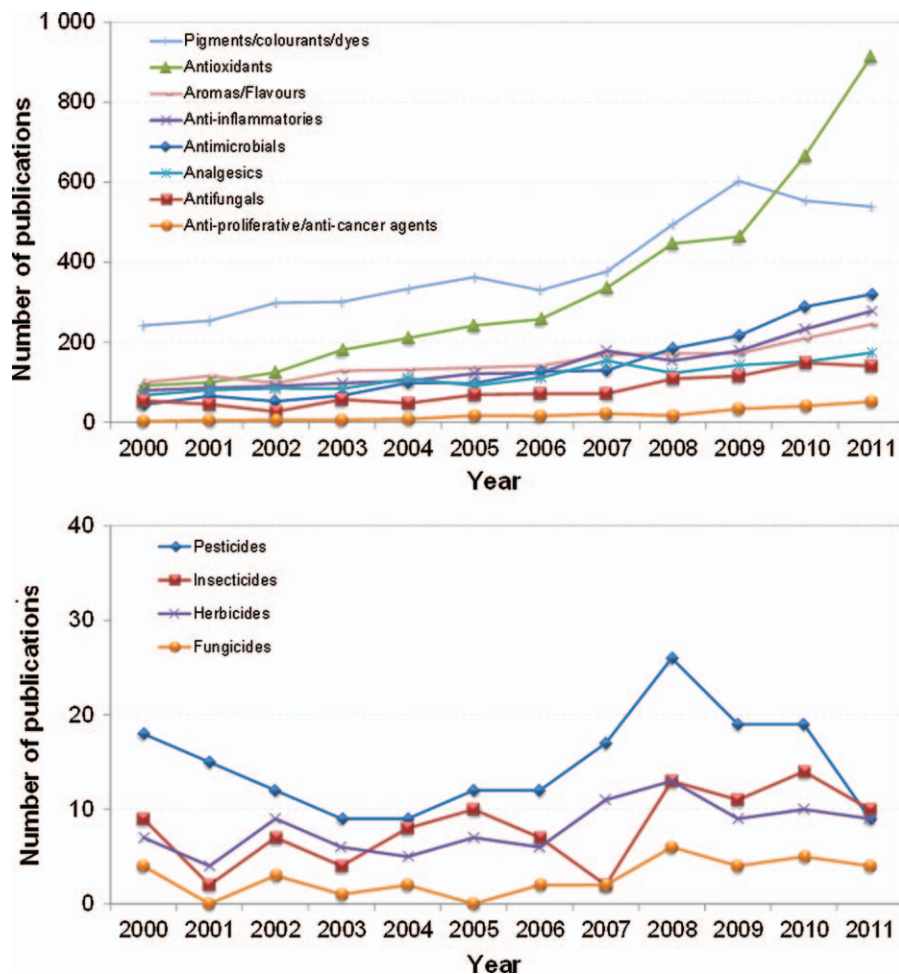


Figure 7.8 Annual number of publications (review and research articles) dealing with natural product extraction: compounds presenting some specific properties or that can be used for some particular applications. Literature search was performed in Scopus, for the 2000–2011 period.⁵

The term ‘pesticides’ was also searched, but in this case, since most of the current pesticides are still of a synthetic origin, the obtained search results presented too many works on the extraction of synthetic pesticides from soils, plants, etc. Therefore, the descriptors ‘pesticides’, ‘insecticides’, ‘herbicides’ and ‘fungicides’ were used together with the descriptors ‘natural’ and ‘extraction’.

For the 2000–2011 period, the works dealing with the extraction of colorants/pigments/ dyes and with antioxidants were the ones that have raised more attention and interest (4000–4700 results), followed by the works on the

extraction of aromas/flavors, anti-inflammatories, antimicrobials, analgesics, and antifungals (960–1820 results), and finally by antiproliferative substances (around 220 results). However, it must be noted that some of the obtained results may be superimposed since, for most of the cases, the extracted target compounds can present multiple biological activities (e.g. simultaneous antioxidant and anti-inflammatory activities) as well as they can be employed for different purposes (e.g. natural colorants or aromas that can be employed in food applications but also may be used as antioxidants or anti-inflammatories in biological applications). These results clearly confirm the previously obtained literature search data in terms of the natural products compound classes: the ones that raised the higher recent interest (phenolics, tannins, essential oils and alkaloids) are those that are well-known as colorants, aromas, antioxidants, anti-inflammatories, antifungals, antimicrobials and antiproliferatives.

In terms of pesticides, works on the extraction of natural insecticides and herbicides were the large majority (around 100 results each) followed by works on the extraction of fungicides (30 results).

For the covered search period, the annual number of publications (review and research articles) for the above referred compounds is presented in Figure 7.8. In general terms, the number of publications dealing with the extraction of these types of substances increased for all the searched descriptors, with the exception of natural pesticides. It is also evident that, in the last 7 years, the most dramatic increases were observed for the works on the extraction of antioxidants, followed by those of antimicrobials, antiproliferatives and anti-inflammatories.

In general terms, it was observed that lipids, phenolics, carbohydrates, essential oils, steroids and alkaloids were the most studied natural products that were obtained from natural sources between 2000 and 2011. Particularly, in the last 7 years a much higher interest in the extraction of tannins, phenolics, essential oils, alkaloids and lipids was observed. These results also denote the most recent trends on the use of natural products for food, nutraceutical, cosmetic, pharmaceutical, agricultural and environmental applications, and as these types of substances present most of the required properties to be used as colorants/pigments/dyes, antioxidants, anti-inflammatories, antimicrobials, analgesics, antifungals and pesticides. In addition to these advantageous properties, some recent consumer trends as well as other safety, sustainability and environmental issues are certainly renewing and increasing the interest in natural products and in their applications.

7.3 Raw Materials

There is an almost infinite number of potential organisms in Nature that can be explored to obtain the target natural products and, in addition, a great part of these potential organisms are still unstudied, unexploited or have not yet been discovered/identified.

Besides, a large number of different parts/tissues of these organisms can be used for these purposes. For example, the extraction of plants can be performed using roots, rhizomes, leaves, stems, flowers, fruits, pods, seeds, barks, peels, rinds, husks as well as on some plant-generated and/or plant-secreted fluids such as saps, resins and latexes.

All these parts/tissues (or fluids) of organisms are always very complex and heterogeneous systems in chemical and in morphological terms. They are organized in distinct and sometimes intricate 3D morphologies and may comprise many different chemical substances (including the target compounds) that surely present distinct chemical/physical (and thus biological) properties. Moreover, these target compounds are usually present in those parts/tissues in different compositions, which may also vary as a consequence of other important factors such as agronomical variability, age/maturation state and edaphic/climatic conditions.

Therefore, all these issues must be considered when choosing a specific raw material to be employed as the source of the foreseen target compounds. In addition, other factors to take into account are the raw material availability, costs, traceability, seasonality, as well as the ecological, environmental and economic impacts that its commercial/industrial utilization may originate. Finally, it is also quite important to evaluate other issues that may affect the use of a specific raw material to obtain natural products, namely the procedures that should be employed for its collection, selection (differences between lots) and storage, or any other pre-treatments/processing steps that may be required (such as drying, grinding, particle size separation, chemical/enzymatic reactions, etc.).

Raw materials mean particle sizes, shapes and porosities will have a strong influence on extraction yields due to the involved mass (and heat) transfer processes which are strongly dependent on the particles specific surface areas and porous structure (namely pore tortuosity). Therefore, an accurate knowledge of these properties is usually essential to estimate and tune some of the operational conditions such as processing time, temperature, solvent/feed ratios (S/F) and solvent flow rates. In addition, for fixed-bed/percolation extraction methods, mean particle sizes (and shapes) will also affect the particle packing, bed porosity and, consequently, the solvent flow and the mass and heat transfer processes.

For example, if these particles are obtained from a plant rhizome (such as ginger or turmeric) they will mostly contain carbohydrates (such as starch and cellulose), proteins, essential oils (terpenoids) and heavy fractions. However, morphologically these particles will mainly present a 3D geometry (usually approaching the spherical geometry) and the target secondary metabolites are synthesized in some specialized internal structures (secretory glands). Therefore, the solvent/solutes convection and diffusion processes are both relevant and much more difficult for this type of internal particle morphology. As a consequence, the resulting extraction efficiencies are usually low. This situation is illustrated in Figure 7.9B, where a hypothetical pore located inside a plant-origin 3D particle (such as particles originated from plant roots, barks

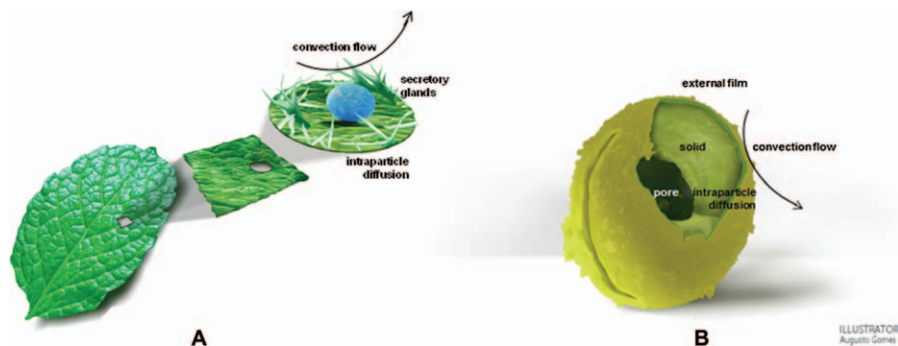


Figure 7.9 Hypothetical plant-origin particles: (A) 2D particle, approaching the planar geometry (leaves, peels, flower petals); (B) 3D particle, approaching the spherical geometry (roots, barks, fruits).

and fruits) is considered. However, for other plant parts/organs such as leaves, flower petals and some fruit peels, particle geometries are usually of the planar type (2D), and the predominant mass transfer phenomenon is convection. In these cases, plant secretory glands are usually located at or near the surface of these plant parts/tissues and the internal porous structures are thus negligible (Figure 7.9A).

The knowledge of raw material general composition may also be quite important since it can help to avoid the negative influence of some substances (such as water, sugars and high molecular weight substances) on the extraction of the envisaged target compounds as well as the co-extraction of undesired compounds. For example, the general composition (w/w, dry basis) of turmeric was identified by Braga *et al.*, where starch represented ~20–34%, proteins were around 10–12% and only 7–8% corresponded to the target oleoresin (essential oils and heavy fractions).⁶ It was also found that the general turmeric composition depended on the employed raw material pre-treatments (drying conditions and mean particle sizes) and on the raw material lot (due to the distinct edaphic-climatic conditions of their production). With this information, the foreseen oleoresin extraction was later designed in order to obtain high oleoresin extraction yields, by using high affinity solvents for the oleoresin substances such as supercritical carbon dioxide, ethanol and isopropanol (or their high-pressure mixtures), as well as to avoid raw material swelling. Another example of the importance of the exact knowledge of the chemical composition and of specific morphologies in different plant parts/tissues concerns the extraction of spilanthol from flowers, stems and leaves of jambu, where it was observed that the highest spilanthol content was found in flower petals and that, at the employed operational conditions, the spilanthol extraction was less efficient from jambu leaves and stems due to specific morphologies of these plant parts.⁷ Several other studies on the different natural products compositions in different plant parts/tissues and the effects of agronomical variability can also be found in literature.^{8–11}

For plant-origin raw materials, some specific descriptors were used, which correspond to several plant parts/tissues: 'roots/rhizomes', 'stems', 'barks', 'leaves/leaf', 'flowers', 'fruits', 'husks', 'peels', 'seeds', 'pods' and 'resins/latexes'. Other descriptors searched were 'residues', 'byproducts', 'pomace' and 'bagasse', since the extraction of natural products from plant agro-industrial wastes is a common and usually advantageous and viable procedure. All searches were carried out using some selected descriptors (in the singular form) in conjunction with the additional descriptor 'extraction'. The results are presented in Figures 7.10 and 7.11.

The largest number of publications on the extraction of natural products from plant raw materials was obtained for roots and rhizomes (around 6230 results), followed by leaves (around 5600 results) and resins, latexes, fruits and seeds (3950–4600 results). Other relevant results were obtained for the extraction from stems, barks and flowers (930–2675 results). Finally, work on the extraction from peels, pods and husks presented a much lower number of publications (170–500 results). In terms of the use of plant agro-industrial

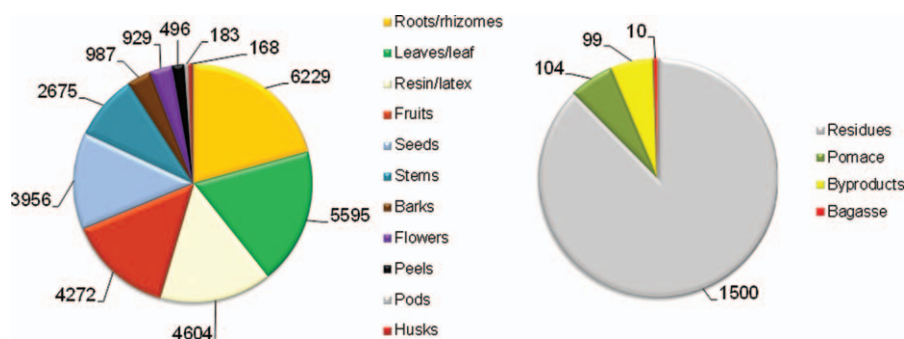


Figure 7.10 Number of publications (review and research articles) dealing with natural product extraction from plant raw materials. Literature search was performed in Scopus, for the 2000–2011 period.⁵

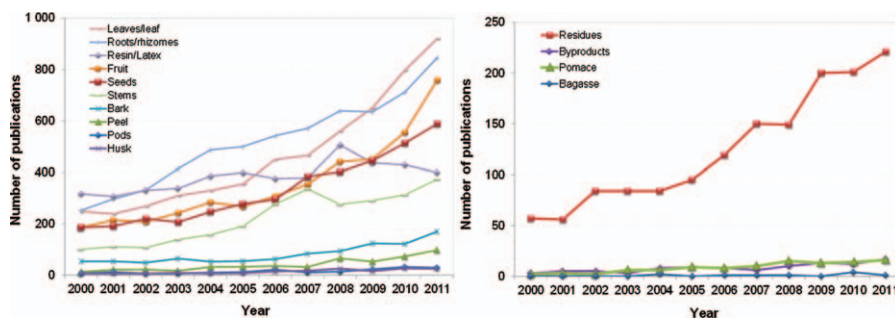


Figure 7.11 Annual number of publications (review and research articles) dealing with natural product extraction from plant raw materials. Literature search was performed in Scopus, for the 2000–2011 period.⁵

residues/wastes (without any imposed search limitations in terms of the employed plants and plant parts), the literature search results clearly led to a much larger number of publications for the term 'residues' (1500 results) if compared to the other search terms employed (10–100 results).

The annual number of publications (review and research articles) dealing with natural product extraction from plant raw materials is presented in Figure 7.11. In general terms, it was observed that the number of publications has increased in the last decade for all the plant parts/tissues. The increase was more pronounced for the extraction from peels, flowers and pods (380–650%), followed by husks, fruits, stems and leaves (270–320% increase). However, in the last 7 years the increase in the number of publications was more pronounced for the extraction from husks, barks, peels, fruits and flowers (175–210% increase). Curiously, despite the fact that the larger number of publications is obtained for the extraction from roots and rhizomes, the annual increase in the number of publications from these plant parts was not as high as that observed for the other plant parts/tissues. Moreover, in the last 7 years this increase was only of around 70%. This means that compared to other plant parts/tissues the interest in the extraction from these plant parts has diminished in the last decade.

On the contrary, it seems that over the last years there was a growing interest in the extraction of natural products from plant residues and wastes. The number of publications on the extraction of plant residues can be even higher if we also consider some of the above referred plant parts as typical residues from the agro-industrial activities (such as peels, pods and husks, or even leaves, seeds and barks).

To try to understand if there are any recent tendencies regarding the extraction of natural products from marine and from microorganism sources, a search was performed using the specific descriptors 'fish', 'algae', 'sponges' and 'plankton' for raw materials of marine sources, and 'bacterium', 'bacteria', 'microalgae' and 'mammalian cells' for microorganism sources. All searches were performed using the additional descriptor 'extraction'. In the case of microorganisms, the descriptor 'metabolite' was also introduced in the search strategies to avoid unrelated publications.

Literature search results are presented in Figures 7.12 and 7.13. As expected, the largest number of publications on the extraction of natural products from marine origins was obtained for the extraction from fishes (around 2570 results), followed by sponges and algae (220–300 results) and, finally by plankton (around 100 results). However, in terms of the annual number of publications for the 2000–2011 period, there was a growing interest in the extraction from algae (around 360% increase) while for fish and plankton this increase was no more than around 160%.

In terms of the extraction of metabolites from microorganisms, results showed that the largest number of publications was obtained for the extraction from bacteria (around 700 results), followed by fungi (around 510 results), microalgae (around 220 results) and, finally, from mammalian cell cultures. In terms of the annual number of publications for the 2000–2011 period, there was

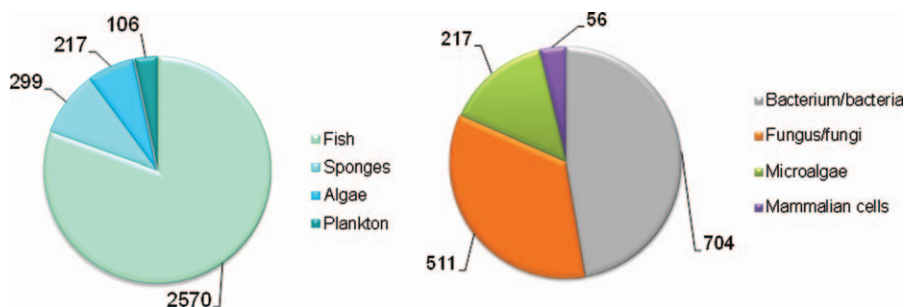


Figure 7.12 Number of publications (review and research articles) dealing with natural product extraction from marine and microorganism origins. Literature search was performed in Scopus, for the 2000–2011 period.⁵

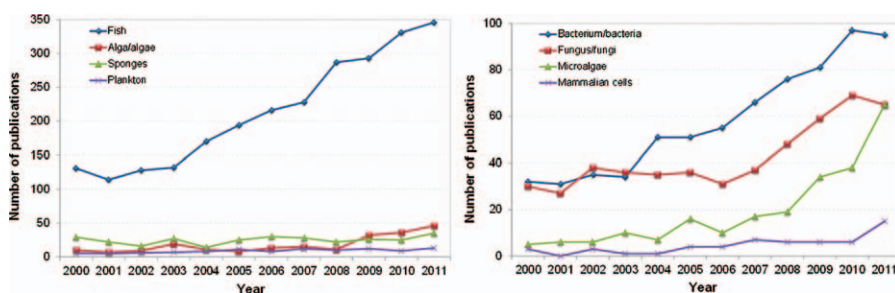


Figure 7.13 Annual number of publications (review and research articles) dealing with natural products extraction from marine and microorganism origins. Literature search was performed in Scopus, for the 2000–2011 period.⁵

a recent growing interest on the extraction from microalgae (1200% increase) and from mammalian cell metabolites (400% increase), while for bacteria and fungi the observed increases were around 200% and 120%, respectively.

In general terms, it may be concluded that the most used natural-origin raw materials in the extraction of natural products for the years between 2000 and 2011 were terrestrial plant-origin raw materials, such as plant roots, rhizomes, leaves, fruits and seeds. It was also observed that, in more recent years, much more attention was given to the extraction of natural products from plant residues/wastes (such as husks, barks, pods and peels). These results somehow confirm the results obtained in Section 7.2 since the most studied plant raw materials and plant parts/tissues seem to be those where higher concentrations of the most studied target compounds can usually be found. Finally, in more recent years there was a rising interest on the extraction from marine organisms as well as from microorganisms, especially fish, algae, bacteria, fungi and microalgae. Despite the extraction of natural products from fish having been studied for a long time, more efforts should be directed to the extraction from other so far unexplored marine sources. The same applies to the extraction of metabolites from microorganisms.

7.4 Extraction Methods

After the selection and the identification of the envisaged target extracts/compounds and of their potential natural sources, it is then necessary to define and to choose the most efficient methodologies to be employed in the extraction process. This selection is usually directly related and even limited by the selection of the most suitable solvents and solvent mixtures (in terms of physicochemical properties, purity/composition and potential risks and toxicity), of additives to be employed (such as enzymes, acids/bases, salts and surfactants), and of the specific operational conditions to be used. These issues will be discussed later.

The selection of the extraction methodologies, as well as of any additional separation/purification methods that may be required, must take in consideration not only the technological/engineering specificities and clear advantages/disadvantages of each technique, but also other quite important issues such as: available technologies; required extraction yield and selectivity; required amounts of final products and their purities; extracts/compounds thermal and chemical stabilities; economic and market issues (direct and indirect processing/manufacturing costs, energy demand, final product pricing, market demand and consumer trends); potential final applications; involved legislation (environmental, trade and industrial legislation) and other specific regulatory concerns (namely for the food, cosmetic and pharmaceutical industry), as well as scale-up and several other production issues that may be relevant. Moreover, in recent years there is a general notion that it is necessary to develop and use 'greener', 'safer' and low-energy consuming extraction, separation and purification methods.

The majority of the chemical, physical and technological/engineering specificities of any potential extraction/separation/purification method are related to crucial factors such as thermophysical properties; thermodynamics, phase equilibria and solubility; potential reactivity and stability; mass, moment and heat transfer processes; and other energetic and/or mechanical issues that may be involved. On the other hand, all these factors are also dependent on some operational conditions such as processing time, composition, temperature, pressure, S/F ratio, flow rate, and other operational and contact modes.

In general terms, an 'ideal' extraction method should preferably: lead to high extraction yields and high purity extracts; employ safe and non-toxic solvents or solvent mixtures; avoid any potential extracts/target compounds degradation; be environmentally and ecologically friendly; meet all required general and specific regulations (environmental, industrial, trade, food, cosmetic and pharmaceutical); be a quick and low-energy consuming method; and evidently, be technologically feasible and economically profitable.

Almost all the currently available methods and equipment for the extraction of natural products from solid, and even from liquid, raw materials are typically solvent-based processes, i.e. they require the use of specific solvents or solvent mixtures which are generally in the liquid or in the supercritical state.

These extraction methods will imply direct contact between the raw material and the solvent (or solvent mixture) which can be performed in two main different ways: by immersion contact (e.g. stirred bed type) or by percolation contact (fixed bed type). The great majority of the available extraction techniques are derived and/or optimized from these two contact manners. Only the traditional mechanical/hydraulic extraction methods (e.g. mechanical presses) do not require the use of solvents. These extraction techniques are largely used to obtain liquid natural extracts/compounds (such as oils and aqueous extracts) and are not applicable for solid target compounds. Nevertheless, these mechanical methods can also be adapted and modified into solvent-based processes by soaking the solid raw materials with liquid solvents.

Figure 7.14 shows the general and most used methodologies for the extraction of natural products. As a result of several specific requirements and of the most recent technological advances, these three basic extraction types can be adapted, modified and/or combined, leading to a wide variety of technological solutions in terms of: operational conditions (temperature, pressure and flow-rate ranges, etc.); operational modes (batch, semi-batch, continuous, sequential, open or reflux conditions, etc.); contact modes (soaking, co-current, countercurrent, closed-circuit, etc.); additional coupled separation/purification methods; stirring conditions; column packing solutions; and thermo-mechanical extraction enhancement (such as the use of ultrasound, microwaves, etc.). Takeuchi *et al.*¹² presented, detailed and provided practical extraction examples (including the corresponding limitations, advantages/disadvantages, specific features, etc.) of most of these extraction methods and of their possible modifications and combinations.

In order to evaluate which have been the most used methods for the extraction of natural products, a literature search was performed covering the years between 2000 and 2011. The search was carried out using some selected descriptors (in the singular form) in conjunction with the additional descriptor 'extraction'. The specific descriptors used correspond to the methodologies that are usually employed for natural product extraction purposes: 'hydro-distillation', 'Soxhlet', 'high pressure', 'supercritical', 'sorpative', 'ultrasound'

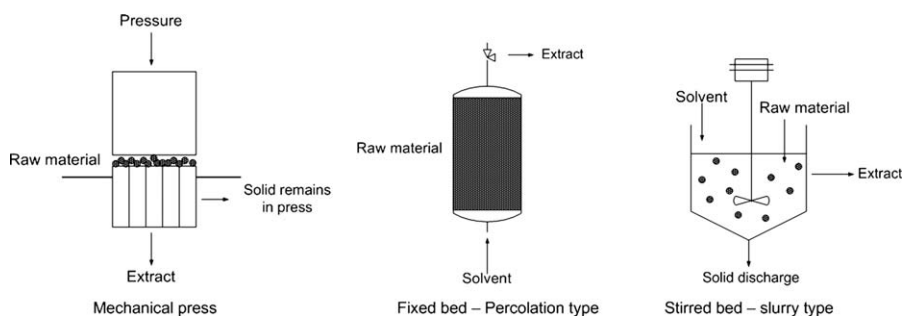


Figure 7.14 General methodologies for the extraction of natural products.

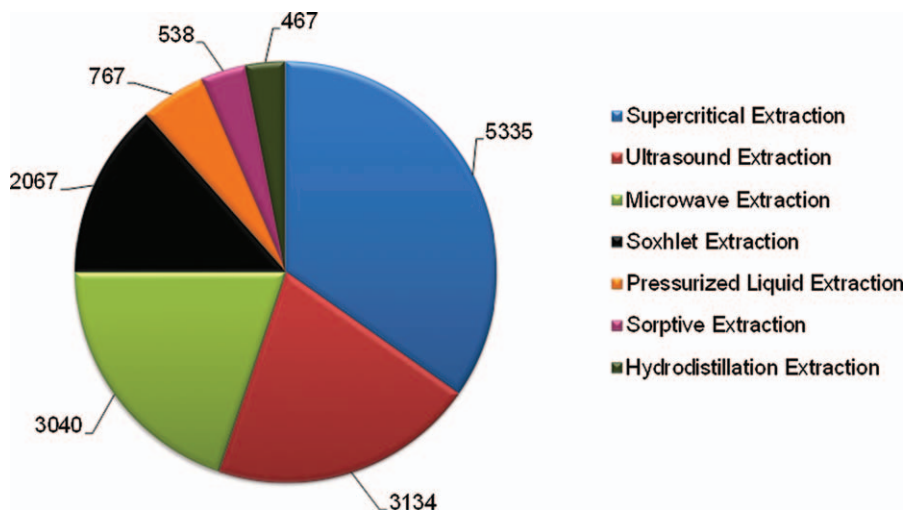


Figure 7.15 Number of publications (review and research articles) dealing with natural product extraction methods. Literature search was performed in Scopus, for the 2000–2011 period.⁵

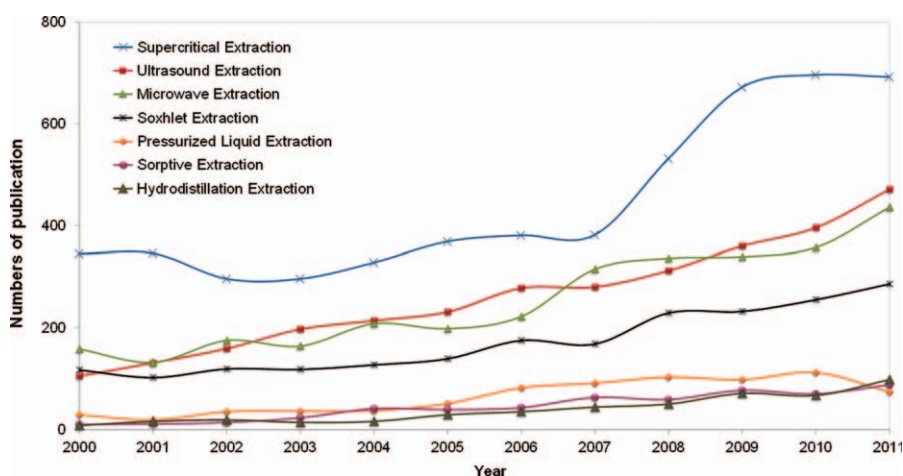


Figure 7.16 Annual number of publications (review and research articles) dealing with natural product extraction methods. Literature search was performed in Scopus, for the 2000–2011 period.⁵

and microwave'. The obtained literature search results, organized by years, are presented in Figures 7.15 and 7.16.

For the 2000–2011 period, the largest number of publications in terms of the most employed natural product extraction method was obtained for works using supercritical extraction (around 5340 results), followed by ultrasound and

microwave (3040–3130 results). Since Soxhlet extraction is a conventional/standard laboratory-scale extraction technique that is frequently used for comparison purposes, it is natural that it is still a quite well used method (around 2070 results). Finally, the less employed methods were pressurized liquid extraction, sorptive extraction and hydrodistillation (470–770 results).

The number of publications has increased in the last decade for all the searched extraction methods. The observed increases were more pronounced for hydrodistillation (1125%), followed by sorptive extraction (780% increase), and then by ultrasound, microwave and pressurized liquid extraction methods (155–350% increase). Despite the fact that the number of works on supercritical and Soxhlet extractions has continuously increased in the last years and that these techniques are amongst the most used extraction methodologies (in absolute terms), their percent increases were much lower for the considered period (101% and 144%, respectively). However, if the last 7 years are considered, it can be observed that the increase in the number of publications was more pronounced for hydrodistillation (238%), followed by sorptive, Soxhlet, ultrasound and supercritical extractions (88–126% increase).

Therefore it is evident that supercritical extraction methods kept the interest of researchers in the last decade, while other methods such as ultrasound, microwave, pressurized liquid and sorptive extraction methods have been getting more attention more recently. These results demonstrate that the most employed methods are certainly being chosen by taking in to consideration safety, environmental and energy consumption concerns. The hydrodistillation and Soxhlet extraction methods results can be probably attributed to their conventional/standard characters as they are usually employed as comparative extraction methods for the other technologies. Finally, the results on the use of microextraction and of sorptive extraction are certainly related to their common use for analytical purposes (e.g. using small amounts of extracts in chromatography).

Furthermore, to improve extraction efficiency and selectivity, additional efforts were made in the development of the most recent techniques, which couple/combine and hyphenate extraction methods, or use extreme and non-conventional extraction conditions.

Although some of these extraction methods are described in great detail in several chapters of this book, some of their most important features/applications will be briefly presented, in terms of the most recent trends and innovations in these methodologies.

7.4.1 Microwave-assisted Extraction

Microwave-assisted extraction (MAE) has drawn significant research and industrial attention over the past 15 years, mainly due to the needs for ‘greener’ and more efficient extraction techniques.^{13,14} One of the greater advantages of MAE over the conventional extraction methodologies is that microwaves can promote the evaporation of raw material residual water, which ultimately ruptures plant cell walls and reduces the resistance to extraction due to internal

diffusion.^{15,16} Since heating occurs in a targeted and selective manner in MAE, there is much less energy loss to the surrounding environment, which makes this process usually considered as more environmentally friendly when compared to conventional solid–liquid extraction methodologies (such as Soxhlet extraction and hydrodistillation). Moreover, since the amount of solvent used is typically much less, this facilitates the post-extraction purification procedures, which may help to preserve the extract quality. Despite the fact that only polar solvents can absorb microwaves, it is also possible to extract low polarity compounds by simply performing a solvent-free MAE. In 2003, Chemat *et al.* proposed the combination of MAE¹⁷ and dry distillation, and this methodology was applied to extract essential oils from various aromatic plants.^{18–20} These authors claimed great advantages in terms of time and energy consumption and also reported some differences in the composition of the essential oils compared to those obtained by hydrodistillation, probably due their lower degradation (by hydrolysis, trans-esterification and/or oxidation).

Other innovations were introduced in the extraction of essential oils using MAE, such as the use of ionic liquids²¹ and of carbonyl iron powder, which is a microwave absorption compound that increases the temperature and pressure faster in the system and avoids the need for soaking the raw materials before extraction.²²

In 2008, Abert Vian and co-workers developed another MAE modification which mainly differs in the way the extract is recovered. In this modified technique, called microwave hydro-diffusion and gravity (MHG), the extract falls out of the microwave reactor (by gravity) and it is continuously cooled by a heat exchanger placed outside the microwave oven.²³ This methodology was employed to extract essential oils and phenolic compounds from diverse plant materials and it was claimed that this environmentally friendly methodology offers net advantages in terms of yield and selectivity, shorter extraction times and better essential oil composition.^{24–26}

The vacuum microwave-assisted extraction method (VMAE) was also recently developed (2009) as an attempt to preserve sensitive and labile compounds since the absence of air inhibits the potential oxidation reactions that may occur. In addition, vacuum also lowers the solvent boiling point and the mass transfer of target compounds from the plant matrix into the solvent is promoted via the negative pressure gradient.²⁷ Wang *et al.* applied the VMAE method for the extraction of phenolic compounds and natural pigments from three Chinese herbs and studied the effect of some experimental conditions (S/F, time, vacuum pressure and temperature) on the extraction yields of a few specific compounds.²⁸ VMAE proved to be more efficient than MAE and conventional solid–liquid extraction in the destruction of sample micro-structure and on decreasing the amount of oxidation of thermo-sensitive compounds. These conclusions were later confirmed by Xiao *et al.*, who used vitamin C (from guava and green pepper) and vitamin E (from soybean and tea leaves) as representative target compounds to evaluate the effect of the VMAE method in the degradation by oxidation of these substances.²⁷

More recently, in 2011, Zill-e-Huma *et al.* coupled the VMAE and the MHG methods and called this new extraction technique vacuum microwave hydro-diffusion and gravity (VMHG).²⁹ This method was applied to the extraction of antioxidant compounds from onion by-products and, once again, the use of vacuum revealed to be positive on the quercetin content in the extracts and on the preservation of their antioxidant activity.

Instead of vacuum, other authors used pressurized inert gas (nitrogen or argon) to prevent the oxidation of active target compounds, from 2009 onwards.^{16,27,30} The results achieved by this protected microwave-assisted extraction (NPMAE or APMAE, respectively, for nitrogen and argon) were similar to those that applied vacuum.

In 2010, a microwave-assisted Soxhlet extraction method was also developed as an attempt to obtain a more efficient Soxhlet-based extraction technique in terms of extraction time and solvent/energy consumption. This method was considered the most interesting improvement of Soxhlet-based extraction techniques among all that have appeared so far.³¹ A review on the evolution of Soxhlet-based methods and equipment was published by Luque de Castro and Priego-Capote.³¹ Some of this equipment is partially or fully automated, presenting some advantages in terms of: the possibility of controlling temperature,³² which is indispensable when thermo-labile compounds are involved; and the possibility of coupling this extraction method to other analytical processes such as high-resolution techniques for the separation or detection of specific compounds.³³ Microwave-assisted Soxhlet extraction has been widely applied to the analysis of environmental samples^{34–36} and food samples.^{37–39}

Microwaves were recently (2010) employed for the first time to assist the enzymatic aqueous extraction of corilagin and geraniin from *Geranium sibiricum* Linne, a traditional medicinal Chinese herb.⁴⁰ At the optimum extraction conditions, Yang and co-workers were able to achieve an increase in the corilagin and geraniin extraction yields of 64% and 73%, respectively, when compared to a control extraction performed using acetone and water in an ultrasonic bath. Considering that the extraction times were significantly reduced and that no chemical decomposition of the extracted phenolic compounds was observed, this methodology was thus considered a 'green' and efficient alternative to extraction procedures using organic solvents.

7.4.2 Ultrasound-assisted Extraction

Another well-established combinatory technique that has known greater popularity especially after 2007 is the so-called ultrasonic microwave-assisted extraction (UMAE). The additional ultrasound waves in the extraction medium induce mechanical, cavitation and thermal effects that can lead to the disruption of cell walls, to particle size reduction, and to enhanced mass transfer across cell walls,^{41–44} without causing significant changes in the structural and functional properties of most target compounds.⁴⁵ All these combined effects boost the extraction efficiency, especially by decreasing the

extraction time and by increasing the extraction yield. This was reported for the extraction of polysaccharides with anti-tumor activities from a fungus (*Inonotus obliquus*);⁴⁶ of lycopene from tomato paste;⁴⁷ and of oils from soybean germ and from cultivated marine microalgae.⁴⁸ Sayar *et al.* applied microwaves and ultrasound as a sample pre-treatment procedure prior to solid-liquid extraction of oil from jatropha seeds. These authors also observed an increase in the extraction yield and a decrease in the extraction time.⁴⁹ Details on the availability and use of the combined equipment of these processes as well as on the corresponding applications were reviewed by Leonelli and Mason.⁵⁰

UMAE has also been used as a sample preparation technique for the rapid characterization of flavonoids in *Spatholobus suberectus*, a widely used herb in traditional medicine, and revealed to present a higher efficiency when compared to other commonly used extraction methods.⁵¹ In 2011, Lu *et al.* used an ionic liquid in the ultrasound microwave-assisted extraction (IL-UMAE) of anthraquinones from rhubarb and concluded that this was an efficient, rapid, simple and 'green' preparation technique.⁵²

The combination of ultrasound and the vacuum distillation technique was performed in 2009 by Da Porto and Decorti for the separation of flavor compounds from spearmint plants.⁵³ This new combined technique achieved a higher extraction yield (0.13%) if compared to the conventional hydro-distillation method (0.04%).

In 2004, Luque-García and Luque de Castro used ultrasound to assist the Soxhlet extraction of total fat from oleaginous seeds. Despite the advantages of this technique when compared to conventional Soxhlet extraction and even when compared to microwave-assisted Soxhlet extraction (in terms of extraction time), it has not been the subject of the same research interest and optimization as the microwave-assisted Soxhlet extraction method.⁵⁴

Ultrasound was also used to assist the aqueous enzymatic oil extraction (AEOE) from distinct plant materials. The original AEOE is an 'eco-friendly' process that has an important drawback: its typical long process time. The use of ultrasonic pre-irradiation in this process can significantly reduce process time, as reported for the extraction of oil from *Jatropha curcas* L. seed kernels, almond and apricot seeds and peanuts.^{55–57} More recently, Long *et al.* used ultrasound to assist the AEOE method on the extraction of flaxseed oil using immobilized enzymes.⁵⁸ The method was optimized and the operational conditions for maximum oil recovery (68%) were established.

Ultrasound was also used in conjugation with supercritical fluid extraction (SFE) in a methodology designated as ultrasound-assisted supercritical fluid extraction (UASFE), used as an attempt to overcome the main mass-transfer resistance and to improve extraction kinetics in SFE. Ultrasound may be used in sample pre-treatment (and prior to SFE), as Klejdus and co-workers⁵⁹ did in the extraction of isoflavones from algae and cyanobacteria, or during the SFE extraction process (placing a transducer inside or outside the SFE extraction vessel). Riera *et al.* noticed significant enhancements in extraction kinetics and yields when the SFE method was ultrasonically assisted for almond oil

extraction (using an ultrasonic transducer inside the extraction vessel).⁶⁰ Hu *et al.* achieved similar results and conclusions when extracting oil and coixenolide from adlay seeds.⁶¹ These authors concluded that UASFE may lead to the reduction of several operational conditions such as temperature, pressure, CO₂ flow rate and extraction time. Gao *et al.* used a pilot-scale extraction equipment (with the ultrasound probe installed in the upper part of the extractor) to study the effect of ultrasound on the extraction of lutein esters from marigold.⁶² Solid particle size, temperature, pressure, CO₂ flow rate and ultrasonic conditions (power, frequency and irradiation time/interval) were studied and the extraction yields of lutein esters were significantly increased by the process.

Balachandran *et al.* employed a UASFE batch method with an ultrasonic transducer external to the extraction cell to study the effects of ultrasound on the extraction from ginger.⁶³ The authors reported a significant increase in the extraction yield due to increased intra-particle diffusion, caused by cellular disruption as a result of the rapid changes in solvent density associated with the pressure fluctuations induced by ultrasonic waves.

7.4.3 High-pressure Liquid Extraction

The ultra-high-pressure extraction method (UPE), also known as high hydrostatic pressure extraction (HHPE), is an emergent technique for the extraction of natural products. Several advantages have been reported for this methodology that typically uses extremely high operational pressures (from 100 up to 600 MPa) and does not require any additional heating (as temperature rises due to compression). The most important advantages that have been reported so far include high extraction yields, reduced processing time, and low solvent and energy consumption. Furthermore, the low process temperatures avoid the thermal degradation of labile compounds and the loss of volatile components. It has been suggested that the responsible phenomenon for such a high performance is the disruption in vegetable tissues (caused by the high hydrostatic pressure), which improves the mass transfer rate of the solvent into the plant material and intracellular product release.^{64,65} This emergent extraction technique was recently applied for the extraction of pectin from navel orange peel,⁶⁶ and for the extraction of phenolic compounds from longan fruit pericarp,⁶⁷ from *Pinus densiflora* root⁶⁸ and from green tea leaves.⁶⁹

The pressurized liquid extraction (PLE) method, also known as pressurized solvent extraction, accelerated solvent extraction or as enhanced solvent extraction, is another emergent technique that differs from UPE as it typically applies elevated temperatures (up to 200 °C). When water is the used solvent, this technique is usually designated as pressurized hot water extraction, sub-critical water extraction or superheated water extraction. The main advantage of this technique over conventional solid-liquid extraction is the use of relatively high densities (since the solvent is in the liquid state, however, at temperatures well above its normal boiling point), which improves solubility and mass transfer of target compounds.⁷⁰ Extraction time and solvent

consumption are thus significantly reduced if compared to other solvent extraction techniques. The possibility of using mixtures of liquid solvents, presenting different polarities and thus having distinct abilities to establish specific interactions with target compounds, as well as the possibility of dissolving several useful extraction additives in these solvents/mixtures, makes the PLE method a versatile extraction technique since it allows the selective extraction of different substances from the same matrix. Additional advantages may also arise from the utilization of the so-called gas-expanded liquids as the extraction solvents as, for example, upon the dissolution of CO₂ in water or in an organic solvent. In this case, the pH decrease in the extraction medium that follows the *in situ* generation of carbonic and alkyl carbonic acid⁷¹ may increase plant cell membrane permeability and thus the extraction yields,^{72,73} as well as lead to the inactivation of unwanted enzymes^{73,74} and microorganisms.⁷⁵

In the last few years, numerous studies have been published on the application of PLE to the extraction of natural products. Oils and lipids were extracted from several vegetable matrices such as pistachio kernels⁷⁶ and leaves of *Orthosiphon stamineus*, a medicinal herb from South East Asia.⁷⁷ Phenolic compounds were also extracted using the PLE method from *Pinus pinaster* bark,⁷⁸ jabuticaba skins⁷⁹ and tara seed coats.⁸⁰ Other PLE applications include the extraction of betulin and other anti-oxidant compounds from birch bark,⁸¹ procyanidins from red grape pomace,⁸² anthocyanins from elderberry pomace,^{83,84} and prenylflavonoids from hops.⁸⁵ A common feature to almost all of these studies is the use of experimental design methodologies in order to study the influence of some process variables (typically temperature, pressure, static extraction time, solvent composition, S/F and particle size) on the extraction yields and extract composition.

7.4.4 Supercritical Fluid Extraction

Supercritical fluids, and supercritical carbon dioxide (SC-CO₂) in particular, have many well known advantages as extraction solvents. However, its industrial application is still somewhat limited, mostly due to the usually required higher investment costs when compared to other extraction methodologies at atmospheric or at relatively low pressures. Nevertheless, this process can be optimized to be economically competitive, regardless of the equipment scale and employed raw materials.^{86,87} This can be achieved by the optimization of several operational conditions such as pressure, temperature, solvent composition, solvent flow rate, extraction time, as well as raw material pre-treatment and post-extraction fractionation/separation procedures. The method can also be improved by coupling it to other techniques such as microwaves and ultrasound.

Despite many works that have dealt with SFE process optimization, these strategies largely depend on the specific raw material to be extracted. Therefore, in order to improve the SFE method in terms of extraction yields and selectivity, some efforts have been made in recent years regarding this subject. Raw

materials can be pre-treated in order to help the subsequent extraction. For example, grape seeds were pre-treated with enzymes in order to degrade some seed cells and thus facilitate the subsequent oil extraction.⁸⁸ Another recent pre-treatment approach was the one reported by Rochová *et al.* in 2008, who used a method called instantaneous controlled pressure-drop process (or DIC process – ‘Détente Instantanée Contrôlée’) in order to modify the original soybean vegetable structure.⁸⁹ This technique consists in keeping the raw material under high-pressure steam, followed by a quick transition into vacuum; this sudden pressure drop causes a bursting evaporation of the remaining moisture, which, consequently, reduces particle size and increases particle porosity, thus leading to a significant increase in mass transfer.

Fractionation and other post-treatment procedures are also viable options to increase process selectivity for some specific compounds, or to remove unwanted substances. For example, Chen *et al.* extracted rice bran oil using SC-CO₂, followed by fractionation/separation method using a multi-stage supercritical fluid deacidification process.⁹⁰ The SFE process led to a total rice bran oil yield of 15.7%, with 3.8% of free fatty acids (FFA). However, the subsequent supercritical deacidification process allowed removing 97.8% of the FFA present in the original extract.^{90–92}

The control of pressure and temperature conditions in order to tune the density of SC-CO₂ was also studied for the SFE extraction/fractionation methods. This type of procedure is not truly novel as several works on the subject were firstly published during the 1990–1999 decade.^{93,94} However, more recently it has been employed for the SFE extraction and fractionation of several raw materials. As an example, it was employed for hop pellets in order to obtain fractions of some specific aromas (oxygenated polar hop essences) to be used in beer production. The major novelty of this work was the use of a multi-cell off-line extraction apparatus containing eight extraction cells. SC-CO₂ densities were varied between 200 kg/m³ and 630 kg/m³, and the subsequent mono- and sesquiterpenes removal from oil was achieved by solid phase extraction (SPE). Extracts (both hop oil and polar hop fractions) presented the required hoppy character when added to beer, as evaluated by sensory panels.⁹⁵

Another procedure that was quite studied in the last decade and that can be used to obtain different extracts/fractions from the same raw material is the solvent change during the SFE process. For example, different extracts were obtained from *Pinus pinaster* bark⁹⁶ by the sequential use of distinct solvents and/or solvent mixtures during the extraction process: phenolic compounds were mostly obtained in a first SC-CO₂ extraction step, while tannin-rich fractions were obtained at a second extraction step using a SC-CO₂ + ethanol (10%, v/v) mixture. A similar approach was applied to the extraction of turmerone and curcuminoids from turmeric. This was performed using a first SC-CO₂ extraction step, followed by a second extraction step with SC-CO₂ + ethanol + isopropyl alcohol.⁹⁷ This strategy was also found to be particularly efficient for the extraction of anthocyanins from elderberry pomace.^{83,84} These authors employed two sequential extraction steps: the first

step was performed with SC-CO₂ to extract low-polarity compounds such as lipids, followed by a second extraction step using an heterogeneous CO₂ + ethanol + H₂O solvent mixture (to obtain anthocyanin-rich extracts). Other similar approaches were reported for the extraction of phenolic compounds (anthocyanins) from grape marc,⁹⁸ for phenolics with antioxidant activity and antiproliferative effect in human colon cancer cells from cherries⁹⁹ and for alkylresorcinols from triticale bran.¹⁰⁰

Recently, besides the other traditionally employed solvent enhancers (such as ethanol, methanol, acetone, etc.), vegetable oils and other natural-origin products were used as co-solvent/modifiers for SC-CO₂ extraction. For example, this approach was used to improve the extraction of lycopene from a mixture of tomato and hazelnut (as raw materials), where the extracted hazelnut oil also acted as a SC-CO₂ modifier.¹⁰¹ Another recent example of this approach was the simultaneous clove/oregano and clove/thyme extraction, where polar monoterpene alcohols (such as carvacrol and thymol) contributed for the SC-CO₂ solubility power enhancement towards the dissolution of several heavier and less soluble compounds.¹⁰² Other examples include the use of essential oils such as limonene to extract fats and oils from different raw materials.^{103–105}

The use of the SFE method in combination with other techniques has also attracted attention in the last decade, especially coupling to ultrasound or microwaves. These techniques may enhance some of the already existing advantages on the use of SFE, thus improving the benefits of using greener technologies to obtain natural products and reducing energy consumption and the use of organic solvents. Staudt *et al.* developed a microwave heating supercritical fluid extraction method (MSFE) in 2003 for the extraction of oils from caraway seeds.¹⁰⁶ The main advantages of this method include short heating times, low energy consumption and sharp temperature profiles.¹⁰⁷ On the other hand, as already referred in Section 7.4.2 on the ultrasound-assisted extraction techniques (UASFE), the use of ultrasound may enhance the mass/heat transfer phenomena that are involved in the SFE method, by promoting stirring at the small-scale and by the physical/mechanical disruption of some raw material cell structures which increases the solvent accessibility to the internal particle structure. As an example of this enhancement, a 30% increase was reported in the extraction yields of oil from particulate almonds when ultrasound was coupled to the SFE method.⁶⁰ SEM micrographs of ginger particles that were submitted to the UASFE method showed clear structural differences from those particles that were not submitted to this technique. This can be due to cavitation collapse, i.e. to the formation, growth and violent collapse of liquid microbubbles that may be present inside the raw material.⁶³

The optimization of SFE processes is a great challenge and mathematical modeling may offer rational approaches to infer about the best operational conditions to be employed as well as for the future development of scale-up procedures. These models are mainly based on thermodynamics (solubility) and mass transfer resistance issues. During the last decade, three different main

approaches have been suggested for SFE mass transfer mathematical modeling: empirical models; models based on the heat and mass transfer analogies; and models based on the differential mass balance integration.^{108,109}

In general terms, these mathematical models usually differ in their specific descriptions of phase equilibrium, of flow patterns and of solute diffusion in the solid phase. For solid–liquid extraction methods the most relevant mass transfer models are the linear driving force model, the shrinking core model, the broken/intact cells model and the combination of the last two models.¹¹⁰ Models can also consider the solid–matrix interactions^{111,112} or not.¹¹³ Due to its simplicity, the Sovová model¹¹³ has been widely employed to describe the SFE method since its initial kinetic curve (which is mostly governed by convection) is very important for industrial applications. Improvements on this model were reported later and the considered solute–matrix interactions were better described.^{114–116} This model has been applied to describe methods using pure SC-CO₂ as well as methods using SC-CO₂ + cosolvent (5 and 10%) mixtures. Talansier *et al.* showed that the Sovová¹¹³ and Goto¹¹¹ models were also robust to describe the kinetic extraction with or without the presence of co-solvents when using co-solvent compositions up to 10% (v/v).¹¹⁷ Over the last years, desorption models have also been used in order to better predict the extraction kinetics at shorter extraction times.^{118–120} Due to the involved mathematical modeling and mathematical computational complexities, different new modeling tools and methods (such as genetic algorithms)^{121,122} and neural networks^{123,124} were proposed in recent years.

Researchers have been more conscientious in the choice of the most efficient extraction methodologies for a particular raw material, as well as in the deeper consideration on the scale-up feasibility of a particular SFE process.^{125,126} Several authors reported methodologies that take into account the process/production costs and agreed that some raw materials may present quite competitive advantages if SFE methods were employed to obtain specific natural products.^{127–129} In these cases, the economic evaluation seems to be an important tool to access the ease of industrialization of SFE processes by knowing all the real costs involved in the process.

7.5 Extraction Solvents and Solvent Mixtures

As already referred, the selection of the most efficient extraction methodologies is generally related to and even limited by the selection of the most suitable solvents and solvent mixtures (in terms of physicochemical properties, purity/composition and potential risks and toxicity), of some additives (such as enzymes, acids/bases, salts and surfactants), and of some particular operational conditions. In addition, the solvents and additives are usually responsible for the ‘green’ and ‘safe’ nature of a specific extraction method. Therefore, we will present some of the most important and recent trends, features and applications of several extraction solvents, solvent mixtures and solvent additives that have been recently employed on the extraction of natural products.

Solvent-based extraction methods are the most widely used due to their inherent versatility, simplicity and effectiveness, which are largely dependent on the thoughtful choice of the solvent(s) used in the process. The selection of the 'best' extraction solvent not only must account for its affinity with the target compounds but should also consider the ease of solvent separation and recovery (from the extract and from the extracted material). This selection is usually based on the solvent's physical properties (such as density, viscosity, interfacial tension, vapor pressure, conductivity and miscibility with other solvents) and on its solvation properties (such as solvent strength, selectivity and solubility of the specific compounds).¹³⁰

The physical properties of the solvent will rule its capacity to wet the solid matrix and to penetrate through matrix pores and capillaries, as well as its recovery capacity since the solvent should be preferably reused. The solvent must also be easily separated from the extract phase to produce solvent-free extracts/compounds. If distillation or evaporation procedures are envisaged, the solvent should not form azeotropes and its latent heat of vaporization should be relatively small in order to avoid any potential extract/target compound thermal degradation.

On the other hand, the solvation properties of the solvent will govern the final extraction yields and the process selectivity towards the target compounds. The solvent powers of commonly used solvents usually increase in the sequence: gases < supercritical fluids (SCFs) < gas-expanded liquids < near critical water < water and organic liquids < ionic liquids.¹³¹ However, their corresponding transport properties usually vary in the opposite way. Therefore, the use of surfactant systems or ionic solvents, such as ionic liquids, is sometimes limited due to their low transport capacities. On the other hand, in some situations the ease of further separation processes and the small number of required extract manipulation procedures may overcome this drawback. Moreover, ideally, the solvents should be non-toxic, non-corrosive, chemically and thermally stable, non-reactive, non-flammable, cheap and harmless to the environment and to human health. Undoubtedly, the toxicity of chemicals towards the human health and the environment is a current and important concern of researchers and industry. Furthermore, this concern is strongly motivated by quite restrictive legislation and by consumer and society awareness to these subjects.

For example, the organic solvents that are commonly used for the extraction and fractionation of target compounds for food applications, such as hexane, methyl acetate, dichloromethane and methanol, are now restricted to maximum residue contents between 1 mg/kg and 10 mg/kg and these values are even much lower in the case of pharmaceutical applications.¹³² Regulations concerning extraction solvents for use in foods are primarily concerned with human health requirements. Several accepted solvents for use in conformity with Good Manufacturing Practice (GMP) are water, propane, butane, propyl acetate, ethyl acetate, ethanol, carbon dioxide, acetone and nitrous oxide.¹³³ Another important issue is that the extraction methods using organic solvents usually present low selectivity towards natural products and, consequently, further

separation and purification steps are normally necessary. This leads to the use of new/more solvents and to higher risks of mass losses and/or degradation of extract/target compounds.

Several approaches have been attempted so far to avoid the use of conventional organic solvents, taking into account the specific selectivity and/or affinity of particular classes of compounds. These approaches will be now briefly detailed.

7.5.1 Extraction Solvent Modification with Additives (Enzymes, H^+/OH^- , Surfactants)

Solvent modification is an approach that has been intensively used over the last few years in order to improve the extraction method selectivity. Whenever possible, priority is usually given to the use of aqueous-based solvents that are modified by adding specific additives or by the optimization of the medium acidity/basicity, ionic strength, etc., to enhance process selectivity/effectiveness.

Enzymes can also be used as additives in solvent extraction processes to enhance the cell wall degradation process. These chemically and morphologically complex membranes contain molecules such as cellulose, hemicellulose, lignin and pectin, and their degradation facilitates the extraction of target compounds. Enzyme-assisted extraction involves the use of an aqueous-organic enzyme solution (usually water and ethanol).^{134,135} Some authors have used enzymes as a pre-treatment procedure, by immersing and soaking the raw material in the enzyme solution, which is further dried and afterwards extracted using solvents such as water or organic solvents.^{136–138} Process optimization includes the choice of the appropriate enzyme(s) and always considers the process conditions that guarantee optimal enzyme activity (such as composition, temperature, pH and ionic strength). To date, several enzymes have been used as solvent extraction enhancers: pectinases, cellulases, glucosidases, galactosidases, xylosidases, and cellobiohydrolases^{136,139} as well as their mixtures.^{134,137} Moreover, these additives can be conjugated with other selective solvents for targeting other specific compounds. This methodology has proved to be efficient for the extraction of seaweeds which are an example of a raw material composed by a complex mixture of sulfated and branched polysaccharides, associated with proteins and ions, and that usually form a physical barrier that reduce extraction efficiencies.¹³⁵ In a recent work, *Penicillium decumbens* cellulase was used to improve the extraction of flavonoids from *Ginkgo biloba* leaves, through a dual functional activity that involved not only enzymatic cell wall degradation but also the increase in the solubility of the target compounds in the water/ethanol/enzyme solution.¹³⁹ Authors observed an increase of almost 100% in flavonoid yields when the enzymatic degradation conditions were optimized.

Several other works have reported the use of acidic and alkaline conditions to extract specific compounds such as alkaloids and anthocyanins. Most alkaloids are present in plants in the form of organic acid salts or as basic compounds that can be extracted by an acid-base extraction method. This

method consists in consecutive liquid–liquid extractions to purify acids and bases from complex mixtures. Belsito *et al.* compared the efficiency of acid, basic and neutral aqueous-based solvents (using water + K_2SO_4 , ethanol + KOH and ethanol + $H_2SO_4 + (NH_4)_2SO_4$, respectively) to extract quinolidizine alkaloids from *S. junceum* flowers.¹⁴⁰ The authors found that alkaline conditions significantly increased (almost 100%) the extraction yield of two (cytosine and *N*-formylcytisine) of the four main alkaloids that exist in this plant, while acidic conditions did not favor the extraction of any of the alkaloids. Shen *et al.* compared the use of enzyme- and alkaline-based aqueous solvents to extract proteins from green tea leaves.¹⁴¹ After the optimization of the extraction process conditions, as additive concentration, temperature, extraction time and S/F ratio, the authors observed higher extraction yields when using alkaline conditions at mild temperature (56.4% using 0.1 M NaOH, 40 °C, 5 h and S/F of 40, v/w) when compared to the enzyme-based solutions (47.8% using 4% of enzyme, pH 8, 60 °C, 4 h and S/F of 35, v/w). Alkaloids have also been extracted from *S. japonica* M. using supercritical carbon dioxide together with alkaline modifiers. Basified methanol/water solutions (with diethylamine, 10%, v/v) were used at concentrations of 1%, 5% and 10% (v/v) as solvent enhancers for the SC- CO_2 extraction at 60 °C and 34 MPa. Alkaloids such as hyoscyamine and scopolamine were extracted in higher yields when using basified methanol/water/SC- CO_2 and if compared to extractions performed with methanol/SC- CO_2 and with carbon dioxide alone, which indicates that alkaloids were extracted in their free basic form.¹⁴²

Hydrocolloids or gums are another class of compounds that can be extracted from some vegetable materials, mostly seeds and tubers, and that present great interest for the food and pharmaceutical industries. Among these substances, galactomannans, xanthan, guar and alginates are the most often extracted compounds. The extraction of these compounds is very sensitive to pH, which not only helps to break vegetable cell walls, but also changes gum viscosity, since these compounds are polyelectrolytes and their solution behavior strongly depends on pH and ionic strength. The extraction of hydrocolloids from durian seeds was reported in the literature using aqueous media at different pH values (from acid to basic conditions) and also at distinct temperatures and S/F. After process optimization, the best extraction conditions were identified as S/F of 35.5 (w/w), 85 °C and pH of 11.9, since they promote protein hydrogen-bond breaking, which enhances the release of the envisaged polysaccharides.¹⁴³ A pectic polysaccharide was also isolated from sweet pepper using a saline solution at low pH (~1.5) that was added with pepsine in order to simulate gastric juice. Despite the low observed yield (~0.28%), the isolated pectin, capsicum CA, presented anti-inflammatory activity, which supports the hypothesis that bioactive pectins may be extracted from food during digestion.¹⁴⁴ Similar work was developed to extract hydrocolloids from other natural sources as cress seed¹⁴⁵ and sugar beet pulp.¹⁴⁶ The latter reported the extraction of two alkaline-soluble polysaccharides (ASP I and ASP II) from sugar beet pulp residues using microwave-assisted extraction (MAE), at

different pH conditions, in order to maximize polysaccharide recovery. The results confirmed the importance of the optimization of the solvent characteristics in the specificity of the employed extraction process.

Another type of additive that can be used to enhance aqueous-based extractions is hydrotropes and/or surfactants. Hydrotropes are highly water soluble low-molecular-weight organic salts that can significantly improve the dissolution of organic compounds in aqueous solutions according to a process usually called hydrotropy. The main advantages of using these additives include their high selectivity, the lower risk of occurrence of emulsification processes and the easy recovery of the solute by dilution with water at mild temperature conditions and of the diluted hydrotropic solution that can be concentrated by evaporation and recycled since hydrotropes are usually stable at higher temperatures, their melting points are high, and they do not produce any toxic effects during evaporation.¹⁴⁷ Most employed hydrotropes include aromatic salts, aromatic alcohols or short-chain soaps, medium and short-chain alkyl polyglucosides and more unusual compounds such as long chain dicarboxylic acids and short-chain amphiphiles derived from ethylene/propylene glycol or glycerol.¹⁴⁸ These molecules present a hydrophilic and a hydrophobic part (like surfactants); however, the hydrophobic part is generally too small to cause spontaneous self-aggregation and micelle formation. Therefore, unlike surfactants, hydrotropes do not self-aggregate after a given critical concentration. Instead, they aggregate in a step-wise aggregation of hydrotrope molecules among themselves first and subsequent co-aggregation of a solute with these hydrotrope aggregates.¹⁴⁹ The self-aggregation is favored by the hydrophobic effect that is governed by the hydrotrope chemical structure and is opposed by the electrostatic repulsions between the charged head groups.¹⁵⁰ Therefore, the solubilization capacity of hydrotropes essentially depends on the nature of the hydrophobic part of the hydrotrope, mainly on its chain length and branching.^{150,151} These additives have been successfully used for the extraction of several phytochemicals from different plant matrices such as curcuminoids,¹⁴⁷ piperine,¹⁵² boswellic acids,¹⁵³ dioscin,¹⁵¹ limonin¹⁵⁴ and citral from *Cymbopogon flexuosus*.¹⁴⁹

On the other hand, amphiphilic surfactant molecules, which have tails/chains and heads of distinct hydrophobic/hydrophilic nature, can form molecular aggregates of colloidal size (micelles). Therefore, these systems may be employed to enhance the aqueous extraction of hydrophobic or non-polar solute target compounds from plant materials and when used at compositions above their critical micellar concentrations (CMC).¹⁵⁵ This is an energetically viable and low cost method that avoids the degradation of thermally unstable compounds since it can be carried out at room temperature without compromising the extraction efficiency. Moreover, it can help to reduce the use of environmental harmful pollutants such as some low-polarity organic solvents. Furthermore, surfactants have competitive extraction yields compared to these organic solvents, and some of them are already approved for food, cosmetic and pharmaceutical applications, as is the case for lecithins, Triton and Tween surfactants. Micelle-mediated separation (MMS) is usually achieved after the

attainment of a specific cloud point concentration (CPC), at a certain temperature, where the solution splits into a surfactant-rich phase and an aqueous-rich phase. This allows the concentration increase of target compounds preferably into the surfactant-rich phase. Phase separation is usually promoted by using non-ionic surfactants that can also respond to temperature changes.^{156–159} The process can be further tuned by the addition of other electrolytes with salting-in effects, that lead to an increase in the CPC values, or with salting-out effects, that force lower CPC values. The extraction kinetics and extraction final yields of micellar extraction to obtain triterpenes from *Salvia triloba* proved to be more efficient than ethanol extraction. The method was even capable of separating some isomeric compounds, thus avoiding further separation and purification steps.¹⁶⁰ Surfactants are thus usually employed to tune and to enhance the solvency capacity of aqueous solutions at a relatively low cost. Furthermore, these substances are usually non-toxic, readily available and environmentally friendly, which motivates their use in several other extraction methods. For instance, Chang *et al.* reported higher extraction efficiencies for some specific flavonoids such as quercetin, quercitrin and rutin from *C. speciosus* flowers by using a surfactant-assisted pressurized liquid extraction (PLE) method.¹⁶¹ The employed anionic surfactant was sodium dodecyl sulfate (SDS). The authors combined this extraction method to micellar electrokinetic chromatography (MEKC) analysis and proposed an efficient organic solvent-free flavonoid extraction-analysis procedure. Sun and Liu reported the extraction of alkaloids (berberine, palmatine and jatrorrhizine), achieving extraction yields higher than 90%.¹⁶² The non-ionic surfactant oligoethylene glycol monoalkyl ether (Genapol X-080) was used in a one-step extraction method as an alternative solvent for microwave-assisted extraction. Memon *et al.* evaluated the extraction efficiency of a phenolic antioxidant (chlorogenic acid, CGA), extracted from *M. laevigata* W. leaves, using a micelle- or surfactant-mediated extraction method.¹⁶³ SDS was employed as the surfactant in combination with microwave irradiation in order to shorten the extraction time. After process optimization, the authors reported a purity of 95.9% for the CGA. In a very recent study, Ulloa *et al.* applied an integrated process using biodegradable non-ionic surfactants as cell disrupters and as organic extractants for the intracellular antioxidants (α -tocopherol, β -carotene and gallic acid) produced by microalgae.¹⁶⁴ Different Tween- and Triton-type surfactants were tested as well as different sodium-based inorganic/organic salts were used as salting-out agents. Among the Tween and Triton X series, Triton X-114 presented the best lytic effect. However, by combining sodium citrate with Tween 20, the authors were able to obtain extraction yields higher than 99% for α -tocopherol and around 60% for β -carotene and gallic acid.

Surfactants were also helpful on the extraction of oils from cruciferous¹⁶⁵ and from palm kernel oil seeds.¹⁶⁶ In these systems, the surfactants reduced the interfacial tension between the aqueous extracting phase and the oils of crushed seeds, thus promoting the oil liberation mechanism and, consequently, their extraction.¹⁶⁷ In a recent study, aqueous-based systems using anionic-extended

surfactants such as sodium linear-alkyl polypropoxylated polyethoxylated sulfates were evaluated as viable alternatives to hexane extraction of corn oil from corn germ.¹⁶⁸ Groups of intermediate polarity were inserted in surfactant molecules between the hydrocarbon tail and hydrophilic head group in order to obtain a smoother transition between the hydrophilic and the hydrophobic regions, which provided a more suitable environment for solubilizing hydrophilic and lipophilic molecules.¹⁶⁷ The authors reported corn oil extraction yields higher than 80%, with chemical compositions similar to that of hexane extracted corn oil. This was achieved using low surfactant concentrations, and carrying out the extractions at room temperature and for short processing times.

7.5.2 Solvent Mixtures and Non-conventional Highly Hydrophobic Organic Solvents

Researchers have also tried to optimize and to rationalize the use of extraction solvents, mainly by combining them in order to obtain solvent mixtures with the required solvency characteristics. As an example, Kim and co-workers designed specific solvent mixtures for the optimal extraction of polyphenols, antioxidant and anti-tyrosinase ingredients from mulberry leaves.¹⁶⁹ Alcohol-water binary solvent mixtures were employed and it was found that the process efficiency was highly dependent on the type of alcohol employed and on the relative composition of these binary solvents. The authors found a correlation between the extraction efficiency of target compounds and the solvent mixture polarity and, as a result, predicted the optimal extraction conditions for a solvent mixture having a solubility parameter above 33.0 [MPa^{1/2}]. Their predictions were also consistent when using other solvent mixtures based on acetone and ethylene glycol.

Along with conventional aqueous and/or organic solvent optimization, researchers have also looked for the advantages of using new and non-conventional solvents. As an example, Hamed evaluated the use of edible oil (sunflower oil) to replace organic solvents in the extraction of antioxidant components from rosemary, thyme and sage and compared these results to those obtained when using methanol, ethanol and *n*-hexane.¹⁷⁰ Although higher antioxidant extraction efficiency was observed for the methanolic extracts, the authors claimed that the differences were not so significant and hence, from a 'greener' perspective, this could represent an interesting alternative to replace several organic solvents. Castillo and co-authors evaluated the use of alternative hydrophobic organic solvents, such as lanolin and cocoa butter, to obtain water-insoluble bioactive compounds (complex tannins, terpenes, and glucoside esters) with high antifungal activity.¹⁷¹ These solvents represented an attractive alternative to obtain natural products for plant fungal control while avoiding the use of synthetic chemical fungicides. There are also reports on the use of terpenes as effective solvents for the extraction of fats and oils from different raw materials.^{103–105}

7.5.3 Ionic Liquids

Ionic liquids (ILs) are molten salts that result from the association of organic cations with organic or inorganic anions. These substances are usually defined as 'designer solvents' since their physicochemical properties can be easily tuned through the appropriate cation/anion combination.¹⁷² These compounds are being intensively proposed as an advantageous alternative to conventional solvents for many separation techniques due to their thermal stability, good dissolving and extracting capacity and due to the possibility of tuning the optimal viscosity and miscibility with either water or organic solvents. Moreover, their low volatility and inflammability/combustibility, wide liquid temperature/pressure range and enhanced criticality safety can lead to extraction processes that are safer than those based on volatile organic compounds.¹⁷³ Recently developed functionalized ionic liquids, that incorporate specific functional groups in their cations and/or anions and can behave as both the organic phase and extracting agent, will further help to suppress some of the problems encountered from extractant/diluent miscibility and to facilitate compound extraction and solvent recovery.¹⁷⁴

Moreover, due to their 'tunability', as well as to their hydrogen-bonding acidity and basicity, ILs can practically cover the whole hydrophilicity/hydrophobicity range, which allows their use for the extraction/separation of a broad range of compounds.¹⁷⁵ The solvatochromic and lipophilicity parameters of different ILs, mainly from the imidazolium family, which is the most tested so far, were recently measured and compared.^{130,176–178} According to these works, the IL polarities/polarizabilities and acidities/basicities are clearly related to their specific chemical structures. Authors also compared IL polarities with those of water, methanol and acetonitrile, and found different ILs with polarities similar to each one of these solvents. IL lipophilicity was measured through the IL-water partition coefficients and by using different stationary phases and aqueous acetonitrile buffered mobile phases.^{178,179} These studies also demonstrated that amine-containing compounds have higher affinity to the ILs whereas acidic compounds have higher affinity to the 1-octanol phase (from the partition coefficient in 1-octanol/water systems). Based on this knowledge, compounds with H-bond donor groups, such as phenols, have been efficiently separated by using the appropriate ILs.^{180,181} Yang and co-authors established a correlation between the separation efficiencies of tocopherol homologues, one of the most important classes of natural bioactive homologues, and the H-bond basicities of several ILs, and clearly demonstrated the essential role of H-bond interactions in the selective extraction of phenolic compounds.¹⁸² This was further confirmed by Dong and co-authors, which studied the hydrophobic IL (1-butyl-3-methylimidazolium hexafluorophosphate, [Bmim][PF₆])/water partition coefficients of three soybean isoflavone aglycones and found that the H-bond acidities of the phenolic hydroxyl groups determine the different H-bond interactions with the employed IL, which leads to different experimental distribution coefficients.¹⁸³

Simulation results also allowed the conclusion that H-bonds are formed between the anions and the phenolic hydroxyls.

Another question that has to be considered when using ILs as extraction solvents is the ultimate separation of the extracted compounds from the IL. Despite all the advantages, the IL's negligible volatilities may be disadvantageous from a purification and regeneration point of view. Since ILs are considered to not evaporate, alternative separation methods to those based on evaporation techniques have to be used and optimized, which represents a major drawback for the use of these solvents. To date, the target compound recovery from IL phases is mostly being done by liquid–liquid extraction methods, which usually implies the use of organic solvents.¹⁸⁴ However, in several cases the use of high pressure/supercritical CO₂ can be employed to extract several target compounds from ILs.¹⁸⁵ Despite the many issues that must be further tested and optimized before using ILs as viable organic solvent substitutes, research in this field has been quite intense as proved by the extensive number of recent publications using ILs as biomaterial dissolution enhancers or as solvents for microwave-assisted extraction processes and/or for separation in aqueous biphasic systems.^{173,184,186–191}

7.5.4 Aqueous Biphasic Systems (ABS)

Aqueous biphasic systems (ABS) consist in the coexistence of two phases in equilibrium that result from the combination of mixtures of hydrophilic compounds (such as some water-soluble polymers, polyethylene glycol, dextran, polypropylene glycol), and of salts (phosphates, sulfates, citrates). Then, at certain critical concentrations, two hydrophilic phases in equilibrium will be formed. These systems may represent greener alternatives to the classical biphasic solvent extraction approaches, which usually make use of an organic phase based on volatile organic solvents, since both phases are mainly composed of water (more than 80% on a molar basis).¹⁹¹ The first ABS systems were obtained from aqueous mixtures of polyethylene glycol (PEG) and dextran, from aqueous mixtures of a polymer and an inorganic salt, and by the co-dissolution of two salts in water.^{192,193} These ABS mixtures were mainly used for the efficient extraction and purification of high value biological compounds such as proteins. The main advantages of this technique include the scaling up feasibility, the process integration capability and its inner biocompatibility.¹⁹⁴ Despite this method not being novel, several new trends and improvements have recently emerged, as the use of new solvents and/or combination of extraction methodologies. Paula *et al.* presented a systematic approach to help on the selection of the key parameters that should be defined to design an efficient ABS extraction system: (i) selection of type of aqueous two-phase systems; (ii) phase-forming salt; (iii) molecular weight of the phase-forming polymer; (iv) system pH; (v) phase composition; (vi) phase volume ratio; and (vii) type and concentration of neutral salts.¹⁹⁵

Polyethylene glycol (PEG) based systems are often used due to its well known benign properties: low volatility, minimal toxicity, and food-safe

nature. Recently it was reported that hydrophilic ILs also allow the formation of ABS systems, presenting some advantages when compared to conventional high-melting inorganic salts: (i) avoid salt crystallization problems; (ii) ‘tunability’ of the ABS method towards a specific process/target compound due to the large number of available hydrophilic ILs; (iii) use as enhancers for the fine additional tuning of already known polymer/conventional salt ABS systems; and (iv) present with low corrosiveness when compared to conventional salts in the typical ABS methods, which enables their use in industrial processes.¹⁹⁶

The success of a biphasic extractive system also depends on the ability to manipulate the properties of each phase involved, in order to obtain the adequate partition coefficients and a high selectivity for specific solutes. Therefore, ILs may also contribute for the tuning of ABS extraction methods. Indeed, two-phase systems composed of different phosphonium-based ILs and water were already successfully used for the extraction of short-chain organic acids (L-lactic, L-malic, and succinic acids).¹¹⁰ Furthermore, aqueous two-phase systems composed of hydrophilic phosphonium-based ILs, K_3PO_4 and water were also studied for the extraction of amino acids, natural colorants and alkaloids.¹⁹³

Despite the advantages, these systems are still being tested and optimized at laboratory scale and are mostly applied for the separation of mixtures of known target compounds. However, considering the good results obtained so far for simplified model systems, the application of the ABS methodology (with or without ILs) may represent a valuable alternative in the near future considering its versatility, high capacity, biocompatible environment, low interfacial tension of phases, high yields, short processing time, low energy consumption and potential to achieve the desired purification and concentration of the product in a single step.¹⁹⁷

ABS were also used to separate betalains, a natural food colorant obtained from beet extract, from sugars.¹⁹⁸ Authors studied the effect of tie-lines, phase volume ratios, neutral salts composition and pH on the partitioning of betalains using a PEG6000/ammonium sulfate ABS extraction method. Results showed that, at optimized conditions, betalains were obtained in the top phase (~75%) and sugars in the bottom phase (>90%). In addition, betalains can be further separated from the polymer phase by an organic–aqueous extraction and the polymer can be reused.

Cismeros and co-authors reported the partitioning of lutein, produced by *Chlorella protothecoides*, in a PEG8000–phosphate ABS and showed that the lutein concentration in the top phase mostly depended on the tie-line length and on the employed PEG molecular weight.¹⁹⁹ The results clearly demonstrated the potential of the ABS process for the development of an industrial biotechnological process for the recovery of intracellular lutein produced by *C. protothecoides*.

Gilda and co-authors reported the application of food-grade polyglycolized glycerides and gelucires to obtain a water-soluble extract of turmeric, which contained several hydrophobic and hydrophilic components with a wide

spectrum of potential biological activities.²⁰⁰ Polyglycolized glycerides are well-defined mixtures of mono-, di- and tri-glycerides and of mono- and di-fatty acid esters of polyethylene glycol, while glucires are commercially available in different compositions of different of mono-, di- and tri-glycerides. Both are commercially available as food-grade excipients and are well known for the improvement of the oral bioavailability of poorly water-soluble drugs. The biological activities of the extracts were compared to the ones of extracts obtained in an organic solvent-based extraction method and in a conventional Soxhlet extraction method, and confirmed the potential benefits associated with the use of polyglycolized glyceride-based systems. Recently, Kulkarni and co-authors evaluated a new process based on the use of benign solvents with different hydrophobicities, namely common alimentary oil, polypropylene glycol and polyethylene glycol, for the selective extraction and fractionation of limonene from orange peels.²⁰¹ The authors optimized an integrated process that consisted in the extraction of limonene from orange peels using polypropylene glycol 240 (PPG) followed by an organophilic pervaporation method that led to the selective recovery of free-of-solvent limonene. Grozdanic and co-authors measured the liquid-liquid equilibria for different aqueous systems containing nicotine and PEG 200 (or ethyl lactate or glycerol) and an IL (1-ethyl-3-methylimidazolium ethyl sulfate) or sodium chloride (or sodium phosphate).²⁰² The objective of the study was to assess the possibility of using environmentally friendly solvents for the extraction/separation of nicotine from its aqueous solutions. The results showed that PEG 200, glycerol and ethyl lactate are good co-solvents for nicotine in water. On the other hand, the tested inorganic salts showed significant salting-out effects in nicotine aqueous solutions even when small amounts were used. However, the tested IL exhibited either a co-solvent (salting-in) effect or an anti-solvent salting-out effect, depending on the used concentration. The authors showed that liquid PEG 200 or ethyl lactate combined with this IL may provide sustainable tunable solvents for neat nicotine and that practically non-volatile solvents, ILs, and inexpensive inorganic salts, such as NaCl and Na₃PO₄, are promising demixing substances for a sustainable handling of aqueous nicotine solutions. Therefore, these results may encourage more research towards the application of these systems for the extraction/purification of other alkaloids and similar molecules.

7.5.5 Tunable Solvents

The main characteristic of this type of solvents is that they may lead to significant changes in several system properties by exposing the solvent to external physical or chemical stimuli. The classical and most commonly used examples of this class of solvents includes the use of tunable high-pressure/supercritical extraction methodologies using carbon dioxide, ethane and pressurized/subcritical water as solvents for the production of solvent-free extracts (when using carbon dioxide or ethane) or for the production of solutions that may be easily concentrated by solvent evaporation and/or by freeze-drying processes (when using water or other co-solvents). In terms of

co-solvents, researchers have been focusing on the use of greener solvents such as water, alcohol (ethanol, isopropanol) or water/alcohol mixtures at different proportions in order to improve the extraction selectivity.^{96,203–205} Despite the well-known advantages associated with the use of these solvents, high-pressure methodologies still have rarely been used at industrial scale and have been applied only for some specific cases.²⁰⁶

Gas-expanded liquids (GXL) are considered tunable systems that consist of homogeneous mixtures of aprotic organics as acetonitrile, dimethyl sulfoxide (DMSO) and tetrahydrofuran (THF) and polar protic solvents as water or PEG that undertake a phase split to form biphasic liquid–liquid mixtures upon the addition of an anti-solvent gas or a SCF.²⁰⁷ Phase split from monophasic to biphasic systems occurs as the result of the difference in the anti-solvent gas/SCF solubility between the aprotic organic solvent and the polar protic solvent. One main drawback of this process is the necessity to use conventional organic solvents to recover the target compounds from the enriched phase. However, to overcome this issue, Donaldson and co-authors explored alternative separation methods such as the supercritical fluid extraction with benign solvents like carbon dioxide.²⁰⁸ These authors developed a tunable solvent method that is capable of using CO₂ at modest pressures to switch from a homogeneous to a heterogeneous system, i.e. by combining PEG with a miscible organic solvent (1,4-dioxane or acetonitrile). The authors employed CO₂ to form a GXL with the organic solvent and this allowed the modification of the properties of this organic solvent and induced the phase separation. PEG-tunable systems have several advantages over the aqueous-based systems mostly due to the enhanced substrate solubility and to their potential application in processes involving water-sensitive compounds or water-unfavorable equilibria. Polyethylene glycol is used in some cases as an alternative to water because it is miscible with most organics and can complex with some ionic compounds, thus being used as a medium for the extraction process as well as an absorbent (phase separation enhancer) for the target compounds.²⁰⁹ The authors proposed the use of this ternary tunable system to carry out homogeneous reactions and heterogeneous separations, leading to the possibility for easy product/catalyst recovery. However, it can be also valuable for the extraction of target compounds mainly from complex vegetal matrices (e.g. lignin and cellulose).

The physical properties of GXLs can be readily tuned by pressure. As already mentioned, CO₂ is the most used anti-solvent for the promotion of phase splitting in these systems. It works as a ‘trigger agent’ since it is almost completely miscible with most organic solvents and is slightly soluble in aqueous media. When the CO₂ pressure increases, lower amounts of water are present in the organic-rich phase and lower amounts of organics are in the aqueous phase. Propane has also been used as the phase split inducer for mixtures of THF/H₂O at pressures lower than the ones applied when using CO₂.²⁰⁷ The use of propane also eliminates the *in situ* carbonic acid formation from the reaction of CO₂ with water, which may present an advantage when extracting acid-sensitive compounds.

In a recent work, Golmakani and co-authors compared two extraction techniques and two green solvents in terms of efficiency to obtain γ -linolenic acid (GLnA) enriched fractions from *Arthrospira platensis* (Spirulina).²¹⁰ These green solvents were ethanol and CO₂ (i.e. CO₂-expanded ethanol). For comparison purposes, a pressurized liquid extraction (PLE) method using an ethanol:ethyl lactate mixture was also employed. The results showed that the performance of the GLX method was intermediate between that observed for the PLE method and the one obtained for the SFE method.

Eckert and co-authors proposed the use of GXLs (CO₂-methanol systems) as a sustainable technique to extract fine chemicals from biorefinery wastes.²¹¹ In this process, CO₂ is used as anti-solvent by decreasing the solubility of lignin in methanol, leading to its precipitation, and consequently enhancing the solubility of some lignin low molecular weight components that are commonly used in the flavor and fragrance industry (vanillin, syringol) as well as pharmaceutical precursors (syringaldehyde). Their concentration in the GXL phase increases as the concentration of the anti-solvent increases until a certain pressure when syringol, vanillin, and syringaldehyde also begin to precipitate. These are high added value chemicals and their removal from biomass using gas-expanded methanol provides an easy and a very cost-efficient way to add sustainability to biorefineries by the low operating costs, the easy recycling of organic solvents, the use of a renewable feedstock, and using a way to produce chemicals without wasteful synthesis.

A further advantage of using these systems is that extractions using CO₂-aqueous and CO₂-alcohol GXLs promote the *in situ* generation of carbonic acid and of alkyl carbonic acid, respectively.⁷¹ This is another way to acidify the solvent medium without adding acidic additives in the process. As an example, for anthocyanin extraction this may represent an important advantage because there will be a temporary reduction in the extraction medium pH value that will increase the anthocyanin stability and the vegetable cell membrane permeability, thus leading to higher diffusivities and extraction yields.^{72,73} Anthocyanins were previously extracted using a ternary system of CO₂-water-ethanol and despite higher anthocyanin yields being obtained using a solvent proportion of CO₂-water-ethanol of 0:80:20% (i.e. without CO₂), higher values of polymeric color was found for the ternary mixtures, which indicates that molecular condensation reactions occurred between anthocyanins, which are known to correspond to enhanced stability and favorable biological activity.^{83,84} This was also reported by the work of Delgado-Vargas and Paredes-López that described the stability of anthocyanins under certain pH conditions and showed that, in acidic conditions, the molecules are more stable, maintaining their red colors.²¹²

Another interesting tunable system consists of the use of reversible ionic liquids (ReVILs), which are formed by the reversible reaction of compounds (with basic nitrogen functionalities) with CO₂ at ambient pressure to form a liquid salt (IL).²¹³ The process results in a step change of some of the solvent properties such as polarity or viscosity, by the structure modification of the molecular liquid precursor. This system was firstly used to extract soybean oil

using *t*-butyl amidine.²¹⁴ As such, this solvent switches from non-polar to polar, as well as from hydrophobic to hydrophilic. In the hydrophobic/non-polar form (prior to reaction with CO₂), *t*-butyl amidine was used to extract oil from soybean flakes with an extraction efficiency similar to that of hexane (the amidine was recovered from the oil into water, by the reaction with carbonated water in order to form a water-soluble bicarbonate salt). After the reaction with CO₂, the amidine became hydrophilic and was then recovered from the soybean oil with 96% efficiency (the amidine was separated from the aqueous solution by bubbling air through to strip the CO₂ and to revert the ionic species into their initial non-polar hydrophobic form). This solvent system provides an alternative to hexane extraction for oil extraction processes and eliminates the necessity for hexane distillation, thus reducing energy requirements and volatile organic emissions. Moreover, it may also help to overcome the challenges associated with product isolation and purification from conventional IL phases.

Finally, another example of a tunable solvent is the use of cyclic unsaturated β,γ -sulfones, which have solvent strengths similar to DMSO and usually offer a switchable solvent separation behavior because they can easily decompose into gases that can be further recovered and reused.²¹⁵ Despite these advantages, these solvents have not yet been extensively applied for the extraction of natural compounds from plant matrices.

7.6 Conclusions and Future Perspectives

We presented and discussed the most recent trends and perspectives on the extraction of natural products. This was made in terms of: (i) the natural origin extracts/target compounds that are currently being more studied and extracted (as well as in their potential applications); (ii) the most recently extracted vegetable raw materials that can be explored as sources for the envisaged target compounds (or on the recent tendencies regarding previously studied vegetable raw materials); (iii) the current and most promising strategies regarding the extraction, separation and purification methodologies that are expected to be further developed and employed on the extraction of natural products (including those already being explored but that can be improved and/or combined/hyphenated); and (iv) the novel/alternative solvents, solvent mixtures and extraction additives that could be beneficially used in the above mentioned extraction methodologies.

A literature search was performed (covering the period between 2000 and 2011) using Scopus search engine looking for published review and research articles. The obtained (numerical) search results may contain some inaccuracies and may be slightly over- or under-estimated, due to the employed search descriptors (that may be too specific or too broad for the performed search), to the indexed articles titles/abstracts/keywords (that may not contain the most adequate terms or keywords), as well as to other indexing/searching service limitations. Nevertheless, we were mainly interested in finding/comparing the general trends on the extraction of natural products with an acceptable level of confidence (and not in obtaining/comparing very accurate data). Therefore, we

consider that the obtained results will reflect the major tendencies observed in the last decade in terms of the extraction of natural products.

Results showed that lipids, phenolics, carbohydrates, essential oils, steroids and alkaloids were the most extracted natural products for the considered period. However, in most recent years a much higher interest in the extraction of tannins, phenolics, essential oils, alkaloids and lipids was observed. These results indicate the most recent trends and higher interest in the application of natural products for food, nutraceutical, cosmetic, pharmaceutical, agricultural and environmental applications, as they present important properties as colorants/pigments/dyes, antioxidants, anti-inflammatories, antimicrobials, analgesics, antifungals and pesticides.

In general terms, the most used natural origin raw materials in the extraction of natural products were terrestrial plant origin raw materials, namely their roots, rhizomes, leaves, fruits and seeds. It was also observed that, in more recent years, there was much more attention paid to the extraction of natural products from plant residues/wastes such as husks, barks, pods and peels. In addition, it seems that there was also a rising interest on the extraction from marine organisms (fish and algae) and microorganisms (bacteria, fungi and microalgae).

In terms of the most employed natural product extraction methods, it was observed that supercritical fluid extraction methods kept the interest of researchers in the last decade, while other methods such as ultrasound, microwave, pressurized liquid and sorptive extraction have also been getting more attention in recent years. Additional attention was paid to coupled/combined and hyphenated extraction techniques as well as in the use of extreme and non-conventional extraction conditions.

Finally, it was also observed a recent interest in using novel extraction solvents (or solvent mixtures) that may improve process safety and sustainability, and/or that may improve extraction yield, selectivity and stability of the target compounds. Moreover, these new solvents should be able to be easily removed, recycled and reused, in order to avoid toxicity and environmental issues, as well as to decrease energy and other operational costs.

In conclusion, this work showed that, in recent years, there was a clear renewed/increased interest in the extraction of natural products and in their advantageous applications, namely as food supplements, phytomedicines, nutraceuticals, cosmetics, natural pesticides, as well as in other industrial, energy and environmental applications. These specific applications are also conditioning the employed extraction methods and the solvents/solvent mixtures to be used by these techniques. It is thus expected that these trends will be maintained in the near future as they were mostly motivated by quite recent consumer demands and by safety, environmental and regulatory issues.

References

1. S. D. Sarker, Z. Latif and A. I. Gray, in *Natural Products Isolation*, 2nd edition, ed. S. D. Sarker, Z. Latif and A. I. Gray, Humana Press Inc., New York, NY, 2006, p. 515.

2. M. A. A. Meireles, in *Extracting Bioactive Compounds for Food Products: Theory and Applications*, ed. M. A. A. Meireles, CRC Press, Boca Raton, 2009, p. 1.
3. B. Harjo, C. Wibowo and K. M. Ng, *Chem. Eng. Res. Des.*, 2004, **82**, 1010.
4. Dictionary of Natural Products, Taylor & Francis, <http://dnp.chemnetbase.com/intro/index.jsp> 2012, last accessed in August, 2012.
5. Scopus search engine, Elsevier B. V., <http://www.info.scopus.com/ees>, for the period between 2000 and 2011.
6. M. E. M. Braga, P. F. Leal, J. E. Carvalho and M. A. A. Meireles, *J. Agric. Food Chem.*, 2003, **51**, 6604.
7. A. M. A. Dias, P. Santos, I. J. Seabra, R. N. C. Júnior, M. E. M. Braga and H. C. de Sousa, *J. Supercrit. Fluids*, 2012, **61**, 62.
8. P. Hutzler, R. Fischbach, W. Heller, T. P. Jungblut, S. Reuber, R. Schmitz, M. Veit, G. Weissenbock and J. Schnitzler, *J. Exp. Bot.*, 1998, **323**, 953.
9. M. Kuras, M. Stefanowska-Wronka, J. M. Lynch and A. M. Zobel, *Ann. Bot. (Oxford, UK)*, 1999, **84**, 135.
10. A. A. Esmelindro, J. S. Girardi, A. Mossi, R. A. Jacques and C. Dariva, *J. Agric. Food Chem.*, 2004, **52**, 1990.
11. S. Cosmulescu, I. Trandafir, G. Achim and A. Baciú, *Not. Bot. Hort. Agrobot. Cluj.*, 2011, **39**, 237.
12. T. M. Takeuchi, C. G. Pereira, M. E. M. Braga, M. R. Maróstica Jr., P. F. Leal and M. A. M. Meireles, in *Extracting Bioactive Compounds for Food Products: Theory and Applications*, ed. M. A. A. Meireles, CRC Press, Boca Raton, 2009, p. 464.
13. V. Mandal, Y. Mohan and S. Hemalatha, *Pharmacogn. Rev.*, 2007, **1**, 7.
14. P. Tatke and Y. Jaiswal, *Res. J. Med. Plant*, 2011, **5**, 21.
15. H. Zhou and C. Liu, *J. Chromatogr. A*, 2006, **1129**, 135.
16. V. Pasquet, J.-R. Chéroutier, F. Farhat, V. Thiéry, J.-M. Piot, J.-B. Bérard, R. Kaas, B. Serive, T. Patrice, J.-P. Cadoret and L. Picot, *Proc. Biochem.*, 2011, **46**, 59.
17. F. Chemat, J. Smadja and M. E. Lucchesi, *European Patent Demand*, 03001183.7, 2003.
18. M. E. Lucchesi, F. Chemat and J. Smadja, *J. Chromatogr. A*, 2004, **1043**, 323.
19. B. Bayramoglu, S. Sahin and G. Sumnu, *J. Food Eng.*, 2008, **88**, 535.
20. O. O. Okoh, A. P. Sadimenko and A. J. Afolayan, *Food Chem.*, 2010, **120**, 308.
21. Y. Zhai, S. Sun, Z. Wang, J. Cheng, Y. Sun, L. Wang, Y. Zhang, H. Zhang and A. Yu, *J. Sep. Sci.*, 2009, **32**, 3544.
22. C. Jiang, Y. Sun, X. Zhu, Y. Gao, L. Wang, J. Wang, L. Wu and D. Song, *J. Sep. Sci.*, 2010, **33**, 2784.
23. M. Abert Vian, X. Fernandez, F. Visinoni and F. Chemat, *J. Chromatogr. A*, 2008, **1190**, 14.

24. N. Bousbia, M. Abert Vian, M. A. Ferhat, B. Y. Meklati and F. Chemat, *J. Food Eng.*, 2009, **90**, 409.
25. N. Bousbia, M. Abert Vian, M. A. Ferhat, E. Petitcolas, B. Y. Meklati and F. Chemat, *Food Chem.*, 2009, **114**, 355.
26. M. Zill-e-Huma, M. A. Vian, J. F. Maingonnat and F. Chemat, *J. Chromatogr. A*, 2009, **1216**, 7700.
27. X. H. Xiao, J. X. Wang, G. Wang, J. Y. Wang and G. K. Li, *J. Chromatogr. A*, 2009, **1216**, 8867.
28. J. X. Wang, X. H. Xiao and G. K. Li, *J. Chromatogr. A*, 2008, **1198**, 45.
29. M. Zill-e-Huma, M. Albert-Vian, M. Elmaataoui and F. Chemat, *J. Food Chem.*, 2011, **105**, 351.
30. A. Casazza, B. Aliakbarian, S. Mantegna, G. Cravotto and P. Perego, *J. Food Eng.*, 2010, **100**, 50.
31. M. D. Luque de Castro and F. Priego-Capote, *J. Chromatogr. A*, 2010, **1217**, 2383.
32. F. Priego Capote, J. L. Luque Garcia and M. D. Luque de Castro, *J. Chromatogr. A*, 2003, **994**, 159.
33. L. E. Garcia-Ayuso, J. L. Luque Garcia and M. D. Luque de Castro, *Anal. Chem.*, 2000, **72**, 3627.
34. R. C. Prados-Rosales, M. C. Herrera, J. L. Luque-García and M. D. Luque de Castro, *J. Chromatogr. A*, 2002, **953**, 133.
35. J. L. Luque-García and M. D. Luque de Castro, *J. Chromatogr. A*, 2003, **998**, 21.
36. T. Zhou, X. Xiao and G. Li, *Anal. Chem.*, 2012, **84**, 5816.
37. R. C. Prados-Rosales, J. L. Luque García and M. D. Luque de Castro, *J. Chromatogr. A*, 2003, **993**, 121.
38. F. Priego-Capote and M. D. Luque de Castro, *Talanta*, 2005, **65**, 81.
39. F. Priego-Capote, J. Ruiz-Jiménez and M. D. Luque de Castro, *Food Chem.*, 2007, **100**, 859.
40. Y. C. Yang, J. Li, Y. G. Zu, Y. J. Fu, M. Luo, N. Wu and X. L. Liu, *Food Chem.*, 2010, **122**, 373.
41. M. Toma, M. Vinatoru, L. Paniwnyk and T. J. Mason, *Ultrason. Sonochem.*, 2001, **8**, 137.
42. M. A. Rostagno, M. Palma and C. G. Barroso, *J. Chromatogr. A*, 2003, **1012**, 119.
43. J. Jian-Bing, L. Xiang-Hong, C. Mei-Qiang and X. Zhi-Chao, *Ultrason. Sonochem.*, 2006, **13**, 455.
44. Z. J. Dolatowski, J. Stadnik and D. Stasiak, *Technol. Alimentaria*, 2007, **6**, 89.
45. A. C. Soria and M. Villamiel, *Trends Food Sci. Technol.*, 2010, **21**, 323.
46. Y. Chen, X. Gu, S. Huang, J. Li, X. Wang and J. Tang, *Int. J. Biol. Macromol.*, 2010, **46**, 429.
47. Z. Lianfu and L. Zelong, *Ultrason. Sonochem.*, 2008, **15**, 731.
48. G. Cravotto, L. Boffa, S. Mantegna, P. Perego, M. Avogadro and P. Cintas, *Ultrason. Sonochem.*, 2008, **15**, 898.

49. S. Sayar, Z. Z. Abidin, R. Yunus and A. Muhammad, *J. Appl. Sci.*, 2011, **13**, 2444.
50. C. Leonelli and T. J. Mason, *Chem. Eng. Process.*, 2010, **49**, 885.
51. X. L. Cheng, J. Y. Wan, P. Li and L. W. Qi, *J. Chromatogr. A*, 2011, **1218**, 5774.
52. C. Lu, H. Wang, W. Lv, C. Ma, P. Xu, J. Zhu, J. Xie, B. Liu and Q. Zhou, *Chromatographia*, 2011, **74**, 139.
53. C. Da Porto and D. Decorti, *Ultrason. Sonochem.*, 2009, **16**, 795.
54. J. L. Luque-García and M. D. Luque de Castro, *J. Chromatogr. A*, 2004, **1034**, 237.
55. S. Shah, A. Sharma and M. N. Gupta, *Bioresour. Technol.*, 2005, **96**, 121.
56. A. Sharma and M. N. Gupta, *Ultrason. Sonochem.*, 2006, **13**, 529.
57. Y. Li, L. Jiang, X. Sui, B. Qi and Z. Han, *Procedia Eng.*, 2011, **15**, 4653.
58. J. Long, Y. Fu, Y. Zu, J. Li, W. Wang, C. Gu and M. Luo, *Bioresour. Technol.*, 2011, **102**, 9991.
59. B. Klejdus, L. Lojková, M. Plaza, M. Šnóblová and D. Štěrbová, *J. Chromatogr. A*, 2010, **1217**, 7956.
60. E. Riera, Y. Golás, A. Blanco, J. A. Gallego, M. Blasco and A. Mulet, *Ultrason. Sonochem.*, 2004, **11**, 241.
61. A. Hu, S. Zhao, H. Liang, T. Qiu and G. Chen, *Ultrason. Sonochem.*, 2007, **14**, 219.
62. Y. Gao, B. Nagy, X. Liu, B. Simándi and Q. Wang, *J. Supercrit. Fluids*, 2009, **49**, 345.
63. S. Balachandran, S. E. Kentish, R. Mawson and M. Ashokkumar, *Ultrason. Sonochem.*, 2006, **13**, 471.
64. R. Z. Chen, S. Q. Zhang and Z. Q. Liu, *Chinese Trad. Herbal Drugs*, 2007, **38**, 1905.
65. J. Xi, D. Shen, Y. Li and R. Zhang, *Food Control*, 2011, **22**, 1473.
66. X. Guo, D. Han, H. Xi, L. Rao, X. Liao, X. Hu and J. Wu, *Carbohydr. Polym.*, 2012, **88**, 441.
67. K. N. Prasad, B. Yang, J. Shi, C. Yu, M. Zhao, S. Xue and Y. Jiang, *J. Pharm. Biomed. Anal.*, 2010, **51**, 471.
68. C. G. Joo, K. H. Lee, C. Park and B. C. Lee, *J. Ind. Eng. Chem.*, 2011, **17**, 712.
69. J. Xi, D. Shen, Y. Li and R. Zhang, *Food Res. Int.*, 2011, **44**, 2783.
70. A. Mustafa and C. Turner, *Anal. Chim. Acta*, 2011, **703**, 8.
71. K. N. West, C. Wheeler, J. P. McCarney, K. N. Griffith, D. Bush and C. L. Liotta, *J. Phys. Chem. A*, 2001, **105**, 3947.
72. N. Türker and F. Erdoğan, *J. Food Eng.*, 2006, **76**, 579.
73. T. Norton and D. Sun, *Food Bioprocess Technol.*, 2008, **1**, 2.
74. S. V. Kamat, E. J. Beckman and A. J. Russell, *Crit. Rev. Biotechnol.*, 1995, **15**, 41.
75. N. Foster, R. Mammucari, F. Dehghani, A. Barrett, K. Bezanehtak and E. Coen, *Ind. Eng. Chem. Res.*, 2003, **42**, 6476.
76. A. Sheibani and H. S. Ghaziaskar, *LWT – Food Sci. Technol.*, 2008, **41**, 1472.

77. F. Pouralinzazar, M. A. C. Yunus and G. Zahedi, *J. Supercrit. Fluids*, 2012, **62**, 88.
78. I. J. Seabra, A. M. A. Dias, M. E. M. Braga and H. C. de Sousa, *J. Supercrit. Fluids*, 2012, **62**, 135.
79. D. T. Santos, P. C. Veggi and M. A. Meireles, *J. Food Eng.*, 2012, **108**, 444.
80. I. J. Seabra, M. E. M. Braga and H. C. de Sousa, *J. Supercrit. Fluids*, 2012, **64**, 9.
81. M. Co, P. Koskela, P. Eklund-kergrén, K. Srinivas, J. W. King, P. J. R. Sjöberg and C. Turner, *Green Chem.*, 2009, **11**, 668.
82. J. K. Monrad, L. R. Howard, J. W. King, K. Srinivas and A. Mauromoustakos, *J. Agric. Food Chem.*, 2010, **58**, 4014.
83. I. J. Seabra, M. E. M. Braga, M. T. P. Batista and H. C. de Sousa, *Food Bioprocess. Technol.*, 2010, **3**, 674.
84. I. J. Seabra, M. E. M. Braga, M. T. P. Batista and H. C. de Sousa, *J. Supercrit. Fluids*, 2010, **54**, 145.
85. A. Gil-Ramírez, J. A. Mendiola, E. Arranz, A. Ruíz-Rodríguez, G. Reglero, E. Ibáñez and F. R. Marín, *Innov. Food Sci. Emerg. Technol.*, 2012, **16**, 54.
86. M. Perrut, *Ind. Eng. Chem. Res.*, 2000, **39**, 4531.
87. G. Brunner, *J. Food Eng.*, 2005, **67**, 21.
88. C. P. Passos, R. M. Silva, F. A. Da Silva, M. A. Coimbra and C. M. Silva, *J. Supercrit. Fluids*, 2009, **48**, 225.
89. K. Rochová, H. Sovová, V. Sobolík and K. Allaf, *J. Supercrit. Fluids*, 2008, **44**, 211.
90. C. Chen, C. Wang, L. Wang, Z. Hong, S. Chen, W. Ho and C. J. Chang, *J. Supercrit. Fluids*, 2008, **45**, 322.
91. N. T. Dunford and J. W. King, *J. Am. Oil Chem. Soc.*, 2001, **78**, 121.
92. L. Danielski, C. Zetzl, H. Hense and G. Brunner, *J. Supercrit. Fluids*, 2005, **34**, 133.
93. E. Reverchon, *J. Supercrit. Fluids*, 1992, **5**, 256.
94. E. Reverchon and G. Delta Porta, *J. Supercrit. Fluids*, 1996, **9**, 199.
95. F. V. Opstaele, K. Goiris, G. De Rouck, G. Aerts and L. De Cooman, *J. Supercrit. Fluids*, 2012, **69**, 45.
96. M. E. M. Braga, R. M. S. Santos, I. J. Seabra, R. Facanali, M. O. M. Marques and H. C. de Sousa, *J. Supercrit. Fluids*, 2008, **47**, 37.
97. M. E. M. Braga and M. A. A. Meireles, *J. Food Process Eng.*, 2007, **30**, 501.
98. T. Vata, M. Škerget and Z. Knez, *J. Food Eng.*, 2009, **90**, 246.
99. A. T. Serra, I. J. Seabra, M. E. M. Braga, M. R. Bronze, H. C. de Sousa and C. M. M. Duarte, *J. Supercrit. Fluids*, 2010, **55**, 184.
100. Y. Athukorala, F. S. Hosseinian and G. Mazza, *LWT – Food Sci. Technol.*, 2010, **43**, 660.
101. L. Ciurlia, M. Bleve and L. Rescio, *J. Supercrit. Fluids*, 2009, **49**, 338.
102. J. Ivanovic, I. Zizovic, M. Ristic, M. Stamenic and D. Skala, *J. Supercrit. Fluids*, 2011, **55**, 983.

103. M. Viot, V. Tomao, C. Ginies and F. Chemat, *Chromatographia*, 2008, **68**, 311.
104. C. D. Tanzi, M. A. Vian, C. Ginies, M. Elmaataoui and F. Chemat, *Molecules*, 2012, **17**, 8196.
105. H. A. G. Durand and F. Durand, Patent US 2009/0306386 A1, 2009.
106. R. Staudt, J. Nitzsche and P. Harting, *Supercritical Fluid Extraction Using Microwave Heating*, 6th International Symposium on Supercritical Fluids, Proceedings of the meeting in Versailles (France), 28–30 April, 2003.
107. H. Sonnenschein, J. Germanusund and P. Harting, *Chem. Ing. Tech.*, 2002, **74**, 270.
108. E. Reverchon and I. De Marco, *J. Supercrit. Fluids*, 2006, **38**, 146.
109. M. A. A. Meireles, G. Zahedi and T. Hatami, *J. Supercrit. Fluids*, 2009, **49**, 23.
110. F. S. Oliveira, J. M. M. Araújo, R. Ferreira, L. P. N. Rebelo and I. M. Marrucho, *Sep. Purif. Technol.*, 2012, **85**, 137.
111. M. Goto, M. Sato and T. Hirose, *J. Chem. Eng. Jpn.*, 1993, **26**, 401.
112. O. J. Catchpole, J. B. Grey and B. M. Smallfield, *J. Supercrit. Fluids*, 1996, **9**, 273.
113. H. Sovová, *Chem. Eng. Sci.*, 1994, **49**, 409.
114. H. Sovová, *J. Supercrit. Fluids*, 2005, **33**, 35.
115. H. Sovová, *J. Supercrit. Fluids*, 2012, **66**, 73.
116. E. Langa, J. Cacho, A. M. F. Palavra, J. Burillo, A. M. Mainar and J. S. Urieta, *J. Supercrit. Fluids*, 2009, **49**, 37.
117. E. Talansier, M. E. M. Braga, P. T. V. Rosa, D. Paolucci-Jeanjean and M. A. A. Meireles, *J. Supercrit. Fluids*, 2008, **47**, 200.
118. E. Reverchon and G. Iacuzion, *Chem. Eng. Sci.*, 1997, **52**, 3553.
119. M. J. Cocero and J. Garcia, *J. Supercrit. Fluids*, 2001, **20**, 229.
120. M. J. Cocero and J. Garcia, *J. Supercrit. Fluids*, 2001, **20**, 245.
121. T. Hatami, S. B. Glisic and A. M. Orlovic, *J. Supercrit. Fluids*, 2012, **62**, 102.
122. T. Hatami, R. N. Cavalcanti, T. M. Takeuchi and M. A. M. Meireles, *J. Supercrit. Fluids*, 2012, **65**, 71.
123. M. Izadifar and F. Abdolahi, *J. Supercrit. Fluids*, 2006, **38**, 37.
124. M. Khajeh, M. G. Moghaddam and M. Shakeri, *J. Supercrit. Fluids*, 2012, **69**, 91.
125. N. Mezzomo, J. Martínez and S. R. S. Ferreira, *J. Supercrit. Fluids*, 2009, **51**, 10.
126. J. M. Prado, G. H. C. Prado and M. A. A. Meireles, *J. Supercrit. Fluids*, 2011, **56**, 231.
127. P. T. V. Rosa and M. A. A. Meireles, *J. Food Eng.*, 2005, **67**, 235.
128. S. R. R. Comim, K. Madella, J. V. Oliveira and S. R. S. Ferreira, *J. Supercrit. Fluids*, 2010, **54**, 30.
129. C. L. C. Albuquerque and M. A. A. Meireles, *J. Supercrit. Fluids*, 2012, **66**, 86.
130. C. F. Poole and S. K. Poole, *J. Chromatogr. A*, 2010, **1217**, 2268.

131. A. Z. Fadhel, P. Pollet, C. L. Liotta and C. A. Eckert, *Annu. Rev. Chem. Biomol. Eng.*, 2011, **2**, 189.
132. V. Jordan and U. Müller, in *Industrial Scale Natural Products Extraction*, ed. H. J. Bart and S. Pilz, Wiley-VCH Verlag GmbH & Co, KGaA, Weinheim, Germany, 2011, p. 291.
133. J. M. Aguilera, in *Solid-Liquid Extraction in Extraction Optimization in Food Engineering*, ed. C. Tzia and G. Liadakis, CRC Press, New York, 2003, e-Book.
134. M. Pinelo, B. Zornoza and A. S. Meyer, *Sep. Purif. Technol.*, 2008, **63**, 620.
135. W. A. Wijesinghe and Y. J. Jeon, *Fitoterapia*, 2012, **83**, 6.
136. H. B. Sowbhagya, P. Srinivas, K. T. Purnima and P. Krishnamurthy, *Food Chem.*, 2011, **127**, 1856.
137. A. Zuurro, M. Fidaleo and R. Lavecchia, *Enzyme Microb. Technol.*, 2011, **49**, 567.
138. Z. Li, X. Wanga, G. Shi, Y. Bo, X. Lu, X. Li, R. Shang, L. Tao and J. Liang, *Sep. Purif. Technol.*, 2012, **86**, 234.
139. S. Chen, X. Xing, J. Huang and M. Xu, *Enzyme Microb. Technol.*, 2011, **48**, 100.
140. E. L. Belsito, G. Chidichimo, M. L. Di Gioia, A. Leggio, A. Ligouri, F. Perri and C. Siciliano, *Chromatographia*, 2008, **68**, 345.
141. L. Shen, X. Wang, Z. Wang, Y. Wu and J. Chen, *Food Chem.*, 2008, **107**, 929.
142. Y. H. Choi, Y. Chin, J. Kim, S. H. Jeon and K. Yoo, *J. Chromatogr. A*, 1999, **863**, 47.
143. B. T. Amid and H. Mirhosseini, *Food Chem.*, 2012, **132**, 1258.
144. S. V. Popov, R. G. Ovodova, V. V. Golovchenko, G. Y. Popova, F. V. Viatyasev, A. S. Shashkov and Y. S. Ovodov, *Food Chem.*, 2011, **124**, 309.
145. H. Karazhiyan, S. M. A. Razavi and G. O. Philips, *Food Hydrocolloids*, 2011, **25**, 915.
146. M. L. Fishman, H. K. Chau, P. H. Cooke, M. P. Yadav and A. T. Hotchkiss, *Food Hydrocolloids*, 2009, **23**, 1554.
147. D. V. Dandekar and V. G. Gaikar, *Sep. Sci. Technol.*, 2003, **38**, 1185.
148. M. Durand, Y. Zhu, V. Molinier, T. Feron and J. M. Aubry, *J. Surfactants Deterg.*, 2009, **12**, 371.
149. M. A. Desai and J. Parikh, *Ind. Eng. Chem. Res.*, 2012, **51**, 3750.
150. V. G. Gaikar and M. M. Sharma, *Sep. Technol.*, 1993, **3**, 2.
151. S. P. Mishra and V. G. Gaikar, *Ind. Eng. Chem. Res.*, 2004, **43**, 5339.
152. G. Raman and V. G. Gaikar, *Ind. Eng. Chem. Res.*, 2002, **41**, 2966.
153. G. Raman and V. G. Gaikar, *Langmuir*, 2003, **19**, 8026.
154. D. V. Dandekar, G. K. Jayaprakash and B. S. Patil, *Food Chem.*, 2008, **109**, 515.
155. K. Kittisak, G. Motonobu, S. Mitsuru, P. Prasert and S. Artiwan, *Sep. Purif. Technol.*, 2009, **66**, 111.

156. K. Kiathevest, M. Goto, M. Sasaki, P. Pavasant and A. Shotipruk, *Sep. Purif. Technol.*, 2009, **66**, 111.
157. Q. Fang, H. W. Yeung, H. W. Leung and C. W. Huie., *J. Chromatogr. A*, 2000, **904**, 47.
158. Z. Shi, J. He and W. Chang, *Talanta*, 2004, **64**, 401.
159. J. Shen and X. Shao, *Anal. Chim. Acta*, 2006, **561**, 83.
160. P. Schneider, S. S. Hosseiny, F. Bischoff, U. Müller, H. J. Bart, K. Schlitter and V. Jordan, *Instrum. Sci. Technol.*, 2011, **39**, 407.
161. Y. Q. Chang, S. N. Tan, J. W. H. Yong and L. Ge, *J. Sep. Sci.*, 2011, **34**, 462.
162. C. Sun and H. Liu, *Anal. Chim. Acta*, 2008, **612**, 160.
163. A. A. Memon, N. Memon and M. I. Bhanger, *Sep. Purif. Technol.*, 2010, **76**, 179.
164. G. Ulloa, C. Coutens, M. Sánchez, J. Sineiro, J. Fábregas, F. J. Deive, A. Rodríguez and M. J. Núñez, *Green Chem.*, 2012, **14**, 1044.
165. L. Ugolini, G. De Nicola and S. Palmieri, *J. Agric. Food Chem.*, 2008, **56**, 1595.
166. A. Naksuk, D. A. Sabatini and C. Tongcumpou, *Ind. Crop Prod.*, 2009, **30**, 194.
167. M. Minana-Perez, A. Gracia, J. Lachaise and J. L. Salager, *Colloids Surf. A*, 1995, **100**, 217.
168. S. I. Kadioglu, T. T. Phan and D. A. Sabatini, *J. Am. Oil Chem. Soc.*, 2011, **88**, 863.
169. J. M. Kim, S. M. Chang, I. H. Kim, Y. E. Kim, J. H. Hwang, K. S. Kim and W. S. Kim, *Biochem. Eng. J.*, 2007, **37**, 271.
170. S. F. Hamed, *J. Appl. Sci. Res.*, 2006, **2**, 567.
171. F. Castillo, D. Hernández, G. Gallegos, M. Mendez, R. Rodríguez, A. Reyes and C. N. Aguilar, *Ind. Crops Prod.*, 2010, **32**, 324.
172. H. Zhao, S. Xia and P. Ma, *J. Chem. Technol. Biotechnol.*, 2005, **80**, 1089.
173. B. Tang, W. Bi, M. Tian and K. H. Row, *J. Chromatogr. B*, 2012, **904**, 1.
174. X. Sun, H. Luo and S. Dai, *Chem. Rev.*, 2012, **112**, 2100.
175. J. G. Huddleston, A. E. Visser, W. M. Reichert, H. D. Willauer, G. A. Broker and R. D. Rogers, *Green Chem.*, 2001, **3**, 156.
176. C. F. Poole, *J. Chromatogr. A*, 2004, **1037**, 49.
177. M. H. Abraham, A. M. Zissimos, J. C. Huddleston, H. D. Willauer, R. D. Rogers and W. E. Acree, *Ind. Eng. Chem. Res.*, 2003, **42**, 413.
178. A. Berthod and S. Carda-Broch, *J. Liq. Chromatogr. Relat. Technol.*, 2003, **26**, 1493.
179. M. Molíková, M. J. Markuszewski, R. Kaliszan and P. Jandera, *J. Chromatogr. A*, 2010, **1217**, 1305.
180. J. Fan, Y. C. Fan, Y. C. Pei, K. Wu, J. J. Wang and M. H. Fan, *Sep. Purif. Technol.*, 2008, **61**, 324.
181. J. Martak and S. Schlosser, *Sep. Purif. Technol.*, 2007, **57**, 483.
182. Q. Yang, H. Xing, Y. Cao, B. Su, Y. Yang and Q. Ren, *Ind. Eng. Chem. Res.*, 2009, **48**, 6417.

183. K. Dong, Y. Cao, Q. Yang, S. Zhang, H. Xing and Q. Ren, *Ind. Eng. Chem. Res.*, 2012, **51**, 5299.
184. X. Ni, H. Xing, Q. Yang, J. Wang, B. Su, Z. Bao, Y. Yang and Q. Ren, *Ind. Eng. Chem. Res.*, 2012, **51**, 6480.
185. C. I. Melo, R. Bogel-lukasika and E. Bogel-lukasik, *J. Supercrit. Fluids*, 2012, **61**, 191.
186. C.-H. Ma, T.-T. Liua, L. Yang, Y.-g. Zua, X. Chen and L. Zhang, *J. Chromatogr. A*, 2012, **1218**, 8573.
187. X. Liu, Y. Wang, J. Kong, C. Nie and X. Lin, *Anal. Methods*, 2012, **4**, 1012.
188. Z.-J. Tan, F.-F. Li, X.-L. Xu and Ji.-M. Xing, *Desalination*, 2012, **286**, 389.
189. W. Bi, M. Tian and K. H. Row, *J. Chromatogr. B*, 2012, **880**, 108.
190. A. K. Ressmann, K. Strassl, P. Gaertner, B. Zhao, L. Greiner and K. Bica, *Green Chem.*, 2012, **14**, 940.
191. M. G. Freire, J. F. B. Pereira, M. Francisco, H. Rodriguez, L. P. N. Rebelo, R. D. Rogers and J. A. P. Coutinho, *Chem. Eur. J.*, 2012, **18**, 1831.
192. F. Spyropoulos, A. Portschi and I. T. Norton, *Food Hydrocolloids*, 2010, **24**, 217.
193. C. L. S. Louros, A. F. M. Claudio, C. M. S. S. Neves, M. G. Freire, I. M. Marrucho, J. Pauly and J. A. P. Coutinho, *Int. J. Mol. Sci.*, 2010, **11**, 1777.
194. J. Benavides and M. Rito-Palomares, *J. Chem. Technol. Biotechnol.*, 2008, **83**, 133.
195. A. J. R. Paula, A. M. Azevedo and M. R. Aires-Barros, *J. Chromatogr. A*, 2007, **1141**, 50.
196. J. F. B. Pereira, A. S. Lima, M. G. Freire and J. A. P. Coutinho, *Green Chem.*, 2010, **12**, 1661.
197. M. Rito-Palomares, *J. Chem. Technol. Biotechnol.*, 2004, **807**, 3.
198. S. Chethana, C. A. Nayak and K. S. M. S. Raghavarao, *J. Food Eng.*, 2007, **81**, 679.
199. M. Cisneros, J. Benavides, C. H. Brenes and M. Rito-Palomares, *J. Chromatogr. B*, 2004, **807**, 105.
200. S. Gilda, M. Kanitkar, R. Bhonde and A. Paradkar, *LWT – Food Sci. Technol.*, 2010, **43**, 59.
201. P. S. Kulkarni, C. Brazinha, C. A. M. Afonso and J. G. Crespo, *Green Chem.*, 2010, **12**, 1990.
202. N. D. Grozdanic, V. Najdanovic-Visak, M. Lj. Kijevcanin, S. P. Serbanovic, M. N. da Ponte and Z. P. Visak, *Fluid Phase Equilib.*, 2011, **310**, 198.
203. I. J. Seabra, M. E. M. Braga and H. C. de Sousa, *J. Supercrit. Fluids*, 2012, **64**, 9.
204. H. A. Martinez-Correa, F. A. Cabral, P. M. Magalhães, C. L. Queiroga, A. T. Godoy, A. P. Sánchez-Camargo and L. C. Paviani, *J. Supercrit. Fluids*, 2012, **63**, 31.

205. S. A. O. Santos, J. J. Villaverde, C. M. Silva, C. P. Neto and A. J. D. Silvestre, *J. Supercrit. Fluids*, 2012, **71**, 71.
206. G. Brunner, *Annu. Rev. Chem. Biomol. Eng.*, 2010, **1**, 321.
207. V. M. Blasucci, Z. A. Husain, A. Z. Fadhel, M. E. Donaldson and E. Vyhmeister, *J. Phys. Chem. A*, 2010, **114**, 3932.
208. M. E. Donaldson, L. C. Draucker, V. Blasucci, C. L. Liotta and C. A. Eckert, *Fluid Phase Equilib.*, 2009, **277**, 81.
209. U. S. Food Drug Admin. 2010. Sect. 172.820. Polyethylene glycol (mean molecular weight 200–9500). Code of Federal Regulations, Title 21, Vol. 3. (CITE: 21CFR172.820).
210. M. T. Golmakani, J. A. Mendiola, K. Rezaei and E. Ibáñez, *J. Supercrit. Fluids*, 2012, **62**, 109.
211. C. Eckert, C. Liotta, A. Ragauskas, J. Hallett, C. Kitchens, E. Hill and L. Draucker, *Green Chem.*, 2007, **9**, 545.
212. F. Delgado-Vargas and O. Paredes-López. *Natural Colorants for Food and Nutraceutical Uses*, CRC Press, Boca Raton, 2003, e-book.
213. P. G. Jessop, D. J. Heldebrant, X. W. Li, C. A. Eckert and C. L. Liotta, *Nature*, 2005, **436**, 1102.
214. P. G. Jessop, L. Phan, A. Carrier, S. Robinson, C. J. Durr and J. R. Harjani, *Green Chem.*, 2010, **12**, 809.
215. P. Pollet, C. A. Eckert and C. L. Liotta, *Chem. Sci.*, 2011, **2**, 609.

CHAPTER 8

Post-extraction Processes: Improvement of Functional Characteristics of Extracts

ÁNGEL MARTÍN, SORAYA RODRÍGUEZ-ROJO,
ALEXANDER NAVARRETE, ESTHER DE PAZ,
JOAO QUEIROZ AND MARÍA JOSÉ COCERO*

Department of Chemical Engineering and Environmental Technology,
University of Valladolid, Spain

*Email: mjcocero@iq.uva.es

8.1 Introduction

Frequently the product of an extraction process cannot be directly used in practical applications. Such product may contain unwanted compounds that must be removed. Of particular importance is the removal of solvents used in the extraction, if these substances cannot be allowed in the final product or due to the cost involved in the removal process. Additionally, the physical properties of the product (particle size and morphology, crystalline structure, etc.) often are as important as the chemical composition of the extract and can play a determinant role on the biological activity and the possible practical applications of the material. Moreover, natural extracts usually are prone to degradation processes and must be formulated with protective compounds. The formulation can also extend the functionalities of the extracts, allowing using

RSC Green Chemistry No. 21

Natural Product Extraction: Principles and Applications

Edited by Mauricio A. Rostagno and Juliana M. Prado

© The Royal Society of Chemistry 2013

Published by the Royal Society of Chemistry, www.rsc.org

them in pharmaceutical, cosmetic or food applications with different objectives. With these goals, the main post-extraction processing techniques are presented in this chapter.

8.2 Purification of Extracts and Elimination of Solvents

Remaining amounts of solvents are usually present in pharmaceuticals and food products as a result of the extraction techniques applied in the production processes. Increasing awareness in society regarding both environmental and human health, has inspired new technological approaches that allow the reduction or (if possible) elimination of solvents from final products. Of course, the best strategy for this purpose is to completely avoid the use of toxic solvents in every processing step, but in the cases where this is not possible, solvent elimination techniques are required.

Organic solvents may accumulate in lipid- and fat-rich cells of the human body, including the nervous system, brain, bone marrow, liver, and body fat,¹ and can cause different detrimental effects to health. Thus, residual solvents have been divided by the U.S. Food and Drug Administration (FDA) into three levels in accordance to their potential risk to the human health^{2,3} as follows:

- Class 1 (solvents to be avoided): ‘Known human carcinogens, strongly suspected human carcinogens, and environmental hazards.’ Benzene, 1,2-dichloroethane and 1,1-dichloroethane are included in this class.
- Class 2 (solvents to be limited): Hexane, pyridine and methanol are part of this group of solvents, defined as: ‘Non-genotoxic animal carcinogens or possible causative agents of other irreversible toxicity such as neurotoxicity or teratogenicity.’
- Class 3 (solvents with low toxic potential): ‘Solvents with low toxic potential to man; no health-based exposure limit is needed.’ Ethanol, acetone, ethyl acetate and dimethyl sulfoxide are included in this list.

The FDA has established the limits of use by industries of these solvents and provides a list that has been recently updated.³ Concentration limits for class 1 solvents range from 2 ppm up to 1500 ppm. In case of class 2 solvents, the limits range from 50 ppm to 3000 ppm. Concentrations below 5000 ppm are considered acceptable for solvents in class 3.

The European Commission has emitted a directive regarding the allowed solvents to be used in the food industry.⁴ Several limits are established according to the process in which the solvent is used. Some solvents are accepted for any use if the process accomplishes good manufacturing practice, these being: propane, butane, ethyl acetate, ethanol, carbon dioxide, nitrous oxide and acetone. The only exception is acetone, whose use in the refining of olive-pomace oil is forbidden.

To eliminate solvents remaining in the products it is necessary to manipulate physicochemical variables, the most common ones being temperature and

pressure. Usually, this involves the change of phase of one (or a group) of components in the initial mixture, which consumes a considerable amount of energy. Indeed, it has been estimated that 60% of the energy consumed in the pharmaceutical industry is due to the use of solvents during the production of the active compounds.⁵

The following section describes the main technologies for solvent elimination in terms of their principles and some applications.

8.2.1 Evaporation of Solvents

Separation of solids from volatile solvents by vaporization is commonly known as evaporation.⁶ This technique requires the change of the solvent component from liquid phase to vapour phase. Therefore, pressure decrease and/or temperature increase can be used to achieve this effect. A common application of evaporation is found in the production of the final particles of some high added value products such as vitamins and pharmaceuticals.^{7,8}

In the case of small production rates a single stage is used; on the other hand multiple-stage evaporators operating at different pressure (and thus temperature) levels are used in facilities designed for larger production capacities, in order to recover the latent heat content of the solvent in the process. Pressure reduction has to be used when the decomposition of the product with temperature is likely.⁹

Different methods of energy supply give rise to new evaporation processes. For example, microwave drying is based on the application of electromagnetic energy; the microwave frequencies provide a faster and more homogeneous evaporation of solvents. It can be combined with vacuum in order to work at temperatures suitable for heat-sensitive products.¹⁰

8.2.2 Freeze-drying

Freeze-drying requires a first step where the temperature of the materials (solutes and solvent) is reduced until they are frozen, followed by a sublimation step carried out via a very high vacuum which is sometimes accompanied by heating (Figure 8.1). During this process the product structure changes as an effect of scaffolds formed as the materials freeze.^{11,12} As a result a rigid structure is obtained that works as a shield to the possible damage to the original product.

Freeze drying is widely applied whenever a thermolabile substance is of interest. It forms part of extraction processes of several products, for instance, antioxidants, tissues, hormones and foods among others.^{13–16}

8.2.3 Reverse Osmosis

During reverse osmosis a net flow of solvent takes place from a concentrated solution to a more diluted solution through a semipermeable membrane.

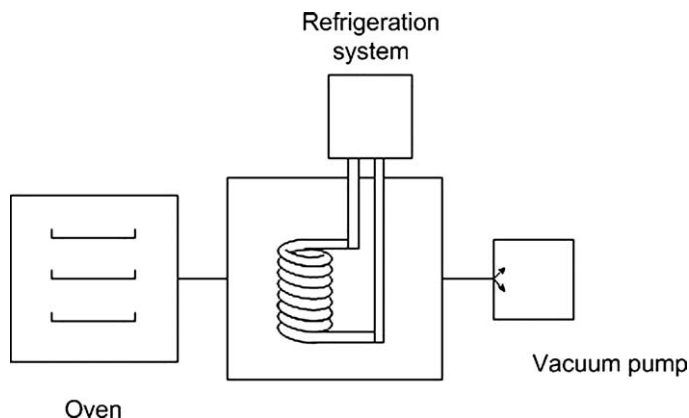


Figure 8.1 Scheme of a freeze drying apparatus.

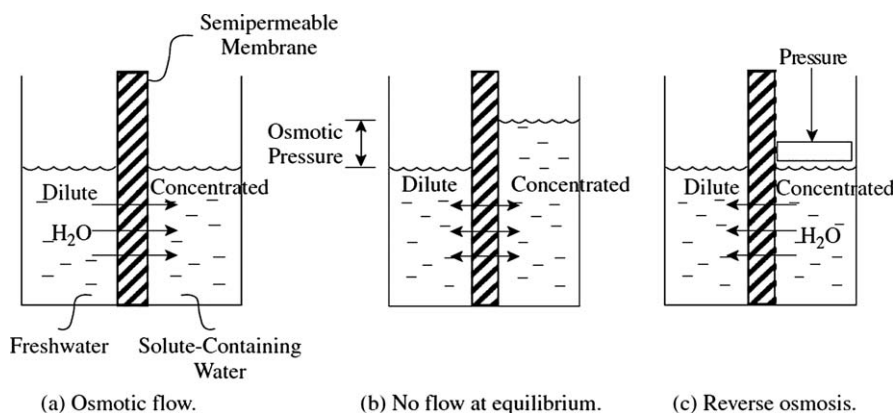


Figure 8.2 Reverse osmosis in principle.

Pressure is applied to the concentrated solution side in order to exceed the osmotic pressure and, as a result, solvent transfer through the membrane exists from the concentrated solution to the diluted side¹⁷ (Figure 8.2). This process requires no phase change and can operate at ambient temperature, which is of great advantage when dealing with especially sensitive materials.¹²

The separation mechanism in the membrane is based on the size, shape, charge and interactions of the compounds with the membranes. Asymmetric cellulose acetate membranes 100 μm thick are commonly used. To apply reverse osmosis a molar mass of the solute greater than 300 Da is usually required.¹⁸ This technique is widely applied in the food industry in processes such as juice concentration.^{12,19} In the pharmaceutical industry it is used to recover active compounds during downstream treatment.^{20–22}

8.3 Particle Size Reduction

Frequently, a main post-extraction step is the production of particles of a specific size and morphology, either by particle size reduction or by precipitation (in general terms, or crystallization if the crystalline product is the target) of the components present in the extract. The basic objectives of this step are to reduce storage volume requirements, and to facilitate the product dosage, improving its bioavailability (i.e. the fraction of the active compound which is effectively absorbed by the human body).

In particular, if the bioavailability is limited by the solubility or dissolution rate of the compound (e.g. in drugs with low water solubility administered orally), the control of particle parameters, such as particle size (PS) and particle size distribution (PSD), are of chief importance. The reduction of particle size increases the dissolution rate of the solid due to the increase of the ratio between the surface area and the volume of particles. Moreover, the saturation solubility of the compound is also increased when particle size is reduced below a critical size of 1–2 μm . For example, the solubility of BaSO_4 in water at 20 °C is 2.2 mg/L for particles above 5 μm , and reaches a value of 2.6 mg/L for particles of 100 nm. For pharmaceutical applications, the reduction of particle size below 1–2 μm allows using different administration routes (i.e. pulmonary, parenteral, etc.). Another parameter that influences the solubility is the solid state of the particles. It is well known that amorphous compounds usually show higher solubility compared to crystalline compounds. Moreover, different polymorphic forms of crystalline compounds can show different solubilities (e.g. caffeine can be produced in two crystalline polymorphs with different solubilities in water). The control of polymorphism in precipitation processes is also a key aspect in the long-term stability of the compounds.²³ Besides, solubility as well as stability of certain drugs can be improved by co-crystallization of the active ingredient with an excipient,²⁴ as for example caffeine co-crystallized with glutaric acid.

Therefore, to reach the highest saturation solubility, the best combination usually is nanometer size of particles and amorphous state. However, a prerequisite for utilization in pharmaceutical products is that the amorphous state can be maintained for the shelf life of the product. If the product in the amorphous state is not stable enough, the usual approach is to co-precipitate it with an amorphous polymer, as will be detailed in the next section.

In this section, the basic principles of the main particle-producing techniques will be presented. As indicated before, the control of particle size is a chief aspect of the process, and particle sizes below 1 μm are required when solubility issues exist. Conventional approaches, such as crystallization or spray drying, usually produce particles in the range from 10 μm to 100 μm (microparticles). For the production of nanoparticles (particles below 1 μm , in pharmaceutical and related disciplines) there are two approaches: bottom-up and top-down methods. The top-down approach mechanically reduces the particle size of micrometric powders obtained by conventional methods to the nanometric range (nanonization). In the bottom-up approach, the product is obtained

directly in the nanometric size from a clear solution or a colloidal solution of the product, after the removal of the solvent.

8.3.1 Top-down Methods

The top-down disintegration is carried out by two main technologies: pearl (or ball) milling and high-pressure homogenization, or a combination thereof.

8.3.1.1 Milling

Ball mills were already used in the first half of the 20th century for the reduction of particle size to the nano range.²⁵ The pearls or balls can be made of ceramics (cerium- or yttrium-stabilized zirconium dioxide), stainless steel, glass or highly cross-linked polystyrene resin-coated beads. Often, particles are suspended in a solvent to facilitate the processing and the recovery of the product (wet milling).

The main disadvantages of this technique are the erosion of material from the milling pearls leading to product contamination, and the adherence of product to the inner surface area of the mill. The erosion can be reduced using coated or polymers beads, as hard polystyrene derivatives.

The milling time is also an issue. The process can last from about 30 minutes to hours or several days depending on many factors such as the surfactant content in the suspension, the hardness of the drug, viscosity, temperature, energy input and of the milling media.

Nevertheless, this process is one of the main particle size reduction techniques developed to the commercial stage. An example is the NanoCrystals technology, patented by Elan Nanosystems (US Patent 5,145,684), where the polymer pearls are moved by a stirrer. The first FDA-approved products using NanoCrystals technology were launched to the market in 2002 and 2003.

8.3.1.2 High-pressure Homogenization

High-pressure homogenization is a top-down method that is carried out with the particles in suspension in a fluid, and it represents an alternative to ball milling, as it reduces significantly particle contamination by erosion. There are two homogenization principles: the microfluidization and the piston-gap homogenizers.

The microfluidization homogenizers are based in particle-to-particle collisions due to changes in flow direction or due to collisions of sub-streams. Products from this technique can contain a relatively large fraction of micro-particles, depending on the hardness of the drug.²⁶

In piston-gap homogenizers the comminution of particles is achieved by cavitation of the solvent, and by collisions among the particles and with the homogenizer walls due to turbulent flow. For this, the solid suspension is driven at high velocity to the homogenizer by a high-pressure pump. The homogenizer consists of a pressure-reduction device, such as an orifice or a valve, which

generates a large pressure drop, of the order of dozens of MPa. After this reduction, pressure must be below the vapour pressure of the suspension fluid at the operation temperature, thus producing the implosion of gas bubbles, or cavitation.

Commonly, the solid is processed in aqueous suspensions using a surfactant, but other dispersion media with reduced water content can also be used (e.g. water–glycerol mixtures for suspensions of intravenous injections), as well as non-aqueous media such as oils or liquid polyethylene glycol (PEG) 400 or 600, for direct filling of soft gelatine capsules with drug nanosuspensions. Moreover, liquefied gases such as carbon dioxide and R-134a have been successfully used for particle size reduction of drugs,²⁷ producing a dry and homogeneous product of high purity upon depressurization to atmospheric pressure after processing. Using such compressed gases as suspension fluids eliminates the additional solvent removal step from the liquid dispersion media, required in wet milling techniques.

8.3.2 Bottom-up Methods

In bottom-up methods, particles can be produced by crystallization/precipitation and solvent evaporation techniques.

8.3.2.1 Crystallization From a Solution

Crystallization is an industrially relevant technique for the production of microparticles by supersaturation of a solution. The three main methods to achieve the supersaturation and crystallization are: (1) elimination of the solvent by evaporation; (2) reduction of the solubility of the solute by controlled cooling of the solution; and (3) addition of a liquid anti-solvent (LAS) to the solution. Of these three methods, LAS addition usually shows the best performance for the production of ultrafine particles, and is frequently used in industry, particularly for pharmaceutical applications.

For the production of nanoparticles by LAS addition, a rapid, uniform and high supersaturation value is needed to produce high nucleation rates and low particle growth. This goal is achieved by using rapid mixing devices (impinging jets, T-mixers, static mixers, multi-inlet vortex mixers, ...), which can be combined with the use of ultrasound. Another challenge is to control particle growth and agglomeration, which is generally obtained by adding polymers or surfactants either to solvent or anti-solvent.²⁸

8.3.2.2 Spray-drying

Spray-drying (Figure 8.3) is a widespread technique in industry to produce micrometric particles from a liquid solution of the active ingredient by solvent evaporation.²⁹ The solvent and solute mixture is atomized into droplets via either a nozzle using compressed gas to atomize the liquid feed (two-fluid nozzle), or a rotary atomizer using a wheel rotating at high speed. Then, a

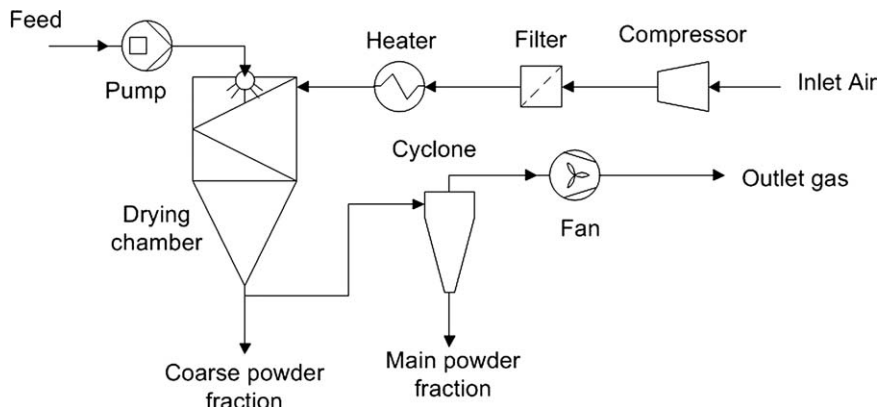


Figure 8.3 Flow diagram of a spray-drying system.

heated compressed gas (140–150 °C; 0.2–0.6 MPa) is brought into contact with the droplets leading to the fast evaporation of the solvent in the chamber, where the temperature is controlled around 80–90 °C in the case of aqueous solutions. The elimination of the solvent causes the formation of particles that fall to the bottom of the chamber. The fine powder is recovered from the exhaust gases using a cyclone or a bag filter.

Commonly, the active ingredient is in aqueous solution and atmospheric air can be used as drying agent. If organic solvents (or emulsions) are sprayed, the gas is substituted by N₂ to avoid the formation of an explosive atmosphere inside the drying chamber. N₂ can also be used if the product is prone to oxidation. The minimum temperature in the spray chamber is selected according to the boiling point of the solvent and the operating pressure in the chamber. In order to reduce this temperature, vacuum spray chambers are commonly used in the soap industry, and are being applied to the food industry.³⁰

Particle size of the product is in the range of 10 to 100 µm. Generally the particle size distribution is quite broad, and it is mainly influenced by the geometry of the nozzle and the initial solution viscosity. Improvement in the reduction of particle size is achieved by reduction of the size of the primary droplets using piezoelectric atomizer technology or ultrasound-assisted atomization. Besides, a nanospray-dryer B-90[®] for lab scale has been developed by Buchi; the particle size reduction is achieved through the combination of a piezoelectric atomizer and the use of laminar gas flow in the drying section. Particles are recovered in an electrostatic collector.³¹

Conventional spray-drying technique is relatively low cost, flexible and leads to the production of high-quality and stable particles, making this technique the most used in the food industry. Nevertheless, processing thermolabile substances can be problematic as the temperature in the chamber must be quite high (80–90 °C) in order to ensure a low moisture content in the final product.

8.3.2.3 *Drying Processes with Enhanced Atomization*

Since the atomization of the solution is a major parameter influencing the particle size and morphology obtained by spray-drying and related techniques, many efforts have been devoted to improve this step. As well as the use of complex atomization nozzles, combined with atomization mechanisms such as vibrations or ultrasound, another important strategy is the use of compressed or dissolved gases as propellants.

One such method is so-called ‘flash-boiling’ atomization.³² This technique consists of dissolving a gas in the solution to be atomized at high pressure (usually, more than 10 MPa). This solution is then depressurized. This causes the sudden release of the dissolved gas as small bubbles, which contribute to improve the atomization of the solution. A parallel strategy is the ‘effervescent atomization’ method,³³ also based in mixing the solution with a compressed gas, but in this case at lower pressures (usually, less than 1 MPa) and with much higher gas to product ratios compared to the flash-boiling method. With these conditions, the gas is not completely dissolved in the solution, and the method is based in achieving an intimate mixing of this biphasic mixture, and then suddenly expanding it through a nozzle. With this, the gas in the biphasic mixture experiences a considerable volumetric expansion, which contributes to break the atomized solution into small filaments and droplets.

8.3.2.4 *Micronization Processes with Supercritical Fluids*

The mechanisms of precipitation and crystallization processes by supercritical fluids (and, particularly, supercritical carbon dioxide, SC-CO₂) are essentially the same as in conventional ones: reduction in the solvent power of the solvent by changes in the operating conditions (pressure and temperature, instead of only temperature as in conventional ‘cooling crystallization’ as the solvent power of SC-CO₂ is related to its density), by the addition of an anti-solvent (SC-CO₂), or by solvent evaporation or extraction. Nevertheless, it is necessary to highlight that in supercritical processes the supersaturation values are, in general, higher and are achieved in a faster way compared to conventional crystallization or LAS addition, due to the favourable transport properties of supercritical fluids. Hence particle size can be reduced to the submicrometre scale.

Other well-known advantages of supercritical fluids for particle precipitation are the reduced use of organic solvents, which are completely avoided in some of the processes, and the mild operating conditions, with pressures in the range of 8 MPa to 20 MPa and temperatures between 35 °C and 60 °C. Also, the post-processing steps, filtering and drying of formed crystals, needed in conventional crystallization process, are avoided as a dry product is directly obtained. Besides, supercritical fluids are interesting for the possibility to easily couple them to extraction processes using supercritical fluid technology. Supercritical micronization processes are often classified into three broad categories: processes with CO₂ as solvent (rapid expansion of supercritical solutions,

RESS), processes with CO₂ as anti-solvent (supercritical anti solvent, SAS, and related processes) and processes with CO₂ as solute (particles from gas saturated solutions, PGSS, and other derived processes).

The RESS process (Figure 8.4) consists of the fast depressurization of a carbon dioxide supercritical solution of the compound of interest through a heated nozzle into a low pressure chamber. This causes a high supersaturation due to the drastic variations of the solubility of many solutes in CO₂ with pressure. Moreover, the increase of supersaturation is extremely fast and homogeneous, since it is transmitted by pressure reduction waves that move at the velocity of sound. Thus a large amount of nucleus of the substrate(s) is produced, enabling it to form very small particles, typically of 0.5–20 µm diameter, with narrow diameter distribution. The particles are collected from the carbon dioxide gaseous stream. The morphology of the resulting solid material depends both on the material structure (crystalline or amorphous) and on the process parameters (temperature, pressure drop, nozzle geometry, dimensions of the atomization vessel).

The main disadvantage of this process is the low solubility of most organic compounds in SC-CO₂, which often makes it impossible to prepare the solution of the compound of interest in SC-CO₂, the difficulties for the scale-up, and the energy cost associated with the recompression of CO₂ for its recirculation in the process. As an example, the precipitation by RESS of caffeine from industrial decaffeination by SC-CO₂,³⁴ is being substituted by near-isobaric processes such as caffeine absorption in water or adsorption in activated carbon.³⁵ Another alkaloid that has been precipitated by RESS is theophylline.³⁴ Alkaloids, due to their crystalline nature, are obtained as needles with a length around 5 µm. Other compounds that are suitable for RESS processing because they have a reasonable solubility in SC-CO₂ are phospholipids such as cholesterol³⁶ or lecithin,³⁷ leading to particles smaller than 1 µm.

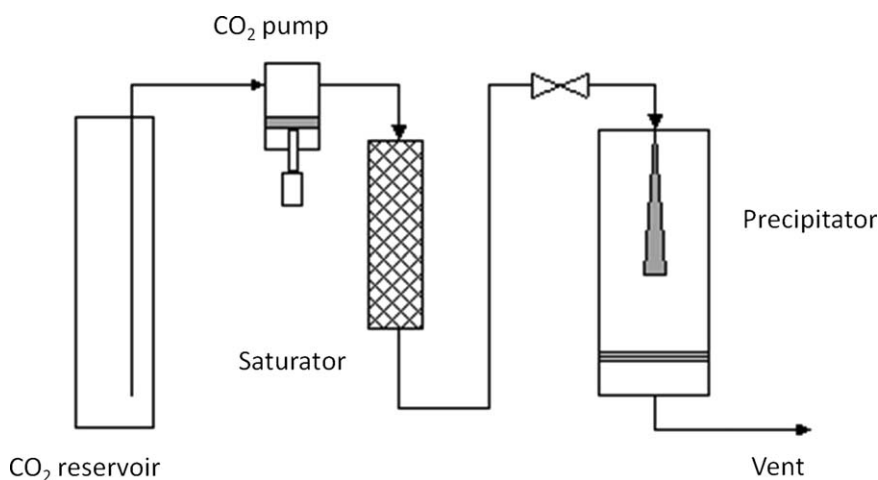


Figure 8.4 Flow diagram of a RESS process.

Different modifications of the RESS process³⁸ have been developed to overcome the main disadvantages, such as RESS-NS (RESS non-solvent), where a co-solvent is used to increase the solubility of the solute in the SC-CO₂, or RESSAS (rapid expansion of supercritical solution into aqueous solution), where the supercritical solution is expanded into an aqueous solution of a surfactant to stabilize the particles and to avoid particle growth and agglomeration. RESSAS has been successfully used to reduce the particle size of naproxen from 0.8 μm by RESS to 0.3 μm .³⁹

Supercritical anti-solvent (SAS) and related techniques (precipitation from a compressed anti-solvent, PCA; aerosol solvent extraction system, ASES; supercritical enhanced dispersion of solutions, SEDS; etc.) exploit the relatively low solubility of organic solid molecules and pharmaceutical compounds in SC-CO₂, compared to the high solubility of volatile organic solvents. The solute of interest is dissolved in a conventional organic solvent to form a solution; afterwards this solution is pressurized and mixed with carbon dioxide. This causes a fast increase in supersaturation, with the subsequent precipitation of particles, due to a simultaneous extraction of the solvent to the supercritical fluid and saturation of the solvent with SC-CO₂ (Figure 8.5). Usually the best results are obtained in conditions in which the organic solvent is completely miscible with carbon dioxide at the precipitation temperature and pressure.⁴⁰ Commonly, a drying cycle is performed at the end of particle precipitation in order to remove traces of un-extracted solvent by passing an amount of SC-CO₂ equivalent to 2–3 times the volume of the precipitation vessel. Typical operating conditions are pressures in the range of 8–15 MPa and temperatures between 35 °C and 50 °C. The particles are collected at the bottom of the precipitation vessel or in on-line filters.

SAS precipitation has been successfully applied to the precipitation of antioxidants from different natural matrices, such as ethanolic extracts of

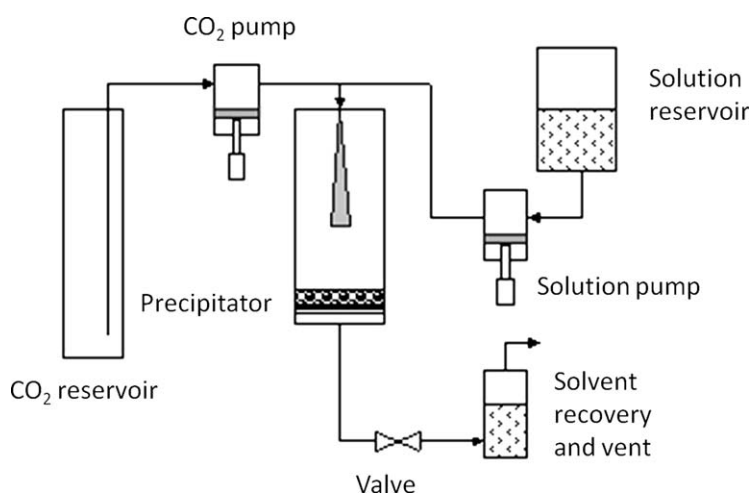


Figure 8.5 Flow diagram of a SAS process.

rosemary leaves.⁴¹ Moreover, the SAS process has shown to be useful to produce pure and stable polymorphs by control of the crystallization kinetics through manipulation of process parameters.^{42,43}

In the particles from gas saturated solutions (PGSS) process, the material to be micronized is saturated with SC-CO₂ in order to decrease the melting temperature, to improve flowing parameters, i.e. lowering the viscosity, and to facilitate the disintegration of this molten solution into tiny droplets after its expansion through a nozzle to atmospheric pressure, as the dissolved CO₂ is released as gas bubbles (Figure 8.6). Therefore, a prerequisite of this technique is to have an active compound (or a carrier material) with a relatively low melting temperature and with a high affinity with CO₂ (or high solubility of CO₂ in the melted material). The droplets produced by the expansion of the gas-saturated solution are rapidly solidified due to the Joule–Thompson effect that accompanies the rapid depressurization of the CO₂, and cooled down to room temperature or below. The product can have different morphologies (sphere, porous spheres or particles and fibres), depending on the nature of the material and the operating parameters, including the pre-expansion (mixing) pressure and temperature and the so called ‘gas to product’ (GTP) ratio (ratio between the flow rate of CO₂ and the flow rate of product). Typical ranges of operating conditions vary from 8 to 15 MPa, 50 to 100 °C and GTP from 1 to 10 kg/kg.

PGSS has been applied to a number of substances,⁴⁴ with solid lipids (e.g. cocoa butter) and semi-crystalline polymers (e.g. polyethylene glycol, PEG) being the more suitable, as they can dissolve big amounts of CO₂ and the molten viscosity is highly reduced. The PGSS process can be operated in discontinuous mode by mixing the two components in a high-pressure stirred vessel, or in continuous mode, mixing a stream of the molten solid and the CO₂ in a static mixer.

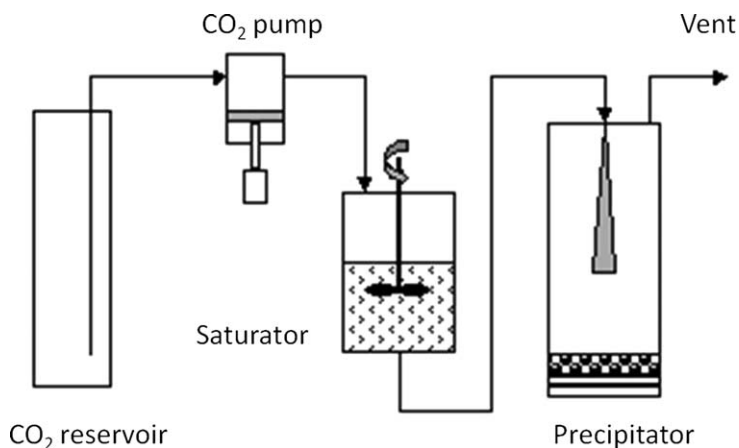


Figure 8.6 Flow diagram of a batch PGSS process.

Finally, some techniques with CO₂ as solute employ the supercritical fluid as a propellant in order to achieve an enhanced atomization similar to the effervescent or flash-boiling mechanisms previously discussed. Several variations of this approach have been described (supercritical assisted atomization SAA, bubble dryer, PGSS-drying, etc.)

The PGSS-drying technique consists of pumping the solution to be dried at ambient temperature into a static mixer, where compressed and preheated carbon dioxide is added ($6\text{ MPa} < P < 10\text{ MPa}$ and $100\text{ °C} < T < 130\text{ °C}$) (Figure 8.7). The residence time in the static mixer is on the order of a few seconds, thus avoiding any degradation by temperature. Afterwards this mixture is rapidly depressurized via a nozzle into a spray tower where fine droplets are formed. This chamber is operated at ambient pressure; the temperature, slightly above room conditions ($40\text{--}60\text{ °C}$), is fixed according to the SC-CO₂-solvent (usually, water) equilibrium and the flow rate of each stream for the final moisture requirements, also avoiding thermal degradation.⁴⁵ The process is very versatile; it allows the processing of organic solutions and emulsions, for both pure component micronization and for product encapsulation, without modifications, and it is carried out in an inert, oxygen-free atmosphere. The technique can be considered as an alternative to spray-drying, with the advantages of lower processing temperatures (enabling to process thermolabile compounds as well as compounds with low melting temperatures) and enhanced atomization and particle size control, and the disadvantage of requiring some high-pressure equipment. An application of this technique is the drying of aqueous green tea extracts.⁴⁶

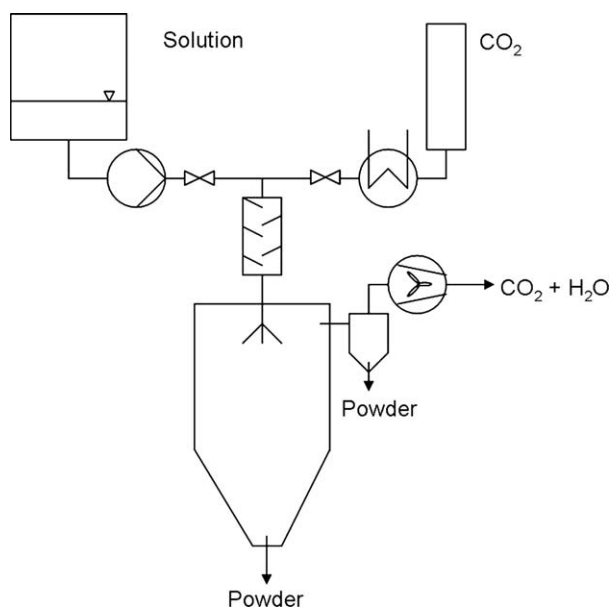


Figure 8.7 Flow diagram of a PGSS-drying process.

8.4 Formulation

The properties of active natural extracts can be enhanced by a formulation with appropriate carrier and co-active materials. There can be many motivations for the formulation of substances, depending on the field of application, although these often come down to three main objectives: protection of the active compound, enhancement of the dosing and the bioavailability, and functionalization of the product.

In recent pharmaceutical applications, the development of micro or nano particulate carriers has been a rich source for innovation and the creation of new products, some of which are already commercialized.⁴⁷ The basic property sought with such formulations is to enhance the bioavailability and the dosing rate of the active compound. If the active compound is administered orally, several carrier materials can be used; they can show different degradation rates or selective sensitivity to the conditions in the gastrointestinal tract, thus allowing tailoring the absorption of the active compound. For intravenous administration of drugs, with a careful design of the nanocarrier it is possible to modify its pharmacokinetic properties, for example making it more transparent to the immune system, thus increasing the *in vivo* longevity of the drug carrier and making it active for a prolonged period. Furthermore, the carrier materials can be used to provide additional functionalities to the product, such as stimuli-sensitive release (pH, temperature, etc.), intracellular delivery, tracer functionality, etc.

Innovative formulations can also open new possibilities in food industries. For example, a formulation can be developed to improve the preservation of flavour and fragrance compounds, which are strongly related to the quality of the product as perceived by consumers.⁴⁸ Formulations can be also used to open new fields of application for natural compounds such as natural colorants or antioxidants, for instance allowing stabilizing liposoluble compounds in water, making it possible to use them in different foods or beverages.⁴⁹ Innovative formulations have also important applications in cosmetics, for example in creams with improved trans-dermal delivery properties.⁵⁰

Different types of formulations have been developed in order to achieve these objectives. As far as dry formulations are concerned, a distinction is usually made between microcapsules, in which a shell of carrier material surrounds a core of active compound, and microcomposites, in which the active material is dispersed within the core material (Figure 8.8). Another important class of formulations is those which maintain a nanostructure in aqueous media. Some examples are micelles, formed by self-assembly of a carrier material with surfactant properties, which forms an inner cavity that can encapsulate active compounds,⁵¹ or liposomes, constituted by carrier materials such as phospholipids that form a bi-layer in water, similar to cell membranes, which can encapsulate materials both in the inner aqueous cavity and inside the bi-layer.⁵²

There is a considerable variety of carrier materials that can be used, depending on the application and the intended functionalities. Table 8.1 presents a few of the most frequently used carrier materials, together with some

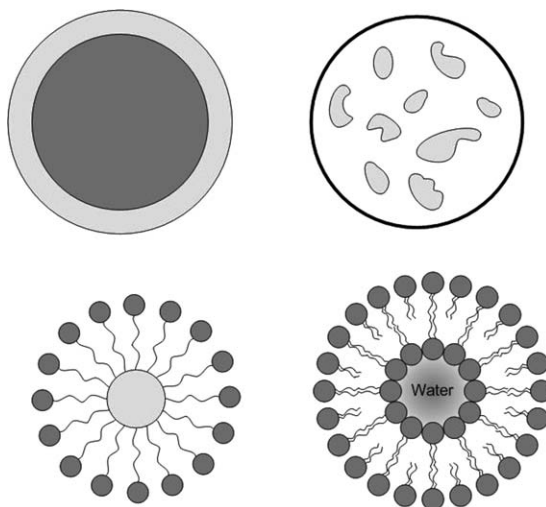


Figure 8.8 Several types of formulations: (a) microcapsules, (b) microcomposites, (c) micelles, (d) liposomes.

Table 8.1 Some frequently used carrier materials.

Carrier material	Remarks
Starch	Widely used to encapsulate flavour and fragrance compounds. Used as fat replacer and emulsion stabilizer. Large variety: modified starches (excellent properties as emulsifiers), maltodextrins (bland in flavour, used to incorporate additives without modifying organoleptic properties), cyclodextrins (emulsifiers and encapsulants), etc.
Lecithin	Emulsifier, forms liposomes in aqueous solutions.
Gums	Emulsifiers, film forming. Gum Arabic widely used due to high solubility in water, low viscosity, emulsification characteristics and good retention of volatile compounds.
Proteins	Good emulsification properties, protection of compounds by steric-stabilizing effects at emulsion interface.
Polyethylene glycol (PEG)	Water soluble and biocompatible (if molecular weight is low). Degradation rates can be adjusted by modification of molecular weight or by blend with other carriers.
Poly(lactic acid) (PLA)	Biodegradable biopolymer derived from lactic acid, can be obtained by fermentation of starch or by chemical synthesis.
Polycaprolactones (PCL)	Low viscosity and melting point (58–60 °C). Glass transition temperature of about –60 °C. Relatively slow degradation rate.
Poly-hyaluronic acid (HYAFF)	Biodegradable and biocompatible. Used in tissue engineering and bone reconstruction. Depending on the levels of esterification, hydrophobicity can be modified.
Poloxamers (Pluronics)	Excellent emulsifying properties, form micelles in aqueous media. Variable properties (e.g. melting point, hydrophilic-lipophilic balance HLB) depending on copolymer ratio. Used with cancer therapy drugs.

of their most relevant properties. In general, the food industry shows a preference for natural carrier compounds that can already be found in food products (starches, gum Arabic, phospholipids, ...), while the pharmaceutical and cosmetics industries use a large variety of both natural materials and synthetic biocompatible polymers.

8.4.1 Solvent Evaporation Method

The solvent evaporation method is one of the most extensively used techniques to produce microcapsules and microcomposites of pharmaceutical compounds, particularly using polylactic acid or polylactic co-glycolic acid as carrier materials.⁵³ The basis of the process is simple: first the polymer is dissolved in a water-immiscible solvent, and the active compound is dissolved or dispersed into this solution. Then, the solution is emulsified forming an oil-in-water (O/W) emulsion, using suitable surfactants and dispersion techniques (stirring, homogenization, ultrasound, etc.). Finally, the organic solvent is evaporated, thus leading to the formation of polymer spheres loaded with the active compound.

Despite the simplicity of the concept, a careful selection of process parameters must be applied in order to control product properties such as particle size, porosity and loading efficiency. One first set of main process parameters are those controlling the droplet size in the emulsion template. Usually, all variations in process parameters leading to a decrease of droplet size in the emulsion produce smaller microspheres, with typical particle sizes in the range 1–100 μm . This includes optimization of surfactant concentration, increase of stirring rate in stirring emulsification, or substitution of stirring by a more efficient emulsification method, decrease of the viscosity of the organic phase by reduction of the polymer concentration, etc. Encapsulation efficiency is controlled to a large extent by the choice of organic solvent, and particularly by the miscibility of the solvent with water. Since in order to remove the solvent, it must first diffuse through the continuous water phase, solvents with high solubility in water can normally be evaporated faster than solvents with low solubility. If the evaporation of the solvent is slower, the precipitation of the polymer and the formation of the particle shell is also slower, and thus the active compound is exposed for a longer period to the water phase and more active compound can be lost by partitioning between the aqueous and organic phases.⁵⁴

A particular challenge is posed by the formulation of water-soluble active compounds such as proteins, that in a single oil-in-water emulsion would be partitioned preferably into the aqueous phase, and therefore outside the microcapsules. Such systems are formulated using double water-in-oil-in-water (W/O/W) emulsions, in which the active compound is dissolved in the aqueous phase and the polymer in the organic phase of the first W/O emulsion.⁵⁵ The first W/O emulsion can also be used to control the porosity of particles, which is higher when the amount of water in the initial emulsion is increased. The modification of the porosity can allow for different drug release methods,

ranging from degradation or erosion of the polymer capsule in low-porosity particles, to diffusion of water through the pores of the particle in systems with higher porosity.

8.4.2 Spray-drying Technique

Spray-drying is one of the most widely used commercial processes for formulation, and particularly for the encapsulation of flavour and fragrance compounds for the food industry.⁴⁸ As in the solvent evaporation method, an emulsion can be fed to the process instead of a homogeneous liquid solution in order to provide an initial template for the final product. Depending on the relative kinetics of precipitation of the active and carrier materials, different morphologies can be obtained, ranging from microcomposites when the kinetics are similar to microcapsules when the solidification of the carrier is slow. It must be noted that during drying, the core of particles usually achieves significantly lower temperatures than the shell,⁵⁶ which is a positive factor for the encapsulation of thermolabile extracts. Some disadvantages are the possible loss of volatiles during the process, as well as the tendency to obtain a significant fraction of active compound in the surface of the particles instead of inside them, which can suffer fast degradation processes and contribute to the loss of properties or off-flavours.

8.4.3 High-pressure Emulsion Techniques

As seen through the two previous techniques, emulsion-template methods are the foundation of many co-precipitation and encapsulation techniques. However, solvent evaporation or spray-drying techniques are limited by the slow kinetics of removal of the organic solvent from the emulsion. Due to the unfavourable kinetics, some undesired results can be obtained, as the already mentioned reduction of the encapsulation efficiency in solvent-evaporation methods if the precipitation of carrier is too slow, or the increase of the amount of superficial, non-encapsulated active compound in spray-drying techniques. Furthermore, as particles are formed slowly over a period comprising considerable changes in the conditions of the medium (remaining organic solvent concentration, temperature, polymer concentration, etc.), some dispersion in the properties of the final product is unavoidable.

The basic idea behind the high-pressure emulsion techniques is to accelerate the kinetics of mixing and heat transfer processes, approaching them to the kinetics of the particle formation processes. With this, a higher homogeneity of the conditions in which particles are formed can be achieved, which results in a better control of the properties of the final product. Moreover, with the intensification of the mixing and heat-transfer processes a reduction of the particle size of the active compound may be achieved, entering into the nanometer range (<100 nm).

Figure 8.9 presents a flow diagram of the high-pressure emulsion process. The process operates with an organic solvent at high temperature (typically,

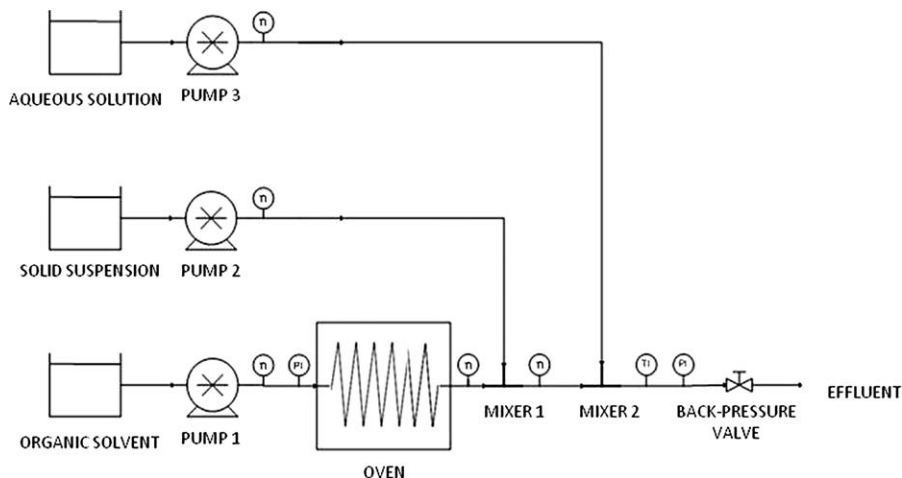


Figure 8.9 Flow diagram of a high-pressure emulsion process.

80–150 °C), in order to increase the solubility of the active compound into the organic solvent and make the process suitable for active compounds with low solubility (or, optionally, to replace the organic solvent by an other that may have more desirable properties, e.g. toxicity, but cannot be normally used due to a low solubility of the active compound). Moderate pressures (in the order of 5 MPa) are applied in order to keep the solvent in the liquid state. Since many active compounds are thermolabile, heating of the organic solution is achieved by direct contact between a suspension of particles of the active compound in cold organic solvent with another stream of preheated organic solvent. Immediately afterwards, the hot organic solution is mixed with a third stream of cold water, which contains the surfactant and carrier materials, as well as other possible additives. By using a suitable mixer, an oil-in-water O/W emulsion is formed in this step, providing the template for the formation of particles. Furthermore, the emulsion is cooled down, reducing the exposure of the product to high temperatures and the possible degradation of the active compound. Finally, particles of active compound are formed in this step by a thermal effect (drastic reduction of solubility by reduction of temperature) rather than by a solvent evaporation method, thus providing the aforementioned intensification of the particle formation process. With this, a suspension of active compound particles in the O/W medium is obtained, which must be further processed in order to remove the organic solvent and, optionally, water, thus precipitating the surfactant and carrier materials and forming dry microcomposites. It must be mentioned that although some active compound particles are formed during these last solvent evaporation processing steps as in a normal solvent evaporation method, the vast majority of the product precipitates before as a consequence of the thermal effect, due to the drastic increase of solubility that can be achieved with the increased temperature (which may be of several orders of magnitude).

This high-pressure emulsion process was originally developed for the formulation of β -carotene and other carotenoids,⁵⁷ and it is already used industrially for this and other applications.

8.4.4 Supercritical Fluid Processes

Several of the supercritical fluid processes previously described can be used to formulate active compounds with a wide variety of natural or bio-compatible carrier materials.⁵⁸ As in the case of the precipitation of pure active compounds, the intense research efforts in this field have not yet been matched by commercial implementation, which still is limited. Among the wide range of supercritical formulation techniques available, some of the most successful are supercritical impregnation (SI), supercritical anti-solvent precipitation (SAS), particles from gas saturated solutions (PGSS) and supercritical extraction of emulsions (SEE).

As shown in Figure 8.10, the supercritical impregnation technique consists of putting into contact supercritical carbon dioxide, the carrier material and the active compound. The process is usually carried out in batch, stirred vessels. Equipment for supercritical extraction can be used for this purpose. This technique takes advantage of the peculiar interactions between supercritical fluids (particularly, supercritical carbon dioxide) and many polymers.⁵⁹ Supercritical CO₂ can reversibly swell and plasticize polymers, thus improving the diffusion of the active compound into the matrix. The impregnation process is controlled by the balance of interactions between polymer, CO₂ and active compound. Depending on this balance, the active compound can just be physically deposited inside the polymer by formation of particles during the depressurization of the supercritical fluid, or it can be either adsorbed or attached to the polymer, if the partition coefficient is favourable enough. The partition mechanism holds a considerable potential, due to the possibility of modifying the partition coefficient by functionalizing the carrier

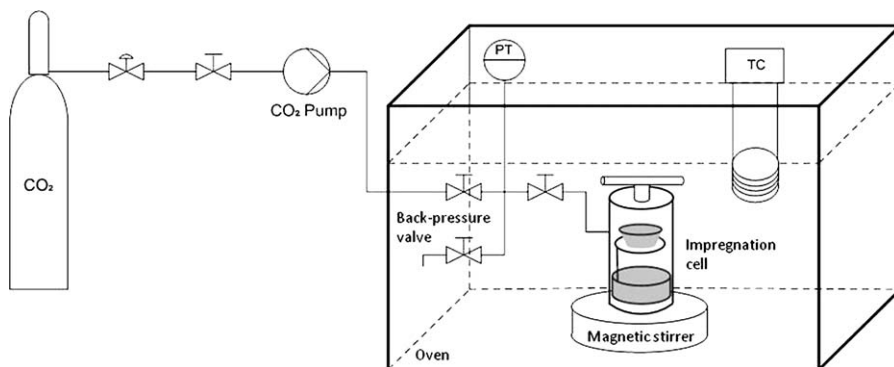


Figure 8.10 Schematic diagram of a supercritical impregnation apparatus.

material or by using suitable active compound forms (e.g. organometallic complexes). Another potential advantage of this technique is that pre-formed carrier material particles can be used. Because the formation of carrier material particles and the impregnation steps are carried out independently, there are more possibilities to control the final morphology than in processes in which both steps are simultaneous.

The supercritical anti-solvent technique maintains the advantages previously discussed of a considerable flexibility and adaptability to a wide range of active and carrier materials, although it still relies on the use of a certain amount of organic solvent. The morphology of the product can be controlled to some extent, but microcomposites are obtained rather than microcapsules in most cases, with a possible influence of the partition processes already described for the supercritical impregnation technique. On the other hand, a considerable range of particle sizes can be covered with this technique, from the nanometer to the micrometer scale (100 nm to 100 μm). Compared to the SAS processing of pure compounds, in the case of co-precipitations the concentration of carrier material as well as the carrier/active compound ratio is a major parameter to be considered. As this ratio is varied, in some cases it is possible to establish whether the carrier solidifies before or after the active compound, and therefore to switch from microcomposite to microcapsule morphologies. It must be mentioned that the interactions of CO_2 with the organic solvent/polymer mixtures can be unfavourable in some cases, as CO_2 can act as a co-solvent which is detrimental for the precipitation of the polymer.⁶⁰

The supercritical extraction of emulsions (SEE) can be considered as an extension of the SAS precipitation in which an emulsion (normally, oil-in-water O/W) is processed rather than a homogeneous solution. As in previous emulsion-based techniques presented, the emulsion provides a template for the formation of particles. Indeed, each droplet of organic solvent of the emulsion behaves as a miniature SAS precipitator. As shown in Figure 8.11, as the process evolves, these droplets are saturated with CO_2 , which leads to the precipitation of the active compound by anti-solvent effect. Afterwards the organic solvent is extracted from the droplets to the supercritical phase.⁶¹ It must be noted that this final extraction step can be rather slow, particularly

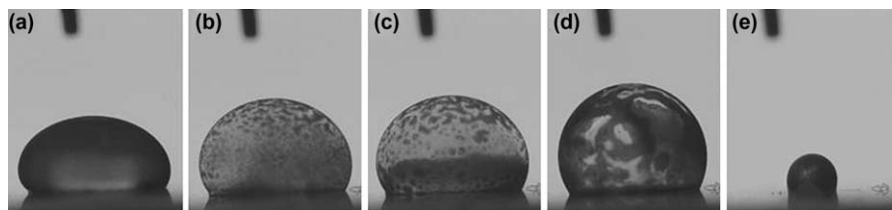


Figure 8.11 β -carotene particle precipitation during an experiment at pressure of 5 MPa and temperature of 35 °C: (a) initial condition, (b) beginning of particle formation, (c, d) particle agglomeration and (e) final condition after the drop detachment from the cell surface.

if the organic solvent is partially miscible with water (e.g. ethyl acetate). A suspension of particles in water is thus obtained, which must be subsequently processed in order to remove the water and to precipitate the carrier material over the pre-formed particles of active compound.

Finally, PGSS techniques are particularly suitable for the encapsulation of natural extracts in low melting point polymers. An example is the encapsulation of essential oils in PEGs.⁶² PGSS-drying can also be used to encapsulate active compounds, which as in previous cases is usually accomplished by the processing of an O/W emulsion.⁶³

8.4.5 Overview

As a summary, Table 8.2 presents some of the main characteristics of the different techniques discussed for particle size reduction and formulation of active materials.

8.5 Case Study: Formulation of β -carotene as a Natural Colorant

The food market demands functional foods and healthy products, using natural additives which provide the final product with a healthy added value.⁶⁴ Functional ingredients, such as carotenoids, fatty acids, natural antioxidants and numerous other compounds, are being extensively used on a great variety of food products.⁶⁵ Carotenoids are some of the most common pigments in nature, the most abundant being β -carotene, lycopene, lutein and zeaxanthin. The main roles of carotenoids in the human diet are as precursors of vitamin A and as antioxidants.⁶⁰ It has been suggested that carotenoids can be beneficial to human health disorders such as cardiovascular diseases, macular degeneration or cataracts.⁶⁶

Since carotenoids are authorized food ingredients, they are widely used in the food, cosmetic and pharmaceutical industries as natural colorants. Industrial carotenoids are usually crystalline powders soluble in oils and organic solvents, but poorly soluble in water. Due to their antioxidant properties, they easily suffer degradation processes in the presence of heat, oxygen or light. In many industrial applications, a mixture of the carotenoid with a biopolymer is used, since covering carotenoids with polymers provides protection against oxidation and degradation processes.⁶⁰ Moreover, the high hydrophobicity of carotenoids makes them insoluble in aqueous systems, and therefore they have a poor intake in the body. To improve their dispersion in water, their coloring strength potential and also to increase their bioavailability during gastrointestinal passage, carotenoid crystals must be formulated.⁶⁷ For applications as natural colorant, it is important to obtain an appropriate color intensity of the formulation, which depends on the properties of the particles (size and crystallinity).⁶⁸

Table 8.2 Overview of particle size reduction and formulation techniques.

<i>Technique</i>	<i>Compatible starting materials</i>	<i>Products</i>	<i>Development</i>
Milling, homogeneization	Wide range of solid food-pharma compounds	Crystalline micro/nano particles, physical mixtures	Commercial
Liquid antisolvent	Mostly pharmaceuticals from organic solutions	Crystalline microparticles	Commercial
Spray-drying	Mostly food products, from aqueous solutions or oil-in-water emulsions	Microparticles, microcapsules filled with solid or liquid active compounds	Commercial
Emulsion evaporation	Active compounds soluble in organic solvents, carrier/surfactant materials soluble in aqueous/organic solvents (other combinations more complex to process)	Micro/nano capsules filled with solid or liquid active compounds	Commercial
RESS	SC-CO ₂ -soluble materials	Nanoparticles, nanocomposites	Laboratory
SAS	Wide range of solid food-pharma products, must be soluble in a volatile organic solvent	Crystalline/amorphous micro/nano particles, microcapsules or microcomposites	Pilot scale/early commercialization
PGSS	Materials or combination of active materials with carrier materials capable of dissolving high concentrations of SC-CO ₂	Microparticles, microcapsules filled with solid or liquid active compounds	Pilot scale/early commercialization
PGSS-drying, SAA and other compressed-gas atomization methods	Solutions (mostly aqueous) capable of dissolving certain amounts of CO ₂	Microparticles, microcapsules filled with solid or liquid active compounds	Laboratory/pilot scale
SEE	Oil-in-water emulsions	Micro/nanocapsules	Laboratory
High-pressure emulsion technique	Organic solutions of food/pharma products with carrier/surfactant materials	Micro/nano composites	Commercial

8.5.1 Formulation of β -carotene by Precipitation from Pressurized Organic Solvent-on-water Emulsions

With the high pressure emulsion technique previously discussed in Section 8.4.3, a study of the formulation of β -carotene using a modified *n*-octenyl succinate (OSA) starch refined from waxy maize as carrier material was carried out.⁶⁸ Formulations were prepared with a process based on the formation of an organic-in-water emulsion with pressurized fluids. As previously discussed, the aim in the conception of this process is to improve the formulation over the conventional emulsion evaporation process, accelerating the mass transfer kinetics to the time scales of the precipitation processes. Ethyl acetate was chosen as organic solvent because it is a generally recognized as safe (GRAS) solvent with low toxicity.

The experimental set-up is shown in Figure 8.12. The experimental apparatus consists of three small storages at ambient pressure, corresponding to the feed of pure organic solvent (ethyl acetate), β -carotene suspension in the same organic solvent, and the aqueous solution of the modified OSA-starch. The stream of the organic solvent is preheated in order to reach the specified operation temperature after mixing with the β -carotene suspension (typically 145 °C). All streams are pressurized with the pumps in order to keep them in the liquid phase at this temperature. The suspension of β -carotene is pumped at ambient temperature. Then, it is mixed with the hot organic solvent stream in a T-mixer; at this point the β -carotene is completely dissolved because the solubility increases with temperature. Shortly afterwards, the β -carotene solution is mixed with the cold aqueous solution of surfactant using another T-mixer, in order to reduce the contact time of β -carotene particles with the hot organic solvent and to avoid the isomerization and degradation of the product. The contact of the hot solution of β -carotene with the cold aqueous solution in

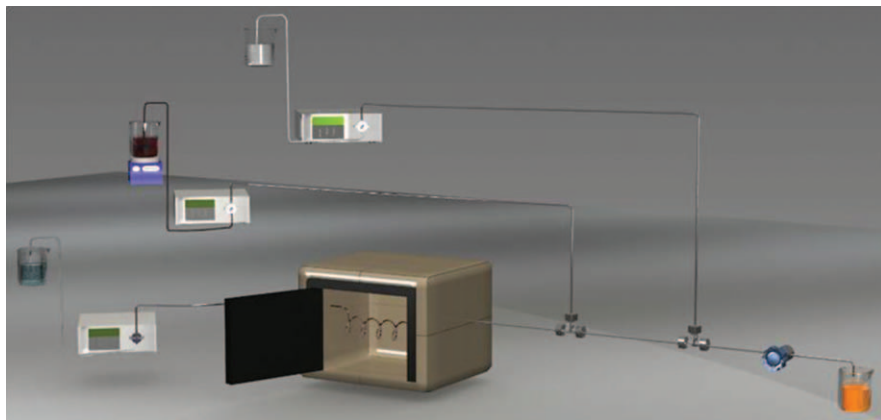


Figure 8.12 Experimental set-up for precipitation of β -carotene from pressurized ethyl acetate-in-water emulsions.

the second T-mixer causes the emulsification of the organic solvent and the precipitation of β -carotene by a combined anti-solvent and cooling effect. Then, the emulsion is collected and the organic solvent is removed by vacuum evaporation in order to produce a suspension of β -carotene nanoparticles in water stabilized with the surfactant.

In this research, the influence of the main process parameters was studied: the concentration of modified OSA-starch and the organic/water ratio. The effect of the concentration of modified OSA-starch dissolution was carried out by varying this concentration from 37 g/L to 367 g/L. In Figure 8.13a and b, the influence of the concentration of surfactant on the percentage of encapsulated β -carotene and micellar particle size is presented.

The results show that the percentage of encapsulated β -carotene is higher when the concentration of modified starch is increased. With regard to the micellar particle size, the main sizes obtained ranged from 200 nm to 600 nm; it is higher when the concentration of surfactant is increased. Although an increase in the micellar particle size is in general disadvantageous for the stability of the suspension, it must be taken into account that the use of high concentrations of starch allows to encapsulate a higher percentage of β -carotene and to obtain a better emulsion stability. As for the effect of the organic/water ratio, it was carried out varying this ratio from 0.6 to 1.3. It is necessary to emphasize that this organic/water ratio has a strong influence on the micellar particle size. When this ratio is increased, the micellar particle size increases as well. On the other hand, the encapsulation efficiency does not show a clear variation, achieving percentages of encapsulated β -carotene of 70–80%. The best results were obtained with low ratios, in the range of 0.65 and 0.73.

The obtained suspension can be further processed by spray-drying or similar techniques in order to obtain a dry product. As shown in Figure 8.14, a homogeneous suspension of β -carotene in water can be easily obtained by rehydration of this formulation.

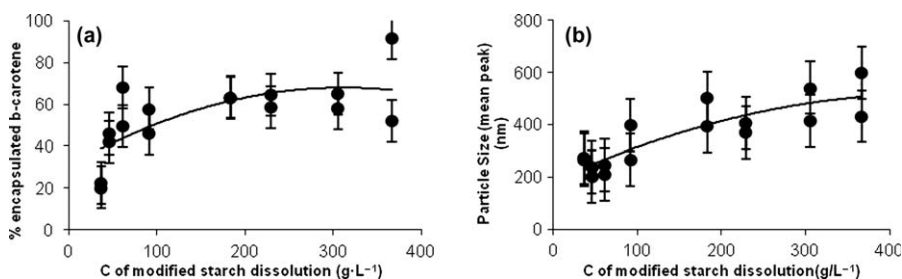


Figure 8.13 Effect of the concentration of modified starch dissolution on (a) the percentage of encapsulated β -carotene and on (b) the micellar particle size.

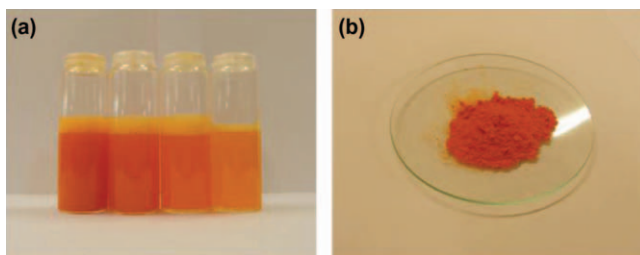


Figure 8.14 Formulations of β -carotene as natural colorant: (a) homogeneous suspensions in water, (b) dry product.

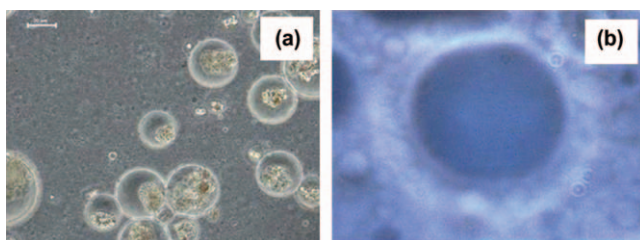


Figure 8.15 Microscopic images of structures formed by hydration of formulations of β -carotene with lecithin: (a) micellar aggregates, (b) multilamellar liposomes.

8.5.2 Formulation of β -carotene with Soybean Lecithin by PGSS-drying

Lecithin can be used as carrier of liposoluble materials due to its capacity to form liposomes in aqueous media. In this research, β -carotene was encapsulated in lecithin by PGSS-drying technique. For this, a dichloromethane-in-water emulsion, containing β -carotene in the organic phase and lecithin in the aqueous phase, was processed.

The influence of the main process parameters was studied: pre-expansion temperature (100–132 °C), pre-expansion pressure (8–10 MPa), and concentration of soybean lecithin (55–72 g/L). Results showed that dry particles of 10–500 μm , constituted by fused spherical particles of less than 10 μm , were obtained, with β -carotene encapsulation efficiencies up to 60%. By hydration of these particles, β -carotene-loaded multilamellar liposomes of 1–5 μm , together with larger micellar aggregates, were obtained. Figure 8.15 shows microscopic images of some of these structures.

8.5.3 Co-precipitation of β -carotene with Polyethylene Glycol by Supercritical Anti-solvent Process (SAS)

In this research, the effect of the main process parameters (temperature, pressure and initial concentrations of PEG and β -carotene), on the

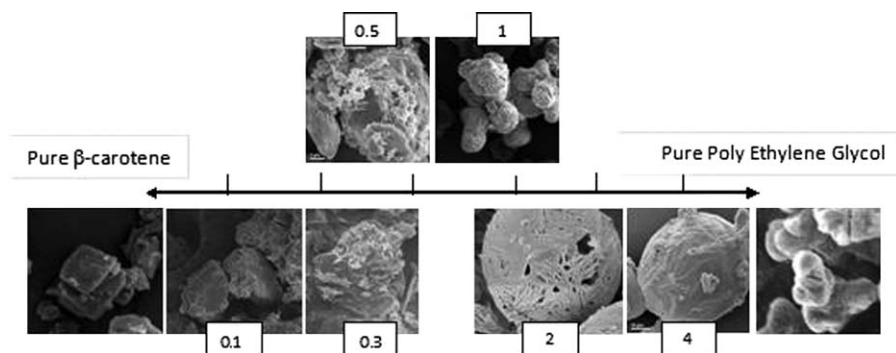


Figure 8.16 Morphology of β -carotene/polyethylene glycol co-precipitates as a function of the ratio between the materials.

co-precipitation of β -carotene and polyethylene glycol (PEG) by SAS process was studied.⁶⁹ Regarding the effect of pressure, the particle size decreases when pressure is increased. With respect to the temperature, no particles were obtained at temperatures higher than 25 °C, and instead, a polymer film was obtained. This could be due to the co-solvent effect of CO₂ on the polymer. Due to this, the temperature was varied from 0 °C to 25 °C. The particle size was in the range of 50 μ m to 200 μ m. However, when a higher PEG/ β -carotene ratio was used, particles with a higher diameter were obtained. As for the concentration of the substances, it has an important effect on the morphology of the co-precipitates. When the concentration of PEG is increased, the coating of β -carotene is better, and after the complete encapsulation of β -carotene particles, a change in the polymer concentration could lead to different morphologies. As shown in Figure 8.16, different morphologies of co-precipitates can be obtained, as hollow spheres, carotenoids particles that are partially covered with relatively small PEG spheres, or smooth surface spherical particles, only by changes in the concentration ratio between the polymer and the carotenoid.

8.5.4 Formulation of β -carotene by Supercritical Extraction from an Emulsion (SEE)

With this technique, the formulation of β -carotene, using modified starch and a blend of Tween 20 and Span 20 as surfactants, was carried out by Mattea *et al.*⁷⁰ This work was later extended to the formulation of lycopene by Santos *et al.*⁷¹

A suspension of organic submicron and nanoparticles with a final concentration of organic solvent as low as 1 ppm was obtained. Particle size distributions obtained in the suspension after supercritical precipitation are directly related to the droplet size distribution of the emulsions, and particle sizes in the range 50–400 nm were obtained. The similarity between particle sizes of the initial emulsion and the final suspension suggests that β -carotene

particles may be encapsulated in surfactant micelles. Process variables like pressure and temperature are related to the capacity to eliminate the remaining organic solvent from the products, but they have a minor effect on the final particle size.

8.6 Conclusions

Several post-extraction and formulation techniques have been discussed in this chapter, which can be used to confer additional properties to a product based on a natural extract. These properties can range from a simple protection of the active compound by encapsulation on a carrier material, to an increase of the bioavailability of poorly soluble compounds by an appropriate design of particle size, morphology and crystallinity, or other highly specific functionalities such as a controlled release, targeted delivery or tracer capabilities. The post-processing techniques can sometimes be advantageously combined to different extraction methods, and they should be considered from the beginning of an integrated product design, together with other factors such as the origin of the extract and its biological activity, or the required extraction and purification processes.

References

1. C. Reichardt and T. Welton, *Solvents and Solvent Effects in Organic Chemistry*, Wiley-VCH, 2011.
2. *FDA Guidance for Industry. Q3C Impurities: Residual Solvents*, U.S. Department of Health and Human Services, Food and Drug Administration, 1997.
3. *FDA Guidance for Industry. Q3C - Tables and list*, U.S. Department of Health and Human Services, Food and Drug Administration, 2012.
4. European Commission, *DIRECTIVE 2009/32/EC on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients (Recast)*, 2009.
5. C. Jiménez-González, A. D. Curzons, D. J. C. Constable and V. L. Cunningham, *Clean Technol. Envir.*, 2004, **7**, 42.
6. G. Towler and R. K. Sinnott. *Chemical Engineering Design: Principles, Practice and Economics of Plant and Process Design*, Elsevier, 2012.
7. P. Watts, M. Davies and C. Melia, *Crit. Rev. Ther. Drug*, 1990, **7**, 235.
8. L. Mu and S. S. Feng, *J. Control. Release*, 2002, **80**, 129.
9. C. Ratti, *J. Food Eng.*, 2001, **49**, 311.
10. C. M. McLoughlin, W. A. M. McMinn and T. R. A. Magee, *Drying Technol.*, 2003, **21**, 1719.
11. A. Mersmann, M. Kind and J. Stichlmair, *Thermal Separation Technology*, Springer Verlag, 201.
12. C. Geankoplis, *Transport Processes and Separation Process Principles (includes unit operations)*, Prentice Hall Press, 2003.

13. K. Ridgway, S. P. D. Lalljie and R. M. Smith, *J. Chromatogr. A*, 2007, **1153**, 36.
14. F. Du, G. Ruan and H. Liu, *Anal. Bioanal. Chem.*, 2012, **403**, 55.
15. M. Díaz-Maroto, M. Pérez-Coello and M. Cabezudo, *Eur. Food Res. Technol.*, 2002, **215**, 227.
16. C. de Torres, M. C. Díaz-Maroto, I. Hermosín-Gutiérrez and M. S. Pérez-Coello, *Anal. Chim. Acta*, 2010, **660**, 177.
17. R. Smith, *Chemical Process Design and Integration*, Wiley, West Sussex, 2005.
18. S. Keith and R. Hughes, *Industrial Membrane Separation Technology*, Springer, 1996.
19. R. Rodrigues, H. Menezes, L. Cabral, M. Dornier, G. Rios and M. Reynes, *J. Food Eng.*, 2004, **63**, 97.
20. K. Kimura, S. Toshima, G. Amy and Y. Watanabe, *J. Membrane Sci.*, 2004, **245**, 71.
21. M. Dudziak and M. Bodzek, *Chem. Pap.*, 2010, **64**, 139.
22. J. H. Al-Rifai, H. Khabbaz and A. I. Schäfer, *Sep. Purif. Technol.*, 2011, **77**, 60.
23. A. A. Thorat and S. V. Dalvi, *Chem. Eng. J.*, 2012, **181–182**, 1.
24. S. Mirza, I. Miroshnyk, J. Heinämäki and J. Yliruusi, *Dosis*, 2008, **24**, 90.
25. J.-U. A. H. Junghanns and R. H. Müller, *Int. J. Nanomed.*, 2008, **3**, 295.
26. C. M. Keck and R. H. Müller, *Eur. J. Pharm. Biopharm.*, 2006, **62**, 3.
27. J. Kluge, G. Muhrer and M. Mazzotti, *J. Supercrit. Fluids*, 2012, **66**, 380.
28. A. A. Thorat and S. V. Dalvi, *Chem. Eng. J.*, 2012, **181–182**, 1.
29. R. Vehring, *Pharm. Res.*, 2008, **25**, 999.
30. Y. Kitamura, H. Itoh, H. Echizen and T. Satake, *J. Food Process. Pres.*, 2009, **3**, 714.
31. X. Li, N. Anton, C. Arpagaus and F. Belleteix, *J. Control. Release*, 2010, **147**, 304.
32. E. Sher, T. Bar-Kohany and A. Rashkovan., *Prog. Energ. Combust.*, 2008, **34**, 417.
33. S. D. Sovani, P. E. Soika and A. H. Lefebvre, *Prog. Energ. Combust.*, 2001, **27**, 483.
34. H. Ksibi, P. Subra and Y. Garrabos, *Adv. Powder Technol.*, 1995, **6**, 25.
35. M. B. King and T. R. Both, *Extraction of Natural Products Using Near-Critical Solvents*, Blackie Academic & Professional, 1993.
36. H. Krober, U. Teipel and H. Krause, *Chem. Eng. Technol.*, 2000, **23**, 763.
37. V. Krukoniš, *Supercritical Fluid Nucleation of Difficult-to-communite Solids*, in AIChE Annual Meeting, San Francisco, 1984.
38. M. Türk and R. Lietzow, *J. Supercrit. Fluids*, 2008, **45**, 346.
39. M. Türk and D. Bolten, *J. Supercrit. Fluids*, 2010, **55**, 778.
40. A. Martín and M. J. Cocero, *Adv. Drug Delivery Rev.*, 2008, **60**, 339.
41. A. Visentin, S. Rodríguez-Rojo, A. Navarrete, D. Maestri and M. J. Cocero, *J. Food Eng.*, 2012, **109**, 9.
42. Á. Martín, K. Scholle, F. Mattea, D. Meterc and M. J. Cocero, *Crystal Growth & Des.*, 2009, **9**, 2504.

43. G. Weber-Brun, Á. Martín, E. Cassel, R. M. Figueiró Vargas and M. J. Cocero, *Crystal Growth & Des.*, 2012, **12**, 1943.
44. A. V. M Nunes and C. M. M. Duarte, *Materials*, 2011, **4**, 2017.
45. A. Martín and E. Weidner, *J. Supercrit. Fluids*, 2010, **55**, 271.
46. D. Meterc, M. Petermann and E. Weidner, *J. Supercrit. Fluids*, 2008, **45**, 253.
47. V. P. Torchilin, *Adv. Drug Delivery Rev.*, 2006, **58**, 1532.
48. A. Madene, M. Jacquot, J. Scher and S. Desobry, *Int. J. Food Sci. Tech.*, 2006, **41**, 1.
49. M. Gonnet, L. Lethuaut and F. Boury, *J. Control. Release*, 2010, **146**, 276.
50. C. C. Müller-Gowmann, *Eur. J. Pharm. Biopharm.*, 2010, **58**, 343.
51. M. C. Jones and J. C. Leroux, *Eur. J. Pharm. Biopharm.*, 1999, **48**, 101.
52. V. P. Torchilin, *Nature Rev. Drug Discovery*, 2005, **4**, 145.
53. P. B. O'Donnell and J. W. McGinity, *Adv. Drug Delivery Rev.*, 1997, **28**, 25.
54. R. Bodmeies and J. W. McGinity, *Int. J. Pharm.*, 1988, **43**, 179.
55. S. Freiberg and X. X. Zhu, *Int. J. Pharm.*, 2004, **282**, 1.
56. J. D. Dziezak, *Food Tech.*, 1988, **42**, 136.
57. D. Horn and J. Rieger, *Angew. Chem. Int. Ed.*, 2001, **40**, 4330.
58. M. J. Cocero, A. Martín, F. Mattea and S. Varona, *J. Supercrit. Fluids*, 2009, **47**, 546.
59. I. Kikic and F. Vecchione, *Curr. Op. Solid State Mat. Sci.*, 2003, **7**, 399.
60. A. Martín, F. Mattea, L. Gutiérrez, F. Miguel and M. J. Cocero, *J. Supercrit. Fluids*, 2007, **41**, 138.
61. F. Mattea, A. Martín, C. Schulz, P. Jaeger, R. Eggers and M. J. Cocero, *AIChE J.*, 2010, **56**, 1184.
62. S. Varona, S. Kareth, A. Martín and M. J. Cocero, *J. Supercrit. Fluids*, 2010, **54**, 369.
63. S. Varona, A. Martín and M. J. Cocero, *Ind. Eng. Chem. Res.*, 2011, **50**, 2088.
64. F. Mattea, A. Martín and M. J. Cocero, *J. Food Eng.*, 2009, **93**, 255.
65. C. I. Moraru, C. P. Panchapakesan, Q. Huang, P. Takjistov, S. Liu and J. I. Kokini, *Food Tech.*, 2003, **57**, 24.
66. H. D. Silva, M. A. Cerqueira, B. W. S. Souza, C. Ribeiro, M. C. Avides, M. A. C. Quintas, J. R. S. Coimbra, M. G. Carneiro-da-Cunha and A. A. Vicente, *J. Food Eng.*, 2011, **102**, 130.
67. H. S. Ribeiro, B. S. Chu, S. Ichikawa and M. Nakajima, *Food Hydrocolloids*, 2008, **22**, 12.
68. E. De Paz, A. Martín, A. Estrella, S. Rodríguez-Rojo, A. A. Matías, C. M. M. Duarte and M. J. Cocero, *Food Hydrocolloids*, 2012, **26**, 17.
69. F. Mattea, A. Martín and M. J. Cocero, *Ind. Eng. Chem. Res.*, 2008, **47**, 3900.
70. F. Mattea, A. Martín, A. M. Gago and M. J. Cocero, *J. Supercrit. Fluids*, 2009, **51**, 238.
71. D. T. Santos, A. Martín, M. A. Meireles and M. J. Cocero, *J. Supercrit. Fluids*, 2012, **61**, 167.

CHAPTER 9

Isolation and Purification of Natural Products

WANG XIAO,* FANG LEI, ZHAO HENGQIANG
AND LIN XIAOJING

Shandong Analysis and Test Center, Shandong Academy of Sciences,
19 Keyuan Road, Jinan, China

*Email: wangxiao2008@yahoo.cn

9.1 Introduction

The term ‘natural products’ usually refers to chemical substances found in nature that possess distinctive pharmacological or biological activities.¹ Natural products cover a wide and diverse range of products, including alkaloids, terpenoids, flavones, lignans and coumarins, among others. Today, with the catalogued biodiversity in the world, natural products are serving as a rich source of chemical diversity, structural diversity and bioactive diversity. They have been the major starting materials for pharmaceutical, cosmetic, flavour and dietary supplement industries.²

Isolation of compounds from natural sources is the most important, difficult and time-consuming step in natural product research and production. It begins with the extraction process and it is followed by various separation processes/techniques that aim to achieve high purity of a single compound or group of compounds for detailed studies, e.g. molecule structure identification, bioactivity test, quality control of natural sources or further industrial production.

Extraction represents the primary step in getting crude extract from natural sources. However, the products obtained by the extraction methods described

RSC Green Chemistry No. 21

Natural Product Extraction: Principles and Applications

Edited by Mauricio A. Rostagno and Juliana M. Prado

© The Royal Society of Chemistry 2013

Published by the Royal Society of Chemistry, www.rsc.org

in the previous chapters are usually mixtures of several components, and often only some of them show bioactivity. The overall objective of purification and isolation is to separate a single compound or group of compounds from other inert constituents and undesired compounds.

In addition, most active components present the following characteristics: (1) low content; (2) coexisting with homologues and structural analogues; (3) thermolabile. Besides, the natural products are usually complex matrices, which implies that isolation and purification of specific components is laborious and difficult.³ For these reasons, the selection of an appropriate strategy using adequate techniques and operational conditions is essential to achieve high yields of target compound(s).⁴

Conventional isolation and purification techniques include solvent partitioning, adsorption, low-pressure chromatography, crystallization, etc. These techniques are often inefficient, so that it is difficult to obtain fractions enriched in the active compounds. Over the past decade, several novel isolation and purification techniques have been introduced and investigated.⁴⁻⁶ These include membrane separation, preparative high-performance liquid chromatography (prep-HPLC), counter-current chromatography (CCC), supercritical fluid chromatography (SFC), etc. These newly introduced techniques have the advantages of being fast, often allowing the isolation of previously inaccessible products and being possible to be scaled up to industrial level. However, it must be emphasized that none of these techniques provides by itself a comprehensive solution to all separation problems, and that the best approach is usually to employ a combination of different techniques. In most cases, it begins with the process of pre-isolation or enrichment followed by various isolation and purification steps. In each step a different technique may be applied.

This chapter focuses on the techniques used for isolation and purification of natural products, with special attention devoted to low molecular weight compounds, and provides the most common strategies used in each step of the process of obtaining target compounds from natural raw materials. After establishing the target compound or group of compounds, the choice of isolation strategy should be seriously considered according to its physico-chemical characteristics, including solubility aspects (i.e. hydrophobicity or hydrophilicity), acid–base properties, molecular charge, stability and size. Designing the most appropriate isolation protocol is necessary to obtain the target compounds smoothly. Figure 9.1 shows a general process of extraction and purification of natural products.

9.2 Pre-isolation or Enrichment

The crude extracts, directly extracted from raw materials via any extraction technique, are very complex and the content of active ingredients is usually low. Generally the crude extract cannot be separated directly by chromatographic techniques, which implies the necessity of an appropriate pre-isolation method or enrichment method according to the characteristics of the target compounds. For example, if the target compounds are macromolecular,

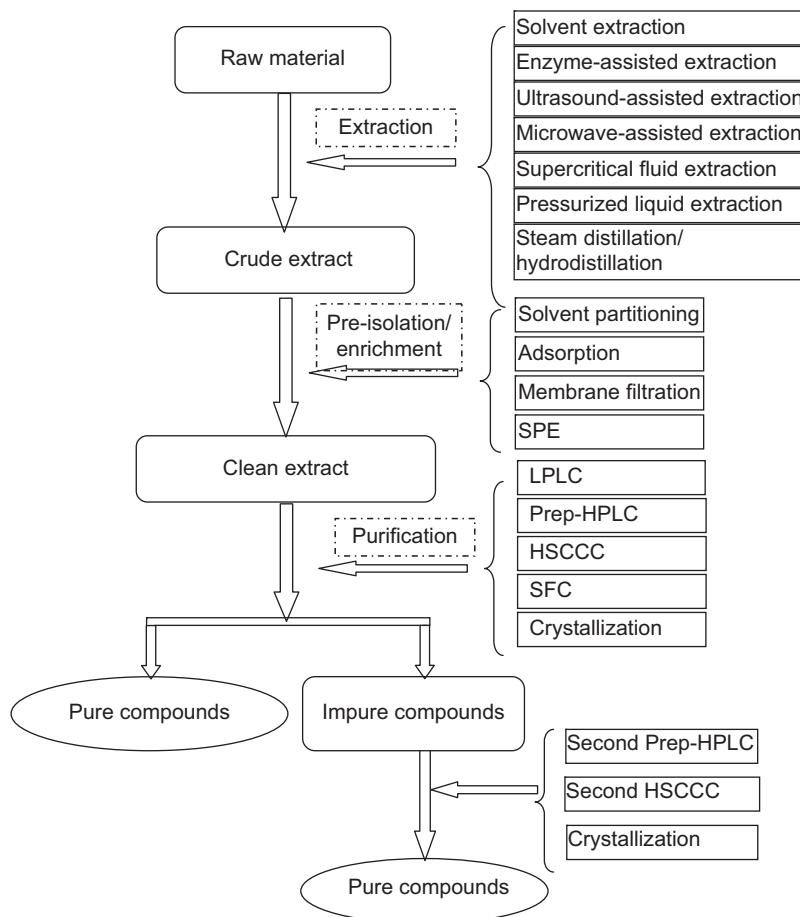


Figure 9.1 The strategy of extraction, isolation and purification of natural products. Reprinted by permission of the publisher from *Separation and Purification Reviews*, **39**(1–2), L. Yin, Y. Li, B. Lu, Y. Jia and J. Peng, *Trends in Counter-Current Chromatography: Applications to Natural Products Purification*, pp. 33–62, 2010, Taylor & Francis Ltd, <http://www.tandf.co.uk/journals>.⁷

membrane filtration may be used to remove small molecules, considered in this case impurities. But when the target compounds are small molecules with different polarities, solvent partitioning or adsorption may be highly effective as an enrichment technique. At present, solvent partitioning, adsorption enrichment and membrane separation are the three treatment methods most commonly used at laboratory and production scales.

9.2.1 Solvent Partitioning

Solvent partitioning is extensively used as the first step for the separation of different groups of compounds from crude extracts. The technique involves the

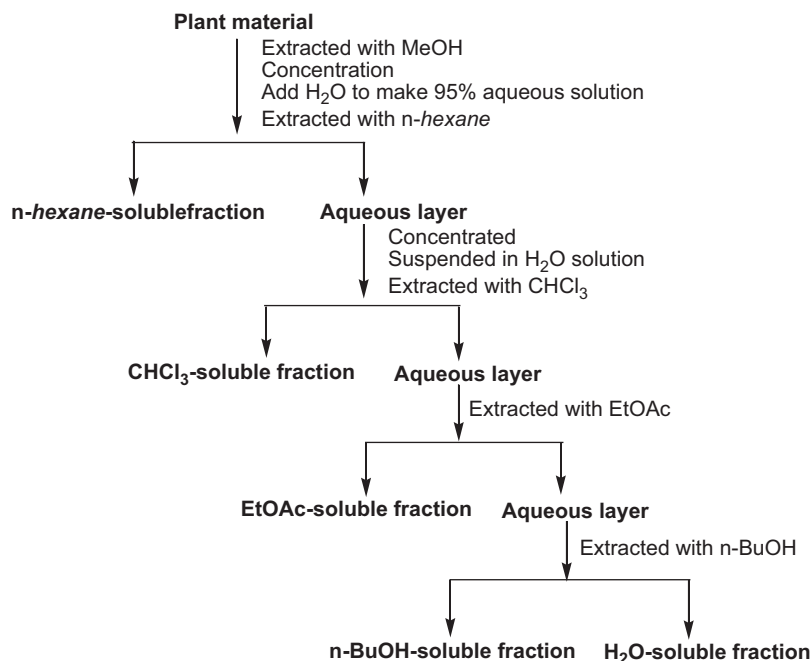


Figure 9.2 The typical partitioning scheme (adapted from Sarket *et al.*).⁸

use of two immiscible solvents in a separating funnel, in which compounds are distributed in the solvents according to their different partition coefficients.

A crude extract is usually an extremely complex mixture containing a wide variety of polar, moderately polar, and non-polar components. According to the varying polarity, compounds present are usually pre-purified by solvent partitioning with solvents of increasing polarity. A typical partitioning process of a natural product extract is the following: the crude extract is first extracted with *n*-hexane/petroleum ether to produce a fraction of non-polar components, such as lipids, terpenoids, and so on. Then the solution is extracted with chloroform (CHCl₃), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH), successfully giving three corresponding fractions. Less polar components are enriched in the CHCl₃ fraction, while moderately polar ones, as monoglycosides, are present in the EtOAc fraction. The polar components, especially the glycosides, are concentrated in the *n*-BuOH fraction. Figure 9.2 shows a typical partitioning scheme. During the solvent partitioning process of a natural product extract, the following should be considered:⁸

1. solvent partitioning with EtOAc may produce acetates from the original natural product, since EtOAc contains a trace amount of acetic acid;
2. the alkaloids tend to be extracted with CHCl₃, because CHCl₃ is a light acidic solvent;
3. *n*-BuOH should be saturated with water before it is used to extract the water layer.

Although the solvent partitioning method has been successfully applied in the separation of different classes of compounds from natural products, it still has disadvantages. For example, separation by solvent partitioning cannot be always performed in a clear cut manner and overlapping of compounds in successive fractions can be found. Moreover, a large amount of toxic solvent is used.

9.2.2 Adsorption Enrichment

Adsorption, in general, is one of the most efficient enrichment methods, and it has a moderate purification effect. So far, adsorption is gaining popularity in pharmaceutical applications and has been successfully applied to industrial refining and purification of bioactive substances such as phenolics,⁹ saponins,¹⁰ flavonoids,¹¹ alkaloids¹² and coumarins,¹³ among other several examples.

In all kinds of adsorption processes resins are widely used to enrich plant secondary metabolites. Macroporous resins can be used to selectively adsorb constituents from aqueous solutions as well as non-aqueous systems through electrostatic force, hydrogen bonding interaction, complexation, size sieving action, etc. They are durable non-polar (polystyrene), moderately polar (ester group) or polar (amide, amidocyanogen, acylamino polystyrene) macroporous polymers with high adsorption capacity. Macroporous resins have many advantages over conventional matrices, including an over 10-fold increase in sample loading capacity, concentration of target components, higher adsorption specificities, easier adsorption, better mechanical strength and re-uses, and lower fluid resistance. It is also important to notice that aqueous ethanol is used to desorb target compounds, and therefore the use of macroporous resins is considered a 'green' technique.

In principle, substances are purified according to their molecular weight, polarity and shape. The effective adsorption of macroporous resins is related to their surface adsorption, electrical properties, sieve classification and hydrogen bond interactions. Therefore, different resin adsorbents can be selected for the preparation of different compounds with special characteristics. The selection of suitable resins is mainly based on the polarities of the chemicals and of the resin, as well as the average pore diameters and surface areas. Table 9.1 contains a list.

Adsorptive macroporous resins have been used for enrichment or separation of numerous constituents from different classes of pharmacologically active natural plants.^{14–18} Concentration and purification of phenolic compounds by adsorption has been reviewed by Soto *et al.*,¹⁸ and several applications are discussed in detail in this work. Also, in this chapter case studies will be used to illustrate the purification process for different compounds classes.

9.2.3 Membrane Separation

Membrane technology is playing an increasingly important role in our society.¹⁹ The general principle of different membrane technologies is based on the selective permeability of the membrane to allow target substances to pass

Table 9.1 Physical properties of macroporous resins.

<i>Resin</i>	<i>Polarity</i>	<i>Structure</i>	<i>Particle size (mm)</i>	<i>Surface area (m²/g)</i>	<i>Moisture content (%)</i>
D101	non-polar	styrene	0.3–1.2	≥ 400	50–55
HPD-100	non-polar	styrene	0.3–1.2	650–700	60–70
D1400	non-polar	styrene	0.3–1.2	≥ 550	55–60
X-5	non-polar	styrene	0.3–1.2	500–600	50–55
FL-3	non-polar	polystyrene	0.3–1.2	80–120	60–70
D4020	non-polar	styrene	0.3–1.2	540–580	60–65
AB-8	weakly polar	styrene	0.3–1.2	480–520	55–60
HP-20	weakly polar	polystyrene	> 0.25	600	65–70
AL-1	weakly polar	polystyrene	0.3–1.0	500–650	55–75
DM130	weakly polar	polystyrene	0.3–1.2	500–550	65–75
FL-2	mid polar	polystyrene	0.3–1.0	120–200	55–65
HPD-600	mid polar	polystyrene	0.3–1.2	550–600	55–65
DM-301	mid polar	styrene	0.3–1.2	≥ 480	60–70
D302	mid polar	polystyrene	0.3–1.2	300	50–55
XDA-8	mid polar	styrene divinyl- benzene	0.3–1.2	≥ 1050	65–70
FL-1	polar	polystyrene	0.3–1.0	100–200	55–70
HPD-500	polar	polystyrene	0.3–1.0	500–550	55–75
HPD-826	polar	hydrogen bond	0.3–1.2	500–600	60–70
AL-2	polar	polystyrene	0.3–1.0	100–150	55–65
ADS-11	polar	sulfonic group	0.3–1.2	190–220	60–70
ADS-31	polar	phenolic hydroxyl	0.3–1.2	≥ 60.9	40–50
XAD-7HP	polar	acrylate	0.56	500–600	45–55
DA201	polar	styrene	0.3–1.2	≥ 150	60–65
NKA-II	polar	styrene	0.3–1.2	160–200	55–65

through the membrane, whilst usually keeping away unwanted substances.¹ Membrane separation processes are generally classified as microfiltration, ultrafiltration, nanofiltration and reverse osmosis, according to the size of particles retained by the membrane (Figure 9.3).^{20,21} Membrane separation processes are pressure-driven separation techniques and various methods, e.g. high pressure, concentration gradient and chemical potential difference, may be adopted as the driving force for this process. Another key feature of the membrane separation process is the selection of a membrane with appropriate pore characteristics.

Compared to traditional separation methods, such as evaporation, extraction and ion exchange, membrane separation presents the advantage of simple equipment, room temperature operation, no phase transition, high selectivity and low energy cost, among others. Membrane separation techniques find a wide range of applications in the purification of natural products.¹ The concentration, separation and purification of target compounds can be

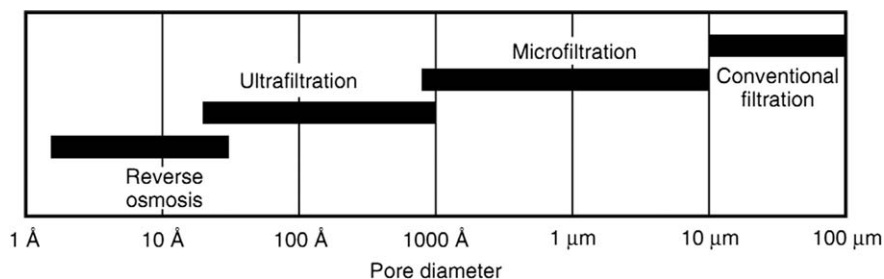


Figure 9.3 Pore size of reverse osmosis, ultrafiltration, microfiltration, and conventional filtration membrane.

Reprinted from *Membrane Technology and Applications*, 3rd edition, R. W. Baker, 2012, with permission from John Wiley & Sons.²¹

achieved by using this technology through choosing the proper membrane, process parameters and mode. Membrane separation technology separates the target compounds in solution by molecular size. The molecular weight of effective components in natural products is often no more than 1,000 g/mol and that of inactive ingredients varies from 1,000 to 1,000,000 g/mol. Selecting suitable membrane filtration allows achieving the selective removal of inactive ingredients and the enrichment of target components. The membrane separation process is carried out at room temperature, especially suitable for thermosensitive material, without the need for adding chemical reagents and conserving energy. At the same time, the separation device has the advantages of simple structure, short process flow, simple operation, easy control and maintenance, and it can effectively reduce the production cost and improve product quality.

One example is the separation of flavonoids from *Ginkgo biloba* by membrane filtration. *Ginkgo biloba* has attracted increasing interest into its potential application in food and dietary supplements due to containing a large number of potentially active components, such as flavonol glycosides (flavonoids) and terpenoids. *Ginkgo biloba* extracts (GBE) have many potential pharmacological and clinical effects. In order to enhance the flavonoid content in GBE crude products, Xu *et al.*²² modified polyvinylidene fluoride (PVDF) ultrafiltration membranes by using KMnO_4 and KOH as oxidant and strong base, respectively, to facilitate the hydrogen fluoride (HF) elimination from PVDF chain, and using polyvinyl pyrrolidone (PVP) aqueous solution as a coating medium. The modified membrane was used to purify the flavonoids from the crude product taking advantage of the hydrogen-bonding effects between flavonoids and the PVP function. Experiments showed that the flux of GBE solution was greatly improved and the flavonoid content in the final product increased from 21.3 wt.% to 34.8 wt.%. It was also observed that the mass transfer of flavonoids decreased with increasing pH value of GBE solution.

Some other applications of different membrane techniques in the primary purification of natural products are listed in Table 9.2.

Table 9.2 Applications of membrane techniques for primary purification of natural products.

<i>Solute</i>	<i>Raw material</i>	<i>Membrane technology</i>	<i>Ref.</i>
oligosaccharides	carbohydrates	ultrafiltration	23
flavonoids	<i>Ginkgo folium</i>	ultrafiltration	24
fructo-oligosaccharides		nanofiltration	25
xylose	hemicellulose hydrolysates	nanofiltration	26
protein	hemoglobin and bovine serum albumin	ultrafiltration	27
<i>R</i> -phycoerythrin	<i>Grateloupia turuturu</i>	ultrafiltration	28
oligosaccharides	chicory rootstock	ultrafiltration and nanofiltration	29
α -lactalbumin	acid casein whey	ultrafiltration	30
ephedrine	<i>Ephedra sinica</i> Stapf	microfiltration	31
toosendanin	azedarach bark	microfiltration and ultrafiltration	32
gypenosides	gynostemma	ultrafiltration	33
gardenia yellow	<i>Gardenia jasminoides</i> Ellis	microfiltration and ultrafiltration	34
mannital	seaweed	ultrafiltration	35

9.2.4 Solid Phase Extraction (SPE)

Solid phase extraction (SPE) was introduced in the early 1970s. It evolved from the combination of solid–liquid extraction with column liquid chromatography technology, and it is largely used for the isolation, purification and concentration of a wide range of extracts from the most diverse sources.³⁶ The basic process of SPE is shown in Figure 9.4.³⁷ Compared to the traditional liquid–liquid extraction, SPE can improve the recovery of the target compounds and separate them from interfering components effectively. SPE can be divided into four categories according to its mechanism: reverse-phase SPE, normal-phase SPE, ion-exchange SPE and adsorption SPE.³⁶ The main SPE sorbents and their characteristics are shown in Table 9.3.

New SPE sorbents are being continuously developed, and new materials such as ionic liquid-modified materials,³⁸ molecularly imprinted polymers³⁹ and carbon nanotubes⁴⁰ are opening new fields of application for this technique, as shown in Table 9.4.

As sample pre-treatment technology, SPE is more and more popularly used in the laboratory. It uses the sorbent absorption capacity to separate the target compounds from interference components. It enhances the analysis capability and increases the recovery rate of the sample. SPE has several advantages, such as simple operation, high efficiency and speed compared, to liquid–liquid extraction. The SPE device is composed of the SPE column packed with sorbents and an auxiliary element. The selection of sorbents is the most important factor when using the SPE method to pre-treat samples. Based on the properties and amount of the target compounds, suitable sorbents and column should be chosen to achieve the best result.

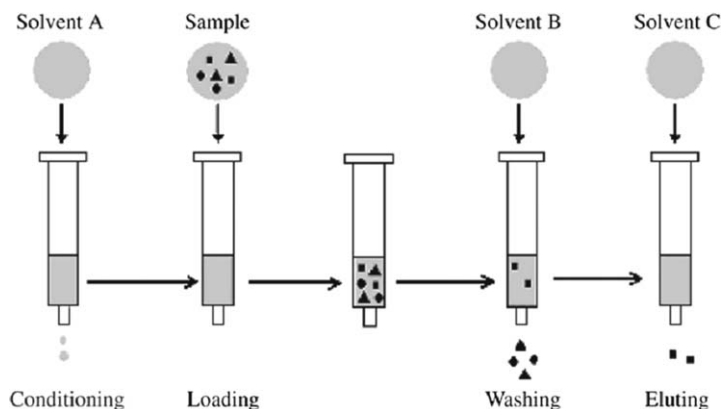


Figure 9.4 The basic process for solid phase extraction. Reprinted from *Journal of Biochemical and Biophysical Methods*, **70**, C. He, Y. Long, J. Pan, K. Li, F. Liu, Application of molecularly imprinted polymers to solid-phase extraction of analytes from real samples, pp. 133–150, 2007, with permission from Elsevier.³⁷

Table 9.3 The main SPE sorbents used in solid phase extraction (SPE) and their characteristics.

<i>SPE sorbents, main solid phases</i>	<i>Characteristics</i>
C ₁₈ and C ₈ silicas	high recovery; more hydrophobic phases containing a minimum amount of residual silanol groups
polar poly(styrene–divinylbenzene) copolymer sorbents	high specific surface areas; stability over the pH range 1–14
ion-pair and ion-exchange sorbents	suitable for ionizable compounds
normal-phase sorbents	suitable for analytes dissolved in samples made of a (usually) non-polar organic solvent

Table 9.4 Application of solid phase extraction (SPE) to new materials.

<i>New SPE sorbents</i>	<i>Sample source</i>	<i>Target compounds</i>	<i>Ref.</i>
<i>N</i> -methylimidazolium modified silica	<i>Salvia Miltiorrhiza</i> Bunge	tanshinones	41
molecularly imprinted polymers	<i>Siegesbeckia pubescens</i> herbal extract	kirenol	42
carbon nanotubes	feed	sulfonamides	43
mixed-mode sorbents	anthocyanin extracts	anthocyanin	44

Today, SPE is widely used as a sample preparation technique for the analysis of different types of materials in the fields of medicine, food science and technology, environmental science and chemistry, among others.^{45–48} It is a relatively consolidated technique with great potential in the analysis and isolation of natural products.

9.3 Purification

With the pre-isolation or enrichment techniques described above, target compounds can be concentrated and enriched in the extracts. However, the 'clean' extract is still quite complex and may contain several different classes of chemical compounds. In order to obtain the bioactive compound(s) with high purity, suitable isolation and purification techniques are needed. In the last decades, several powerful purification technologies have been developed, such as prep-HPLC, HSCCC and SFC, among others. However, each of these technologies has unique characteristics and a particular application for which it is best suited, and none of them is certainly suitable for the separation of all the natural products. To solve the complicated problem of the isolation and purification of natural products, the combination of different purification technologies is usually adopted, according to the nature of the components present in the extract and the target compounds.

The principles, characteristics of various purification technologies and some applications in the purification of natural products will be introduced briefly.

9.3.1 Chromatographic Techniques

Chromatography is the most widely used separation technique, where the components in a sample mixture are separated according to differences in their distribution between the mobile phase and stationary phase. Individual components are distinguished by their equilibrium constants, *i.e.* their ability to participate in common intermolecular interactions in the two phases.⁴⁹ Repeated sorption/desorption events during the movement of the sample components along the stationary phase result in useful separation when there is adequate difference in the strength of the physical interactions for the sample components in the two phases. The basic mechanisms of chromatographic separations are shown in Figure 9.5.

The main instrumentation system components for chromatography usually include solvents, pump, chromatography column and detector. Through more than one hundred years of development, chromatography has been transformed from an essentially batch technique into an automated, instrumental method, such as HPLC. While analytical HPLC is useful for obtaining information about sample mixtures and does not rely on their recovery, the aim of preparative HPLC is to isolate and purify the target compounds using large columns, high pressure, large sample loading and high flow rate.

Apart from prep-HPLC, low-pressure liquid column chromatography (LPLC) and high-speed counter-current chromatography (HSCCC) are often used to purify the target compounds. In this section, we introduce these three useful chromatographic methods in detail.

9.3.1.1 Low-pressure Liquid Column Chromatography (LPLC)

Low-pressure liquid column chromatography (LPLC) depends on the particle size of the stationary phase and the resulting operating pressure of the packed

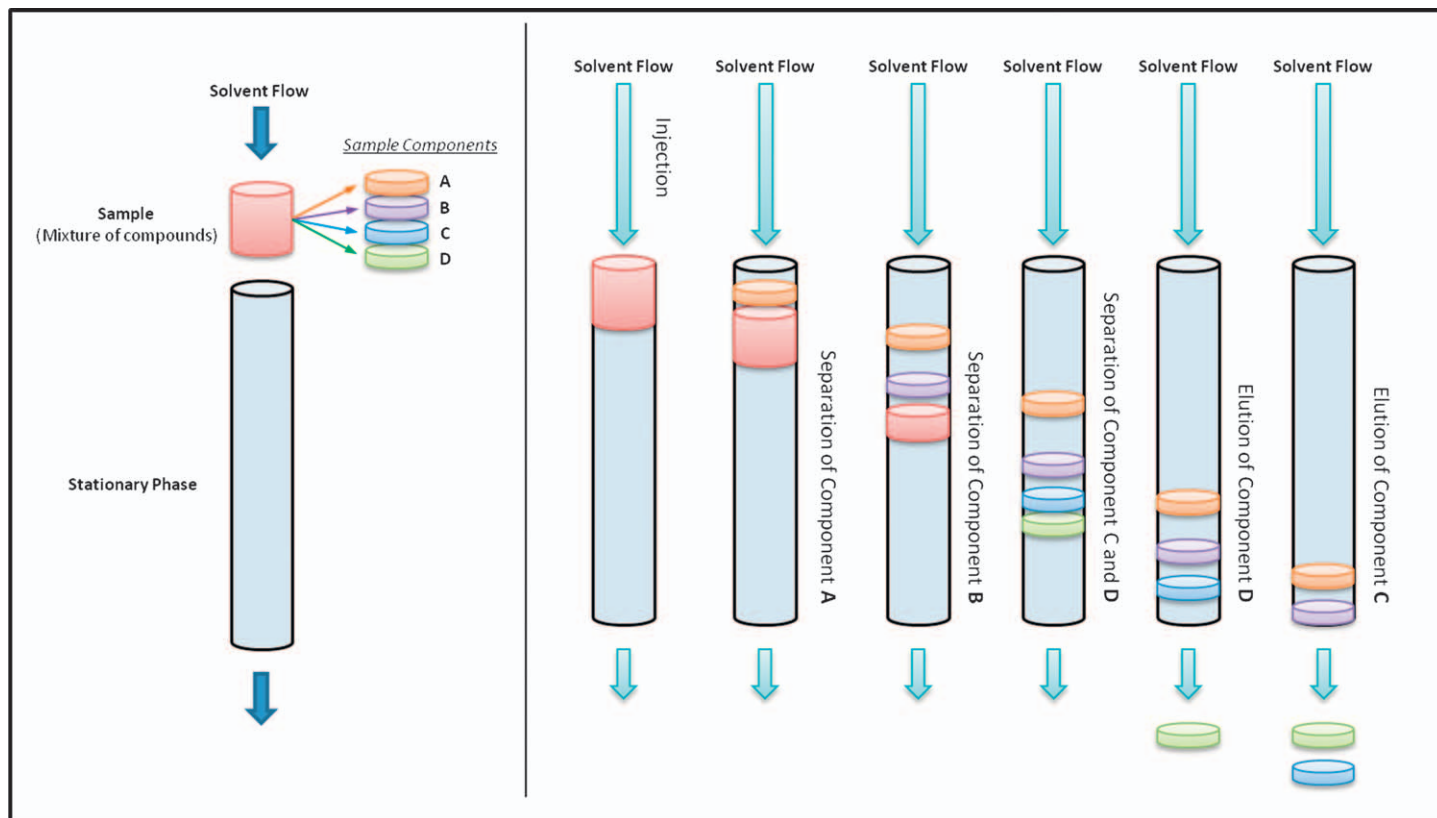


Figure 9.5 The basic mechanism of chromatographic separations (adapted from Harvey).⁴⁹

column. The LPLC is run with 40–200 μm particles at a flow rate that does not generate significant increase in the system pressure, which remains close to the atmospheric pressure. Compared to high/medium-pressure liquid column chromatography (HPLC/MPLC), LPLC produces lower resolution of separation and longer run times, so it is commonly used in the separation of compounds from crude extracts to produce large number of fractions. Adsorption and size exclusion are some of the principles of separation in LPLC, according to the nature of the stationary phase.⁵⁰

The separation of compounds based on adsorption results mainly from the adsorption affinity of the target molecules for the surface of the stationary phase and their solubility in the mobile phase. Silica gel is the most widely used stationary phase and has the advantage of excellent capacity for both linear and nonlinear isothermal separations and complete inertness towards labile compounds. Because silica gel is a typical polar sorbent whose surface is weakly acidic, there is a tendency towards preferential adsorption of strongly basic molecules such as alkaloids. Therefore, the main use of this type of column is for polar compounds.

Bonded-phase silica gel, which is derived from silica gel by chemically modifying its physical properties and chromatographic behaviour, can be either a non-polar (reversed-phase) packing material or one of intermediate polarity (bonded normal phase), such as C_8 , C_{18} and aminopropyl.⁵¹ Other stationary phases based on absorption principle include alumina and polystyrene. Alumina is a porous polymer of aluminium oxide that can be produced with acidic, basic or neutral surface, based on the pH of the final wash of the synthetic absorbent. There is a specific utility for each kind of alumina, e.g. the acidic alumina ($\text{pH} \approx 4.0$) is used for the separation of carboxylic acids; the basic alumina ($\text{pH} \approx 10.0$) and the neutral alumina ($\text{pH} \approx 7.0$) are used for basic and non-polar compounds, respectively. Currently, alumina is rarely used for purification purposes due to its ability to catalyse a variety of different reactions. Polystyrene gel can be used as adsorbent in reversed-phase LPLC, which is cheaper than bonded-phase silica gel and suitable for the separation of tannins and macromolecules.

The separation of compounds based on size exclusion results mainly from the size of these molecules. The process is shown in Figure 9.6.⁵² Smaller molecules are retained for more time in the stationary phase than bigger molecules, due to longer travel distance inside the particle. The packing materials of size exclusion stationary phases include polyacrylamide, polysaccharide and dextran, all of which are good for the separation of labile natural products. The gels made of polyacrylamide are hydrophilic and essentially free of charge, with particle sizes ranging from 45 μm to 180 μm , and are suitable for the separation of macromolecules, e.g. carbohydrates, peptides and tannins. Polymers made of carbohydrates are inert three-dimensional networks having functional ionic groups attached by ether linkages to glucose units of polysaccharide or dextran chains to produce anion and cation exchangers, as well as gel filtration resins. The typical brand of this packing material is Sephadex, which is one of the most extensively used gels in natural products separation.

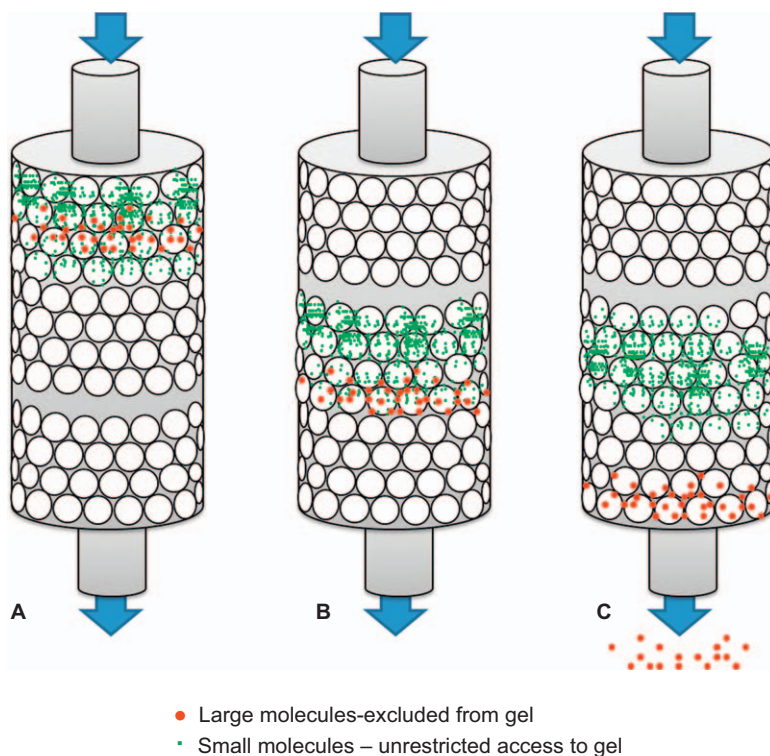


Figure 9.6 The process of gel chromatography (adapted from Braithwaite and Smith):⁵² (A) mixture applied to the top of the column; (B) partial separation; (C) complete separation; excluded substance emerges from the column.

Mobile phase solvents used for LPLC may present a wide range of polarity, starting from *n*-hexane, passing through methanol and up to water. The choice of the solvent depends on the type of LPLC operation and the intended outcome, i.e. the type of compounds to be isolated. For example, for normal-phase adsorption LPLC, the commonly used solvents are *n*-hexane, chloroform, dichloromethane, ethyl acetate and methanol, and for reversed-phase adsorption LPLC, water, methanol and acetonitrile are extensively used. The choice of appropriate stationary phase and mobile phase is crucial for obtaining optimum separation of components, maximizing the recovery of solutes and avoiding irreversible adsorption of solutes onto the packing material.

The column can be developed for the elution of samples using various methods. For example, when the particle size of the packing material is larger than 60 μm , gravity elution is easy to run; the mobile phase is poured on the top of the open column and allowed to flow naturally under gravity. When the particle size of the packing material is in the 40–60 μm range, positive pressure can be applied on the top of the column to accelerate the flow rate and achieve

better resolution, which is called flash chromatography (FC).⁵³ Another way to use pressure is to apply a vacuum at the end of the column, which is called vacuum liquid chromatography (VLC).⁵⁴

9.3.1.2 Preparative High-performance Liquid Chromatography (Prep-HPLC)

In recent years, with the drop of its cost, preparative high-performance liquid chromatography (prep-HPLC) has been the basis of the preparative separation of natural products. Compared to other 'low pressure' column chromatographic systems, prep-HPLC presents some advantageous features. The particle size of prep-HPLC is smaller, of 3–10 μm , and the high surface area results in a technique with high power of resolution. Moreover, it can be connected to several detectors, such as diode array, refractive index, fluorescence, etc.

The system of prep-HPLC consists of the same parts as the analytical HPLC. They both are made of a number of essential parts, including system controller, pumps, degasser, autosampler, guard column, column, detectors and fraction collectors,⁵⁵ shown in Figure 9.7. On the other hand, there are some differences between analytical HPLC and prep-HPLC. The aim of analytical HPLC is to get information about the sample, whereas the goal of prep-HPLC is to isolate the target compounds. The functions and constituents of the prep-HPLC system are:

- system controller – the controller of the whole prep-HPLC and operations that refer to flow rate, composition of solvents in binary, ternary and quaternary systems for isocratic or gradient modes, fraction collections, detection parameters and data presentation;
- pump – used to pump the solvent into the system column at 5–100 mL/min, depending on the preparative scale, with minimal pulsing;

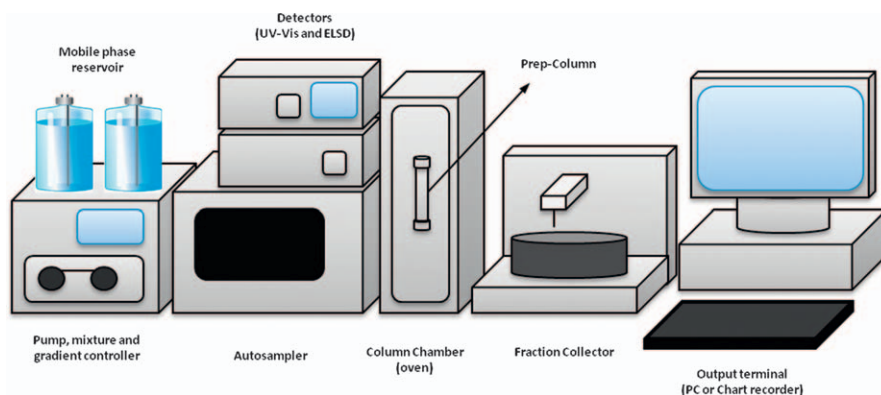


Figure 9.7 A typical prep-HPLC system (adapted from Latif and Sarker).⁵⁵

- degasser – applied to remove the air from the mobile phases to avoid poor separation resolutions caused by the air dissolved in the solvent;
- autosampler – comprises injection loop, syringe and sample carousel, and it is used for the injection of the sample previously dissolved in a proper solvent into the column;
- guard column – used to protect the column from particulate matter before the dissolved sample is injected into the column;
- column – the core part of the separation process of prep-HPLC. The samples are subjected to columns filled with various stationary phases. The resolution of complex mixtures can be achieved using suitable solvent systems;
- detectors – used to detect the compounds eluted from the column and to determine the collection of the fractions. Ultraviolet/visible (UV-vis) and refractive index (RI) are the most commonly used detectors. UV-vis detectors are used to detect compounds that can absorb electromagnetic radiation under wavelengths of 200–600 nm. RI detectors are rare general-purpose detectors that exploit light-scattering of compounds to detect them in the effluents. One disadvantage of RI detectors is that the change of the solvent results in drift noises, thus, this type of detector can not be used in gradient elution;
- fraction collectors – usually operated and programmed in an automated way to collect fractions containing the compounds separated in the column and identified in the detector.

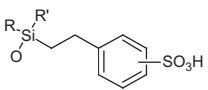
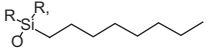
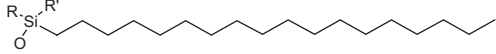
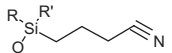
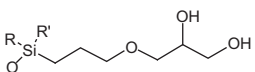
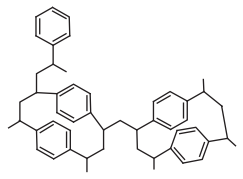
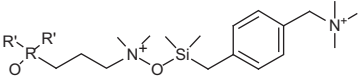
There are four main chromatographic techniques for the purification of samples obtained from natural products: normal phase, reversed-phase, gel permeation and ion-exchange, according to the type of stationary phase. Deciding which technique to use depends on the compatibility of the extract with the different columns. Table 9.5 shows the different types of stationary phases available and the respective separation techniques they use.⁵⁵

Because the crude samples from natural sources are usually very complex, when they are directly subjected to prep-HPLC, its separation resolution is usually poor, which results in shortened life-span of the separation column. Therefore, other efforts are usually done for the pre-purification of the crude samples.

Preparative isolation of pure compounds by prep-HPLC involves analytical HPLC, which means that the ‘method development’ is first carried out using an analytical HPLC system, and when the method has been established, the sample is scaled-up to the prep-HPLC system.⁵⁶

With the development of stationary phases, preparative separations of natural products are now prevalent by prep-HPLC. Marston⁵⁶ summarized the applications of prep-HPLC to the preparative separation of compounds from natural products that are difficult to separate. The preparative separation of other compounds, such as alkaloids, malto-oligosaccharides, etc., was also reported.^{57,58} Prep-HPLC has become a powerful chromatography tool to separate bioactive compounds due to its superexcellent efficiency and high

Table 9.5 Stationary phases commonly used in preparative high-pressure liquid chromatography (prep-HPLC).

Stationary phase	Structure	Technique
benzenesulfonic acid		strong cation exchange
C ₈		reversed phase
C ₁₈		reversed phase
CN (cyano)		normal and reversed phase
diol		normal and reversed phase
polymeric polystyrene divinyl benzene		reversed phase
quaternary ammonium		strong cation exchange
silica		normal phase

recovery.^{55,56} Successful cases of the preparative separation of pure compounds by prep-HPLC will be presented in Section 9.4.

9.3.1.3 High-speed Counter-current Chromatography (HSCCC)

Conventional High-speed Counter-current Chromatography. Modern high-speed counter-current chromatography (HSCCC), a form of CCC introduced by Ito and co-workers in the early 1980s, is now accepted as an efficient preparative technique, and it is widely used for the separation and purification of various chemical and pharmaceutical compounds, especially natural products.^{59,60} This chromatographic technique consists of a liquid–liquid method, where the stationary phase is liquid, so that no solid phases are used. It relies on the partition of the solute between two immiscible solvents to achieve its separation.^{61,62} HSCCC benefits from a number of advantages when compared to the more traditional solid–liquid separation methods: (i) no irreversible adsorption; (ii) complete recovery of injected sample; (iii) tailing minimized; (iv) low risk of sample denaturation; (v) low solvent

consumption; and (vi) favourable economics, since once the initial investment in an instrument has been made, no expensive columns are required and only common solvents are consumed.⁶³

HSCCC is mainly a preparative purification technique, where crude extracts or semi-pure fractions can be chromatographed with sample load ranging from milligrams to multigrams. Although the efficiency of CCC cannot match that of HPLC, the high selectivity and high retention of the HPLC stationary phase make the CCC method a valid alternative or complementary technique to HPLC, and a powerful preparative chromatographic tool. The volume of stationary phase in CCC can be as high as 80%, while the stationary phase content in HPLC is only about 40% of the volume of the column. Another advantage of CCC is the ability to reverse the flow direction and, therefore, to interchange the mobile and stationary phases.^{63,64} It is gaining popularity as a separation tool applied to natural products.

A typical HSCCC is shown in Figure 9.8. It consists of a mobile phase reservoir, a pump, an injection valve, a column, detectors, a fraction collector and a data processor. It is very similar to an HPLC unit, except for the column. The design principle of HSCCC column is shown in Figure 9.9. A long tube (usually over 100 m in length) is wound around a spool-shaped holder to form multiple coiled layers. The holder is rotating and at the same time the coil (multiple coiled layers) is revolving. The liquid stationary phase is held in an inert, coiled tubular column by a centrifugal force field, while the immiscible mobile phase flows through the column, as shown in Figure 9.10.^{65–67}

The selection of the two-phase solvent system is the most important parameter to be optimized, and it also is the most difficult step. It is estimated that about 90% of the entire work in HSCCC is spent on this stage.⁶⁷ A suitable two-phase solvent system requires the following considerations:⁶⁸

- for ensuring a satisfactory retention of the stationary phase, the settling time of the solvent system should be considerably shorter than 30 s;
- for an efficient separation, the partition coefficient (K) value of the target compounds should be close to 1, and the separation factor between two components ($\alpha = K_{D2}/K_{D1}$, $K_{D2} > K_{D1}$) should be larger than 1.5. In

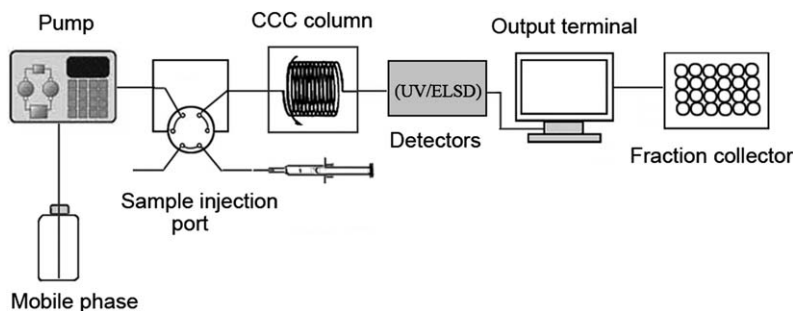


Figure 9.8 A typical HSCCC system.

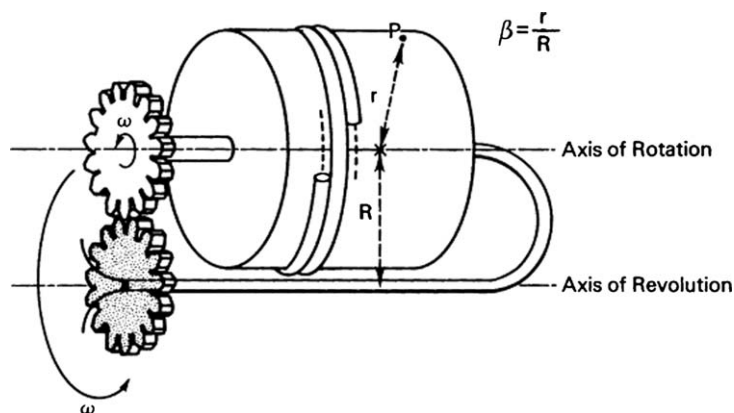


Figure 9.9 The design principle of type J coil planet centrifuge for high-speed CCC. Reprinted from *Journal of Chromatography A*, **244**(2), Y. Ito, J. Sandlin, W. G. Bowers, High-speed preparative counter-current chromatography with a coil planet centrifuge, pp. 247–258, 1982, with permission from Elsevier.⁶⁵

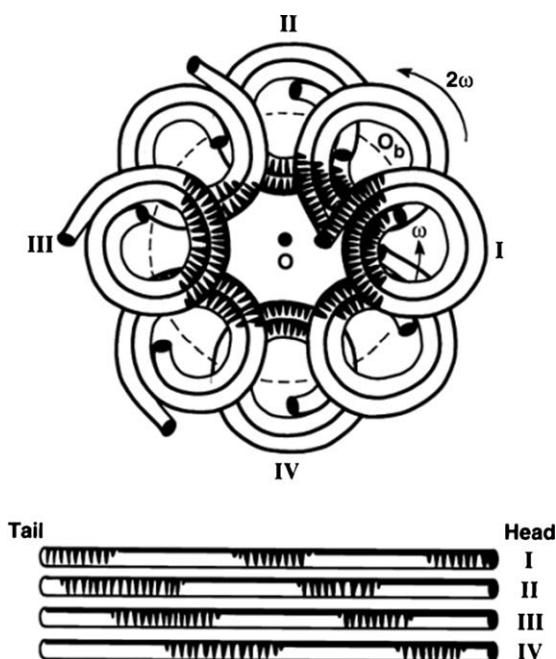


Figure 9.10 Hydrodynamic distribution of two phases in a spiral tube in type J synchronous planetary motion. Reprinted from *Journal of Chromatography A*, **1065**(2), Y. Ito, Golden rules and pitfalls in selecting optimum conditions for high-speed counter-current chromatography, pp. 145–168, 2005, with permission from Elsevier.⁶⁷

general, small K_D values result in poor peak resolution, while large K_D values tend to produce excessive band broadening. The K_D value usually can be determined by HPLC method. After partitioning, samples of the two solvent phases, of the upper and lower layers, are analysed by HPLC. From the two chromatograms, the K_D value of each compound is determined by the ratio of the peak areas or heights of the corresponding peaks.

Either phase of the two-phase solvent system can be used as the mobile phase. However, when the lower phase is used as the mobile phase, the retention of the stationary phase is usually more stable, but it should be pumped into the column in the head-to-tail elution mode.⁶⁹ If the upper phase is used as the mobile phase, it should be pumped into the column in the tail-to-head elution mode. The sample solution is usually prepared by dissolving the crude sample in either phase or in a mixture of the two phases, and the injection volume is usually less than 5% of the total column capacity.

In the HSCCC separation procedure, the coil column is first entirely filled with the upper phase of the solvent system. Then the apparatus is rotated at a suitable speed (usually 800–1000 rpm), while the lower phase is pumped into the column. After the mobile phase front emerges a hydrodynamic equilibrium is established in the column, and the sample solution can be then injected through the injection valve. The effluent of the column is continuously monitored by a detector, most commonly a UV detector. Peak fractions are collected according to the elution profile. The retention of the stationary phase relative to the total column capacity is computed from the volume of the stationary phase collected from the column after the separation is completed.

The versatility of HSCCC makes it an ideal method to isolate bioactive natural products. Up to now, many types of natural products including phenolics, flavonoids, alkaloids and coumarins, have been successfully isolated. Table 9.6 shows some natural compounds isolated by HSCCC. Numerous publications on the theories, principles, designs and practical applications are available.^{67,116,117} Based on the polarity and solubility of natural products, the suitable solvent systems for different types of compounds are summarized in Figure 9.11.

pH-Zone-refining Counter-current Chromatography. pH-Zone-refining counter-current chromatography (CCC) was developed by Ito⁶⁷ in the 1990s as a novel preparative-scale separation technique. It is a type of liquid–liquid partition chromatography that uses a basic (or acid) retainer in the stationary phase to retain the analytes in the column and an acid (or basic) eluter to elute the analytes according to their pK_a values and hydrophobicities. The greatest advantage of this separation technique is the large sample loading capacity, which exceeds 10-fold that of HSCCC for the same separation column. In addition, the technique yields highly concentrated fractions, concentrating minor compounds, and allowing the separation to be monitored by the pH of the effluent when there are no chromophores.^{63,67,116}

Table 9.6 Application of high-speed counter-current chromatography (HSCCC) to separate different classes of natural compounds.

Sample sources	Target compounds	Solvent System (v/v)	Ref.
Alkaloids			
<i>Capsicum frutescens</i> (hot pepper)	dihydrocapsaicin, capsaicin, nordihydrocapsaicin	CCl ₄ –MeOH–H ₂ O (4:3:2)	70
<i>Tripterygium wilfordii</i> Hook F.	wilfortrine, wilfordine, wilforgine, wilforine	Pet–EtOAc–EtOH–H ₂ O (6:4:5:8)	71
<i>Corydalis yanhusuo</i>	dehydrocorydalin, palmatine, coptisine, columbamine	CCl ₄ –MeOH– 0.2 M HCl aqueous solution (7:3:4)	72
<i>Evodia rutaecarpa</i> (Juss.) Benth	evodiamine, rutaecarpine, evocarpine, 1-methy-2-[(6Z,9Z)]-6,9-pentadecadienyl-4-(1 <i>H</i>)-quinolone, 1-methyl-2-dodecyl-4-(1 <i>H</i>)-quinolone	Hex–EtOAc–MeOH–H ₂ O (5:5:7:5)	73
<i>Aconitum coreanum</i>	guanfu base P, guanfu base G, guanfu base F, atisine, guanfu base A, guanfu base I	Hex–EtOAc–MeOH–0.2 M aqueous solution (1:3.5:2:4.5)	74
<i>Sophora flavescens</i> Ait.	matrine, oxysophocarpine, oxymatrine	CHCl ₃ –MeOH– 2.3 × 10 ^{−2} M potassium dihydrogen phosphate aqueous solution (27.5:20:12.5)	75
<i>Coptis chinensis</i> Franch	palmatine, berberine, epiberberine, coptisine	CHCl ₃ –MeOH–0.2 M HCl (4:1.5:2)	76
Flavonoids			
<i>Oroxylum indicum</i> (L.) Kurz	chrysin, baicalein, baicalein-7- <i>O</i> -glucoside, baicalein-7- <i>O</i> -diglucoside, chrysin-7- <i>O</i> -glucuronide, baicalein-7- <i>O</i> -glucuronide, chrysin-diglucoside	CHCl ₃ –MeOH–H ₂ O (9.5:10:5)	77
<i>Taraxacum mongolicum</i>	isoetin-7- <i>O</i> -β-D-glucopyranosyl-2'- <i>O</i> -α-L-arabinopyranoside, isoetin-7- <i>O</i> -β-D-glucopyranosyl-2'- <i>O</i> -α-D-glucopyranoside, isoetin-7- <i>O</i> -β-D-glucopyranosyl-2'- <i>O</i> -α-D-xylopyranoside	EtOAc– <i>n</i> -BuOH–H ₂ O (2:1:3)	78
<i>Hypericum japonicum</i> Thumb	isoquercitrin, quercitrin, quercetin-7- <i>O</i> -rhamnoside	EtOAc–EtOH–H ₂ O(5:1:5) (gradient elution: from 120 min, the flow rate increases from 1.0 mL/min to 2.0 mL/min)	79

Table 9.6 (Continued)

Sample sources	Target compounds	Solvent System (v/v)	Ref.
<i>Lysimachia christinae</i> Hance	kaempferol-3- <i>O</i> - β -D-glucopyranosyl, (2 $\rightarrow$ 1)- α -L-rhamnopyranoside, kaempferol-3- <i>O</i> - β -D-glucopyranoside, kaempferol-3- <i>O</i> - α -L-rhamnopyranoside	EtOAc–MeOH–H ₂ O (50:1:50)	80
<i>Pueraria lobata</i>	3'-hydroxypuerarin, puerarin, 3'-methoxypuerarin, puerarin-xyloside, daidzin	EtOAc– <i>n</i> -BuOH–H ₂ O (2:1:3)	81
<i>Citrus reticulata</i> Blanco	nobiletin, tangeretin, 3,5,6,7,8,3',4'-heptamethoxyflavone, 5-hydroxy-6,7,8,3',4'-pentamethoxyflavone	Hex–EtOAc–MeOH–H ₂ O (1:0.8:1:1)	82
<i>Radix astragali</i>	calycosin-7- <i>O</i> - β -D-glucoside, ononin, (6a <i>R</i> , 11a <i>R</i>)-9,10-dimethoxypterocarpan-3- <i>O</i> - β -D-glucoside)	Hex–EtOAc– <i>n</i> -BuOH–MeOH–H ₂ O (0.5% TFA) (1:2:1:1:5), <i>n</i> -BuOH–EtOAc–MeOH–H ₂ O (0.5% TFA) (2:3:1:1:5), CHCl ₃ –MeOH–H ₂ O (4:3:2)	83
<i>Vaccinium myrtillus</i>	cyanidin-3- <i>O</i> -sambubioside, delphinidin-3- <i>O</i> -sambubioside	MtBE– <i>n</i> -BuOH–MeOH–H ₂ O–TFA (1:4:1:5:0.01)	84
Quinones			
<i>Salvia miltiorrhiza</i>	dihydrotanshinone I, cryptotanshinone, methylenetanshinone, tanshinone I, anshinone, danshenxinkun B	Hex–EtOH–H ₂ O (10:5.5:4.5), (10:7:3)	85
<i>Lithospermum</i> <i>erythrorhizon</i> Sieb. et Zucc	β -hydroxyisovalerylshikonin, acetylshikonin, isobutyrylshikonin	Pet–EtOAc–MeOH–H ₂ O (5:5:8:2)	86
<i>Rubia cordifolia</i>	tectoquinone, 1-hydroxy-2-methylanthraquinone, mollugin	Pet–EtOH–diethyl ether–H ₂ O (5:4:3:1)	87
<i>Catsia tora</i> L.	1,2,6-trihydroxy-7,8-dimethoxy-3-methylanthraquinone, 1,2,6,8-tetrahydroxy-7-methoxy-3-methyl-anthraquinone, 2-hydroxy-1,6,7,8-teramethoxy-3-methylanthraquinone, 6-dihydroxy-1,7,8-trimethoxy-3-methylanthraquinone, 1,2-dihydroxy-6,7,8-tri-methoxy-3-methylanthraquinone	Hex–EtOAc–MeOH–H ₂ O (4:1:3:2)	88

<i>Rheum palmatum</i> L.	rhein, emodin, aloe-emodin, chrysophanol	TBME–THF–H ₂ O (2:2:3)	89
<i>Polygonum multiflorum</i>	emodin, chrysophanol, rhein, gallic acid	Hex–EtOAc–MeOH–H ₂ O (3:7:5:5)	90
Phenylpropanoids			
<i>Caulis Ionicerae</i>	caffeic acid, chlorogenic acid, luteolin	EtOAc–EtOH–H ₂ O (4:1:5)	91
<i>Peucedanum praeruptorum</i> Dunn.	qianhu coumarin D, Pd-Ib, peucedanocoumarin I, peucedanocoumarin II, (+)-praeruptorin A, (+)-praeruptorin B, (+)-praeruptorin E	Pet–EtOAc–MeOH–H ₂ O (5:5:6:4)	92
<i>Psoralea corylitolia</i> L.	psoralen, isopsoralen	Hex–EtOAc–MeOH–H ₂ O (1:0.7:1:0.8)	93
<i>Angelica dahurica</i> (Fisch. ex Hoffm) Benth. et Hook	imperatorin, oxypeucedanin, isoimperatorin	Hex–EtOAc–MeOH–H ₂ O (1:1:1:1), (5:5:4.5:5.5)	94
<i>Notopterygium forbesii</i> Boiss	notopteron, isoimperatorin	Pet–EtOAc–MeOH–H ₂ O (5:5:4.8:5), (5:5:5:4)	95
<i>Schisandra Chinensis</i> (Turcz) Baill	schisandrin, schisantherin	Hex–EtOAc–MeOH–H ₂ O (22:8:20:20)	96
<i>Arctium lappa</i>	arctiin	EtOAc– <i>n</i> -BuOH–EtOH–H ₂ O (5:0.5:1:5)	97
<i>Magnolia officinalis</i>	honokiol, magnolol	Hex–EtOAc–MeOH–H ₂ O (1:0.4:1:0.4)	98
<i>Salvia miltiorrhiza</i> Bge.	salvianolic acid A, salvianolic acid B	Hex–EtOAc–MeOH–H ₂ O (3:6:6:10)	99
Terpenoids			
<i>Rhizoma atractylodis Macrocephalae</i>	atractylon, atractylenolide III	Pet–EtOAc–EtOH–H ₂ O (4:1:4:1)	100
<i>Artemisia dalailamae</i> Kraschen	taraxeryl acetate, coumarins	CHCl ₃ –MeOH–H ₂ O (2:2:1)	101
<i>Momordica charantia</i>	goyaglycoside-e, momordicoside L, goyaglycoside-a, momordicoside K	MtBE– <i>n</i> -BuOH–MeOH–H ₂ O (1:2:1:5), (1:3:1:5)	102
<i>Cyperus rotundus</i>	α-cyperone	Hex–EtOAc–MeOH–H ₂ O (1:0.2:1.1:0.2)	103
<i>Triperygium wilfordii</i> Hook.f	triptonide, isoneotriptophenolide, hypolide, triptophenolide, triptonoterpene methyl ether VI	Hex–EtOAc–MeOH–H ₂ O (3:2:3:2)	104
<i>Aucklandia lappa</i> Decne	costunolide, dehydrocostuslactone	Pet–MeOH–H ₂ O (5:6.5:3.5)	105
<i>Schisandra chinensis</i> (Turcz.) Baill	deoxyschisandrin, γ-schisandrin	Hex–MeOH–H ₂ O (35:30:3)	106

Table 9.6 (Continued)

Sample sources	Target compounds	Solvent System (v/v)	Ref.
<i>Gardenia jasminoides</i> Ellis	geniposide	EtOAc- <i>n</i> -BuOH-H ₂ O (2:1.5:3)	107
<i>Glycyrrhiza uralensis</i>	liquiritigeni, isoliquiritigenin	Hex-EtOAc-MeOH-ACN- H ₂ O (2:2:1:0.6:2)	108
<i>Cistanches salsa</i> (C.A. Mey) G. Beck	2'-acetylacteoside, phenylethanoid glycosides (PhGs) acteoside	EtOAc- <i>n</i> -BuOH-EtOH-H ₂ O (4:0.6:0.6:5)	109
<i>Rhodiola sachalinensis</i> A. Bor	salidroside	EtOAc- <i>n</i> -BuOH-H ₂ O (3:2:5)	110
Saponins			
<i>Dioscorea nipponica</i> Makino	dioscin	Hex-EtOAc-EtOH-H ₂ O (2:5:2:5)	111
<i>Rhizoma dioscoreae</i>	diosgenin, linoleic acid, linolenic acid	Hex-EtOAc-EtOH-H ₂ O (1:1:1.4:0.6)	112
<i>Panax quinquefolium</i> L.	ginsenosides	EtOAc- <i>n</i> -BuOH-H ₂ O (1:1:2)	113
<i>Panax ginseng</i>	ginsenoside-Rb ₁ , notoginsenoside-R ₁ , ginsenoside-Re, ginsenoside-Rg ₁	Hex- <i>n</i> -BuOH-H ₂ O (3:4:7)	114
<i>Panax notoginseng</i>	asiaticoside, madecassoside	CHCl ₃ -EtOH- <i>n</i> -BuOH-H ₂ O (7:6:3:4)	115

CCl₄=carbon tetrachloride; CHCl₃=trichloromethane; EtOAc=ethyl acetate; EtOH=ethanol; Hex=hexane; HCl=hydrochloric acid; MeOH=methanol; MtBE=methyl *tert*-butyl ether; *n*-BuOH=*n*-Butanol; Pet=petroleum ether; TBME=*tert*-butyl methyl ether; TFA=trifluoroacetic acid; THF=tetrahydrofuran.

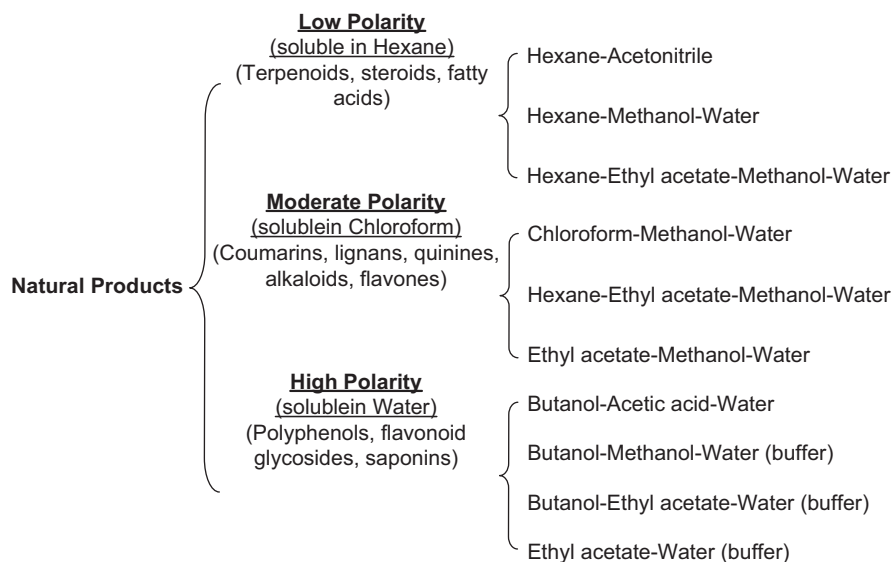


Figure 9.11 Solvent systems with different polarities for HSCCC of natural products.

In pH-zone-refining CCC, successful separation of target compounds requires the careful search for a suitable two-phase solvent system. For the basic compounds, a suitable two-phase solvent system should provide ideal partition coefficient (K_D) values in both acidic ($K_{D_{acid}} \ll 1$) and basic ($K_{D_{basic}} \gg 1$) media. On the contrary, for the separation of acidic compounds, the partition coefficient values in acidic medium should be $K_{D_{acid}} \gg 1$ and in basic media $K_{D_{basic}} \ll 1$. Compared to the conventional HSCCC, the selection of a pH-zone-refining CCC solvent system is relatively simple. Most ionic compounds can be successfully separated using the solvent system composed of methyl tertiary butyl ether (MtBE)–acetonitrile–water at volume ratio of 1:0:1–2:2:3. If the target compounds are highly polar, *n*-butanol can be used to replace part of MtBE in the solvent system. If the target compounds are hydrophobic, a solvent system composed of *n*-hexane–ethyl acetate–methanol–water can be used and the volume ratio can be selected from 5:5:5:5 to 10:0:5:5. The rules of selecting solvent systems have been clearly described by Ito in the literature.⁶⁷ Table 9.7 shows some examples of separation of natural products by pH-zone-refining CCC. Some successful cases are also presented in case study section.

9.3.1.4 Supercritical Fluid Chromatography (SFC)

Supercritical fluid chromatography (SFC) takes advantage of supercritical fluid extraction and chromatography separation, and has become competitive with conventional methods for separating valuable constituents of natural products

Table 9.7 Application of pH-zone refining counter-current chromatography (CCC) to separate natural compounds.

Class	Sample source	Target compounds	Solvent System (v/v)	Ref.
alkaloids	<i>Picralima nitida</i> Stampf Th. et H. Dur.	alstonine	MtBE–ACN–H ₂ O (2:2:3)	118
	<i>Catharanthus roseus</i>	vindoline, catharanthine, vincaleukoblastine	MtBE–ACN–H ₂ O (4:1:5)	119
	<i>Corydalis decumbens</i>	protopine, tetrahydropalmatine, bicuculline	MtBE–ACN–H ₂ O (2:2:3)	120
	<i>Huperzia serrata</i>	huperzines A, huperzines B	MtBE–ACN–H ₂ O (4:1:5)	121
	<i>Aconitum sinomontanum</i> Nakai	lappaconitine	MtBE–THF–H ₂ O (2:2:3)	122
	<i>Nelumbo nucifera</i> Gaertn (lotus)	liensinine, isoliensinine, neferine	MtBE–H ₂ O (1:1), Hex–EtOAc–MeOH–H ₂ O (5:5:2:8)	123
	<i>Nelumbo nucifera</i> Gaertn (lotus)	<i>N</i> -nornuciferine, nuciferine, roemerine	Pet–EtOAc–MeOH–H ₂ O (5:5:2:8)	124
	<i>Sophora flavescens</i> Ait	sophocarpine, matrine	MtBE–H ₂ O (1:1)	125
	<i>Stephania yunnanensis</i>	isocorydine, corydine, tetrahydropalmatine, N-methylasimilobine, anonaine	MtBE–ACN–H ₂ O (2:2:3)	126
	<i>Peganum harmala</i> (Harmel)	harmine, harmaline	MtBE–THF–H ₂ O (2:2:3)	127
organic acids	<i>Echinacea Purpurea</i>	cichoric acid	MtBE–CH ₃ CN–H ₂ O (4:1:5)	128
	<i>Salvia miltiorrhiza</i>	salvianolic acid B	MtBE–H ₂ O (1:1)	129

ACN = acetonitrile; EtOAc = ethyl acetate; MeOH = methanol; MtBE = methyl *tert*-butyl ether; Pet = petroleum ether; THF = tetrahydrofuran.

in recent years along with the development of chromatography techniques.¹³⁰ Supercritical fluids, used as the mobile phase in this technique, have useful physical properties, such as low viscosity and high diffusivity, which results in remarkably faster mass transport when compared to common organic solvents. The solvent strength and the separation efficiency of the mobile phase can be improved by adding modifiers to the supercritical fluid. Almost all stationary phases used in HPLC and GC can be used in SFC. There are two types: capillary supercritical fluid chromatography (cSFC), which is similar to GC; and packed-column supercritical fluid chromatography (pSFC), which is similar to HPLC.¹³¹

SFC has the advantage of low operating temperatures, high diffusivities of the solutes, high throughput, low consumption of organic solvents and wide range of applicability. Therefore, it is a very useful tool for the preparation of thermolabile compounds and complex extracts. It has considerable potential as an alternative instrumental preparative technique complementing HPLC and gas-liquid chromatography (GLC).¹³² Because of the unique properties of SFC, it developed rapidly and received more and more attention in natural products research, where constituents are complex and thermolabile most of the time. The manipulation of various parameters is possible, such as mobile phase, stationary-phase type, temperature and pressure, making the separation of complex samples possible. Recently there have been a number of reports on the application of SFC for isolation and purification of natural products, presented in Table 9.8.

Due to the particularity of the mobile phase, SFC has an important position in the research and development of natural products. As SFC develops, it will play a more important role in the separation of bioactive compounds, particularly those with low volatility and those that are thermolabile.

9.3.2 Crystallization

Crystallization refers to the process where the solute automatically precipitates from the solution to form a new phase. Solubility and saturation are the basis of the crystallization process. Very small solute particles precipitate in a supersaturated solution, forming a crystal nucleus. After the formation of this nucleus, the solute enters into its surface, depending on diffusion effects, so as to let the nucleus continuously grow into a full crystal. Integrated crystallization processes involve three basic steps: (i) formation of a supersaturated solution; (ii) nucleation; (iii) crystal growth. The supersaturation of the solution is the driving force of the formation of solute crystals.²⁰

Usually there are seven methods in the preparation of a supersaturated solution: evaporation, cooling, chemical reactions, salting-out, isoelectric point, composite and azeotropic distillation. A number of reports on the application of crystallization for the isolation and purification of natural products are listed in Table 9.9.

Table 9.8 Application of supercritical fluid chromatography (SFC) to separate natural compounds.

Sample source	Target compounds	SFC condition	Ref.
rosemary extract	antioxidant and antimicrobial compounds	CO ₂ modified by 10% of ethanol	133
flavonol isomers mixture	flavonol isomers	CO ₂ modified by ethanol containing 0.5 % (v/v) phosphoric acid	134
<i>Artemisia annua</i> L.	artemisinin	C ₁₈ column (9.4 mm × 250 mm I.D., 5 μm), CO ₂ velocity of 22 g/min, column temperature and pressure of 40 °C and 11 MPa	135
tuna oil	DHA ester, EPA ester	Kromasil 10-C ₁₈ columns 10 × 250 mm, and Kromasil 5-C ₁₈ , 10 × 250 mm; pressure, 14.5 MPa; temperature 65 °C; mobile-phase linear velocity 1.9 mm/s	136
tocopherol mixture	tocopherol homologues	ODS column (250 mm × 4.6 mm i.d., 5 μm), pressure, 20 MPa; temperature 70 °C; mobile-phase flow-rate 750 mL/min	137
spinach leaf extracts	carotene, xanthophyll, chlorophyll	Zorbax ODS column; Mobile phase: 3% (v/v) propanol in CO ₂ ; pressure: 27.6 MPa; temperature: 40 °C; flow rate: 2.0 mL/min	138
cell culture extract	taxol	Lichrosphere diol column: 250 × 4.6 mm; 5 μm; gradient from 8 to 35 % methanol in carbon dioxide, 2 mL/min, 30 °C, 15 MPa	139

Table 9.9 Application of crystallization to separate natural compounds.

Sample source	Target compounds	Crystallization method	Ref.
<i>Flos Sophorae</i> extract	rutin	cold water crystallization	140
	oleic acid and linoleic acid	methanol solvent crystallization	141
phytosterol mixtures	stigmasterol and β-sitosterol	solvent crystallization	142
<i>Artemisia annua</i>	artemisinin	two-step anti-solvent crystallization	143
<i>Malania oleifera</i> oil	nervonic acid	solvent crystallization	144
<i>Tagetes patula</i> L.	pigment	three-solvent crystallization	145
<i>Flos sophorae</i>	rutin	solvent crystallization	146

9.4 Case Studies

9.4.1 Isolation of Saponins from *Clematis chinensis*

Clematis chinensis Osbeck (Ranunculaceae) is widely distributed in the south of China. According to the Chinese Pharmacopoeia I, the roots and rhizomes of

C. chinensis, *C. hexapetala* Pall., and *C. mandshurica* Rupr. are collectively termed 'Weilingxian', a traditional Chinese herbal drug that is commonly used as an anti-inflammatory, antitumor, and analgesic agent. However, previous phytochemical investigations have revealed that the chemical constituents of these three species are quite different. To explore the similarities in the chemical constituents and bioactivity of the tissues from these three species, the chemical constituents of *C. chinensis* were studied, and seven new triterpene saponins, clematochinenosides A–G were obtained, together with known saponins.¹⁴⁷ The structures of the new triterpene saponins are shown in Figure 9.12.

A 50% EtOH extract of the dried roots and rhizomes of *C. chinensis* (4 kg) was suspended in H₂O and successively extracted with petroleum ether (PE), EtOAc and *n*-BuOH. The *n*-BuOH-soluble fraction was subjected to column chromatography over porous polymeric resin (D101), silica gel and C₁₈ silica gel. Seven new triterpene saponins, clematochinenosides A–G, were isolated, together with 10 known saponins, cirensenoside O, kizutasaponin K, ciwujianoside C, kizutasaponin K, hederasaponin B, kizutasaponin K, huzhangoside B, huzhangoside D, clematichinenoside C and clematichinenoside B. The roadmap for the extraction and separation processes is shown in Figure 9.13.

9.4.2 Isolation of Tritoniopsins A–D from *Cladiella krempfi*

Soft corals from South China Sea have been extensively studied by Chinese marine natural product chemists and have yielded a plethora of steroids and terpenoids, the latter mainly including diterpenoids. It has been suggested that such secondary metabolites are probably involved in the defensive mechanisms of the animals, which appear to be relatively free from predation. Four diterpenes, tritoniopsins A–D (Figure 9.14), have been isolated from *C. krempfi* collected in the South China Sea.¹⁴⁸

The frozen sample of *C. krempfi* was extracted with acetone. The acetone extracts were concentrated, and the residue was fractionated between H₂O and diethyl ether. The ethereal extract was subjected to Sephadex LH-20 chromatography with CHCl₃/MeOH (1:1) to result in five fractions, from A to E. Tritoniopsins A–D were obtained in fractions B and D after purification with silica gel column chromatography using light petroleum ether and an increasing amount of Et₂O, and subsequently with C₁₈ reversed-phase HPLC. The roadmap for the processes of extraction and separation is shown in Figure 9.15.

9.4.3 Isolation of *cis*-Clerodane-type Furanoditerpenoids from *Tinospora crispa*

Currently more than 1000 plant species are used as folk medicines throughout the world. In Malaysia, extracts from *Tinospora crispa* Miers are often used to treat malaria and filariasis. To isolate the active compounds for

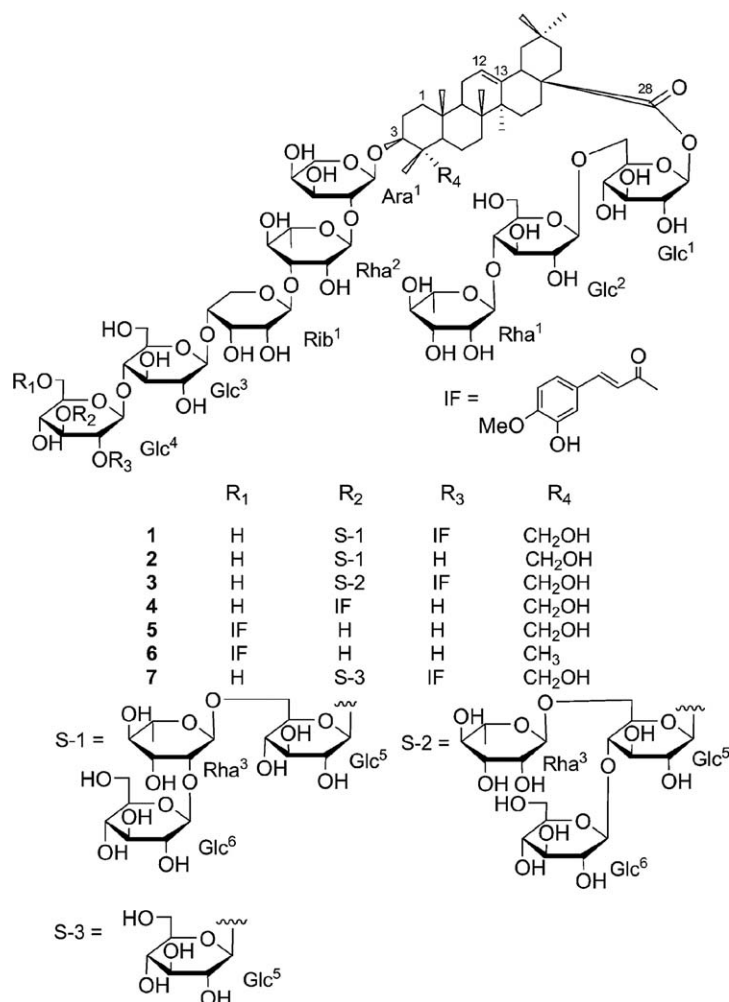


Figure 9.12 The new triterpene saponins from *C. chinensis*.

Reprinted with permission from *Journal of Natural Products*, **73**, Q. Fu, K. Zan, M. Zhao, S. Zhou, S. Shi, Y. Jiang and P. Tu, Triterpene saponins from *Clematis chinensis* and their potential anti-inflammatory activity, pp. 1234–1239, 2010, copyright 2010 American Chemical Society.¹⁴⁷

further pharmacological studies, the BuOH-soluble fraction of an EtOH extract of *T. crispata* was chromatographed repeatedly using centrifugal partition chromatography (CPC), Sephadex LH-20, and a reversed-phase Lobar column. Six *cis*-clerodane-type furanoditerpenoids, borapetosides A–F were obtained (Figure 9.16).

Fresh vines of *T. crispata* were ground in a blender, and the resultant mass was stirred with water (30 L) and 95% EtOH (40 min × 2) in sequence. The extract was partitioned between water and *n*-BuOH. The *n*-BuOH-soluble fraction was

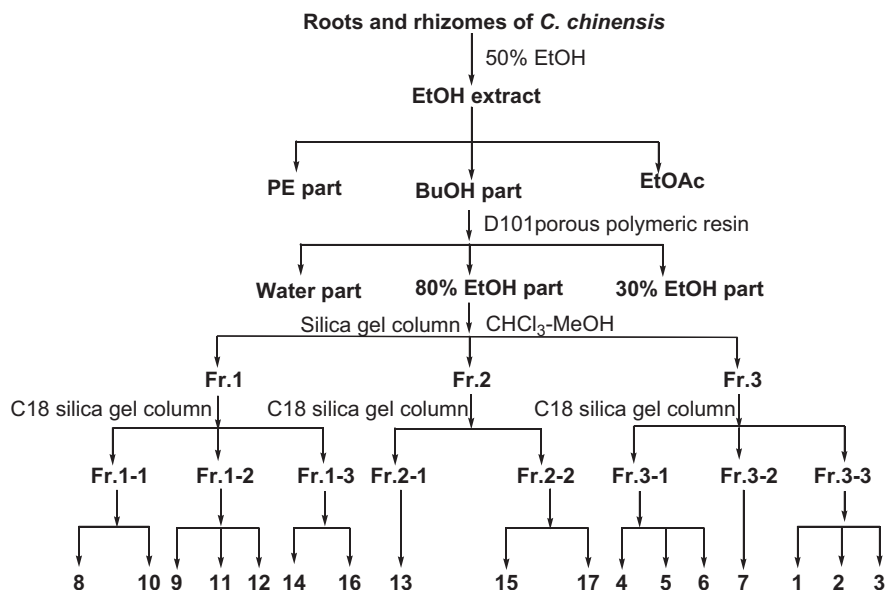


Figure 9.13 The roadmap of extraction and separation of compounds from *C. chinensis*. 1–7: clematichinenosides A–G; 8: cirenenoside O; 9: kizutasaponin K; 10: ciwujianoside C; 11: kizutasaponin K; 12: hederasaponin B; 13: kizutasaponin K; 14: huzhangoside B; 15: huzhangoside D; 16: clematichinenoside C; 17: clematichinenoside B.

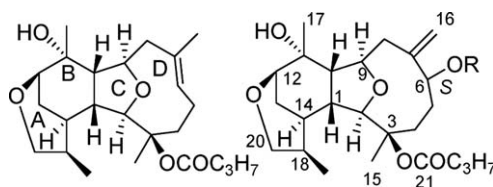


Figure 9.14 The new diterpenes from *Cladiella krempfi*. Reprinted with permission from *Journal of Natural Products*, **74**, M. L. Ciavatta, E. Manzo, E. Mollo, C. A. Mattia, C. Tedesco, C. Irace, Y.-W. Guo, X.-B. Li, G. Cimino and M. Gavagnin, Tritoniopsins A–D, Cladiellane-based diterpenes from the South China Sea Nudibranch *Tritoniopsis elegans* and its prey *Cladiella krempfi*, pp. 1902–1907, 2011, copyright 2011 American Chemical Society.¹⁴⁸

fractionated via a Sanki CPC, using $\text{CHCl}_3 - \text{MeOH} - \text{H}_2\text{O}$ (10:10:5) as a delivery system at a flow rate of 3 mL/min and rotation speed of 800 rpm. An aliquot of fraction B was separated in a RP-18 Lobar column, eluted with 30% $\text{MeOH}(\text{aq})$ to MeOH , and in a Sephadex LH-20 column, and yielded compounds A–D. Fraction D yielded compounds E and F by using RP-18 Lobar column chromatography and Sephadex LH-20. The roadmap for the extraction and separation is shown in Figure 9.17.

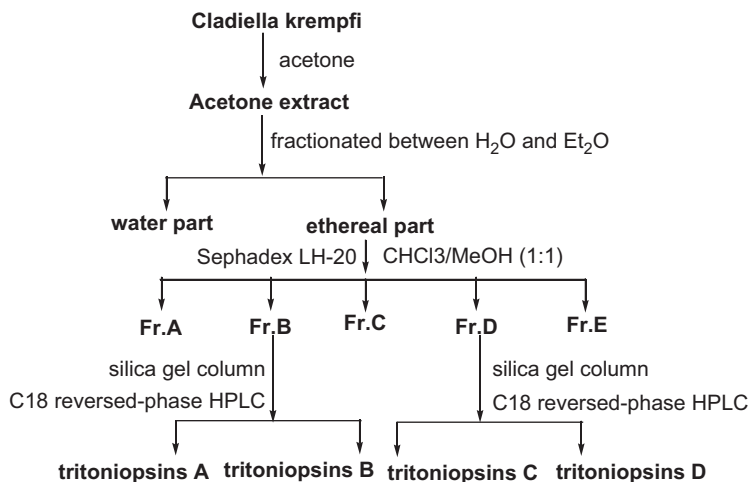


Figure 9.15 The roadmap of extraction and separation of tritoniopsins from *Cladiella krempfi*.

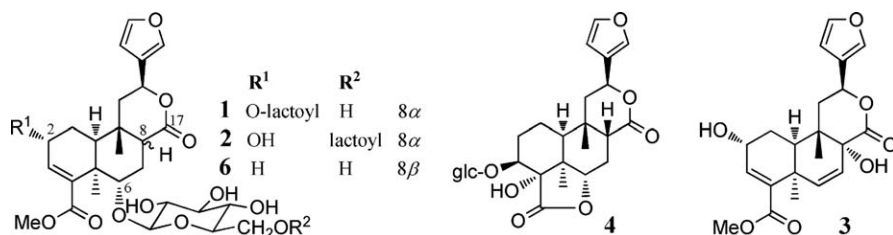


Figure 9.16 The new *cis*-clerodane-type furanoditerpenoids from *Tinospora crispa*. Reprinted with permission from *Journal of Natural Products*, **75**, S.-H. Lam, C.-T. Ruan, P.-H. Hsieh, M.-J. Su and S.-S. Lee, Hypoglycemic diterpenoids from *Tinospora crispa*, pp. 153–159, 2012, copyright 2012 American Chemical Society.¹⁴⁹

9.4.4 Isolation of Flavonoids from *Paeonia suffruticosa*

The flowers of *Paeonia suffruticosa* Andr. are used in Chinese folk medicine for the treatment of diseases related mainly to irregular menstruation and dysmenorrhea. In order to get better understanding of its pharmacological function and to further exploit this important plant resource, Wang *et al.*^{117,150} developed an efficient method for the preparative isolation and purification of seven flavonoids (Figure 9.18) from *P. suffruticosa* Andr.

Fresh flowers (5 kg) of *P. suffruticosa* Andr. were extracted with 95% aqueous ethanol. Then the extract was evaporated to dryness under reduced pressure and dissolved with water. After filtration, the aqueous solution was successively extracted with water-saturated light petroleum (b.p. 60–90 °C),

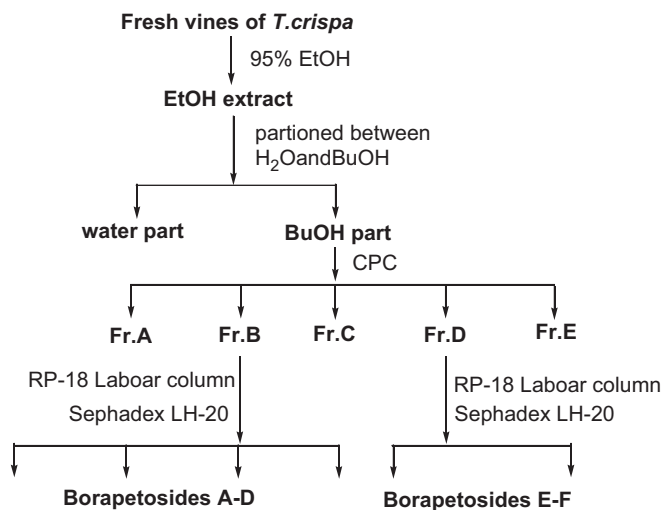


Figure 9.17 The roadmap of the extraction and separation of borapetosides from *Tinospora crispa*.

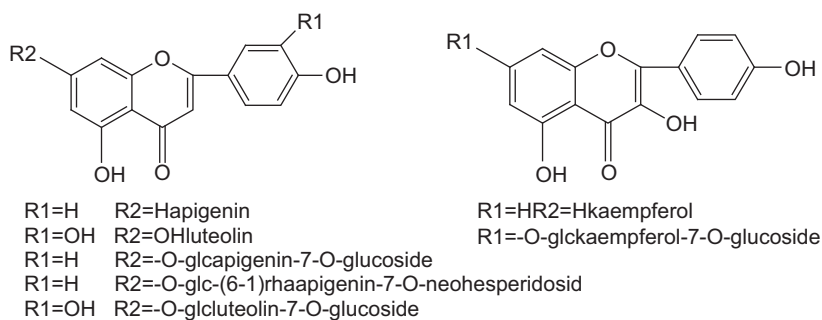


Figure 9.18 The flavonoids from *P. Suffruticosa*.¹¹⁷

ethyl acetate and *n*-butanol. The flavonoid compounds were found to be in the ethyl acetate extracts after HPLC analysis. The ethyl acetate extracts were separated by polyamide chromatography eluted with ethanol–H₂O; the flavonoids were divided into flavonoid glycosides (30% ethanol effluent) and flavonoid aglycones (95% ethanol effluent). Both fractions were further separated by HSCCC. As a result, 5 mg of apigenin-7-*O*-neohesperidoside (94% purity), 4 mg of luteolin-7-*O*-glucoside (97% purity), 9 mg of apigenin-7-*O*-glucoside (97% purity) and 2.5 mg of kaempferol-7-*O*-glucoside (96% purity) were obtained from 40 mg of extract recovered in the 30% effluent. Three flavonoid aglycones (apigenin, luteolin and kaempferol), with purities over 96%, were obtained from the 95% effluent by HSCCC with the solvent system composed by chloroform–methanol–water (5:3:2, v/v). The roadmap of extraction and separation of the *P. suffruticosa* flowers is shown in Figure 9.19.

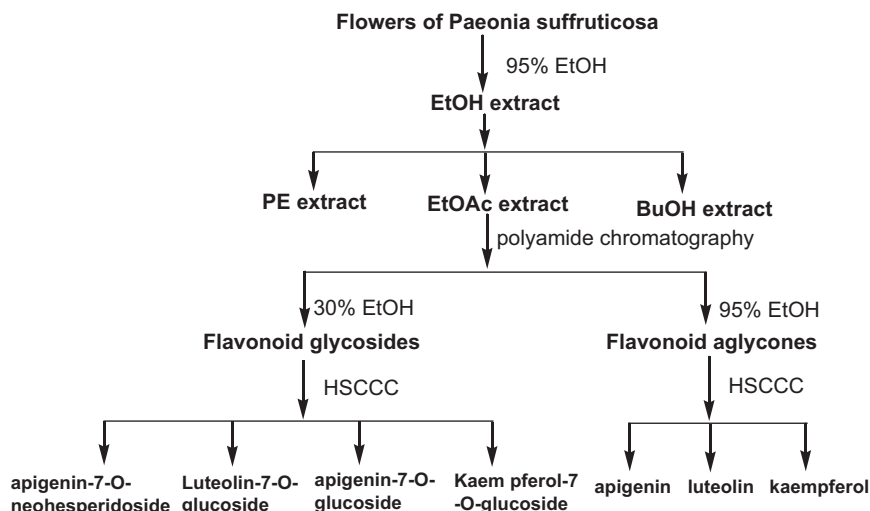


Figure 9.19 The roadmap of extraction and separation of the flowers of *P. suffruticosa*.

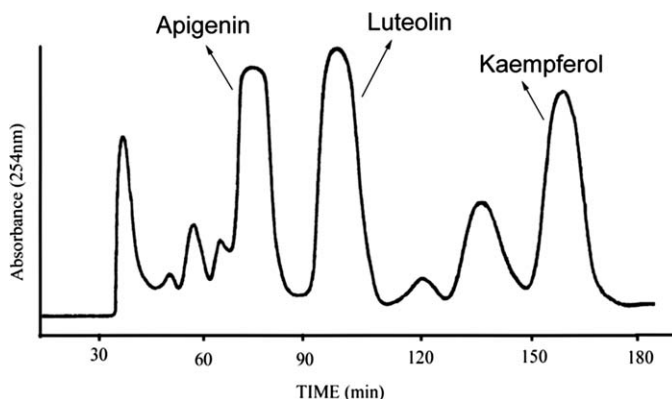


Figure 9.20 HSCCC chromatogram of the 95% effluent (adapted from Zhang and Wang).¹¹⁷ HSCCC conditions: solvent system, chloroform–methanol–water (5:3:2, v/v); revolution speed, 800 rpm; flow rate, 2 mL/min; sample size, 150 mg; retention of the stationary phase, 65%.

The HSCCC chromatograms of 95% ethanol effluent and 30% ethanol effluent are shown in Figures 9.20 and 9.21, respectively.

It is unlikely that a single solvent system can be used to obtain different types of compounds from crude extracts with HSCCC, since the extract of a plant, microbe or animal matrix contains a complicated mix. Therefore, it is often necessary to initially fractionate the crude extract into various discrete fractions containing a group of compounds of similar polarities or molecular sizes before the HSCCC.

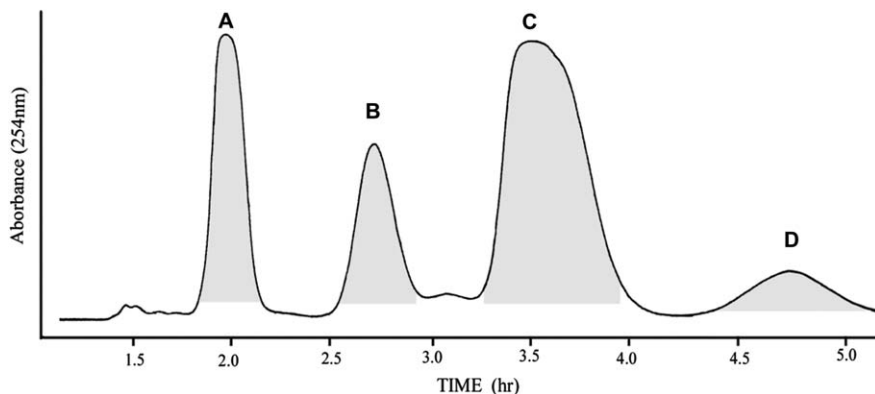


Figure 9.21 HSCCC chromatogram of 30% ethanol effluent. HSCCC conditions: solvent system, ethyl acetate–ethanol–acetic acid–water (4:1:0.25:5, v/v); revolution speed, 800 rpm; flow rate, 1.5 mL/min; sample size, 40 mg; retention of the stationary phase, 34%. A: apigenin-7-*O*-neohesperidoside; B: luteolin-7-*O*-glucoside; C: apigenin-7-*O*-glucoside; D: kaempferol-7-*O*-glucoside. Reprinted from *Journal of Chromatography A*, **1075**, X. Wang, C. Cheng, Q. Sun, F. Li, J. Liu and C. Zheng, Isolation and purification of four flavonoid constituents from the flowers of *Paeonia suffruticosa* by high-speed counter-current chromatography, pp. 127–131, 2005, with permission from Elsevier.¹⁵⁰

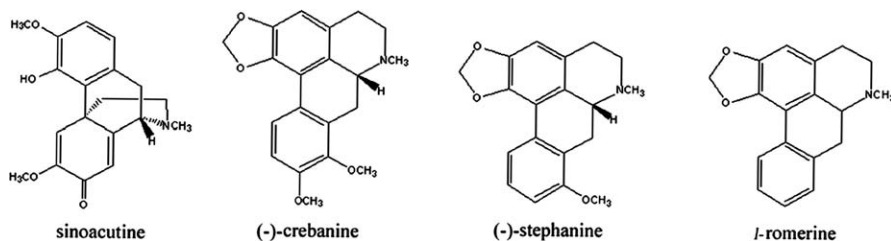


Figure 9.22 The chemical structure of alkaloids from *Stephania kwangsiensis*. Reprinted from *Journal of Chromatography B*, **879**, H. Dong, Y. Zhang, L. Fang, W. Duan, X. Wang and L. Huang, Combinative application of pH-zone-refining and conventional high-speed-counter-current chromatography for preparative separation of alkaloids from *Stephania kwangsiensis*, pp. 945–949, 2011, with permission from Elsevier.¹⁵¹

9.4.5 Isolation of Alkaloids from *Stephania kwangsiensis*

The rhizome of *Stephania kwangsiensis* H. S. Lo (Menispermaceae) is a well-known Chinese herbal medicine; its major active constituents are alkaloids (Figure 9.22). The conventional isolation methods are often tedious, requiring multiple chromatography steps, so pH-zone-refining CCC was combined to conventional HSCCC to separate and purify the alkaloids from this plant.¹⁵¹ First, pH-zone-refining CCC was successfully performed with a two-phase solvent system, *n*-hexane–ethyl acetate–methanol–water (3:7:1:9, v/v), with

10 mM triethylamine in the organic stationary phase and 5 mM hydrochloric acid in the aqueous mobile phase, which resulted in two fractions, one containing 370 mg of sinoacutine with high purity and the second one containing 600 mg of a mixture of three other alkaloids, from 2.0 g of the crude extract (Figure 9.23). The mixture was separated by conventional HSCCC with the two-phase solvent system composed of *n*-hexane–ethyl acetate–methanol–water (7:3:6:4, v/v), which resulted in 42 mg of (–)-crebanine, 50 mg of (–)-stephanine and 30 mg of *l*-romerine, with high purities, from 150 mg of mixture of alkaloids (Figure 9.24). The roadmap of extraction and separation of compounds from *S. kwangsiensis* is shown in Figure 9.25. The results from this study indicate that combining pH-zone-refining CCC and conventional HSCCC can improve the separation efficiency of alkaloids from plants.

9.4.6 Isolation of Psoralen and Isopsoralen from *Psoralea corylitolia*

Psoralea corylitolia L. (Buguzhi in Chinese) is one of the most popular traditional Chinese medicines. This herb is used to treat a wide variety of diseases, including impotence, seminal emission, cold pain in the loins and knees, frequent urination and enuresis due to kidney deficiency. The major active constituents of *P. corylitolia* are psoralen and isopsoralen (Figure 9.26). Wang *et al.*¹⁵² reported a simple and efficient method to extract and purify psoralen and isopsoralen from *P. corylitolia*. The compounds were first extracted by supercritical CO₂. The optimized SFE conditions were pressure of 26 MPa, temperature of 60 °C and sample particle size of 40–60 mesh. The yield of the preparative SFE was 9.1% and the combined yield of psoralen and isopsoralen was 2.5 mg/g of dry seeds. A crude sample of 150 mg was separated by preparative HSCCC with a two-phase solvent system composed by *n*-hexane–ethyl acetate–methanol–water (1:0.7:1:0.8, v/v) (Figure 9.27); the fractions were analysed by HPLC. The separation produced 39 mg and 40 mg of psoralen and isopsoralen with purities of 99.2% and 99.0%, respectively, in 180 min.

Combining SFE and HSCCC shows great advantages in enriching and isolating psoralens. The results of this study clearly demonstrate that combining SFE and HSCCC can provide a rapid and efficient method for the separation of low polar compounds from natural sources.

9.4.7 Isolation of Six Isoflavones from *Semen sojae praeparatum* by Prep-HPLC

Semen sojae praeparatum is a famous traditional Chinese medicine, which has been used to decrease myocardial oxygen consumption, to improve micro-circulation and to cure tumours and osteoporosis. Pharmacological tests revealed that the major bioactive constituents of *S. sojae praeparatum* are isoflavones. Qu *et al.*¹⁵³ isolated six isoflavones from *S. sojae praeparatum* by prep-HPLC: genistein, genistin, daidzein, daidzin, glycitein and glycitin. The

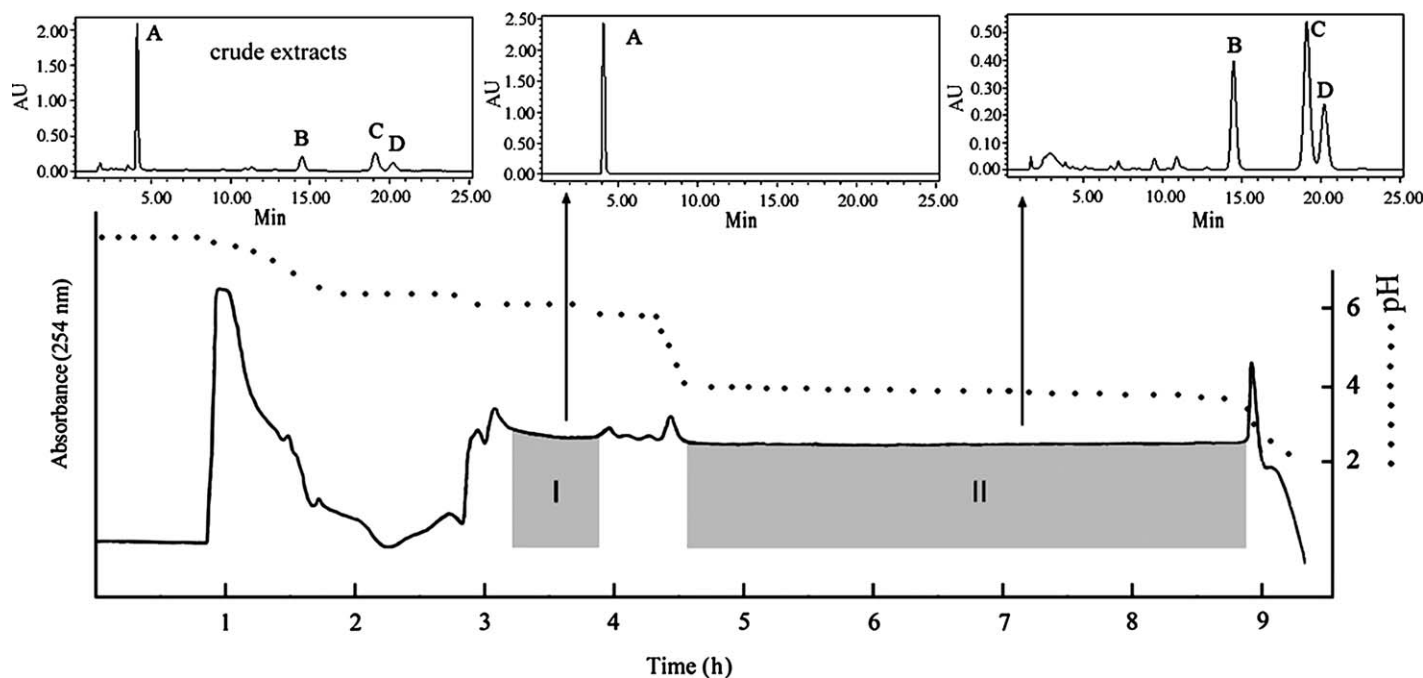


Figure 9.23 pH-Zone-refining CCC chromatogram of the *Stephania kwangsiensis* extract. HSCCC conditions: solvent system: *n*-hexane–ethyl acetate–methanol–water (3:7:1:9, v/v), 10 mM TEA in the upper organic stationary phase and 5 mM HCl in the lower aqueous phase; sample size: 2.0 g; UV detection wavelength: 254 nm. A: sinoacutine; B: (–)-crebanine; C: (–)-stephanine; D: *l*-romerine.

Reprinted from *Journal of Chromatography B*, **879**, H. Dong, Y. Zhang, L. Fang, W. Duan, X. Wang and L. Huang, Combinative application of pH-zone-refining and conventional high-speed-counter-current chromatography for preparative separation of alkaloids from *Stephania kwangsiensis*, pp. 945–949, 2011, with permission from Elsevier.¹⁵¹

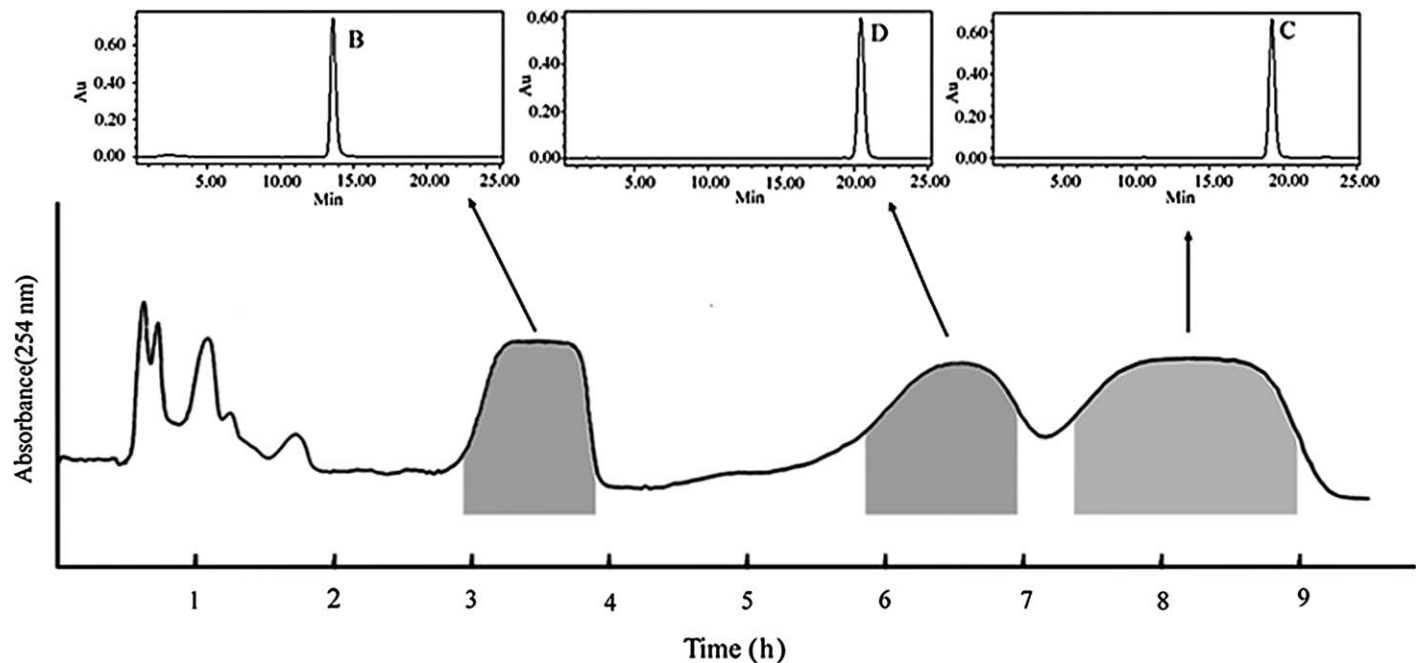


Figure 9.24 Conventional HSCCC separation of fraction II. HSCCC conditions: solvent system: *n*-hexane–ethyl acetate–methanol–water (7:3:6:4, v/v); revolution speed: 800 rpm; flow rate: 1.5 mL/min; sample size: 150 mg; UV detection wavelength: 254 nm; retention of stationary phase: 80%. Reprinted from *Journal of Chromatography B*, **879**, H. Dong, Y. Zhang, L. Fang, W. Duan, X. Wang and L. Huang, Combinative application of pH-zone-refining and conventional high-speed-counter-current chromatography for preparative separation of alkaloids from *Stephania kwangsiensis*, pp. 945–949, 2011, with permission from Elsevier.¹⁵¹

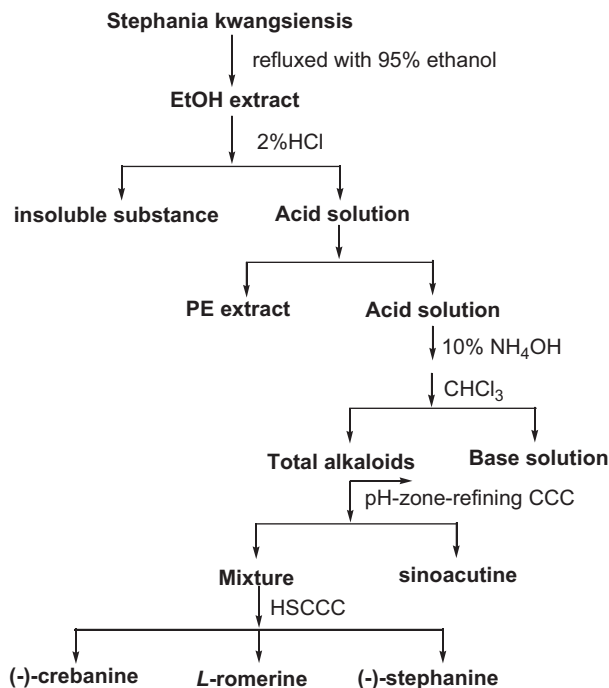


Figure 9.25 The roadmap of extraction and separation of *Stephania kwangsiensis*.

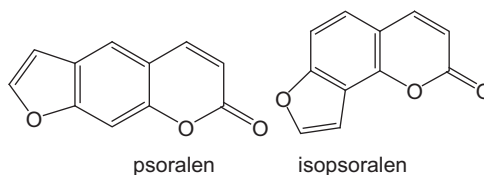


Figure 9.26 Chemical structures of psoralen and isopsoralen. Reprinted from *Journal of Chromatography A*, **1055**, X. Wang, Y. Wang, J. Yuan, Q. Sun, J. Liu and C. Zheng, An efficient new method for extraction, separation and purification of psoralen and isopsoralen from Fructus Psoraleae by supercritical fluid extraction and high-speed counter-current chromatography, pp. 135–140, 2004, with permission from Elsevier.¹⁵²

extraction and separation process is shown in Figure 9.28. After removing the oil by supercritical fluid extraction (SFE), 400 g of the powder was refluxed with 2000 mL of 70% aq. EtOH. The extracts were concentrated to remove EtOH and to remain dissolved in water; the aqueous phase was charged on 250 g D101 macroporous resin. The column was eluted with 1200 mL of water and then with 1650 mL of 40%, 1800 mL of 75% and 750 mL of 95% EtOH to obtain two fractions. Each fraction was purified by prep-HPLC using YWG C₁₈ (20.0 mm × 250 mm, i.d. 10 μm) (Hanbon Science and Technology CO., Ltd.

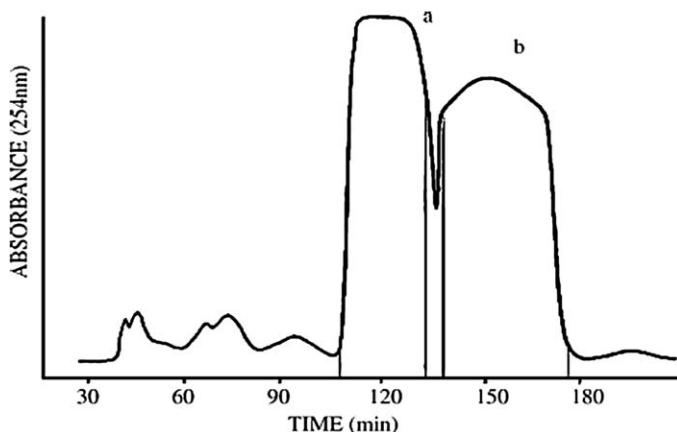


Figure 9.27 Chromatogram of the *Psoralea corylifolia* crude extract by preparative HSCCC. HSCCC conditions: column, multilayer coil of 1.6 mm i.d. PTFE tube with a total capacity of 230 mL; revolution speed: 800 rpm; solvent system: *n*-hexane–ethyl acetate–methanol–water (1:0.7:1:0.8, v/v); flow-rate: 1.5 mL/min; detection: 254 nm; sample size: 160 mg; injection volume: 10 mL; retention of the stationary phase: 70.0%; a: psoralen; b: isopsoralen.

Reprinted from *Journal of Chromatography A*, **1055**, X. Wang, Y. Wang, J. Yuan, Q. Sun, J. Liu and C. Zheng, An efficient new method for extraction, separation and purification of psoralen and isopsoralen from Fructus Psoraleae by supercritical fluid extraction and high-speed counter-current chromatography, pp. 135–140, 2004, with permission from Elsevier.¹⁵²

Jiang Su Province, China) column. The conditions were as follows: mobile phases, MeCN–water–AcOH (25:75:2 and 35:65:2 v/v/v); flow rates, 3.5 and 4.5 mL/min; detection, 260 nm.

9.4.8 Isolation of Anthocyanins from Eggplant

Anthocyanins are natural pigments that can be found in many flowers and fruits. Compared to other synthetic food colorants, anthocyanins do not present any apparent adverse effects on human health. Research has shown that eggplants contain large amount of anthocyanins. Finorini¹⁵⁴ separated two main anthocyanins from eggplant by prep-HPLC. Eggplant skin (87 g) was extracted with 250 mL of formic acid in water (5%, v/v) overnight at 4 °C. After filtration, 10 g of the extract was adsorbed on a Sep-Pak Vac C₁₈ cartridge (Waters, Milford, MA, USA). Figure 9.29 shows the chromatogram of a raw eggplant extract. At first, the column was washed with 20 mL of HCl (pH = 1), and then washed with 25 mL of methanol (containing 0.1% HCl). As a result, 0.48 g of pigment mixture was obtained. A portion of 5 mg of the pigment mixture was subjected to the prep-HPLC using a Bondapark C₁₈ guard insert column (250 mm × 10 mm i.d.). The chromatography was run as follows: 0–4 min, 25% methanol; 4–25 min, 25–35% methanol; 25–30 min,

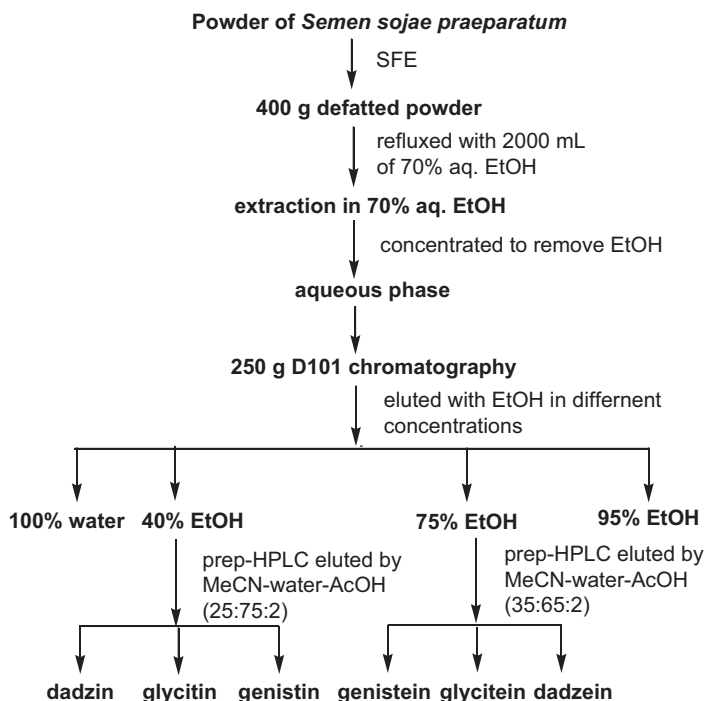


Figure 9.28 The roadmap of extraction and separation of *Semen sojæ praeeparatum*.

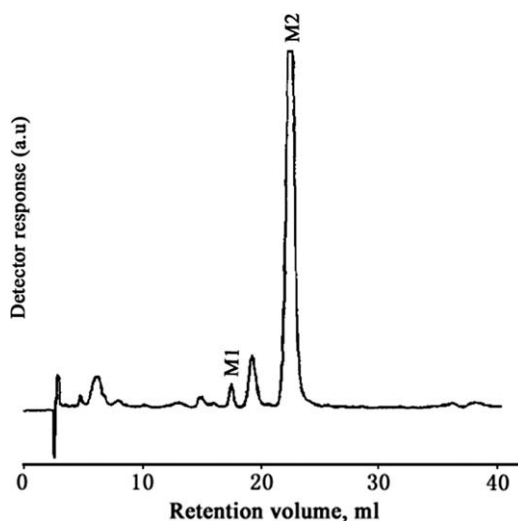


Figure 9.29 Chromatograms obtained with the analytical column for eggplant extract. M1: delphinidin-3,5-diglucoside acylated; M2: delphinidin-3-arabinoside. Reprinted from *Journal of Chromatography A*, **692**, M. Fiorini, Preparative high-performance liquid chromatography for the purification of natural anthocyanins, pp. 213–219, 1995, with permission from Elsevier.¹⁵⁴

35–50% methanol; 30–35 min, 50% methanol. At last, two fractions including delphinidin-3,5-diglucoside acylated and delphinidin-3-arabinoside with high purities were obtained (Figure 9.30). The author also isolated other types of anthocyanins from strawberry, elderberry and radish using prep-HPLC.¹⁵⁴

9.4.9 Isolation and Purification of Flavonoid and Isoflavonoid from *Sophora japonica*

Gel chromatography has been applied to the isolation and purification of bioactive compounds. Qi *et al.*¹⁵⁵ established a method for the isolation and

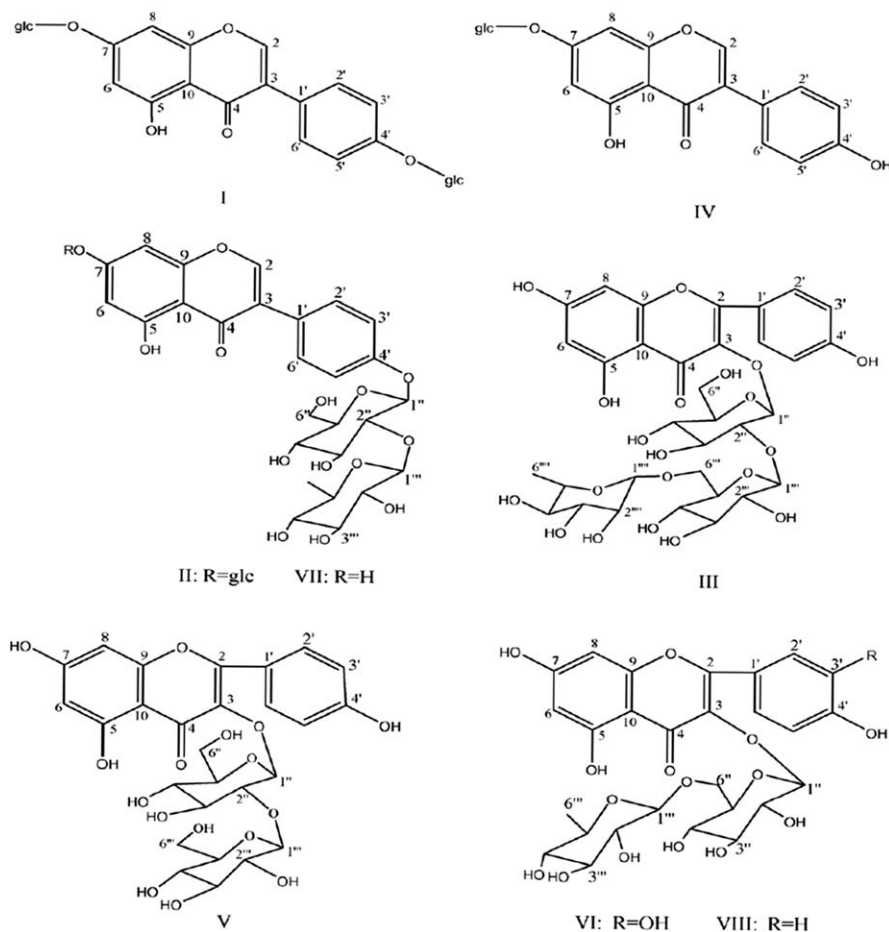


Figure 9.30 Chemical structure of target compounds from *Sophora japonica*. Reprinted from *Journal of Chromatography A*, **1140**, Y. Qi, A. Sun, R. Liu, Z. Meng and H. Xie, Isolation and purification of flavonoid and isoflavonoid compounds from the pericarp of *Sophora japonica* L. by adsorption chromatography on 12% cross-linked agarose gel media, pp. 219–224, 2007, with permission from Elsevier.¹⁵⁵

purification of flavonoid and isoflavonoid compounds (Figure 9.31) in extracts of the pericarp of *S. japonica* using adsorption chromatography on the 12% cross-linked agarose gel Superose 12. Twenty five grams of the powder were extracted with 200 mL of 95% ethanol by ultrasonication for three times. The crude extract yield was 7.2 g. It was then loaded in a D-101 macroporous resin column (35 cm × 3.4 cm, volume of 170 mL), which was successively eluted with 3400 mL distilled water, 3400 mL 20% ethanol and 3400 mL 40% ethanol. The 20% ethanol effluent was collected and evaporated to dryness at 60 °C under

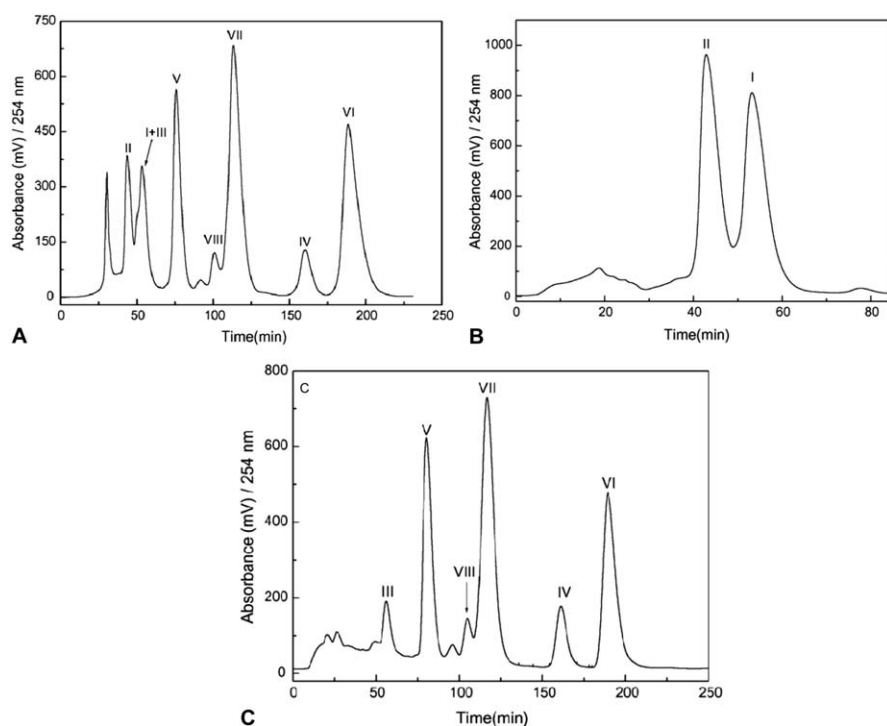


Figure 9.31 Chromatograms on Superose-12 column. (A) Crude sample; (B) sample A; (C) sample B. Mobile phase: 40% methanol; flow rate: 0.5 mL/min; detection wavelength: 254 nm. I: genistein-7,4'-di- O - β -D-glucoside; II: genistein-7- O - β -D-glucopyranoside-4'- O -[(α -L-rhamnopyranosyl)-(1-2)- β -D-glucopyranoside]; III: kaempferol 3- O - α -L-rhamnopyranosyl-(1-6)- β -D-glucopyranosyl-(1-2)- β -D-glucopyranoside; IV: genistein-7- O - β -D-glucopyranoside; V: kaempferol-3- O - β -D-sophoroside; VI: quercetin-3- O - β -L-rhamnopyranosyl-(1-6)- β -D-glucopyranoside; VII: genistein-4'- β -L-rhamnopyranosyl-(1-2)- α -D-glucopyranoside; VIII: kaempferol-3- O - β -L-rhamnopyranosyl-(1-6)- β -D-glucopyranoside. Reprinted from *Journal of Chromatography A*, **1140**, Y. Qi, A. Sun, R. Liu, Z. Meng and H. Xie, Isolation and purification of flavonoid and isoflavonoid compounds from the pericarp of *Sophora japonica* L. by adsorption chromatography on 12% cross-linked agarose gel media, pp. 219–224, 2007, with permission from Elsevier.¹⁵⁵

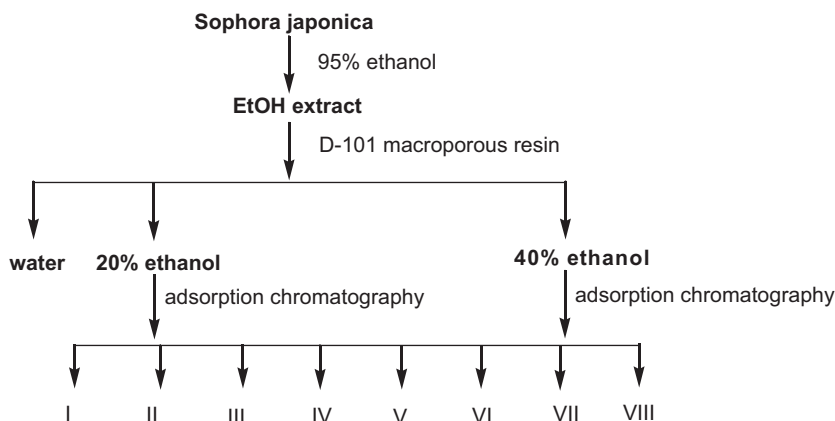


Figure 9.32 The roadmap of extraction and separation of *Sophora japonica*. I: genistein-7,4'-di-*O*- β -D-glucoside; II: genistein-7-*O*- β -D-glucopyranoside-4'-*O*-[(α -L-rhamnopyranosyl)-(1 $\rightarrow$ 2)- β -D-glucopyranoside]; III: kaempferol-3-*O*- α -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranoside; IV: genistein-7-*O*- β -D-glucopyranoside; V: kaempferol-3-*O*- β -D-sophoroside; VI: quercetin-3-*O*- β -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside; VII: genistein-4'- β -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -D-glucopyranoside; VIII: kaempferol-3-*O*- β -L-rhamnopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside.

vacuum and about 1.6 g powder was obtained (sample A). The 40% ethanol effluent was collected, evaporated to dryness and about 3.0 g powder was obtained (sample B). Samples A and B were then separated by adsorption chromatography on Superose 12 with 40% methanol as the mobile phase. As shown in Figure 9.31, eight compounds, including four types of flavonoids and four kinds of isoflavonoids, were obtained. The retention of the flavonoids and isoflavonoids in Superose 12 is based on a mixture of hydrogen bonding and hydrophobic interactions between the hydroxyl groups of aglycone and the residues of the cross-linking reagents used in the manufacturing process of Superose 12. The roadmap of extraction and separation is shown in Figure 9.32.

9.5 Conclusions

Natural products are playing an increasingly important role in the pharmaceutical, cosmetic, flavour and dietary supplement industries nowadays. In recent years, with the development of a variety of modern separation techniques such as prep-HPLC, HSCCC, SFC, etc., a large amount of new compounds with new skeleton structures and high bioactivities have been found and utilized. At the same time, what we specially noticed is that it is very difficult to obtain pure compounds from the complex natural sources with a single method. The best approach is usually to employ a combination of

various separation techniques and use multi-step isolation procedures. Meanwhile, a good separation strategy is also necessary for the efficient purification of natural products. It is of great importance to further study isolation and purification technologies and their application to the research of natural products. With the development of modern separation techniques, more and more natural compounds will be investigated and applied in pharmaceutical, cosmetic, flavour and dietary supplement industries.

References

1. J. Li and H. A. Chase, *Biotechnol. Lett.*, 2010, **32**, 601.
2. M. S. Reisch, *Chem. Eng. New*, 2001, **79**, 23.
3. A. Marston and K. Hostettmann, *Nat. Prod. Rep.*, 1991, **8**, 391.
4. O. Stichler, *Nat. Prod. Rep.*, 2008, **25**, 517.
5. A. L. Harvey, *Drug Discovery Today*, 2008, **13**, 894.
6. J. Li and H. A. Chase, *Biotechnol. Lett.*, 2010, **32**, 601.
7. L. H. Yin, Y. N. Li, B. N. Lu, Y. J. Jia and J. Y. Peng, *Sep. Pur. Rev.*, 2010, **39**, 33.
8. S. D. Sarker, Z. Latif and A. I. Gray, in *Natural Products Isolation*, ed. J. M. Walker, Methods in Biotechnology, Humana Press, Totowa, NJ, 2005.
9. L. Z. Lin, H. F. Zhao, Y. Dong, B. Yang and M. M. Zhao, *Food Chem.*, 2012, **130**, 417.
10. L. H. Yin, Y. W. Xu, Y. Qi, X. Han, L. N. Xu, J. Y. Peng and C. K. Sun, *Chem. Eng. J.*, 2010, **165**, 281.
11. Z. Y. Zhao, L. L. Dong, Y. L. Wu and F. Lin, *Food Bioprod. Process.*, 2011, **89**, 266.
12. G. P. Pi, P. Ren, J. M. Yu, R. F. Shi, Z. Yuan and C. H. Wang, *J. Chromatogr. A*, 2008, **1192**, 17.
13. Z. F. Wei, Y. G. Zu, Y. J. Fu, W. Wang, C. J. Zhao and M. Luo, *Chem. Eng. J.*, 2011, **172**, 864.
14. C. Y. Chen, Y. G. Zu, Y. J. Fu, M. Luo, C. J. Zhao, W. Wang, B. S. Zhao, J. Li and T. Efferth, *Biochem. Eng. J.*, 2011, **58**, 84.
15. E. Nie, J. G. Luo, X. B. Wang, X. Wan and L. Y. Kong, *Sep. Pur. Technol.*, 2009, **65**, 243.
16. J. Li, Z. Chen and D. Di, *Food Chem.*, 2012, **132**, 268.
17. L. Wang, L.-H. Gong, C.-J. Chen, H.-B. Han and H.-H. Li, *Food Chem.*, 2012, **131**, 1539.
18. M. L. Soto, A. Moure, H. Dominguez and J. C. Parajo, *J. Food Eng.*, 2011, **105**, 1.
19. M. Pinelo, G. Jonsson and A. S. Meyer, *Sep. Purif. Technol.*, 2009, **70**, 1.
20. M. Cooke and C. F. Poole (eds), *Encyclopedia of Separation Science*, Academic Press, Michael Cooke, 2000.
21. R. W. Baker, *Membrane Technology and Applications*, 3rd edn., John Wiley & Sons, Hoboken, NJ, 2012.

22. Z. H. Xu, L. Li, F. W. Wu, S. J. Tan and Z. B. Zhang, *J. Membr. Sci.*, 2005, **246**, 137.
23. M. J. Playne and R. Crittenden, *Bull Int. Dairy Fed.*, 1996, **313**, 10.
24. T. Yu and H. Qian, *J. Wuxi University of Light Industry*, 2004, **23**, 55.
25. W. Li, J. Li, T. Chen and C. Chen, *J. Membr. Sci.*, 2004, **245**, 123.
26. E. Sjöman, M. Mänttari, M. Nyström, H. Koivikko and H. Heikkilä, *J. Membr. Sci.*, 2008, **310**, 268.
27. M. Feins and K. K. Sirkar, *J. Membr. Sci.*, 2005, **246**, 137.
28. C. Denis, A. Massé, J. Fleurence and P. Jaouen, *Sep. Pur. Technol.*, 2009, **69**, 37.
29. T. Kamada, M. Nakajima, H. Nabetani and N. Saglam, *Eur Food Res. Technol.*, 2002, **214**, 435.
30. A. Muller, G. Daufin and B. Chaufer, *J. Membr. Sci.*, 1999, **153**, 9.
31. R. M. Shen, B. Hu, Y. Zhang, Y. Xia, Y. Chen and D. J. Wan, *Chem. Bioeng.*, 2008, **25**, 58.
32. L. D. Wang, P. Cui, X. Lu, L. Tong and L. L. Yao, *J. Shandong University (Engineering Science)*, 2011, **41**, 154.
33. K. C. Yi, P. X. Yue and H. Li, *J. Beijing Technology and Business University*, 2010, **28**, 23.
34. Y. Y. Li and Y. X. Gao, *Chin. Food Sci.*, 2006, **27**, 113.
35. D. M. Xue, B. N. Wang, H. W. Liu, Q. Z. Feng, X. Q. Xu and Z. Q. Pei, *Technol. Water Treat.*, 2002, **28**, 284.
36. M. C. Hennion, *J. Chromatogr. A*, 1999, **856**, 3.
37. C. Y. He, Y. Y. Long, J. L. Pan, K. A. Li and F. Liu, *J. Biochem. Biophys. Methods*, 2007, **70**, 133.
38. L. Vidal, M. L. Riekkolaa and A. Canals, *Anal. Chim. Acta*, 2012, **715**, 19.
39. F. G. Tamayo, E. Turiel and A. Martín-Esteban, *J. Chromatogr. A*, 2007, **1152**, 32.
40. M. L. Ravelo-Pérez, V. A. Herrera-Herrera, J. Hernández-Borges and M. Á. Rodríguez-Delgado, *J. Chromatogr. A*, 2010, **1217**, 2618.
41. M. Tian, H. Yan and K. H. Row, *J. Chromatogr. B*, 2009, **877**, 738.
42. F. F. Chen, R. Wang and Y. P. Shi, *Talanta*, 2012, **89**, 505.
43. G. Z. Fang, J. X. He and S. Wang, *J. Chromatogr. A*, 2006, **1127**, 12.
44. S. T. Simmons, Commercial Applicability of an Innovative Anthocyanin Purification Technique, Utilizing Mixed-Mode Solid-Phase Extraction, Master Thesis, Ohio State University, *Food Sci. Nutr.*, 2012. http://etd.ohiolink.edu/view.cgi?acc_num=osu1332267706. Last accessed on 02/21/2013.
45. Y. T. Yue, X. F. Zhang, M. H. Yang, Z. Ou-Yang and H. B. Liu, *Chromatographia*, 2010, **72**, 551.
46. L. Tong, P. Li, Y. Wang and K. Zhu, *Chemosph.*, 2009, **74**, 1090.
47. R. Loos, G. Locoro, S. Comero and S. Contini, *Water Res.*, 2010, **44**, 2325.
48. A. Wilkowska and M. Biziuk, *Food Chem.*, 2011, **125**, 803.
49. D. Harvey, *Modern Analytical Chemistry*, McGraw Hill, 2000.

50. C. S. Wu, *Handbook of Size Exclusion Chromatography and Related Techniques*, Marcel Dekker, New York, 2003.
51. S. J. Uddin, J. A. Shilpi, M. Middleton, M. Byres, M. Shoeb, L. Nahar and S. D. Sarker, *Nat. Prod. Res.*, 2007, **21**, 663.
52. A. Braithwaite and F. J. Smith (eds.), *Chromatographic Methods*, 5th edn., Kluwer Academic Publishers, 1999.
53. J. Turnock, S. Cowan, A. A. Watson, B. Bartholomew, C. Bright, Z. Latif, S. D. Sarker and R. J. Nash, *Biochem. Syst. Ecol.*, 2001, **29**, 209.
54. E. M. Murphy, L. Nahar, M. Byres, M. Shoeb, M. Siakalima, M. Rahman, A. I. Gray and S. D. Sarker, *Biochem. Syst. Ecol.*, 2004, **32**, 203.
55. Z. Latif and S. D. Sarker, in *Natural Products Isolation*, ed. S. D. Sarker and L. Nahar, 2012, Lavoisier.
56. A. Marston, *Phytochemistry*, 2007, **68**, 2785.
57. L. Qu, G. Fan, J. Peng and H. Mi, *Fitoterapia*, 2007, **78**, 200.
58. M. Fiorini, *J. Chromatogr. A*, 1995, **692**, 213.
59. C. X. Zhao and C. H. He, *J. Sep. Sci.*, 2006, **29**, 1630.
60. S. Yao, Y. Li and L. Y. Kong, *J. Chromatogr. A*, 2006, **1115**, 64.
61. Y. Ito and R. L. Bowman, *Science*, 1970, **167**, 281.
62. A. Marston and K. Hostettmann, *J. Chromatogr. A*, 2006, **1112**, 181.
63. L. Fang, Y. Liu, B. Yang, X. Wang and L. Huang, *J. Sep. Sci.*, 2011, **34**, 2545.
64. I. A. Sutherland, J. Muytjens, M. Prins and P. L. Wood, *J. Liq. Chromatogr. Rel. Technol.*, 2000, **23**, 2259.
65. Y. Ito, J. L. Sandlin and W. G. Bowers, *J. Chromatogr. A*, 1982, **244**, 247.
66. Y. Ito, in *High-Speed Countercurrent Chromatography*, ed. Y. Ito and W. D. Conway, Vol. 132, Chemical Analysis Series, New York, Wiley Interscience, Chapter 1, 1996.
67. Y. Ito, *J. Chromatogr. A*, 2005, **1065**, 145.
68. Y. Ito, in *Countercurrent Chromatography, Theory and Practice*, ed. N. B. Mandava and Y. Ito, Marcel Dekker, New York, Chapter 4, 1988.
69. H. Li and F. Chen, in *Bioactive Natural Products – Detection, Isolation, and Structural Determination*, ed. R. J. Molyneux and S. M. Colegate, CRC Press, 2007.
70. F. W. Li, Y. L. Lin, X. Wang, Y. L. Geng and D. J. Wang, *Sep. Pur. Technol.*, **64** 304.
71. X. OuYang, M. Jin and C. He, *Sep. Pur. Technol.*, 2007, **56**, 319.
72. S. Tong, J. Yan and J. Lou, *J. Liq. Chromatogr. Rel. Technol.*, 2005, **28**, 2979.
73. R. Liu, X. Chu, A. Sun and L. Kong, *J. Chromatogr. A*, 2005, **1074**, 139.
74. Q. Tang, C. Yang, W. Ye, J. Liu and S. Zhao, *J. Chromatogr. A*, 2007, **1144**, 203.
75. J. Ling, G. Zhang, Z. Cui and C. Zhang, *J. Chromatogr. A*, 2007, **1145**, 123.
76. F. Yang, T. Zhang, R. Zhang and Y. Ito, *J. Chromatogr. A*, 1998, **829**, 137.

77. Y. Yuan, W. Hou, M. Tang, H. Luo, L. Chen, Y. Guan and I. Sutherland, *Chromatographia*, 2008, **68**, 885.
78. S. Shi, Y. Zhang, Y. Zhao and K. Huang, *J. Sep. Sci.*, 2008, **31**, 683.
79. J. Peng, G. Fan and Y. Wu, *J. Liq. Chromatogr. Rel. Technol.*, 2006, **29**, 1619.
80. Y. Wei, Xie and Y. Ito, *J. Liq. Chromatogr. Rel. Technol.*, 2008, **31**, 443.
81. X. Cao, Y. Tian, T. Zhang, X. Li and Y. Ito, *J. Chromatogr. A*, 1999, **855**, 709.
82. X. Wang, F. Li, H. Zhang, Y. Geng, J. Yuan and T. Jiang, *J. Chromatogr. A*, 2005, **1090**, 188.
83. W. Xiao, L. Han and B. Shi, *J. Chromatogr. B*, 2009, **877**, 697.
84. Q. Du, G. Jerz and P. Winterhalter, *J. Chromatogr. A*, 2004, **1045**, 59.
85. H. Li and F. Chen, *J. Chromatogr. A*, 2001, **925**, 109.
86. L. Feng, H. Ji, H. Gu, D. Wang, J. Cui, R. Liu and J. Zhai, *Chromatographia*, 2009, **70**, 1197.
87. R. Liu, Y. Lu, T. Wu and Y. Pan, *Chromatographia*, 2008, **68**, 95.
88. Y. Xie, Y. Liang, H. Chen, T. Zhang and Y. Ito, *J. Liq. Chromatogr. Rel. Technol.*, 2007, **30**, 1475.
89. S. Tong and J. Yan, *J. Chromatogr. A*, 2007, **1176**, 163.
90. S. Yao, Y. Li and L. Kong, *J. Chromatogr. A*, 2006, **1115**, 64.
91. Z. Wang, J. Wang and Y. Sun, *et al, Sep. Pur. Technol.*, 2008, **63**, 721.
92. Z. Hou, D. Xu, S. Yao, J. Luo and L. Kong, *J. Chromatogr. B*, 2009, **877**, 2571.
93. X. Wang, Y. Wang, J. Yuan, Q. Sun, J. Liu and C. Zheng, *J. Chromatogr. A*, 2004, **1055**, 135.
94. Y. Wei and Y. Ito, *J. Chromatogr. A*, 2006, **1115**, 112.
95. F. Yang, T. Zhang, Q. Liu, G. Xu, Y. Zhang, S. Zhang and Y. Ito, *J. Chromatogr. A*, 2000, **883**, 67.
96. T. Huang, Y. Shen and P. Shen, *J. Liq. Chromatogr. Rel. Technol.*, 2005, **28**, 2383.
97. X. Wang, F. Li, Q. Sun, J. Yuan, T. Jiang and C. Zheng, *J. Chromatogr. A*, 2005, **1063**, 247.
98. X. Wang, Y. Wang, Y. Geng, F. Li and C. Zheng, *J. Chromatogr. A*, 2004, **1036**, 171.
99. Y. Sun, H. Zhu, J. Wang, Z. Liu and J. Bi, *J. Chromatogr. B*, 2009, **877**, 733.
100. C. Zhao and C. He, *J. Sep. Sci.*, 2006, **29**, 1630.
101. F. Yang, Q. Ou and W. Yu, *J. Liq. Chromatogr. Rel. Technol.*, 1995, **18**, 395.
102. Q. Du and J. Yuan, *J. Liq. Chromatogr. Rel. Technol.*, 2005, **28**, 1717.
103. X. Shi, X. Wang and D. Wang, *et al, Sep. Sci. Tech.*, 2009, **44**, 712.
104. A. Peng, R. Li and J. Hu, *et al, J. Chromatogr. A*, 2008, **1200**, 129.
105. A. Li, A. Sun and R. Liu, *J. Chromatogr. A*, 2005, **1076**, 193.
106. T. Huang, P. Shen and Y. Shen, *J. Chromatogr. A*, 2005, **1066**, 239.
107. T. Zhou, G. Fan, Z. Hong, Y. Chai and Y. Wu, *J. Chromatogr. A*, 2005, **1100**, 76.

108. C. Ma, G. Li, D. Zhang, K. Liu and X. Fan, *J. Chromatogr. A*, 2005, **1078**, 188.
109. L. Lei, F. Yang, T. Zhang, P. Tu, L. Wu and Y. Ito, *J. Chromatogr. A*, 2001, **912**, 181.
110. H. Li and F. Chen, *J. Chromatogr. A*, 2001, **932**, 91.
111. L. Yin, L. Xu, X. Wang, B. Lu, Y. Liu and J. Peng, *Chromatographia*, 2010, **71**, 15.
112. Y. Xu, X. Han, D. Dong, L. Xu, Y. Qi, J. Peng and L. Zhan, *J. Sep. Sci.*, 2008, **31**, 3638.
113. J. Wang, H. Bai, C. Liu and L. Li, *Chromatographia*, 2010, **71**, 267.
114. Q. Du, G. Jerz, R. Waibel and P. Winterhalter, *J. Chromatogr. A*, 2003, **1008**, 173.
115. B. Diallo, R. Vanhaelen-Fastré and M. Vanhaelen, *J. Chromatogr. A*, 1991, **558**, 446.
116. A. Berthod (ed.), *Countercurrent Chromatography – the Support – Free Liquid Stationary Phase*, Elsevier, Amsterdam, 2002.
117. T. Zhang and X. Wang, *High-speed Chromatography Technology*, China Chemical Press, 2011.
118. Y. Ito, C. O. Okunji, M. M. Iwu and P. L. Simth, *J. Liq. Chromatogr. Relat. Technol.*, 2005, **28**, 775.
119. J. H. Renault, J. M. Nuzillard, G. L. Crouérou, P. Thépenoer, M. Zèches-Hanrot and L. L. Men-Olivier, *J. Chromatogr. A*, 1999, **849**, 421.
120. X. Wang, Y. Geng, F. Li, X. Shi and J. Liu, *J. Chromatogr. A*, 2006, **1115**, 267.
121. A. Toribio, E. Delannay, B. Richard, K. Plé, M. Zèches-Hanrot, J. M. Nuzillard and J. H. Renault, *J. Chromatogr. A*, 2007, **1140**, 101.
122. F. Q. Yang and Y. Ito, *J. Chromatogr. A*, 2001, **923**, 281.
123. X. Wang, J. Liu, Y. Geng, D. Wang, H. Dong and T. Zhang, *J. Sep. Sci.*, 2010, **33**, 539.
124. Z. J. Zheng, M. L. Wang, D. J. Wang, W. J. Duan, X. Wang and C. C. Zheng, *J. Chromatogr. B*, 2010, **878**, 1647.
125. F. Q. Yang, J. Quan, T. Y. Zhang and Y. Ito, *J. Chromatogr. A*, 1998, **822**, 316.
126. R. L. Hu, X. J. Dai, Y. B. Lu and Y. J. Pan, *J. Chromatogr. B*, 2010, **878**, 1881.
127. X. Wang, Y. L. Geng, D. J. Wang, X. G. Shi and J. H. Liu, *J. Sep. Sci.*, 2008, **31**, 3543.
128. X. Wang, Y. Geng, F. Li, Q. Gao and X. Shi, *J. Chromatogr. A*, 2006, **1103**, 166.
129. X. Wang, Y. Geng, F. Li and X. Shi, *J. Chromatogr. A*, 2006, **1115**, 267.
130. G. Guiochon and A. Tarafder, *J. Chromatogr. A*, 2011, **1218**, 1037.
131. A. Rajendran, O. Kräuchi, M. Mazzotti and M. Morbidelli, *J. Chromatogr. A*, 2005, **1092**, 149.
132. P. Ramírez, M. R. García-Risco, S. Santoyo, F. J. Señoráns, E. Ibáñez and G. Reglero, *J. Pharm. Biomed. Anal.*, 2006, **41**, 1606.

133. Y. M. Choo, A. N. Ma, H. Yahaya, Y. Yamauchi, M. Bounoshita and M. Saito, *J. Am. Oil Chem. Soc.*, 1996, **73**, 523.
134. Z. M. Liu, S. Q. Zhao, R. N. Wang and G. H. Yang, *Chin. J. Chromatogr.*, 1997, **15**, 288.
135. F. Pang, H. Lv, Y. Liu and M. Zhang, *J. Chem. Eng. Chinese Universities*, 2010, **24**, 569.
136. M. Alkio, C. Gonzalez, M. Jäntti and O. Aaltonen, *J. Am. Soil Chem. Soc.*, 2000, **77**, 315.
137. C. W. Jiang, Y. W. Yang, Q. L. Ren and P. D. Wu, *Chin. J. Anal. Chem.*, 2003, **31**, 1337.
138. B. D. Hui, *Chin. Food Sci.*, 2005, **26**, 162.
139. T. A. Berger, *J. Chromatogr. A*, 1997, **785**, 3.
140. Y. Xu and Z. Shi, *Strait Pharm. J.*, 2010, **22**, 49.
141. G. Cui, S. Jia, C. Zhang and S. Wu, *J. Petrochemical Uni.*, 2007, **20**, 12.
142. W. Xu, Y. Huang, J. Qian, O. Sha and Y. Wang, *Sep. Pur. Technol.*, 2005, **41**, 173.
143. H. Qu, K. B. Christensen, X. C. Fretté, F. Tian, J. Rantanen and L. P. Christensen, *Chem. Eng. Technol.*, 2010, **33**, 791.
144. D. Y. Xiong, X. M. Liu, W. G. Li and M. J. Liu, *J. Guangxi University (Nat. Sci. Ed.)*, 2004, **29**, 85.
145. R. Zhang and J. Xing, *Food Sci. Technol.*, 2007, **5**, 187.
146. Y. S. Tu and Z. Q. Shi, *Strait Pharm. J.*, 2010, **22**, 49.
147. Q. Fu, K. Zan, M. B. Zhao, S. X. Zhou, S. P. Shi, Y. Jiang and P. F. Tu, *J. Nat. Prod.*, 2010, **73**, 1234.
148. M. L. Ciavatta, E. Manzo, E. Mollo, C. A. Mattia, C. Tedesco, C. Irace, Y. W. Guo, X. B. Li, G. Cimino and M. Gavagin, *J. Nat. Prod.*, 2011, **74**, 1902.
149. S. H. Lam, C. T. Ruan, P. H. Hsieh, M. J. Su and S. S. Lee, *J. Nat. Prod.*, 2012, **75**, 153.
150. X. Wang, C. Cheng, Q. Sun, F. Li, J. Liu and C. Zheng, *J. Chromatogr. A*, 2005, **1075**, 127.
151. H. J. Dong, Y. Q. Zhang, L. Fang, W. J. Duan, X. Wang and L. Q. Huang, *J. Chromatogr. B*, 2011, **879**, 945.
152. X. Wang, Y. Wang, J. Yuan, Q. Sun, J. Liu and C. Zheng, *J. Chromatogr. A*, 2004, **1055**, 135.
153. L. Qu, G. Fan, J. Peng and H. Mi, *Fitoterapia*, 2007, **78**, 200.
154. M. Fiorini, *J. Chromatogr. A*, 1995, **692**, 213.
155. Y. Qi, A. Sun, R. Liu, Z. Meng and H. Xie, *J. Chromatogr. A*, 2007, **1140**, 219.

CHAPTER 10

Scale-up of Extraction Processes

JULIAN MARTÍNEZ* AND LUIZ PAULO SALES SILVA

School of Food Engineering, University of Campinas (FEA/Unicamp) – Rua Monteiro Lobato 80, Cidade Universitária Zeferino Vaz, 13083-862, Campinas-SP, Brazil

*Email: julian@fea.unicamp.br

10.1 Introduction

The extraction methods presented in the previous chapters have been extensively studied by the scientific community. Hundreds of materials, procedures and process parameters are reported in published books, articles and patents, based on results obtained at laboratory extraction procedures. On the other hand, few results are found for pilot plant scale processes, and even less at the industrial scale. Moreover, the works that explore the relationships between small-scale and large-scale extraction processes are, in most cases, limited to specific raw materials, products and process conditions, which make their generalization unviable. Therefore, the definition of universal scale-up criteria is a really hard task. In this chapter we certainly will not establish scale-up rules valid for every type of extraction. However, if we carefully analyze each process parameter, and how it can affect the extraction yield and kinetics, at least the finding of some possible scale-up procedures can be closer.

We will deal mainly with extraction processes with pressurized solvents (liquid, gaseous or supercritical), exploring the main factors affecting their yield and kinetics, and how they can vary from laboratory to industrial scale. Nevertheless, most of the factors and criteria that we analyze may surely be explored in other types of extraction, such as liquid–liquid and solid–liquid, and others explored in this book.

RSC Green Chemistry No. 21

Natural Product Extraction: Principles and Applications

Edited by Mauricio A. Rostagno and Juliana M. Prado

© The Royal Society of Chemistry 2013

Published by the Royal Society of Chemistry, www.rsc.org

10.2 Fundamental Aspects of Scale-up Operations

10.2.1 What is Scale-up?

Before beginning to explore the factors involved, possibilities and limitations of processes on an industrial scale, it is necessary to state as clearly as possible what we are looking for. This means scale-up should be defined. A vast role of definitions may be given to scale-up. A very simple definition, for example, is the mere choice of industrial equipment from catalogs, which is often the procedure adopted by process industries. However, if the objective is to establish process parameters and conditions, as well as to propose and to project large-scale units, we must restrict ourselves to another definition, which is also simple:

Scale-up is the task of producing an identical, if possible, process result at a larger production rate than the previously accomplished.¹

Here we analyze this definition, part by part.

1. 'The previously accomplished' is a process already known, since it was performed and its results were obtained at analytical, or being more generalist, small scale. Therefore, performing scale-up requires previous information about the process.
2. 'An identical process result' is the information that was achieved at the small-scale process, such as yield, chemical composition and quality of a product. This means that, through scale-up, those same characteristics should be obtained, with none or the least modifications as possible.
3. 'A larger production rate' tells that the same product obtained at small scale must be produced at large scale, but with amount per hour, day or year higher than those achieved at small scale. It can, additionally, be established 'how much' higher those rates should be, which we will call the 'scale-up factor'.

It is important to highlight that we are considering that increasing production rates implies using larger equipment, or even more equipment than in small scale. In both cases the total capacity of the large-scale unit will be higher. Thus, it is necessary to predict the large-scale equipment requirements to increase the production rate as much as desirable. The way these two factors are related is the heart of the scale-up procedure: the scale-up criteria.

A thermodynamic approach can also be given to the scale-up concepts. In classical thermodynamics the properties of a system are divided between extensive and intensive. Extensive properties are those that depend on the system size, such as mass, volume and energy, whilst intensive properties, like concentration, density and viscosity do not depend on the system's size.² Concerning scale-up, the goal is to conserve the values of most 'intensive data' of the process, by increasing the 'extensive data' with appropriate criteria.

In this chapter, we will deal initially with the definition of scale-up given in this section. The reader may claim that it would also be interesting to predict processes at different conditions from those performed at small scale, and we agree with that. An approach considering such issue will be presented in Section 10.4.3.

10.2.1.1 Defining Scale-up in Extraction Processes

Now that we have a clear and general definition of scale-up in process engineering, let us apply it to the specific process we are interested in: extraction. We will discuss scale-up in extraction from solid raw materials, using either liquid or supercritical solvents. In both cases, a typical extraction curve can be obtained at analytical scale, by measuring the extract yield (mass ratio between extract and solid feed) as a function of time, or even of solvent amount used. A general illustration of an extraction curve is given in Figure 10.1.

The scale-up objective is, then, to reproduce exactly the same curve at larger scale, by preserving some intensive extraction parameters used in analytical scale, and increasing other parameters with defined criteria. In extraction processes, the intensive parameters to be preserved can be:

- temperature (T);
- pressure (P) (if using supercritical or pressurized solvents);
- solvent velocity (v);
- Extraction bed shape (length to diameter ratio (L/D)).

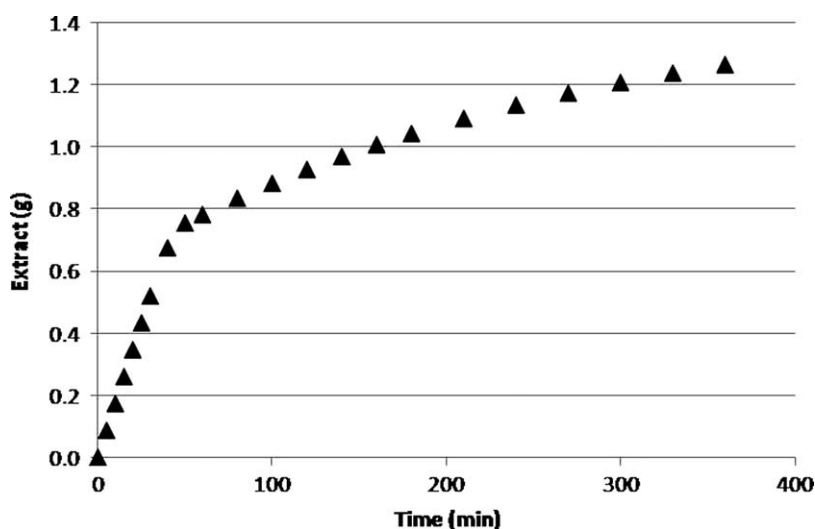


Figure 10.1 Typical extraction curve.

To scale-up the extraction, some extensive parameters that must increase are:

- solid feed (F);
- solvent flow rate (Q);
- extraction bed dimensions – length (L) and diameter (D).

Finally, it is necessary to establish what results are wanted to be reproduced, such as:

- extraction yield (X_0);
- extraction velocity (mass transfer rate);
- physical and chemical properties of the extract (composition, viscosity, density. . .);
- extract quality (flavor, aroma).

Among this information, extraction yield and velocity may be obtained from the curve, and additional tests and measures must be performed to evaluate the others.

10.2.2 Scale-up Criteria

The definition and classification of scale-up criteria can be obtained from the information listed in the previous section. Summarizing, a process can be defined if it is exactly known what is the product and its required properties, and when the operations needed to achieve this product are well-established at a small scale. Based on that, primary and secondary scale-up criteria can be defined.¹ Figure 10.2 illustrates the scheme of primary and secondary scale-up criteria.

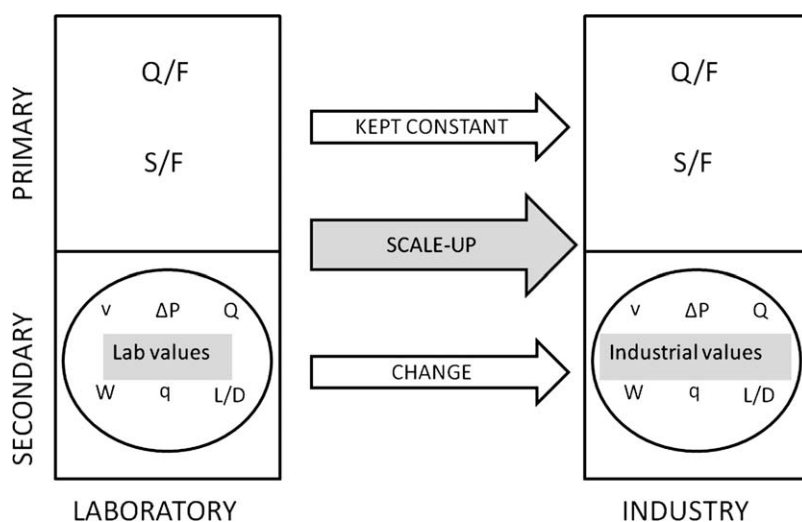


Figure 10.2 Scheme of primary and secondary extraction scale-up criteria: F = solid feed; S = solvent mass; Q = solvent flow rate; v = solvent velocity; ΔP = friction loss; q = heat transfer rate; L = extractor length; D = extractor diameter; W = stirring power.

10.2.2.1 Primary Scale-up Criteria

A primary scale-up criterion is a process parameter, or even a set of process parameters, that leads to the required process result, that is, the product and its properties, independently of the scale adopted. Thus, when performing scale-up, it is necessary to adopt exactly those parameters. If some process parameters are changed there is the risk of modifying the result. Then, the challenge of scale-up is to find which are the parameters that, when conserved, guarantee the same result.

Heading to extraction processes, the desired result is a product with well-defined chemical composition and yield. To more specific applications, parameters such as viscosity, thermal properties, aroma, flavor and diverse biological activities can be included as parts of the expected result. The primary scale-up criteria may be some of those listed in Section 10.2.1.1: temperature, pressure and solvent velocity. Note that it is possible, theoretically, to keep these three values constant from small to large scale. Nevertheless, and unfortunately, this is not possible with all the process data.

10.2.2.2 Secondary Scale-up Criteria

Some physical and mechanical changes may occur in a process due to scale modification. Since such effects cannot be avoided, they should be at least well understood, in a way that the process engineer will be able to control them using the primary scale-up criteria and, consequently, to achieve the required result. The way through which those changes must be conducted is defined as the secondary scale-up criteria. Here are some examples of changes that result from scale-up in extraction.

1. Heat transfer, which is needed to attain and keep the process temperature during the extraction (it should be remembered that temperature is one of the primary criteria). In extraction experiments at analytical scale, temperature may be controlled by simple devices, such as thermostatic baths, electrical resistances, or even by placing the extractor inside an oven. Moreover, since the total volume to be heated is in the range of cubic centimeters, its internal thermal resistance is not a hard hurdle to be overcome. Otherwise, when heating an industrial extraction column that may have over one cubic meter, the low scale heating devices are not appropriate. Hot water or vapor lines are required, and the heating fluid generally flows through a jacket that involves the extractor. Thus, the heat-transfer mechanisms change with scale-up. Indeed, heat loss is expected to be proportionally higher at large scale, because of longer piping, and the impossibility to achieve the same process control as in analytical experiments will result in more heat loss from the extractor to the environment. All these changes in the process characteristics require a detailed study on how the heat transfer can be increased when scaling-up the extraction process. And, of course, such an increase must not be simply proportional to the extractor's increase.

2. Stirring inside the extractor. It is well known that the extraction rate depends on the convective mass transfer from the solid substrate to the solvent, and such convection can be enhanced through different ways of stirring, to produce turbulence in the fluid phase. The mass transfer mechanisms of extraction will be explored in more depth in Section 10.4.1. Even so, it is needless to say that those effects are much more easily achieved in analytical scale, where small extractors may be placed over magnetic stirring devices, or even high-pressure fluids promote the desired turbulence. As well as in heat transfer, to produce equivalent effects in large scale is a hard task, which certainly cannot be achieved using the same tools as in the laboratory. Again, other devices are required, and probably the power increase needed to operate such devices will be proportionally different from the increase in the process scale. Thus, studies on the scale-up criteria of stirring are surely needed.
3. Solvent distribution. Every extraction process is based on the contact between a solvent and a substrate, from where the target compounds (the extracts) are removed. Then, a well designed process must assure that the solvent will contact all the solid feed. Figure 10.3a shows a diagram of an extractor, where the arrows represent the flow strains of the solvent entering it.

Note that some parts of the substrate, located at the bottom near the extractor wall, are not reached by the solvent strains, because of the inefficient distribution at the inlet. If the solvent does not reach all the substrate, part of the soluble compounds will remain unextracted and the yield will be lower than expected. This phenomenon is certainly not desired, so there must be mechanisms to prevent it and to promote uniform distribution of the solvent, as illustrated in Figure 10.3b. The nature of these mechanisms may vary from small to large scale. In the

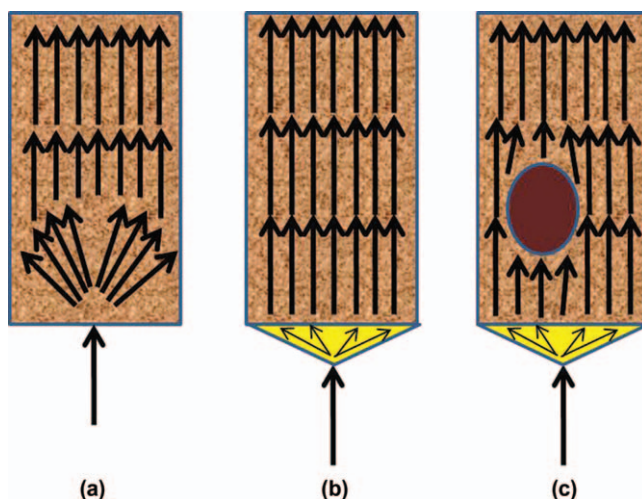


Figure 10.3 Solvent distribution inside an extractor.

laboratory, for example, inert glass spheres can be used at the extractor inlet, creating a region where the solvent may spread uniformly through the entire column radius. But glass spheres are unviable at industrial scale, so other ways to distribute the solvent flow must be adopted, leading even to new accessories. Again, a secondary scale-up criterion is required to establish how the uniform solvent distribution will be scaled-up.

Another problem that may affect the solvent distribution is the placement of the substrate inside the extractor. While at analytical scale such work is performed manually, in an industry this step must be automated. The big challenge of this step is to keep the extraction bed uniform, that is, with the same apparent density and porosity at all its regions along the extractor. The extraction bed's void volume is the space where the solvent will flow, so it is necessary to preserve the porosity in order to keep the same flow pattern from small to large scale. Furthermore, excessive compaction of the substrate must be avoided, because it can lead to the formation of preferential pathways to the solvent, which may not reach all the extractable material, as shown in Figure 10.3c. When the extractor is fed manually, the operator can control this parameter by tuning his own strength, but at industrial scale this can be a critical issue to handle, and certainly another secondary criterion applies.

4. Velocity effects. Mass transfer in packed beds is controlled by diffusion, which may occur inside the solid particles or throughout the solvent, and by convection in the fluid phase. As already stated when agitation was discussed, the degree of convection is intimately related to the movement of the solvent phase. Besides agitation, this movement can be enhanced by increasing the solvent flow rate, which may indirectly result in increased velocity. Classical equations have been widely used to estimate convective mass transfer coefficients using dimensionless numbers, as Reynolds and Schmidt,³ or in a simpler way, as direct function of velocity.⁴ A typical structure of a mass transfer correlation is given in Equation 10.1.

$$\text{Sh} = \frac{k_f d_p}{D} = a + b \text{Re}^c \text{Sc}^d \quad (10.1)$$

where:

$$\text{Re} = \frac{\rho v d_p}{\mu} \quad (10.2)$$

$$\text{Sc} = \frac{\mu}{\rho D} \quad (10.3)$$

Where Sh = Sherwood dimensionless number; k_f = convective mass transfer coefficient (m/s); d_p = particle diameter (m); D = solute diffusion coefficient in the solvent phase (m^2/s); Re = Reynolds dimensionless number; Sc = Schmidt dimensionless number; a , b , c , d = empirical parameters; μ = solvent viscosity (Pa.s); ρ = solvent density (kg/m^3); v = solvent velocity (m/s).

This means that the mass transfer rate of extraction processes is effectively affected by solvent velocity, and such influence should also be considered in scale-up. When moving from analytical to industrial scale, the extractor size will be bigger, and so will be the solvent flow rate. Now, recalling that velocity is the ratio between solvent flow rate and the extractor sectional area (see Equation 10.4), an increase in velocity is reasonable to be expected in scale-up, and it can be necessary to look back to correlations such as Equation (10.1), which is itself a secondary scale-up criterion.

$$v = \frac{\dot{V}}{A} \quad (10.4)$$

where $\dot{V}$ = volumetric solvent flow rate (m^3/s); A = extractor's sectional area (m^2).

5. Friction is present in every system containing continuous flow equipment, pipelines and their accessories. Several methods are classically used by engineers to estimate the pressure decay due to friction effects. The Fanning friction factor, f_f , is related to the pressure loss in pipes as shown in Equation (10.5), for incompressible laminar flow⁵

$$\frac{\Delta P}{\rho g} = 32 \frac{\mu v L}{\rho g d^2} = 2f_f \frac{L}{d} \frac{v^2}{g} \quad (10.5)$$

where ΔP = pressure decay (Pa); g = gravity acceleration (m^2/s); L = pipe length (m); d = pipe diameter (m). Rearranging Equation (10.5) we have an explicit expression for f_f , which is given in Equation (10.6):

$$f_f = 16 \frac{\mu}{\rho v d} = \frac{16}{\text{Re}} \quad (10.6)$$

For turbulent flow, the analysis becomes more complex and the behavior is not linear. In this case, charts can be used to estimate f_f . Anyway, our focus is to observe that, since the pressure decay in pipelines depends on the flow velocity and pipe length and diameter, the changes of v , L and d with scale-up will lead to significant differences in ΔP , affecting the extraction results. Again, it is important to define a scale-up criterion to take these changes into account. The pipeline effects of scale-up are not the unique important issue in ΔP . Industrial-scale equipment also require more complex sets of connections, valves, filters and other accessories that generate friction and, consequently, pressure decay. Thus, the differences in pipeline accessories, as well as in the extractor itself, must also be considered into the secondary scale-up criterion.

6. Flow rate of the extraction solvent will obviously be increased in scale-up, and much of its implications coincide with the issues discussed in the velocity and friction effects. Besides those points, very high solvent flow rates can result in mechanical drag. Thus, possibly some non-soluble material could be removed from the substrate, and the process yield would be higher than that predicted from analytical-scale experiments, although the contrary effect may be observed in concentration of some components

of the extract due to the dilution factor. This effect is hard to evaluate, since drag depends not only on flow rate, but also on the substrate particle characteristics. Even so, this cannot be ignored as a potentially important scale-up criterion.

7. Extraction bed geometry is crucial in industrial extraction processes, and represents a key point for scale-up purposes. When performing analytical extractions in the laboratory, the physical characteristics of the extractor are not important issues. Instead, the main goal is to stabilize all process conditions and to measure the extraction yield as precisely as possible. Except if previously planned, the extractor shape and dimensions are not a reason to worry. Then, many analytical extraction columns have very high length and low diameter. There are some reasons for considering the L/D ratio a fundamental scale-up criterion. In an industrial extraction the obvious goal is to achieve the highest yield as possible in the lowest time, that is, big extraction rates. So the equipment must be handled to enhance all positive effects of the process parameters, and to minimize their negative effects. The negative effects that can be controlled through the extractor's geometry are radial diffusion, free convection and extraction bed compaction. Radial diffusion of solute through the solvent phase will exist since a concentration gradient is developed in this direction, which depends on how the solvent is spread into the extractor. A non-uniform distribution will promote preferential contact of the solvent with certain parts of the substrate, so these regions will have higher solute concentrations than those regions with less contact. Thus, a gradient will be created, and the solute will be forced to move through undesired directions (it should be remembered that it is desired for the solute to move strictly towards the extractor's outlet). Inadequate compaction of the extraction bed also leads to gradients in other directions than axial. The radial diffusion can be minimized by limiting the extractor's diameter-to-length ratio. Short length beds are definitely not recommended, as seen in Figure 10.4a.

On the other hand, very long extractors may lead to two other problems. First, friction losses will be enhanced. Moreover, as extractors are usually placed vertically, it is important to analyze how gravity can act. In a vertical extractor where the solvent flows upwards, as long as the solvent extracts the soluble compounds, the extract concentration increases and so does the fluid phase density. Thus, a density gradient is developed through the axial direction, inducing free convection, as shown in Figure 10.4b. For guidance, the Grashof dimensionless number is recalled in Equation (10.7), where it can be noticed that free convection is enhanced with the referred density gradient.⁵ Mass transfer correlations using the Grashof number are often used to estimate free convective coefficients in several processes.

$$\text{Gr} = \frac{\rho g (\Delta \rho) d_p^3}{\mu^2} \quad (10.7)$$

where g = gravity acceleration (m^2/s); $\Delta \rho$ = variation in fluid phase density (kg/m^3); Gr = Grashof dimensionless number.

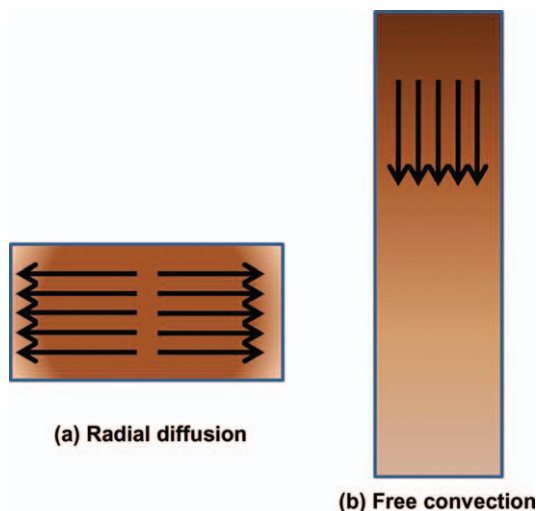


Figure 10.4 Effects of various extraction bed geometries – the darker the tonality, the higher the extract concentration in the solvent.

The longer is the extraction bed, the higher will be the density gradient, and, since such a gradient induces downwards mass transfer, free convection acts against the extraction sense, being then a negative factor of the process. Summarizing, the effects of bed geometry on radial diffusion, free convection and substrate compaction cannot be neglected, and secondary scale-up criteria should be considered to deal with these phenomena during scale-up.

In this section a long list of process parameters and combinations of parameters that affect the results of extraction have been discussed. With this information it is possible to begin to figure how complex and, worse, unpredictable, an industrial extraction can be. The next section deals with some more process data. But the reader is already invited to reflect about how feasible it would be to find out all the scale-up criteria suggested up to now. How much data would be necessary to establish all the correlations needed and the scale-up rules with reasonable confidence? This discussion is important to mark the limits between theoretical and practical scale-up.

10.3 Factors Involved

Extraction from solid matrices has been extensively studied by the scientific community, and there are several applications in food, cosmetic, pharmaceutical and chemical industries for various purposes. In most cases the process consists in a solid matrix containing the target compounds put in contact with a solvent, which removes the compounds it is able to solubilize under specific conditions. Besides solubility, other process parameters are important, like solvent flow, substrate physical properties and extractor

dimensions. In this section we give an overview on some process factors that must be considered when choosing a solvent and extraction conditions, or even when scaling-up an already studied process. Special attention is dedicated to extraction processes with high-pressure solvents, such as supercritical fluid (SFE) and pressurized liquid extractions (PLE).

10.3.1 Solubility

Every extraction process is a unit operation based on the solubility of one or more components in a solvent, which can be pure or a mixture of two or more substances. First of all, solubility obviously depends on the chemical affinity between solutes and solvent, thus the choice of the last is fundamental to attain the desired product. As an example, polar solvents tend to solubilize polar compounds, while non-polar solvents solubilize non-polar compounds. Moreover, solubility can be affected indirectly by other extraction process parameters, as pressure and temperature.

In extraction with compressible solvents (gases and supercritical fluids), solubility is a direct function of the solvent density⁶ (it is well known that liquids are often better solvents than gases). Since in these solvents density is affected by pressure and temperature, as can be shown either experimentally or using equations of state, the solubility may vary considerably with these two process parameters.^{7–10} Based on the solvent density, solubility must increase with pressure and decrease with temperature in compressible solvents.

Temperature can also play an opposite role on solubility, when considering the influence of the solute vapor pressure. This property increases with temperature, so the equilibrium concentration of solutes in the ‘gaseous’ phase, which corresponds to solubility, should also increase (Raoult’s law equation shows this trend in a simple form² – see Equation 10.8).

$$Y^* = \frac{xP_{\text{vap}}(T)}{P} \quad (10.8)$$

Where Y^* = solubility (fluid phase equilibrium concentration) (kg solute/kg solvent); x = solid (substrate) phase equilibrium concentration (kg solute/kg solution); P_{vap} = vapor pressure of the solute (Pa), which depends on temperature; T = extraction temperature (K); P = extraction pressure (Pa).

These conflicting effects of temperature on solubility appear in some SFE processes.^{11,12} Moreover, other solvent properties can be modified with temperature, such as the dielectric constant, which tunes the polarity of a substance. This is considerably important in pressurized water extractions.¹³ Therefore, adjusting the process temperature may also change the solubility of some compounds in a specific solvent.

When looking forward to large-scale processes, where economic issues become important, solubility must be taken into account as a factor that influences the driving forces for mass transfer from substrate to solvent. Many mathematical models (some of which will be described in Section 10.4.1) used to explain extraction kinetics use the concentration gradient between solvent

phase and substrate surface as the driving force for mass transfer. Indeed, equilibrium used to be assumed on the substrate surface, where solute concentration equals its solubility. Therefore, the concentration gradient increases as solute concentration in the solvent phase decreases, indicating that low solvent phase concentrations are preferable to assure high mass transfer rates. One way to keep low solvent phase concentration is to use high solvent flow rates, making residence time low enough to keep solute concentration far from saturation. However, this leads to the increase of operational costs for solvent preparing and recycling, as well its consumption. Therefore, keeping a relatively high solute concentration in the solvent, despite reducing mass transfer rates, may represent operational economy. These issues must be considered in scale-up, through the evaluation of the costs involved with purchase, preparing and recycling the solvent in contrast with the required extraction rates.

10.3.2 Solvent Flow Rate

The choice of solvent flow rate plays an important role in the equilibrium relationships between solvent phase and the thin solvent film that involves the substrate, which can be interpreted as a mass boundary layer. Thus, the gradient between equilibrium and fluid phase concentration may be the main driving force of the extraction process. Determining the solvent flow rate affects the solute concentration in the solvent inside the extractor, and, therefore, the magnitude of the driving force. Very high solvent flow rates result in high concentration gradients, which can make mass transfer in the fluid phase fast, even at a point where the influence of solvent flow rate could be neglected. In this case, the process is controlled by intraparticle diffusion, and any increase in solvent flow rate would mean a useless additional cost.

The influence of solvent flow rate exists in situations where its value is low enough to ensure that intraparticle diffusion will keep the equilibrium concentration of solute at the solid surface. Then, mass transfer can be affected by the solvent velocity, and its increase might be recommended to accelerate the extraction process. In these situations the process is controlled by fluid phase convection and solubility.

10.3.3 Substrate Properties

Extraction yields and kinetics obviously depend on the properties of the raw material. Thus, the preparation of the substrate for extraction is of paramount importance in order to maximize yield and extraction rate. Besides drying, which is often recommended to reduce the undesirable water content in the product, the control of particle size must be always taken into account.

In Fick's diffusion laws,³ it can be noticed that particle size affects the mass transfer kinetics because of its direct relationship with the length of diffusive pathways. In this sense, the natural operation to reduce such barriers would be to mill the solid raw material into the smallest size as possible, reducing the

obstacles to diffusion of solvent into the particle, and of solute + solvent out of the particle pores. On the other hand, excessive particle reduction can lead to compacting, which, in practice, means that blocks of substrate will be formed, through which the solvent will not penetrate. These are called 'preferential pathways'. Since the solvent does not contact the solute inside these blocks, extraction yield is reduced. Another way to control the existence of preferential pathways is by controlling the bed porosity, which should not be excessively low.

Concerning scale-up, the discussion must be addressed to the means that can be used in industrial scale to reduce particle size, and also to fill the extraction columns with the raw material. This process step is surely much more complicated than in the laboratory, where extraction columns are filled manually and the time of this procedure is not a critical issue. Industrial extraction plants usually have two or more extractors, in such a way that one is being reconditioned while others are operating. Thus, the packing of the extraction bed must be completed within an extraction cycle time, and must also avoid the formation of compact regions through where the solvent would not flow. Automated procedures must be used in this step, in order to make the extraction bed as uniform as possible.

10.3.4 Extraction Bed Geometry

An extraction bed is defined as the substrate placed inside the column, where the process happens. Its 'geometry' concerns the length (L) and diameter (D), as well as the ratio between these two dimensions. Laboratory-scale columns and beds are often dimensioned without concerning spatial effects, since their sizes are considered small enough to avoid their influences. But in large-scale processes some effects may appear, which should be considered, and some care must be taken to prevent them and to reproduce the laboratory and pilot plant behavior in larger scales.

Extraction columns are usually installed in a vertical position, which means that the solvent flows upwards across the substrate in the same direction as gravity. Thus, gravitational force acts and potentially affects fluid dynamics and mass transfer. The role of gravity in mass transfer is expressed as natural or free convection. If an extraction bed is long, significant concentration gradients may appear along its length, since there is pure solvent at the inlet and a concentrated mixture at the outlet. Since the solution density increases with concentration, gravitational effects will lead to back mixing, and the extraction rate will be reduced. Therefore, in some situations long extraction beds are not recommended.

Radial effects should also be prevented. They can appear when the solvent distribution at the column inlet is not uniform. In laboratory columns, whose diameter is not much higher than the solvent pipelines, such a problem is less evident. For industrial extractors a distribution system is often needed to guarantee that the solvent spreads equally through all the substrate bed. Based

on the axial and radial issues presented, L/D ratios from 5 to 7 are usually recommended for industrial extractors.

10.4 State of the Art

Extraction processes are extensively studied by research groups around the world, mainly in chemistry, chemical and food engineering departments of universities. The scientific production of those groups can easily be found as papers in specialized journals, academic books and others. Most of the published works report studies on extraction procedures from new sources, or with some innovative methods, validated in laboratory scale, since the facilities of the research groups rarely include pilot or industrial scale equipment. Therefore, as the reader will observe, many 'scale-up' works have a strong theoretical basis, concentrated on modeling and simulation of large-scale processes based on laboratory results. The state of the art will be presented in this sense, trying to remark on the limitations that may appear in this type of procedure. Some large-scale issues commented on in the previous sections will be cited again as examples of how process simulation based on small scale cannot be entirely followed.

10.4.1 Models for Extraction Processes

Modeling is a quite ancient activity in basic and applied sciences. Even unconsciously we model data every day for diverse purposes, from preparing cookery recipes to predicting monthly expenses. In these and many other situations we take some known data and try to express them by a simpler means, and sometimes we attempt to predict other data using those we have got. The objectives of mathematical modeling can be summarized in three parts.

1. Simplify the information. Sometimes it is necessary to express a huge set of experimental data, which would demand some pages of tables or graphics. This tedious task can be simplified by some mathematical form to represent all those data, in a way that many pages are presented in a few lines. Even in modern times, when computers can store enormous amounts of data, the use of models to express some information is still very practical.
2. Comprehend a process. To model a process is to represent real data with some mathematical expression, which can be a single equation, a set of equations or even numerical solutions of differential equations. In all these cases, modeling consists in finding some parameters that make the selected mathematical expression to represent the real data as well as possible, by minimizing an objective function. Nevertheless, for comprehending the process, the calculated parameters must have a meaning, instead of being a simple number. In other words, the objective of

modeling is to explain the process using the values of model parameters, and for that the model must have a strong theoretical basis.

3. Predict a process. This is probably the most ambitious goal of modeling processes. Apart from fitting well to available experimental data, a mathematical model can be used to simulate the same process in longer time, or even in different conditions; scale-up is included in this feature. If the model enables one to predict how the extraction will behave in an industrial unit, with higher solvent flow rates, greater feeds and so on, the best that a model can give will be achieved.

Mathematical models based on the transport phenomena involved, or even with merely empirical basis, are useful tools in scale-up of extraction. Modeling extraction curves can help to comprehend the process kinetics, through the definition of extraction rates, steps, time and even parameters with strong physical meaning that may be useful to estimate the behavior of large-scale procedures.

10.4.1.1 Empirical Models

Empirical models consist basically in mathematical equations used to represent the extraction behavior, i.e. the curve expressing extract mass or yield as function of time or solvent used. An empirical curve does not provide any information about the transport mechanisms that control the process. Thus, when an empirical model is fitted to an experimental extraction curve, the adjusted parameters do not have any physical meaning. They are only 'numbers'. A typical empirical model is presented by Esquivel *et al.*,¹⁴ which was conceived to fit SFE curves with two parameters, as stated in Equation (10.9).

$$E = X_0 F \left(\frac{t}{b + t} \right) \quad (10.9)$$

where X_0 = extraction yield (kg extract/kg raw material); F = substrate feed (kg); t = extraction time (s); b = model parameter (s); E = extract mass (kg).

Since there is no phenomenological basis in this model, no physical meaning can be attributed to the parameter b . Therefore, the behavior of b with other process conditions cannot be predicted using the theoretical basis. This does not mean that an empirical model like that of Equation (10.9) cannot be used for scale-up purposes. If the model is applied to several analytical- and industrial-scale extractions, and a correlation can be established to represent b as function of some process conditions, even models without phenomenological basis may be helpful, although their parameters still remain meaningless.

10.4.1.2 Models with Theoretical Basis

Models based on transport mechanisms deserve great attention, since their use can provide useful information to be applied in scale-up and their parameters

have well-defined physical meaning. These models are elaborated from diverse interpretations of mass transfer and equilibrium inside the extraction bed. The departing point for the conception of such models is the mass balance, which must be developed in both solvent and solid phases of the extractor. This sentence shows that an extraction system consist of the two mentioned phases. In fact, some models apply a mass balance inside the solid pores, but since it is not the objective to describe complex modeling, the approach will be restricted to a biphasic system.

The mass balance equations for an extraction process performed inside a cylindrical column must, *a priori*, take into account every possible phenomenon occurring, such as fluid-phase convection, diffusion through the solvent, inside the substrate particles or pores, equilibrium relationships and concentration gradients in all possible directions. However, based on the previous knowledge about the process, the departure will be from the balance in the axial coordinate of the extractor for the solvent and the solid phases, considering that the greatest mass transfer will be in that direction. Equations (10.10) and (10.11) express the mass balances in the solvent phase and in the solid phase of the extraction bed, respectively.

$$\frac{\partial Y}{\partial t} + v \frac{\partial Y}{\partial z} = \frac{\partial}{\partial z} \left(D \frac{\partial Y}{\partial z} \right) + \frac{J(X, Y)}{\varepsilon} \quad (10.10)$$

$$\frac{\partial X}{\partial t} = \frac{\partial}{\partial z} \left(D_{\text{ef}} \frac{\partial X}{\partial z} \right) + \frac{J(X, Y)}{1 - \varepsilon} \frac{\rho}{\rho_S} \quad (10.11)$$

where Y = extract concentration in the solvent (kg extract/kg solvent); t = time coordinate (s); v = solvent axial velocity (m/s); z = axial coordinate (m); D = solute diffusion coefficient in the solvent (m^2/s); $J(X, Y)$ = interfacial mass transfer flux (s^{-1}); ε = extraction bed porosity; X = extract concentration in the substrate (kg extract/kg raw material); D_{ef} = effective diffusion coefficient of the extract in the solid (m^2/s); ρ = solvent density (kg/m^3); ρ_S = solid density (kg/m^3).

The approach of Equations (10.10) and (10.11) has already neglected some possibly important aspects of extraction that were mentioned in Section 10.2, such as radial diffusion, substrate particle size and changes in velocity and solvent density, which could be important to the scale-up. Those issues could be dealt with, but this would increase the complexity of the modeling to a level where maybe it would not be worth it. Indeed, modeling always has imprecision, so excessive concerns about being extremely realistic may be useless to the final result. Once solved analytically or numerically, the mass balance equations provide extraction curves that may represent extract mass or yield as a function of time or solvent used, such as the curve presented in Figure 10.1.

If one searches in the scientific literature for models to represent extraction processes, tens or maybe hundreds will be found, with lots of different approaches, simplifications and adjustable parameters. Although almost all

those models have an excellent theoretical background, very few are effectively applied for processes apart from those from which they were conceived. But, when looking for universal scale-up criteria, models that can be applied to different extraction processes and conditions should be used. Considering that, the broken and intact cell model of Sovová,¹⁵ originally formulated to fit SFE curves of vegetable extracts, will be the model used to explore scale-up in this chapter. In the following lines a brief description of this model is presented.

The raw material pre-treatment performed before forming the extraction bed involves, among other processes, the milling of the vegetable structure from which the solute is to be recovered. This procedure aims, firstly, to reduce the solid particle size in a way that the contact surface between solid and solvent is increased. However, another effect of milling is the breaking of the cell structures that contain the solute, in such a way that part of the solute becomes free to contact the solvent. On the other side, part of the cells remains unbroken even after the pre-treatment, so their content will be less accessible to the solvent. Based on this approach, the total extractable yield, which is called X_0 , can be divided in two parcels: (1) the easily accessible solute X_p , coming from the broken cells; and (2) the solute in the unbroken cells, X_k . The model proposes that the free solute is extracted prior to the solute inside the cells, thus the extraction process can be divided in three steps, illustrated in Figure 10.5.

The mass balances presented in Equations (10.10) and (10.11) can be simplified by neglecting some terms considered insignificant: solute accumulation in the

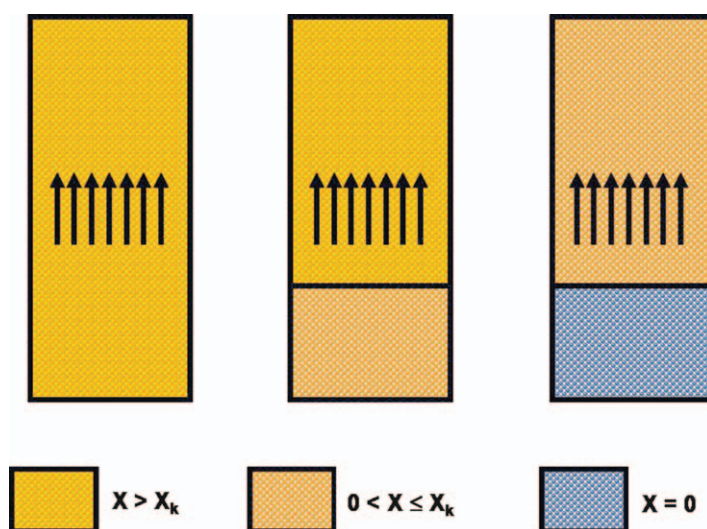


Figure 10.5 Steps of the extraction from solid substrates according to the broken and intact cell model.¹⁵

solvent phase; axial diffusion in the solvent phase and axial effective diffusion in the solid phase. As a result, the simplified mass balances are those of Equations (10.12) and (10.13).

$$v \frac{\partial Y}{\partial z} = \frac{J(X, Y)}{\varepsilon} \quad (10.12)$$

$$\frac{\partial X}{\partial t} = \frac{J(X, Y)}{1 - \varepsilon} \frac{\rho}{\rho_s} \quad (10.13)$$

with the initial and boundary conditions:

$$X(z, t = 0) = X_0 \quad (10.14)$$

$$Y(z = 0, t) = 0 \quad (10.15)$$

The interfacial mass transfer flux, $J(X, Y)$ is expressed as Equations (10.16) and (10.17), depending on the availability of free solute:

$$J(X > X_k, Y) = k_f(Y^* - Y) \quad (10.16)$$

$$J(X \leq X_k, Y) = k_s \left(1 - \frac{Y}{Y^*} \right) \quad (10.17)$$

where k_f (s^{-1}) and k_s (s^{-1}) are the mass transfer coefficients in the solvent and solid phases, respectively. The analytical or numerical resolution of these equations leads to the modeled extraction curves, which must be adjusted to experimental data, through the determination of the model parameters X_k , k_f and k_s . Deep details about the modeling procedures and mathematical algorithms will not be discussed. Instead, model parameters, which can be used for scale-up purposes, will be focused on.

10.4.2 Some Examples of Scale-up Criteria in Extraction Processes

It is well-established that the main challenge of scaling-up an extraction process is to predict what will happen at an industrial scale, given that one only has results obtained in laboratory, or at most in a pilot plant. Although it is desirable to predict processes at various conditions from the small scale results, a first step, which is surely less ambitious, can be adopted. Thus, the very first question to be answered is: 'What must be kept constant in order to reproduce the laboratory extractions in large scale?'

The word 'reproduce' means that the objective is to obtain, at a large scale, exactly the same extraction yield and kinetics that were observed in the laboratory. Therefore, one is induced to suppose that all the thermodynamic and mass transfer parameters must be conserved. But how can this be achieved?

The answer to this question involves a deep knowledge about the factors that might limit an extraction process, which may be of thermodynamic or mass transfer nature.

10.4.2.1 Processes Limited by Solubility

Every extraction process involves compounds being transferred from a substrate to a solvent. Evidently, the solubility of those compounds in the solvent is a key parameter, as explained in Section 10.3.1. Therefore, special attention must be given to solubility, since this property can limit the yield and velocity of the process. In simple words, solubility is the highest solute concentration that a solvent can have, at certain conditions (in supercritical extraction, such conditions are essentially pressure and temperature). This means that, if in a given process there is more solute than the solvent can extract, then the solvent will become saturated and unable to dissolve any additional soluble compounds. Therefore, the solute concentration in the solvent will be equal to its solubility, but never more than that.

Considering the same substrate, solvent and extraction conditions, if a process is limited by solubility there is a ratio between solute and solvent mass that characterizes that situation. Moreover, recalling that the solute concentration in the substrate also depends on process conditions, the ratio between substrate and solvent mass must be defined as the factor to be considered. The same behavior must be reproduced at both small and large scales, because the mentioned ratio is an intensive variable, i.e. independent of the quantities involved. In summary, when an extraction process is limited by solubility, the ratio between substrate and solvent mass must be conserved from laboratory to industrial scale in order to reproduce identical extraction curves, giving the same yield and rates.¹⁶

This situation may be observed in real processes in some particular cases, as follows.

1. Low solvent flow rates. If the extraction is performed with low solvent flow rate, the residence time of the solvent inside the extractor may be long enough to promote large contact time between solvent and substrate, leading to saturation.
2. High amount of solute. Some substrates have high concentration of extractable compounds, which demand huge amounts of solvent for their recovery. Oilseeds and other materials with high lipid content are the most typical example of those substrates. In such situations it may be technically impossible to avoid saturation, and the extraction rate becomes limited by the extract solubility. To avoid saturation the solvent flow rates should be so high that its use would be technically or economically unfeasible.

Example 10.1

Let us consider the extraction of peach almond oil using supercritical carbon dioxide (CO₂) as solvent, at 15 MPa and 40 °C. Mezzomo *et al.*¹⁷ measured the extract's solubility (Y^*) and global yield (X_0) under these conditions, finding 0.003 kg oil/kg CO₂ and 0.177 kg oil/kg substrate, respectively. The process takes 2 hours with a solvent flow rate (Q) of 0.06 kg/h, totalizing 0.12 kg of CO₂ used (S). The substrate mass (F) is 0.1 kg.

Calculating the ratio between substrate and solvent mass there is:

$$\frac{F}{S} = \frac{0.1}{0.12} = 0.83 \frac{\text{kg substrate}}{\text{kg CO}_2}$$

Now, using the global yield, the ratio between extractable oil and solvent mass can be calculated:

$$\frac{X_0 F}{S} = \frac{0.177 \times 0.1}{0.12} = 0.1475 \frac{\text{kg oil}}{\text{kg CO}_2}$$

Notice that the ratio calculated above is much higher than the solubility of the extract in CO_2 at the process conditions. Therefore, it would be impossible to obtain all the extractable oil using only 0.12 kg of solvent, since saturation would be achieved. Figure 10.6 illustrates qualitatively an extraction curve for this process.

The inclination of the curve shown in Figure 10.6a can be interpreted as the extract concentration in the solvent. Along the process, where the extraction curve is linear, extract concentration is constant, and, in this particular case, must be equal to its solubility, and never more than that. Thus, it becomes evident that solubility limits the process velocity, avoiding the increase of the extraction rate, as shown in Figure 10.6b, where the extraction curve is plotted against time. A straight line along all the process, as shown in Figure 10.6, means that the solvent remains saturated. However, if intraparticle diffusivity is low enough, a decreasing-rate step will appear after the constant extraction rate period. It should also be observed that the total amount of extractable material is 0.0177 kg, which is obtained by multiplying X_0 and F , but in 2 hours the yield was only 0.0036 kg. Supposing that all the extract is to be recovered, a simple calculation leads to the total required extraction time. First, the total solvent mass needed can be determined:

$$S = \frac{X_0 F}{Y^*} = \frac{0.0177}{0.003} = 5.9 \text{ kg CO}_2$$

And, if the solvent flow rate of 0.06 kg/h, as proposed, is kept constant, the required extraction time would be:

$$t = \frac{S}{Q} = \frac{5.9}{0.06} = 98.3 \text{ h}$$

The required time is absurdly high, illustrating how the low solvent flow rate retards the process. It is easy to demonstrate that increasing Q would decrease proportionally the required extraction time.

Now, let us scale-up this process to 100 kg of substrate, that is, a thousand-time scale-up, remembering that it is necessary to respect the scale-up criterion for this situation, by keeping constant the substrate to solvent ratio. Thus,

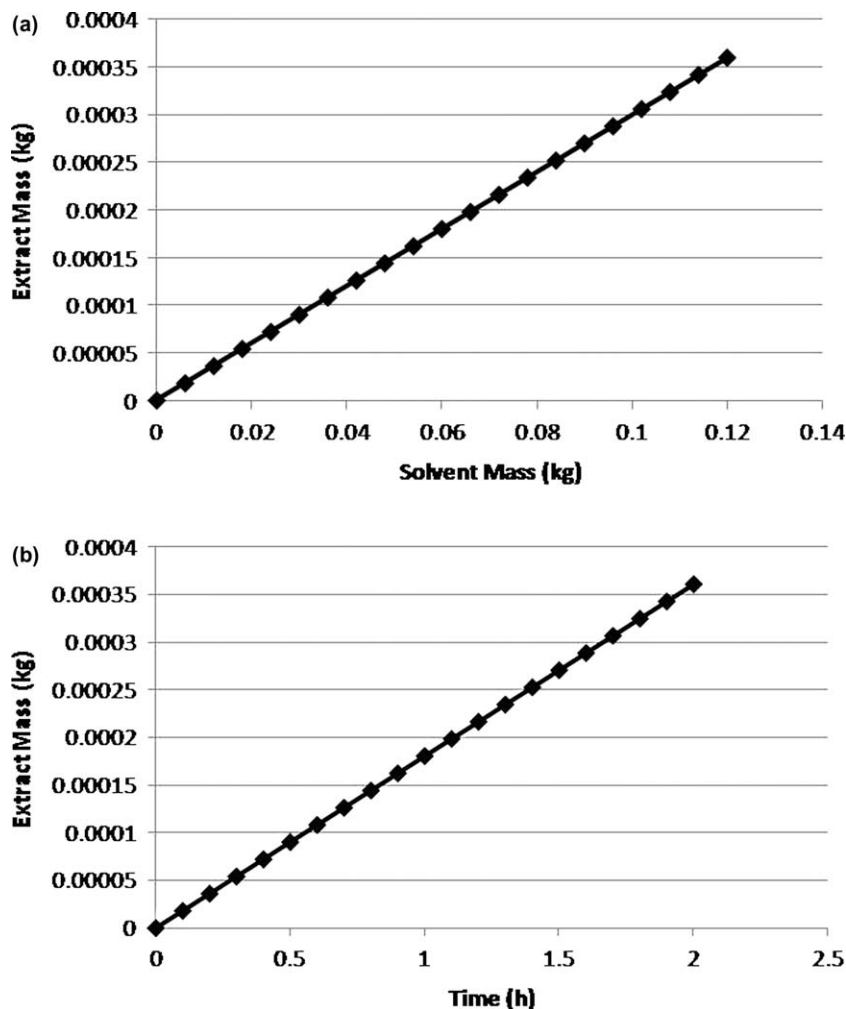


Figure 10.6 Extraction curve of peach almond oil at 15 MPa, 40 °C and 0.06 kg/h of CO₂.

120 kg of CO₂ must be used to obtain the same yield of the laboratory scale process. The solvent flow rate of this extraction can be chosen, and it will determine the time needed to achieve the desired yield. Even so, the maximum flow rate is limited by the technical aspects of the industrial equipment.

Figure 10.7 shows the extraction curves on a large scale, simulated using the S/F scale-up criterion, and using a solvent flow rate of 120 kg/h.

First of all, it can be noticed that Figures 10.6a and 10.7a illustrate proportionally identical extractions, showing how scale-up would be if the S/F ratio was conserved. The slope of the straight line in Figure 10.7a corresponds to solubility, as it does in Figure 10.6a. Comparing the lines of Figures 10.6b

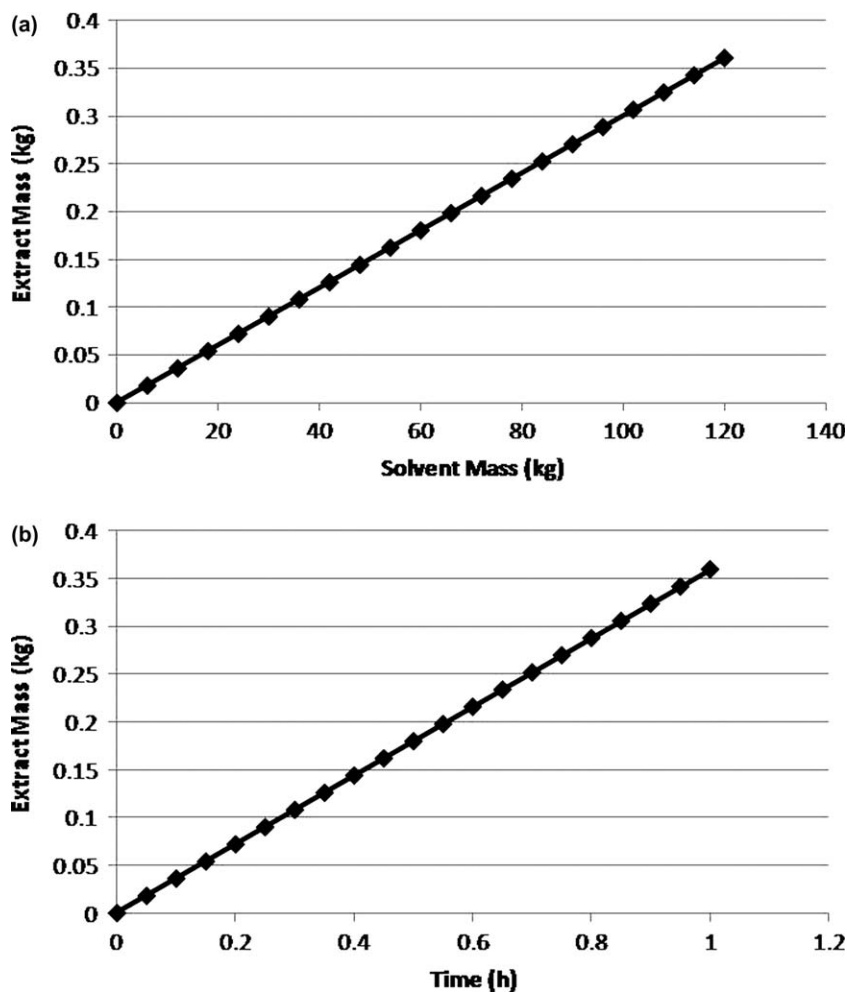


Figure 10.7 Extraction curve of peach almond oil at 15 MPa, 40 °C and 120 kg/h of CO₂.

and 10.7b, it can be noticed that the main difference resides on the total extraction time, which is reduced to half. This reduction is possible by doubling the solvent flow rate, in a way that the ratio between substrate and solvent mass is not modified. Thus, increasing Q is necessary to achieve higher yields, although this would reflect in higher operational cost. Even so, yields near X_0 would be obtained only with extremely high S/F ratios, which are probably unfeasible.

10.4.2.2 Processes Limited by Diffusion

In order to analyze extraction processes limited by diffusion it must be assumed that, for such extractions, solvent saturation is never achieved. Thus, there will

always be a driving force to convective mass transfer, due to the concentration gradient between solvent and solvent–substrate interface, which is the equilibrium concentration.

If the concentration gradient is significant, and if high convective coefficients are guaranteed by controlling flow rate and turbulence, the solute transfer from the interface to the solvent bulk is expected to be fast. On the other hand, intraparticle diffusion is necessary to transport the solute from the substrate to the interface, and the mass transfer resistance to such a mechanism is much higher than that of convection. In other words, the diffusive process must supply solute to the interface with a rate that assures the needed concentration gradient for external convection. Since intraparticle diffusion is the mechanism that demands more time in those extractions, it is considered that these processes are limited by diffusion.

For processes limited by diffusion the substrate mass must be related to the solvent flow rate, since this ratio assures that the solvent residence time inside the extraction bed is conserved. Then, the concentration gradients from small to large scale are kept constant, in a way that the mass transfer mechanisms are reproduced identically in both laboratory and industrial processes. Summarizing, the scale-up criterion for processes limited by diffusion is to keep the ratio between substrate mass and solvent flow rate (Q/F) constant. This scale-up criterion has been effective in some extractions with supercritical carbon dioxide as solvents, from vegetal and animal substrates.^{17–20}

Example 10.2

The supercritical extraction from peach almond will be explored again, but in different conditions than those considered in Example 10.1. Now, the process at 25 MPa and 40 °C will be used, where the extract solubility in CO₂ is 0.013 kg solute/kg solvent and the global yield is 0.191 kg extract/kg substrate. The solvent flow rate in small scale is 0.6 kg/h. The substrate mass used in this process is 0.012 kg. Then, the ratio between solvent flow rate and substrate is:

$$\frac{Q}{F} = \frac{0.6}{0.012} = 5 \frac{\text{kg CO}_2}{\text{kg substrate.h}}$$

In order to verify if the solvent is saturated in this process, the oil concentration during the constant extraction rate period can be calculated, where such a concentration achieves its highest value. According to Mezzomo *et al.*,¹⁷ the CER period is 0.56 h. From this value the total CO₂ amount used is obtained:

$$S = Q \times t_{\text{CER}} = 0.6 \text{ kg/h} \times 0.56 \text{ h} = 3.38 \text{ kg CO}_2$$

The oil mass extracted in the CER period can also be calculated, by multiplying the extraction rate in this period, which is 1.068×10^{-4} kg/h, by the CER time:

$$M_{\text{CER}} = \dot{M}_{\text{CER}} \times t_{\text{CER}} = 1.068 \times 10^{-4} \text{ kg/h} \times 0.56 \text{ h} = 6.02 \times 10^{-5} \text{ kg oil}$$

Finally, dividing M_{CER} by S , the extract concentration is obtained:

$$Y_{\text{CER}} = \frac{M_{\text{CER}}}{S} = \frac{6.02 \times 10^{-5}}{3.38} = 1.78 \times 10^{-5} \frac{\text{kg oil}}{\text{kg CO}_2}$$

It can be noticed that the value of Y_{CER} is far lower than the extract solubility at the given conditions, which ensures that the solvent will never be saturated during the process. Therefore, this extraction is not limited by solubility. Otherwise, the intraparticle diffusion from inside the solid particles to their surface must be the slow step, which limits the amount of solute available to CO_2 , and hence retards the process.

The described extraction was scaled-up by Mezzomo *et al.*,¹⁷ who preserved the ratio between substrate and solvent flow rate, by increasing each of these parameters five times. Figure 10.8 shows both small-scale and scale-up curves.

It can be observed, in Figure 10.8, that similar extraction curves are obtained at different scales when the substrate to solvent flow rate ratio (Q/F) is conserved. Thus, this scale-up criterion can be taken as valid for this situation, where diffusion is the limiting mechanism. The accentuated decrease in extraction rate, which begins at about 50 minutes, shows how intraparticle diffusion is a slow process and, even when the solvent is quite far from being saturated, the extract concentration in it cannot achieve higher levels.

Extraction curves adjusted using the described broken and intact cell model¹⁵ are also illustrated in Figure 10.8. First, it is important to notice that this model provides precise fits to experimental data, allowing to reliably analyze the behavior of the adjusted parameters. In this case the concern is about the mass

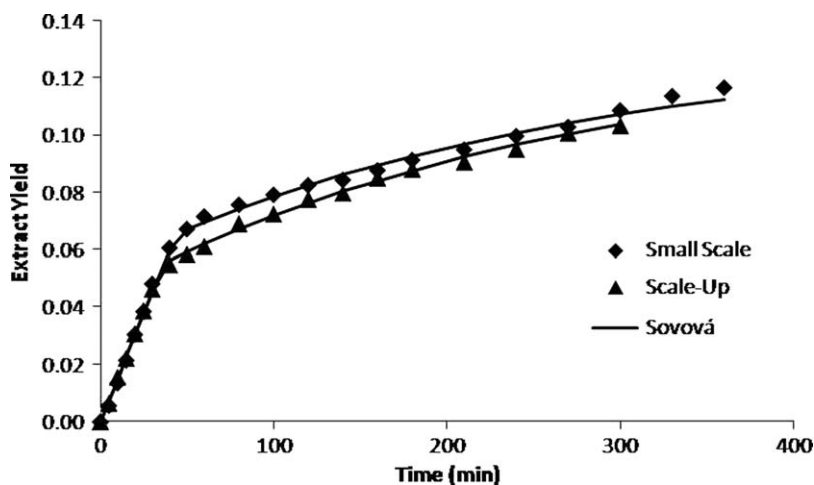


Figure 10.8 Experimental and modeled SFE curves from peach almond at different scales, with constant ratio between substrate mass and solvent flow rate.

transfer coefficients in both fluid (k_f) and solid (k_s) phases. Mezzomo *et al.*¹⁷ obtained the following values for those parameters:

- small scale: $k_f = 2.67 \times 10^{-3} \text{ s}^{-1}$, $k_s = 6.67 \times 10^{-5} \text{ s}^{-1}$;
- scale-up: $k_f = 2.24 \times 10^{-3} \text{ s}^{-1}$, $k_s = 5.93 \times 10^{-5} \text{ s}^{-1}$.

The value magnitudes of both coefficients are preserved, and probably a statistical analysis would not detect significant differences between the values at small scale and scale-up curves. This type of result may encourage one to use mass transfer coefficients obtained while modeling laboratory experiments to predict the behavior of extraction at larger scales. Indeed, the model itself considers implicitly that these parameters will be kept constant if the Q/F ratio is conserved. Nevertheless, one must be cautious when considering the possible scale-up factors listed in Section 10.3 which may be sources of changes in the process dynamics. Such factors will become more evident in the case study presented in Section 10.5.

10.4.3 Scale-up Correlations

The previous section presented feasible scale-up criteria that allow us to predict extraction processes at larger scales, provided that some relations are conserved for certain parameters. Nevertheless, it is often interesting to predict processes at conditions completely different from those tested in the laboratory. In such situations there can be no data, not even ratios between process data, that are kept constant. Thus, methods are necessary to calculate process parameters, such as mass transfer coefficients, at variable conditions. The use of mass transfer correlations as secondary scale-up criteria may be the solution to this challenge.

Relations between model parameters have been proposed in several works, based on substrate or solvent properties or process conditions, such as flow rate, velocity and column dimensions. These relations, when well-established, allow us to represent more than one single extraction process in simple equations, as dimensionless correlations.

The particular objective of a scale-up correlation is to represent, and then to predict, the behavior of one or more mass transfer parameters that describe a process. Although many factors might affect extraction kinetics, scale-up correlations often take into account only the most representative mechanisms, which are able to describe the process in a satisfactory way. Concerning extraction from solid materials in packed columns, with solvent flow, fluid phase convection is responsible for the greatest part of the extraction; therefore correlations for convective coefficients have been extensively explored. Table 10.1 summarizes the published correlations for the solvent phase convective coefficient in extraction processes.

It can be noticed that many different correlations are proposed to predict the convective coefficient in extraction from solid substrates in packed beds. In this

Table 10.1 Some correlations for the convective coefficient in extraction processes.

<i>Correlation</i>	<i>Ref.</i>
$ZQ = 4.10\nu^{0.66}$	21
$Sh = 0.206Re^{0.8}Sc^{0.33}$	22
$Sh = 0.00084Re^{0.4299}Sc^{0.8783}$	23
$k_f = 0.00002\nu^{0.32}$ and $k_f = 0.00005\nu^{0.16}$	24

k_f = convective mass transfer coefficient; Q = solvent flow rate; Re = Reynolds dimensionless number; Sc = Schmidt dimensionless number; Sh = Sherwood dimensionless number; ν = solvent velocity; Z = dimensionless mass transfer parameter.

field a universal scale-up criterion is almost impossible to find, and each published correlation works well for restricted situations, close to those used in the experiments that originated them. To compile this vast set of data and find a unique scale-up rule is a tough challenge for future researchers.

10.4.4 Configurations of Industrial Units

In the beginning of this chapter it was pointed that there are several characteristics of industrial-scale operations that are never reproduced at analytical procedures. This is not due to the negligence of researchers, but to a real limitation of what can be done in a laboratory. One must be aware of this when scaling-up extractions – once the analytical processes were performed, how do we imagine they would be done in industry? What additional equipment, accessories and configurations would be necessary to put the plan into practice? In this section some typical configurations of extraction systems working at an industrial scale and some research studies done on this field are reviewed. Supercritical fluid extraction will be given special attention.

10.4.4.1 Operation Modes

As with many other processes in chemical and food engineering, extraction can be performed in batch, semi-continuous or continuous modes, depending on the strategies used to feed the substrates and solvents into the equipment. In a batch extraction, both solvent and substrate are fed into vessels and the mixture can be stirred to increase mass transfer. After a defined residence time, the mixture is removed and the extract is separated from the solvent. Batch extractions have their use limited to processes with small rates, thus scale-up is not a critical issue for this mode.

Most analytical extraction procedures dealt with in this chapter can be classified as semi-continuous or quasi-continuous.²⁵ This means that the solid substrate is loaded into the extractor as a batch, and the solvent flows continuously through the extraction bed for a defined time, with fixed flow rate, leaving the extractor with solute to be separated by a further step. Semi-continuous extractors can even operate in series, with the solvent passing

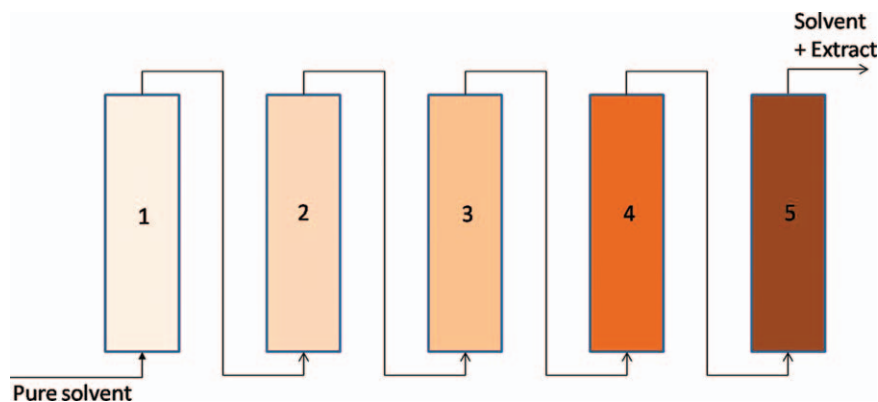


Figure 10.9 Semi-continuous extractors in series; the solute concentration in the substrate increases from extractor 1 to 5.

through more than one substrate bed. The idea of this configuration, illustrated in Figure 10.9, is to promote high concentration gradients, in order to keep mass transfer rates high.

Finally, continuous extraction may also be performed at an industrial scale, with the solid substrate being fed into the extractor and also discharged continuously. To make continuous solid feed viable, conveying systems are required, leading to additional process parameters that are not considered at analytical scale.

10.4.4.2 Working Principles

Industrial extraction processes can work in one or more stages. In single-stage extractions there is a unique vessel and the solvent flows through it for an optimal residence time in order to remove the soluble components at the desired rate and with the desired yield. Single-stage extraction can be operated in batch or semi-continuous modes.

Multi-stage extractions require more than one extractor, and the solvent flows in countercurrent mode. That is, the pure solvent enters the extractor that contains the most exhausted substrate, and passes through a series of extractors, ending at the unextracted substrate. As illustrated in Figure 10.9, this scheme provides high concentration gradients between solvent and solid phases, enhancing mass transfer. For scale-up purposes, additional piping, accessories and extraction columns are required. Few research works are published about this configuration, since such schemes are not so easy to reproduce in the laboratory. However, the work of Nuñez *et al.*²⁶ can be highlighted; they used robust mathematical modeling to simulate the extraction behavior in a three-column unit. In this design two extractors operate while the third is being reconditioned, resulting in a continuous process, as show in Figure 10.10. The piping and valve design allows the operator to easily modify the operation mode.

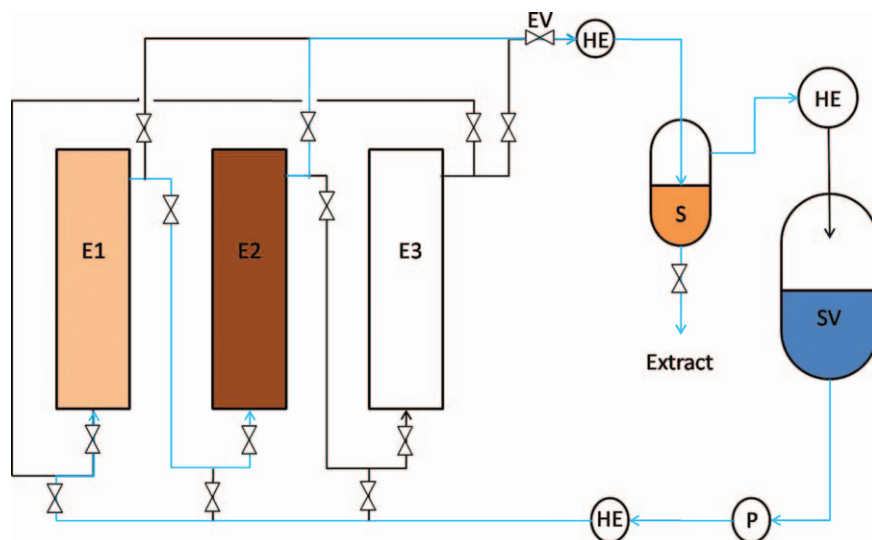


Figure 10.10 Multi-stage unit with three extractors – dotted lines indicate the solvent flow in the illustrated configuration. HE = heat exchangers; P = pump; EV = expansion valve; SV = solvent vessel; S = separator; E1 and E2 = extractors operating; E3 = extractor being reconditioned.²⁶

In every configuration for industrial-scale extraction the solvent is recovered and used for various extraction cycles. After the extraction the product is separated from the solvent by diverse methods and the solvent is recycled. Solvent recycling is not a common procedure at the analytical scale, therefore this is one more change in the process nature that must be considered in scale-up. First, specific equipment and additional piping and accessories are needed to install a recycling line, leading to new factors that may affect the process as a whole. Second, some ratio of solvent loss must always be taken into account, since the separation step, as in every equilibrium problem, leaves solvent traces in the extract. In addition, the recycled solvent itself may carry traces of solute in it. Thus, its power to remove additional extract in the next cycles will be gradually reduced. In other words, solubility is a process parameter that decreases as long as the solvent is recycled into an extraction unit. In analytical extractions pure solvents are always used, so solubility is conserved. This is one more parameter whose behavior changes from small to large scale, and these changes may be important to be evaluated in some way.

10.4.5 Some Published Works on Scale-up of Extraction Processes

In the last years, many researchers have been searching for scale-up criteria, or even for correlations that would allow predicting large-scale extraction processes from laboratory data. Table 10.2 presents some published works in

Table 10.2 Some published works on scale-up of extraction processes.

Process	Product	Scale-up criterion or correlation	Ref.
SFE	clove oil and ginger oleoresin	constant Q/F	27
SFE	cashew nut oil	constant Q/F	28
SFE	clove and vetiver extracts	constant Q/F	18
SFE	chamomile extract	constant v and d_p	29
SFE	peach almond oil	constant Q/F	17
SFE	clove oil and sugarcane filter cake	constant S/F	19
	policosanol		
SFE	striped weakfish oil	constant Q/F	20
UAE	rosemary extract	constant S/F	30
UAE	apple pomace extract	constant S/F	31
SLE	various	constant residence time	32
MAE	soybean and rice bran oils	constant S/F	33

F = feed; Q = solvent flow rate; MAE = microwave-assisted extraction; SFE = supercritical fluid extraction; SLE = solid-liquid extraction; UAE = ultrasound-assisted extraction.

this field, where it can be observed that finding universal criteria for scale-up is far from being a simple task, and works mostly divide themselves between using S/F or Q/F as the scale-up criterion.

10.5 Case Study: Supercritical CO₂ Extraction from Red Pepper

This section presents a set of experimental results obtained for the scale-up of an extraction process. The process consisted in supercritical extraction from *Capsicum frutescens* peppers using, as solvent, carbon dioxide. Extractions were performed at laboratory and pilot scale, and their curves are compared in terms of yield and kinetics, also using mathematical modeling.

10.5.1 Experimental Procedures

10.5.1.1 Materials

Fruits of *Capsicum frutescens* peppers, locally known as ‘malagueta’, were purchased in a local market in Campinas, southeastern Brazil. The fruits were cleaned, oven-dried at 65 °C for 22 hours, and ground in a knife mill to reduce their particle size, in order to increase mass transfer during the extraction. Carbon dioxide with 99.9% purity (White Martins, Campinas, Brazil) was used as solvent. The SFE pressure and temperature were 15 MPa and 40 °C, respectively.

10.5.1.2 Extractions

Two SFE units were used in the present study. A Spe-ed SFE unit (Applied Separations, Allentown, PA) with a 300 cm³ column was employed for the

laboratory (small scale) extractions. This unit contains a back pressure valve to control the pressure, a pump to drive the liquid solvent into the extraction vessel, which is placed in an oven where temperature is set to the required value. Solvent flow rate is regulated by a micrometer valve after the extractor. This valve also works as the expansion valve of the line, so it is heated at 130 °C to avoid deposition of ice due to the effect of the isenthalpic expansion. The extract is separated from the CO₂ after the expansion by reducing pressure to atmospheric, and collecting it in glass recipients.

For the scale-up experiments, a pilot unit (Thar Technologies, model SFE-2X5LF-2-FMC, Pittsburgh, USA) with a 5150 cm³ column was employed. As well as the Spe-ed SFE unit, this equipment has a back pressure valve to control the pressure and a pump to drive the liquid solvent into the extraction vessel, which is involved in a heating mantle that maintains it at the required temperature. Immediately before the extraction vessel, a heat exchanger ensures that the solvent is at the set temperature. Solvent flow rate is regulated by the pump which is controlled by software. A set of three separators connected in series is used to separate the extract from the CO₂. During the experiments, the first separator worked at 8 MPa and 40 °C, the second one at 5 MPa and 30 °C and the last one at 3 MPa and 30 °C. Differently from the bench equipment Spe-ed unit, the extract cannot be collected continuously. As a result, for each point of the kinetics experiment, the solvent flow rate must be interrupted, maintaining the extraction vessel pressurized, and the separators depressurized, so that the extract can be collected. Afterwards, the experiment can be restarted. As a result, the operation of this equipment to obtain extraction curves generates small periods of static extraction, whose influence is not significant.³⁴

The extracts were collected at defined times, and each sample was weighed in order to build the SFE curves, representing yield versus time. In the pilot unit, ethanol was used to help remove the extract which remained stuck on the separator's surface. Afterwards, ethanol was removed through evaporation under vacuum at 50 °C. Both laboratory- and pilot-scale extractions were performed in duplicate.

Table 10.3 shows the process parameters for laboratory and pilot extractions, needed to define the scale-up criteria and to apply the mass transfer model to fit the SFE curves. All these data were determined experimentally, except CO₂ density, which was obtained from the literature.³⁵

The scale-up criterion adopted in this study was to keep constant the ratio between solvent flow rate and substrate feed (Q/F), as stated in Section 10.4.2.2, considering that diffusion would be the controlling mechanism of this process. From the data of Table 10.3 that ratio can be calculated to be 0.0165 (kg CO₂)/(kg solid.s).

10.5.1.3 Mathematical Model

The broken and intact cell model of Sovová¹⁵ was applied to fit both laboratory- and pilot-scale extraction curves. A multiple-fitting approach was adopted to fit each pair of duplicates simultaneously, leading to a unique set of

Table 10.3 Process parameters for laboratory and pilot scale SFE of *Capsicum frutescens* pepper at 15 MPa and 40 °C.

	Laboratory	Pilot
F (kg)	0.10001 ± 0.00001	1.99 ± 0.01
d_p (mm)	0.34 ± 0.02	0.34 ± 0.02
extractor height (cm)	12.54	61.4
bed height filled with glass spheres (cm)	3.34	15.0
L (cm)	9.2	46.4
D (cm)	5.42	10.34
L/D	1.7	4.5
extractor volume (cm ³)	289.33	5155.84
bed volume (cm ³)	212.26	3879.47
Q (kg/s)	$(1.65 \pm 0.02) \times 10^{-4}$	0.0033
ρ_s (kg/m ³)	1320 ± 10	1320 ± 10
ρ (kg/m ³)	780.23	780.23
Y^* (kg solute/kg CO ₂)	0.003166	0.003166
X_0 (kg solute/kg feed)	0.069 ± 0.003	0.069 ± 0.003

F = solvent feed; d_p = particle diameter; L = extraction bed height; D = extraction bed diameter; Q = solvent flow rate; ρ_s = solid density; ρ = solvent density; Y^* = extract solubility in the solvent; X_0 = extraction yield.

parameters for each scale. A derivative-free routine³⁶ was used to fit the model to the experimental data. The objective function (f) to be minimized was the sum of squared errors.

10.5.2 Results and Discussion

Tables 10.4 and 10.5 present the experimental data for the SFE at laboratory and pilot scale, respectively, as well as the modeled curves. In Table 10.6 the values of the objective function and the adjusted model parameters for both scales are reported. The experimental and modeled curves are illustrated in Figure 10.11.

As stated in Section 10.2, in a scale-up procedure one expects to achieve in the large scale the same process behavior obtained in laboratory. By observing Figure 10.11 it is possible to notice that the scale-up of *Capsicum frutescens* pepper did not succeed in achieving such a goal. The SFE curves obtained at laboratory and pilot scale are quite different. Moreover, the values of the modeled parameters shown in Table 10.6 reveal some differences between both processes. In this sense, it should be remembered that, for both scales, the applied model fitted very well to the experimental data, so the values of the adjusted parameters can be discussed. Among those parameters, the most outstanding differences appear in the constant extraction period (t_{CER}) and in the convective mass transfer coefficient (k_f). On the other hand, the values of X_k , the solute ratio inside the cells, is similar for both scales, as expected since this value depends only on the SFE pressure and temperature, particle diameter and pre-treatment procedures, which were the same.

Table 10.4 Experimental and modeled kinetic data for the laboratory scale SFE from *Capsicum frutescens* pepper at 15 MPa and 40 °C.

Time (min)	M_{CO_2} (kg)	Extraction (a)		Extraction (b)		Model	
		$F = 0.100012 \text{ kg}$		$F = 0.1000065 \text{ kg}$			
		Extract (g)	Yield (%)	Extract (g)	Yield (%)	Extract (g)	Yield (%)
0	0.0000	0.0000	0.00	0.0000	0.00	0.0000	0.00
5	0.0495	0.1097	0.11	0.0935	0.09	0.1094	0.11
10	0.0990	0.2571	0.26	0.2265	0.23	0.2199	0.22
15	0.1485	0.3894	0.39	0.3507	0.35	0.3303	0.33
20	0.1980	0.5228	0.52	0.4746	0.47	0.4408	0.44
30	0.2970	0.7872	0.79	0.7270	0.73	0.6617	0.66
45	0.4455	1.1633	1.16	1.0636	1.06	0.9930	0.99
60	0.5940	1.4848	1.48	1.3746	1.37	1.3243	1.32
75	0.7425	1.7484	1.75	1.6411	1.64	1.6557	1.66
90	0.8910	1.9751	1.97	1.8913	1.89	1.9705	1.97
110	1.0890	2.2485	2.25	2.1785	2.18	2.3492	2.35
135	1.3365	2.5630	2.56	2.5539	2.55	2.7457	2.75
155	1.5345	2.7584	2.76	2.7690	2.77	2.9857	2.99
180	1.7820	3.0323	3.03	3.0527	3.05	3.1782	3.18
210	2.0790	3.3022	3.30	3.3416	3.34	3.3667	3.37
240	2.3760	3.5332	3.53	3.5906	3.59	3.5465	3.55
270	2.6730	3.7349	3.73	3.8130	3.81	3.7178	3.72
300	2.9700	3.9260	3.93	4.0177	4.02	3.8810	3.88

Table 10.5 Experimental and modeled kinetic data for the pilot scale SFE from *Capsicum frutescens* pepper at 15 MPa and 40 °C.

Time (min)	M_{CO_2} (kg)	Extraction (a)		Extraction (b)		Model	
		$F = 2 \text{ kg}$		$F = 1.9895 \text{ kg}$			
		Extract (g)	Yield (%)	Extract (g)	Yield (%)	Extract (g)	Yield (%)
0	0.0	0.0000	0.00	0.0000	0.00	0.0000	0.00
15	3.0	2.5474	0.13	3.0480	0.15	4.6524	0.23
30	6.0	7.1697	0.36	8.0876	0.41	9.3123	0.47
45	9.0	12.4535	0.62	13.8230	0.69	13.9721	0.70
90	18.0	28.7807	1.44	29.6527	1.49	27.9515	1.40
135	27.0	42.3356	2.12	43.5346	2.19	41.9310	2.10
180	36.0	54.2386	2.71	55.1462	2.77	55.2949	2.77
240	48.0	70.0592	3.50	69.7438	3.51	70.6436	3.54
300	60.0	83.9220	4.20	82.7470	4.16	82.9168	4.31

As observed in Table 10.6, t_{CER} is considerably higher at the pilot extraction. Physically, this means that more time was spent to extract all the easily available solute. Since this step is controlled only by convection, the convective coefficient would be expected to be lower at pilot scale, and it effectively is. The possible reasons that led to those differences should be discussed.

Table 10.6 Model parameters and objective function for the SFE from *Capsicum frutescens* pepper at 15 MPa and 40 °C in laboratory and pilot scales.

Parameter	Laboratory	Pilot
t_{CER} (min)	82	145
X_k (kg solute/kg feed)	0.0413	0.0400
k_f (s^{-1})	1.08×10^{-3}	6.95×10^{-4}
k_s (s^{-1})	1.90×10^{-5}	4.30×10^{-5}
f	3.93×10^{-7}	2.51×10^{-5}

t_{CER} = constant extraction rate period; X_k = solute in the unbroken cells; k_f = mass transfer coefficient in the solvent phase; k_s = mass transfer coefficient in the solid phase; f = objective function.

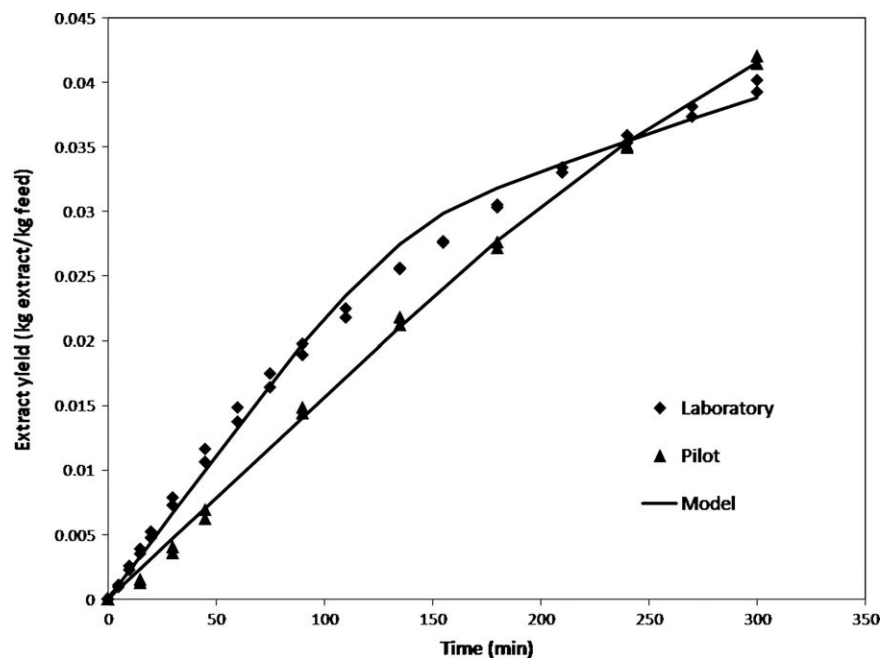


Figure 10.11 Experimental and modeled SFE curves from *Capsicum frutescens* pepper at 15 MPa and 40 °C at laboratory and pilot scales.

1. Bigger extraction beds require additional care on packing. They are not as controllable as laboratory extractors, thus the risk of irregular packing and forming of preferential paths to the solvent is increased. If preferential paths were formed, the solvent would be unable to reach all the easily available solute, and the system would work as the Q/F ratio was higher than expected. As a result, the extraction rate is lower than expected, reflecting in the higher t_{CER} and lower k_f , when comparing to the laboratory extraction.

2. The separation methods of the equipment are different. In small scale the pressure is suddenly reduced to atmospheric through an expansion valve, and all the extract is collected in a unique collector. Minimal extract residues remain in the equipment pipelines. In the pilot equipment, due to the high flow rates, the use of separators with gradual pressure reduction is needed to avoid ice formation from isenthalpic expansion. Significant deposition of extract on the separator's walls was observed, and the recovery of this extract was done using ethanol as solvent. Therefore, an additional separation step was adopted to evaporate the ethanol under vacuum and, then, to obtain the solvent-free extract. The complexity of the separation procedure at the pilot extraction may lead to higher extract losses, even of pepper oleoresin that remains stuck on the separators, or evaporation of volatile compounds together with ethanol. Thus, the recovery of extract may have occurred in rates lower than those potentially feasible at the performed conditions, leading to the differences in the SFE curves, t_{CER} and k_{f} .
3. Another scale-up criterion to be considered is the extraction bed geometry (see Sections 10.2.2.2 and 10.3.4). As mentioned before, L/D ratios from 5 to 7 are usually recommended for industrial extraction, in order to achieve the best yields and rates. In Table 10.3 the ratios of both laboratory and pilot scale are shown, and they are 1.7 and 4.5, respectively. Here, the pilot scale configuration is closer to that recommended for industrial processes, and it can be possible to determine what worked better at pilot SFE to relate with such a difference. Table 10.6 shows that the solid phase mass transfer coefficient, k_{s} , is higher in the pilot extraction. Indeed, in Figure 10.11 a clear trend to achieve higher yield at pilot scale than in laboratory can be noticed, if both curves are extrapolated. In this sense, high L/D ratio seems to be positive for the diffusive period of extraction, where radial diffusion should be prevented. Moreover, in such a configuration the enhanced contact between solvent and substrate may have helped to accelerate diffusion.
4. The effect of the solvent flow rate may also be important in scale-up. First, Q is related to the residence time of the solvent inside the extraction bed, which gives an indication of the contact time between solvent and solute. As stated in Section 10.2.2.1, contact time is important to promote the mass transfer mechanisms of extraction. In this sense, excessively high solvent flow rates could reduce the contact time to levels where part of the solute would not be recovered. Another possible effect of high solvent flow rates is the mechanical drag of some insoluble compounds from the solid, which would lead to yields higher than those predicted.

10.6 Conclusion

Predicting extraction processes at the industrial scale will always be one of the most important tasks for food and chemical engineers. After many decades of study, the theoretical basis of extraction is well-established, since hundreds of

works on thermodynamic and mass transfer aspects of extraction have been published. Even so, the challenge of reproducing in industry what was achieved in the laboratory remains for further generations. In this chapter a vast revision of the important parameters of the extraction process was conducted, looking at how they may vary from small to large scale, giving some clues to engineers that aim to propose scale-up criteria. Then, it was shown that a classical scale-up criterion, such as keeping Q/F , can be satisfactory for a limited range. Nevertheless, the case study has shown that when stepping from laboratory- to pilot-scale extractions, the nature and intensity of some mass transfer phenomena are inevitably modified. Thus, process engineers must work hard to comprehend and to predict the influence of each change of process characteristics that accompany the move from laboratory to pilot plant and industrial procedures. The adequate scale-up of a process is of paramount importance to a further and not less important step, which is cost estimation.

References

1. K. J. Valentas, L. Levine and J. P. Clark, *Food Processing Operations and Scale-up*, Marcel Dekker Inc., New York, NY, 1991.
2. S. I. Sandler, *Chemical, Biochemical, and Engineering Thermodynamics*, John Wiley & Sons Inc., Hoboken, NJ, 2006.
3. R. B. Bird, W. E. Stewart and E. N. Lightfoot, *Transport Phenomena*, John Wiley & Sons Inc., New York, NY, 2007.
4. A. K. K. Lee, N. R. Bulley, M. Fattori and A. Meisen, *J. Am. Oil Chem. Soc.*, 1986, **61**, 921.
5. J. R. Welty, C. E. Wicks, R. E. Wilson and G. L. Rorrer, *Fundamentals of Momentum, Heat and Mass Transfer*, John Wiley & Sons Inc., Hoboken, NJ, 2008.
6. G. Brunner, *Gas Extraction: An Introduction to Fundamentals of Supercritical Fluids and Their Application to Separation Processes*, Darmstad, Steinkopff, Germany, 1994.
7. J. Mendez-Santiago and A. S. Teja, *Ind. Chem. Eng. Res.*, 2000, **39**, 4767.
8. V. M. Rodrigues, E. M. B. D. Sousa, A. R. Monteiro, O. Chivone-Filho, M. O. M. Marques and M. A. A. Meireles, *J. Supercrit. Fluids*, 2002, **22**, 21.
9. J. C. Francisico and B. Sivik, *J. Supercrit. Fluids*, 2002, **23**, 11.
10. V. F. Cabral, W. L. F. Santos, E. C. Muniz, A. F. Rubira and L. Cardozo-Filho, *J. Supercrit. Fluids*, 2007, **40**, 163.
11. N. Mezzomo, B. R. Mileo, M. T. Friedrich, J. Martínez and S. R. S. Ferreira, *Biores. Tech.*, 2010, **55**, 132.
12. S. Mazzutti, S. R. S. Ferreira, C. A. S. Riehl, A. Smania, Jr., F. A. Smania and J. Martínez, *J. Supercrit. Fluids*, 2012, **70**, 48.
13. C. C. Teo, S. N. Tan, J. W. H. Yong, C. S. Hew and E. S. Ong, *J. Chromatogr. A*, 2010, **1217**, 2484.
14. M. M. Esquivel, M. G. Bernardo-Gil and M. B. King, *J. Supercrit. Fluids*, 1999, **16**, 43.

15. H. Sovová, *Chem. Eng. Sci.*, 1994, **49**, 409.
16. M. Perrut, *Ind. Chem. Eng. Res.*, 2000, **39**, 4531.
17. N. Mezzomo, J. Martínez and S. R. S. Ferreira, *J. Supercrit. Fluids*, 2009, **51**, 10.
18. J. Martínez, P. T. V. Rosa and M. A. A. Meireles, *The Open Chem. Eng. J.*, 2007, **1**, 1.
19. J. M. Prado, G. H. C. Prado and M. A. A. Meireles, *J. Supercrit. Fluids*, 2011, **56**, 231.
20. A. C. Aguiar, J. V. Visentainer and J. Martínez, *J. Supercrit. Fluids*, 2012, **71**, 1.
21. M. G. Bernardo-Gil and M. Casquilho, *AiChE J.*, 2007, **53**, 2980.
22. J. Puiggene, M. A. Larrayoz and F. Recasens, *Chem. Eng. Sci.*, 1997, **52**, 195.
23. J. Shi, Y. Kakuda, G. Mittal and Q. Pan, *J. Food Eng.*, 2007, **78**, 33.
24. E. P. Carvalho, F. Pishnitchenko, N. Mezzomo, S. R. S. Ferreira, J. M. Martínez and J. Martínez, *Comput. Chem. Eng.*, 2012, **40**, 148.
25. R. Eggers and P. T. Jaeger, in *Extraction Optimization in Food Engineering*, ed. C. Tzia and G. Liadakis, Marcel Dekker Inc., New York, NY, 2003.
26. G. Nuñez, C. A. Gelmi and J. M. Del Valle, *Comput. Chem. Eng.*, 2011, **35**, 2687.
27. P. T. V. Rosa and M. A. A. Meireles, *J. Food Eng.*, 2005, **67**, 235.
28. R. N. Patel, S. Bandyopadhyay and A. Ganesh, *J. Chromatogr. A*, 2006, **1124**, 130.
29. P. Kotnik, M. Skerget and Z. Knez, *J. Supercrit. Fluids*, 2007, **43**, 192.
30. L. Paniwnyk, H. Cai, S. Albu, T. J. Mason and R. Cole, *Ultrasonics Sonochem.*, 2009, **16**, 287.
31. M. Virost, V. Tomao, C. Le Bourvellec, C. M. G. C. Renard and F. Chemat, *Ultrasonics Sonochem.*, 2010, **17**, 1066.
32. E. Simeonov, I. Seikova, I. Pentchev and A. Mintchev, *Ind. Chem. Eng. Res.*, 2004, **43**, 4903.
33. B. G. Terigar, S. Balasubramanian, C. M. Sabliov, M. Lima and D. Boldor, *J. Food Eng.*, 2011, **104**, 208.
34. J. M. Prado, I. Dalmolin, N. D. D. Carareto, R. C. Basso, A. J. A. Meireles, J. V. Oliveira, E. A. C. Batista and M. A. A. Meireles, *J. Food Eng.*, 2012, **109**, 249.
35. <http://webbook.nist.gov/chemistry/fluid/> (accessed in August, 2012).
36. M. J. D. Powell. *Subroutine BOBYQA*, Department of Applied Mathematics and Theoretical Physics, Cambridge University, 2009.

CHAPTER 11

Integration of Pressurized Fluid-based Technologies for Natural Product Processing

DIEGO T. SANTOS, MARIA T. M. S. GOMES,
RENATA VARDANEGA, MAURICIO A. ROSTAGNO
AND M. ANGELA A. MEIRELES*

LASEFI/DEA/FEA (School of Food Engineering)/UNICAMP (University of Campinas), R. Monteiro Lobato, 80; 13083-862, Campinas, SP, Brazil

*Email: meireles@fea.unicamp.br

11.1 Introduction

As discussed in the previous chapters, pressurized liquids and sub/supercritical fluids can be very efficient in extracting a wide range of bioactive compounds from natural sources. The complexity of natural product matrices, the need of isolating specific bioactive compounds and the high costs involved are prompting the development of new strategies to improve the whole process. One of these developments is the concept of integrating different stages into one single on-line operation.¹

Usually, several different processes are required for the production of highly concentrated extracts. The most important processes involved in the production of extracts from natural products are the extraction of target compounds, their purification, the elimination of the solvent and their stabilization by encapsulation and particle formation. Most of these processes are performed sequentially and one process cannot start before the preceding has

RSC Green Chemistry No. 21

Natural Product Extraction: Principles and Applications

Edited by Mauricio A. Rostagno and Juliana M. Prado

© The Royal Society of Chemistry 2013

Published by the Royal Society of Chemistry, www.rsc.org

been completed. Depending on the raw material and on the desired characteristics of the final product, different processes and techniques may be used.

As it is a complex process with several factors involved, the production of extracts from natural sources can be a challenge. The high operational and investment costs involved, due to several pieces of equipment, long process time and associated labor and utilities, are translated to the manufacturing costs of the extracts, which is one of the main challenges to be faced by the industry.² In this context, the use of process integration can be explored to address these problems and set the basis for a modern and efficient natural product industry. However, it is necessary to have adequate knowledge of the processes involved in order to explore their characteristics at most and to improve the overall process.

The processing of most natural products for the production of extracts involves the use of one or more solvents (liquid, supercritical or a mixture of both) in a sequential manner using different processes. Therefore it presents a high potential to implement the concept of process integration. In most cases, pressurized fluid technology (pressurized liquids and sub/supercritical fluids) can be used to replace traditional methods and fully integrate the processes from extraction to solvent evaporation and particle formation. In fact, pressurized fluid technology is not considered as an alternative to single procedures; the full potential of this technology can only be achieved by using an integrated approach.^{3,4}

There are several potential applications of this concept to natural products processing. In the next sections, the use of pressurized fluid-based technologies in integrated systems will be discussed, as well as the possibility and perspectives of coupling different processes for the production of extracts from natural sources. Finally, a case study dealing with the extraction and stabilization of bixin-rich extract from annatto seeds employing pressurized fluid technology will be presented to illustrate the concept of integrating different processes in one single on-line operation.

11.2 Sequential Extraction using Different Process Conditions or Techniques

Sequential extraction is a well-known procedure that can be useful to improve the process selectivity and the recovery of different types of extracts from the same raw material. Depending on the raw material, it is possible to perform successive extractions employing different solvents or process conditions (pressure and/or temperature) to selectively extract different classes of compounds. Pressurized liquids and supercritical fluids present several characteristics that can be explored to achieve this objective.

In general, supercritical CO₂ can be employed for extracting non-polar to moderately polar phytochemicals, while water, ethanol and other polar organic solvents are better for extracting polar compounds. For the extraction of moderately polar phytochemicals using supercritical CO₂ it is usually necessary to add a co-solvent, such as ethanol or another organic solvent. The amount

and type of co-solvent depends on the matrix and the polarity of the target compounds, with concentrations ranging from 1% to 90% of the total solvent mass.^{3–5}

There are several reports available in the literature where a sequential extraction strategy was adopted for a comprehensive extraction of different compound classes from the same raw material. In several cases, supercritical fluid extraction (SFE) using CO₂ is employed in a first extraction step to extract lipophilic compounds and later a more polar solvent (liquid solvent or modified CO₂) also under pressure is employed in order to extract polar compounds (Table 11.1).

To illustrate the principle we can take as an example a study found in the literature dealing with grape seeds. In this case, the grape seeds were initially extracted using pure supercritical CO₂, which removed over 95% of the oil present. In a sequential extraction step, the residue was re-extracted using subcritical CO₂ modified with methanol (40%); this step removed over 79% of catechins and epicatechins present. In the last extraction step, polyphenolic dimers/trimers and procyanidins were extracted from the residue using pure methanol. Each extraction step was carried out using different conditions and produced a different extract with unique composition and characteristics. Furthermore, the whole process was carried out on a single instrumental extraction system, demonstrating the potential of pressurized fluid technology to implement the sequential extraction strategy.¹²

This is only one of the possible approaches to adopt this strategy, but depending on the raw material different processes can be combined or replaced. For example, in the case of *Ginkgo biloba* leaves, the raw material was extracted twice using 70% ethanol (under reflux) and after the evaporation of the solvent, the extract was further extracted using supercritical CO₂. The first extraction step was used to recover flavonoids and terpenoids while the second extraction step (SFE at 300 MPa, 60 °C, using CO₂ + 5% ethanol as modifier) was used for purification of the extract. Using the second extraction step (SFE) to replace conventional methods has several advantages: not only does it replace additional purification processes, where large amounts of solvents such as acetone and chloroform and different purification columns are used (see chapter 9), but it also leads to higher extract yields and products with higher concentration of flavonoids and terpenoids.¹³

The main interest in the use of the sequential extractions is to fully explore the potential of the raw material to produce different types of extracts. But it can also be used as a tool to eliminate undesirable components of the raw material and to improve extraction yields of target compounds. The extraction of grape skins with supercritical CO₂, for instance, improved the subsequent recovery of polyphenols from the residue using 50% ethanol–water mixture at 60 °C under atmospheric pressure.¹⁴ The removal of non-polar components by the CO₂ increased the yields of the second step by 2–3 times when compared to the single-step conventional extraction.

Obviously, the sequential extraction strategy can be used for off-line processing or for combining techniques. However, combined processes using

Table 11.1 Applications of sequential processes for the extraction and purification of natural products.

<i>Raw material</i>	<i>Components/ compounds</i>	<i>Process</i>	<i>Observations</i>	<i>Ref.</i>
Stevia leaves	glycosides	First step: pretreatment of the leaves by SFE. Second step: extraction of the stevia glycosides by SFE using CO ₂ as solvent and water and/or ethanol as co-solvent.		6
Jabuticaba (<i>Myrciaria cauliflora</i>)	anthocyanin pigments and lipophilic compounds	First step: PLE using ethanol to obtain polar compounds including anthocyanin pigments. Second step: SFE with CO ₂ to recover low polarity compounds.	Fractionated extractions of jabuticaba skins were successfully performed, producing two valuable extracts with antioxidant activities. The extract from the first step was rich in anthocyanin pigments, and the extract from the second step was rich in lipophilic compounds including essential oils and less polar flavonoid compounds.	7
Turmeric (<i>Curcuma longa</i> L.)	curcuminoids	First step: SFE using CO ₂ at 22.5 MPa and 35 °C. Second step: SFE using CO ₂ and 50% ethanol or isopropyl alcohol at 30 MPa and 30 °C.	The SFE allowed to obtain a volatile oil fraction using pure CO ₂ and a curcuminoids fraction using co-solvents.	8
Elderberry pomace (<i>Sambucus nigra</i> L.)	anthocyanins	First step: SFE using CO ₂ as solvent at 21 MPa and 40 °C. Second step: ESE using diverse CO ₂ /ethanol/water solvent mixtures (0–90%, 0.5–100%, 0–95%, v/v/v) at 21 MPa and 40 °C.	Higher extract yields, anthocyanin contents and antioxidant activities were obtained with the presence of water, both in the raw material and in the solvent mixture. The CO ₂ dissolved in the ESE solvent mixture favored either anthocyanin content or antioxidant activity, which were not directly related. Compared to the literature data for elderberries and grapes, these fractions had higher anthocyanins contents. From the results, it is proposed adding economic value to this agroindustrial residue by using solvents and techniques 'generally regarded as safe' in the food and pharmaceutical industries.	9, 10

Jambú flowers, leaves and stems (<i>Spilanthes acmella</i>)	spilanthol	First step: SFE using CO ₂ as solvent at 25 MPa and 50 °C. Second step: ESE using CO ₂ as solvent and ethanol, water and their mixtures as co-solvent at 25 MPa and 50 °C.	The first step extracted most of the spilanthol while the second step removed only small amounts of spilanthol that still remained in the vegetal matrix. Higher extraction yields, total phenolic compounds and compounds with high antioxidant activity were obtained when using organic/polar solvents as enhancers, as was the case of ESE (H ₂ O) and ESE (EtOH + H ₂ O) from flowers and ESE (H ₂ O) from leaves.	11
Chardonnay grape seeds	oils, polyphenols and procyanidins	First step: SFE using CO ₂ . Second step: subcritical CO ₂ modified with methanol. Third step: ESE using methanol.	Pure supercritical CO ₂ removed over 95% of the oil from the grape seeds. Subcritical CO ₂ modified with methanol extracted monomeric polyphenols, whereas pure methanol extracted polyphenolic dimers/trimers and procyanidins. At optimum conditions, 40% methanol-modified CO ₂ removed 79% of catechin and epicatechin from the grape seed. The third step provided a dark red solution shown via electrospray ionization HPLC-MS to contain a relatively high concentration of procyanidins.	12
<i>Ginkgo biloba</i> leaves	flavonoids and terpenoids	First step: two cycles of extraction with 70% ethanol for 2 h under reflux. Second step: SFE of the extract obtained in the first step using CO ₂ with modifier (ethanol) under different conditions of temperature (50–80 °C), pressure (10–30 MPa) and concentration of modifier (1–5%).	The combination of steps provides an efficient and economical mean for obtaining flavonoids and terpenoids from <i>Ginkgo biloba</i> leaves. At the most favorable experimental conditions of 30 MPa, 60 °C, and carbon dioxide containing 5% ethanol as modifier, the yield of GBE powder was 2.1% (based on the air-dry weight of <i>Ginkgo biloba</i> leaves) compared to a yield of only 1.8% by conventional solvent extraction.	13

CO₂ = carbon dioxide; ESE = enhanced solvent extraction; EtOH = ethanol; H₂O = water; PLE = pressurized liquid extraction; SFE = supercritical fluid extraction.

pressurized fluids in both extraction steps can take advantage of automation and on-line control of the process.¹⁵ Additionally, there is no material discharge after the first extraction, as it allows both extractions to be carried out in the same vessel eliminating a unit operation, thereby reducing costs. Furthermore, several components of SFE and PLE systems are basically the same and combined equipment can take advantage of this aspect to reduce investment cost.

There are many possibilities for the application of the process integration concept. It can be assumed that the adequacy and economic feasibility of the implementation of a sequential extraction scheme are basically determined by the raw material used, the products obtained and additional costs associated with the use of two extraction processes. Therefore, from an economic perspective, it is necessary to balance the manufacturing costs with the expected benefits for each product. The economics of extraction processes is presented in more detail in Chapter 12.

11.3 On-line Fractionation/Purification

11.3.1 On-line Separators: Fractionation by Changes in Temperature and Pressure

SFE is a technique that has several unique characteristics that can be used to separate different compound classes depending on their physicochemical characteristics. One of these characteristics is that each extracted compound dissolved in the supercritical fluid is soluble only under certain conditions; if these conditions change, the fluid loses its ability to dissolve this compound, which leaves the solution and precipitates. Using this feature, SFE consists basically of two steps: (1) extraction of the soluble substances from the natural raw material by the supercritical solvent and (2) separation of these compounds from the supercritical solvent by its expansion. The separation of extracted compounds and the fluid is achieved by decompression of the mixture inside a collection vessel. Since many supercritical fluids are gases at room temperature, this step is relatively simple.

On the other hand, the characteristics of supercritical fluids, especially their drastic changes in solubility with small changes in temperature and pressure around the critical point, can be used to selectively precipitate some components of the extract while keeping other components dissolved in the fluid. Using this concept, it is possible to separate different compound classes present in the extracts by gradually changing the temperature and/or pressure of the supercritical fluid exiting the extraction vessel. To selectively separate specific components from the solution (extracted compounds and fluid) multiple separator vessels operating at different conditions of temperature and/or pressure can be coupled on-line to the extraction vessel (Figure 11.1). The solute-solvent mixture is separated in the separators by rapidly reducing the pressure, increasing the temperature, or both, which induces the selective precipitation of different compound classes as a function of their different solubility in the supercritical fluid.⁵ If properly designed, it is possible to have

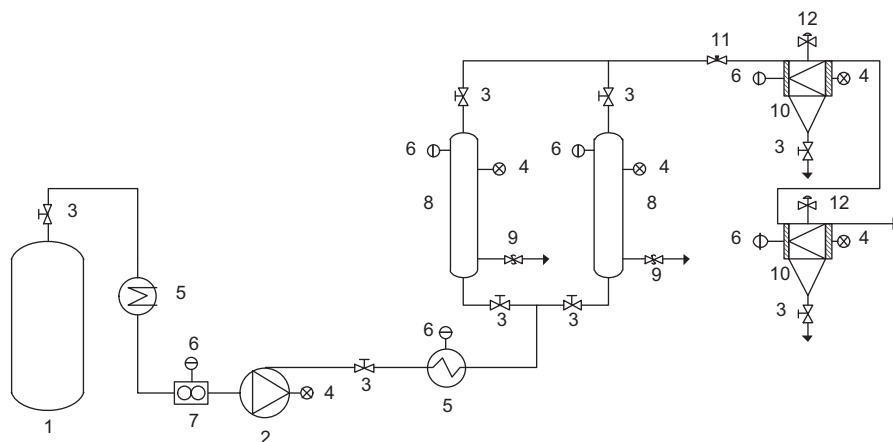


Figure 11.1 Flow diagram of SFE process with two separators. (1) CO₂ reservoir; (2) CO₂ pump; (3) blocking valves; (4) manometers; (5) heat exchanger; (6) temperature controller; (7) flow totalizer; (8) extractors; (9) security valves; (10) separators; (11) micrometric valve; (12) back pressure regulator.

sophisticated extraction systems containing two or more separators, which allow for fractionating the extract into several fractions.

Currently, the use of SFE coupled to on-line fractionation for the extraction of bioactive compounds from natural sources has increased considerably. Different approaches and several applications have been successfully used.^{5,16–24} The classical example of this strategy is the separation of waxes from essential oils. For such applications, the extraction temperature and pressure are usually set to achieve a high CO₂ density, which allows maximizing the extraction of both waxes and essential oils. By taking advantage of the different solubility exhibited by waxes and essential oil compounds at temperatures around 0 °C in liquid CO₂ it is possible to separate them using two separators. Under these conditions, the solubility of waxes is near to zero while essential oils compounds remain completely miscible. Therefore, waxes solubilized during supercritical extraction can be precipitated in the first separator set at 0 °C whereas essential oil compounds can be collected in the second separator maintained at higher temperature and lower pressure. In the last separator the large pressure reduction induces the change of CO₂ to the gaseous state; in this state it can be completely eliminated from the solid or liquid extract, and can still be recycled in the process.^{25–27}

Another classical example is the extraction and subsequent fractionation of different compounds from hops (*Humulus lupulus*). In this case, SFE can be used to enrich and to fractionate the essential oil and the bitter principles of hops, both of which contribute to the flavor of beer. The sequential fractionation of the extract by stepwise reducing the pressure may be used to produce two different extracts with unique properties (green and yellow extracts).¹⁶

Essentially, the changes in the thermodynamic equilibrium between the fluid and the extracted components caused by the modification of the temperature and/or pressure of the medium inside the separator can be explored for the purification of a wide range of natural products. Its usefulness will depend on several factors, including the components of the extract and conditions used (temperature, pressure, flow rate, equipment design, etc.). In some cases, it has been proven to be a highly effective strategy, while in other processes it has limited applications. However, it can be considered as a potential alternative for a pre-purification step providing the first separation of the extract components. Furthermore, since for the recycling of the CO₂ it is necessary to use a minimum pressure, usually around 6 MPa, using separators under controlled temperature and pressure can be translated to reduced operational costs.

11.3.2 On-line Extraction and Adsorptive Purification Processes

Extracts obtained from natural products usually are complex mixtures of different types of components from the raw material. Due to the often low concentration of target compounds and high amounts of undesirable co-extracted material, additional steps are usually necessary to either enrich the extract or to separate specific components from the mixture. The most used techniques for a highly efficient separation are based on the selective adsorption of the target compounds on an appropriate adsorbent. The main techniques of this type of separation are chromatography (liquid, gas and supercritical) and solid-phase extraction (SPE). Besides high efficiency and speed, these techniques have the additional advantage of allowing coupling to other processes, such as extraction. Initially developed for relatively large-scale chemical processing applications, the on-line coupling of extraction to purification with adsorptive processes has also been used for natural products. Basically it can be explored to selectively retain target compounds and remove undesirable co-extracted components or to selectively remove target compounds while retaining co-extracted components.

Since extraction and purification can be performed using a solvent under pressure, they are natural candidates to adopt the process integration concept. It is technically possible to have combinations of all the different types of these techniques (PLE, SFE, HPLC, SFC, SPE), and their applications range from analytical separation and analysis to industrial separation of highly valuable compounds. Examples of the application of this concept for the extraction and purification of extracts from natural sources are presented in Table 11.2.

Due to the physicochemical properties of supercritical fluids, i.e. lower viscosity and higher diffusion coefficients than liquids, combined with higher solubility than in the vapor phase, SFE offers a number of advantages to integrate adsorptive separation processes. Furthermore, thermal methods are very sensitive and efficient, although they are limited either by the thermal stability of the solute or by the adsorbents used for pre-concentration.⁴³ There are several examples of the coupling of SFE and chromatographic techniques

Table 11.2 Applications of on-line processes for the extraction and purification of natural products.

<i>Raw material</i>	<i>Components/ compounds</i>	<i>Process</i>	<i>Observations</i>	<i>Ref.</i>
seed oil soybean flakes and rice bran	tocopherols	Techniques: SFE-SFC Scale: preparative SFE: Solvent: supercritical CO ₂ Pressure: 25 MPa Temperature: 80 °C SFC: not specified	Total tocopherol recovery and enrichment was found to be a critical function of the mass ratio of CO ₂ /seed charge. Approximately 60% of the available tocopherols in soyflakes can be recovered in the SFE step, yielding enrichment factors of 1.83–4.33 for the four tocopherol species found in soybean oil. Additional enrichment of tocopherols can be achieved in the SFC stage, ranging from 30.8 for δ-tocopherol to 2.41 for β-tocopherol.	28
placentas of <i>Capsicum</i> fruits	capsaicinoids (capsaicin, dihydrocapsaicin, and nordihydrocapsaicin)	Techniques: SFE-SFC Scale: analytical SFE: Sample: 2–80 mg + 0.1 mL of methanol Solvent: CO ₂ Flow rate: 5 mL/min Pressure: 20 MPa Temperature: 40 °C Extraction time: 5 min SPE: Column: silica gel, 50 × 4.6 mm SFC: Stationary phase: C1 (150 × 4.6 mm) Mobile phase: CO ₂ (5 mL/min) and ethanol (0.3 mL/min) Temperature: 40 °C Detection: photodiode-array detector (200–400 nm)	The recovery rate of trapping capsaicin by SPE was 92.1% and the CV was 7.9% (<i>n</i> = 5). Since capsaicinoids are mucous membrane irritants, the SFE-SFC method, which involved fewer manual operations than the extraction–HPLC method, seemed preferable. It was also faster.	29

Table 11.2 (Continued)

Raw material	Components/ compounds	Process	Observations	Ref.
corn bran	removal of oil to obtain fractions enriched with free sterols and ferulate-phytosterol esters	Techniques: SFE-SPE-SFC Scale: preparative SFE: Sample: 175 g Solvent: supercritical CO ₂ Flow rate: 5 L/min Pressure: 34.5 MPa Temperature: 40 °C SFC: Sorbent: 24 g of the amino-propyl 3 steps 1: CO ₂ at 69.0 MPa/80 °C 2: 10 mol% EtOH/CO ₂ , 34.5 MPa/40 °C 3: 15 mol% EtOH/CO ₂ , 34.5 MPa/40 °C	SFE-SFC of corn bran produced a fraction enriched over 4-fold in free sterols and 10-fold in ferulate-phytosterol esters, suggesting that such a scheme could be used industrially to produce a functional food ingredient. The extraction yield of the available oil was 96%.	30
tomatoes, fruit and several food products	lycopene	Techniques: SFE-HPLC Scale: analytical SFE: Sample: 5–61 mg Solvent: supercritical CO ₂ Static extraction: 10 min/90 °C, 40 MPa with 100 µL methanol as modifier Dynamic extraction: 5 min/90 °C, 40 MPa Flow rate: 1.5 mL/min HPLC: Isocratic: 90% acetonitrile/10% methyl- <i>tert</i> -butyl ether Flow rate: 1 mL/min Column: C18 monolithic column (100 × 4.6 mm) Detection: UV-vis	The main advantage of the system is the reliability. The whole analysis takes place in a closed system, so that degradation of lycopene due to atmospheric oxygen and UV light is avoided.	31

soybean flour	isoflavones (genistin, daidzein and genistein)	Techniques: SFE-SPE Scale: analytical SFE: Sample: 1.0 g Solvent: CO₂ and modifier (10 mol% of 70% methanol) Flow rate: 1.0 mL/min Pressure: 20–36 MPa Temperature: 40–70 °C. Extraction time: 3×30 min SPE: ODS trap was rinsed with 1.5 mL of methanol at a flow rate of 0.5 mL/min	The highest extraction yield of genistin and genistein were obtained at 70 °C/20 MPa, although it was lower than for UAE and Soxhlet methods. The highest extraction yield of daidzein was obtained at 50 °C/36 MPa, which was higher than for UAE and Soxhlet methods.	32
hop pellets	phenolic compounds (hydroxybenzoic and hydroxycinnamic acids, quercetin and kaempferol glycosides)	Techniques: PLE-SPE-HPLC Scale: analytical PLE: Sample: 1 g mixed with 2 g diatomaceous earth Temperature: 60 °C Solvent: acetone: water (4:1, v/v) Extraction time: 10 min SPE: PLE extracts were diluted with water prior to SPE to reduce the acetone content. The cartridge was washed with water after application of the sample and DMF-water (85:15, v/v) used for elution	PLE delivered highly concentrated extracts, was much faster than manual extraction and reduced subsequent time-consuming steps like solvent evaporation, thus minimizing the possibility of alteration and degradation of sample compounds.	33
malt samples	proanthocyanidins	Techniques: PLE-SPE-HPLC Scale: analytical PLE: Sample: 4 g mixed with 1.8 g of diatomaceous earth Temperature: 60 °C Pressure: 100 MPa	The combined techniques reduced time and manual work to a minimum compared to manual methods. 20 samples can be processed within 24 h in respect to eight samples with the manual method. The recovery of five main malt proanthocyanidins was 97 %, with a	34

Table 11.2 (Continued)

Raw material	Components/ compounds	Process	Observations	Ref.
green grape	pesticides (lindane, vinclozolin, quinalphos, procymidone, endosulfan, sulfate and tetradifon)	Solvent: acetone:water (80:20, v/v) Static extraction time: 10 min Extraction volume: 14 mL SPE: SPE cartridge was conditioned with 7 mL water. The crude extract was diluted with 24 mL water and the cartridge was washed with 8 mL water and 1 mL DMF-water (85:15, v/v). The adsorbed analytes were eluted with 2 mL of the latter solvent. HPLC: Column: RP-18 column 150 × 4.6 mm Mobile phases: (A) NaH₂PO₄ 0.02 M, pH 3.4 and (B) acetonitrile-NaH₂PO₄ 0.05 M (2:1) Detection: scanning detector (280 nm) Techniques: PHWE-MMLLE-GC-MS PHWE: Sample: 50 mg Flow rate: 1 mL/min Temperature: 120 °C Extraction time: 40 min MMLLE: Membrane: porous polypropylene membrane Porosity: 0.4 µm Elution carried out with a flow rate of 0.2 mL/min for 45 s, leading to 0.150 mL of extract volume	reproducibility of 5%. This new instrumental coupling is thought to reduce time and costs along with improved results for a broad range of solid sample materials. The role of MMLLE is to clean and concentrate the extract before on-line transfer to the GC via a sample loop and an on-column interface using partially concurrent solvent evaporation. The results were in good agreement with those obtained by conventional methods.	35

roots of <i>Scutellaria baicalensis</i> Georgi	flavonoids (baicalin, baicalein and wogonin)	GC-MS: Carrier gas: helium at 150 kPa Detection: flame ionization detector (FID) at 300 °C Techniques: UAE-HPLC Scale: analytical UAE: Sample: 6 mg Solvents: methanol, ethanol, mixtures of methanol or ethanol-water Flow rate: 0.5 to 3.0 mL/min Ultrasonic power: 0, 50, 75, 100 and 150 W HPLC: Mobile phase: water and acetonitrile with 0.1 % phosphoric acid Flow rate: 1 mL/min Detection: UV (280, 360 and 400 nm)	The extraction yields were 73.8–131.5 mg/g for baicalin, 6.8–15.9 mg/g for baicalein and 4.4–14.3 mg/g for wogonin. The extraction yields of flavonoids obtained by the proposed method are comparable to those obtained by dynamic microwave-assisted extraction, static ultrasonic extraction and reflux extraction.	36
Lamiaceae herbs (basil, oregano, rosemary, sage, spearmint and thyme)	phenolic acids (caffeic, chlorogenic, ferulic, gallic, <i>p</i> -coumaric, syringic and vanillic acids)	Techniques: UAE-SPE-HPLC Scale: analytical UAE: Sample: 4–6 mg Solvent: 60% ethanol Flow rate: 0.25 mL/min Temperature: 45 °C Extraction time: 15 min SPE: Strong anion exchange (SAX) sorbents (30 mm × 2.1 mm I.D.) treated with methanol Solid-phase trap: methanol 2 min (2 mL/min) and 10 mM acetic acid 5 min (1 mL/min)	The extra sample clean-up step involving trapping the analytes to strong anion exchange material decreased interference from the matrix and improved the separation, allowing UV detection.	37

Table 11.2 (Continued)

Raw material	Components/ compounds	Process	Observations	Ref.
<i>Lycicnotus pauciflorus</i> Maxim	flavonoid (nevadensin)	HPLC: Column: C18 (75 mm × 4.6 mm, 2.5 μm) Flow rate: 1.0 mL/min Gradient: 0 min 5% B, 2 min 5% B, 6 min 25% B, 13 min 40% B, 26 min 40% B. Eluent A: 0.5% acetic acid (v/v) in water. Eluent B methanol Detection: UV (280 nm) Techniques: DMAE-HSCCC DMAE: Sample: 15 g Solvent: methanol Ratio liquid: solid: 30:1 (v, w) Flow rate: 10 mL/min Microwave power: 200 W HSCCC: Two-phase solvent system composed of n-hexane–ethyl acetate–methanol–water (7:3:5:5, v/v/v/v)	13 mg of nevadensin were isolated from 15.0 g original sample by HSCCC with five times sample injection in 12 h, and the isolation yield of nevadensin was 0.87 mg/g. The mean purity of nevadensin was higher than 98.0%. This on-line method was effective and fast for high-throughput isolation of nevadensin from <i>L. pauciflorus</i> Maxim.	38
<i>Andrographis paniculata</i> Nees	diterpenoids (andrographolide and dehydroandrographolide)	Techniques: DMAE-HPLC Scale: analytical DMAE: Solvent: 60% aqueous methanol Flow rate: 1.0 mL/min Microwave power: 80 W Extraction time: 6 min HPLC: Column: C18 (250 mm × 4.6 mm, 5 μm) Mobile phase: 65% aqueous methanol Flow rate: 1.0 mL/min Detection: UV (225 nm)	Mean recoveries for andrographolide and dehydroandrographolide were 97.7% and 98.7%, respectively. Compared to ultrasonic extraction used in the Chinese pharmacopoeia, the proposed method was demonstrated to obtain higher extraction yield in a shorter time. In addition, only small quantities of solvent (5 mL) and sample (10 mg) were required.	39

<i>Garcinia mangostana</i>	xanthones, α -mangostin and γ -mangostin	Techniques: MAE-HSCCC Scale: analytical MAE: Sample: 5 g Solvent: 95% ethanol Liquid:solid ratio: 10:1 (v, w) Temperature: 70 °C Extraction time: 10 min HSCCC: Solvent: petroleum ether–ethyl acetate–methanol–water (0.8:0.8:1:0.6, v/v) LC: Column: silica gel (100.0 mg/10.0 mm long $\times$ 2.0 mm)	Under optimal conditions, 75 mg of α -mangostin and 16 mg of γ -mangostin were obtained from 360 mg dried extract of <i>G. mangostana</i> within 7 h with purity over 98% in one-step separation.	40
tea	caffeine	Technique: DMAE-LC Scale: analytical DMAE: Sample: 15 mg Solvent: 50% ethanol aqueous Flow rate: 1.0 mL/min Microwave power: 70 W LC: Column: silica gel (100.0 mg/10.0 mm long $\times$ 2.0 mm)	The recovery of caffeine in the tea samples is in the range of 88.2–99.3%. A silica gel column connected with the extraction vessel was used to remove chlorophyll in tea.	41
<i>Flos Carthami</i>	safflower yellow	Techniques: DMAE-UV Scale: Analytical DMAE: Sample: 2–5 mg Solvent: Water, methanol, ethanol, mixtures of methanol–water and mixtures of ethanol–water Microwave power: 20–100 W Flow-rate: 0.6–1.6 mL/min UV Detection: 401 nm	Optimized conditions: Sample: 3 mg; Solvent 60% Methanol; Microwave power: 60 W; Flow-rate: 1.0 mL/min; Extraction time: 4 min; Yield of safflower yellow (%) 11.35. The extraction yield obtained with DMAE was higher than produced by the reference method. The process is monitored on-line, thus the approach is useful for establishing the necessary time to complete the extraction.	42

CO₂ = carbon dioxide; DMAE = dynamic microwave-assisted extraction; DMF = dimethyl formamide; EtOH = ethanol; GC-MS = gas chromatography-mass spectrometry; HPLC = high-performance liquid chromatography; HSCCC = high-speed counter-current chromatography; LC = liquid chromatography; MMLLE = microporous membrane liquid–liquid extraction; PHWE = pressurized hot water extraction; SFC = supercritical fluid chromatography; SFE = supercritical fluid extraction; SPE = solid phase extraction; UAE = ultrasound-assisted extraction; UV = ultraviolet–visible detector.

for on-line extraction, fractionation and analysis, including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC) and supercritical fluid chromatography (SFC).⁴⁴

In most combined processes, the compounds to be separated are dissolved in the supercritical solvent, such as carbon dioxide, which is forced through a packed-bed adsorption column. The desired compounds are retained in the column by the adsorbent and later eluted by a small amount of solvent (or fluid) leading to a concentrated extract. The adsorbent is then regenerated by the pure solvent. This process is a separation based on different adsorptive interactions of the species with the adsorbent, combined with column hydrodynamics and mass-transfer characteristics. SFE combined with an adequate analytical instrument is especially useful if complex samples have to be analyzed, which helps to improve both selectivity and sensitivity of the analytical method.⁴⁵

SFE-SFC seems to be the most logical system for combined and integrated extraction, fractionation, identification and quantification of bioactive compounds from natural sources, since the extraction solvent and the chromatographic mobile phase are in the same physical state. Successful applications of SFE-SFC have been reported for various compounds from different matrixes.^{28–30} For instance, combined supercritical fluid extraction and supercritical fluid chromatography (SFE-SFC) was applied to the simultaneous extraction and analysis of capsaicinoids from the placentas of Capsicum fruits.²⁹ The combination of these techniques has been extensively studied and a general scheme of the integrated process is shown in Figure 11.2A. Notice that since this is a process for extraction and purification of natural products at industrial scale and the purpose is not identification of compounds, no detectors are included in the system.

Basically, separation of the components of the extracts can be achieved by the coupling of the adsorption column before or after the back pressure regulator valve. In the case of the system presented in the Figure 11.2A, the SFE extract stream is directed through the pressure-reducing regulator prior to its deposition onto the chromatographic column. The pressure reduction affects the ability of the fluid to dissolve the extract, which is concentrated at the top of the column without breaking through it. In addition, this gradual reduction of the pressure avoids freezing of the regulator due to the Joule–Thompson expansion effect. After a given volume of CO₂ is used for the extraction of the raw material, the process is terminated and the extraction cell is bypassed. The CO₂ stream is then directed into the column for fractionation of the SFE extract by the selective desorption of retained components from the column. Chromatographic conditions (flow rate, pressure, temperature, co-solvent) can be different from those used for the extraction to provide a selective adsorption of the retained components. After the elution of target compounds is achieved, the adsorbent bed is reconditioned between the runs or during the extraction process.⁴⁶

In contrast, when the chromatographic column is coupled on-line before the back pressure regulator valve, the whole system (extraction and

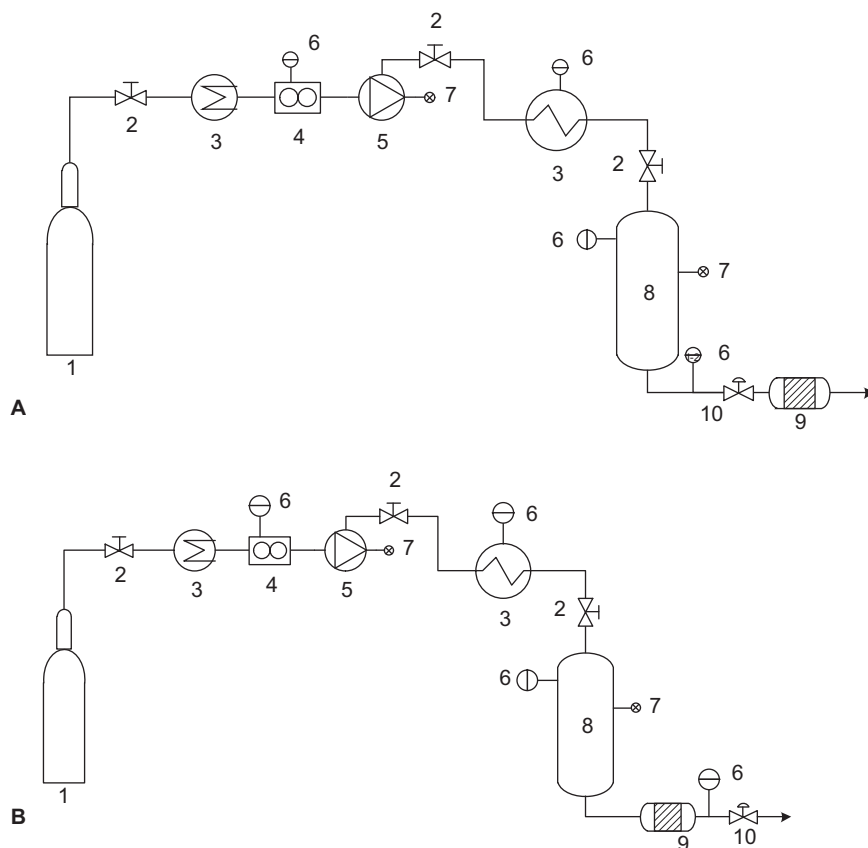


Figure 11.2 A simple flow schematic of a SFE process, showing two types of separation modes: (A) solvent collection and (B) solid-phase trapping. 1: CO₂ reservoir; 2: Blocking valve; 3: Heat exchanger; 4: Flow totalizer; 5: CO₂ pump; 6: Temperature controller; 7: Manometer; 8: Extraction vessel; 9: Chromatographic column; 10: Back pressure regulator valve.

chromatographic column) is operating under the same pressure and using the same solvent (Figure 11.2B). As long as the chromatographic column is able to retain the extract components, the same extraction solvent can be used. After the extraction, the retained extract is later eluted with different conditions and the column is regenerated using fresh solvent. However, the temperature of the chromatographic column can be different from the extraction vessel temperature, which may be useful to promote an adequate separation of the extract components. It is noteworthy that in most cases the temperature of the extraction vessel may be lower than the temperature of the chromatographic column in order to promote reduction of the supercritical fluid density and reduce its ability to dissolve the components of the extracts. Another back pressure regulator valve may also be used after the column, allowing a different pressure for the chromatographic separation compared to the extraction process.

The on-line coupling of extraction techniques and fractionation by adsorptive processes can also be performed using pressurized liquid solvents, such as water, ethanol, ethyl acetate and other organic solvents. Extraction using pressurized liquids has been termed in several different ways, including subcritical solvent extraction, pressurized solvent extraction (PSE), pressurized liquid extraction (PLE) and accelerated solvent extraction (ASE). Pressurized liquids have been successfully used for the extraction of several bioactive compounds from different plants.⁴⁷ A major advantage of PLE over conventional solvent extraction methods conducted at atmospheric pressure is that pressurized solvents remain in a liquid state well above their boiling points, allowing high temperature extraction. Higher extraction temperatures can improve the target compound solubility in the solvent and its desorption from the raw material matrix.⁴⁸ PLE is an attractive alternative because it is usually more efficient and has lower solvent consumption than conventional extraction techniques. PLE enables the rapid extraction (usually completed in less than 30 min) of bioactive compounds in a closed and inert environment under high pressures (no higher than 20 MPa) and temperatures of 25–200 °C.⁴⁹

For the separation and purification of components of an extract obtained by PLE, the most logical approach is the coupling to liquid chromatography since the extraction solvent and the chromatographic mobile phase are in the same physical state. The same aspects previously discussed for SFE-SFC also apply to PLE-HPLC coupling. The basic concept is the retention of the compounds extracted from the raw material by the stationary phase. However, in the case of PLE-HPLC, the extraction solvent/mobile phase characteristics are not heavily influenced by pressure. The main factors influencing the retention of the compounds is the type of stationary phase, the temperature and the type of liquid solvent used. Once the extraction is completed, the compounds retained by the column are eluted using a different solvent composition. In both cases (SFE-SFC and PLE-HPLC), another chromatographic column can be coupled to the outlet of the trapping column to perform the separation/detection of individual components of the extract.

On the other hand, a solid-phase extraction (SPE) column can be used instead of a chromatographic column for the on-line trapping of compounds present in an extract. SPE is an adsorptive based technique with the same principles of chromatography. It is highly efficient and selective, and has been used for concentrating and purifying a wide range of analytes from the most diverse crude extracts.³³

SPE is usually performed in five successive steps. First, the sorbent is activated with an organic solvent. The solid sorbent is conditioned using the same solvent of the extraction process, through which the extract is passed. While target compounds are retained by the sorbent, undesirable co-extractives are removed with the solvent from the sample. Retained impurities may be washed from the solid sorbent with an appropriate solvent. The final step consists in the elution of target compounds with a small amount of an appropriate solvent.⁵⁰ The nature and characteristics of the adsorbent,

the target compounds and the solvent used are the main factors influencing the separation. Another important aspect is the load capacity of the adsorbent.⁵¹

On-line coupling of PLE or SFE to SPE offers the possibility of highly reproducible extraction and purification in only one process, requiring a minimum of manual work. An important advantage of coupling high pressure processes is that the pressure used in the extraction step allows pumping the extract to the purification and analysis steps. In addition, these coupled processes save time. The combination of PLE to other techniques, such as SPE and HPLC, has been successfully used for the extraction and purification of different types of compounds present in a wide range of raw materials. An automated sample preparation by PLE coupled to SPE for liquid chromatography/mass spectrometry, for instance, was used for the investigation of polyphenols in the brewing process.³³ The extraction efficiency was higher compared to manual extraction and the extract presented a significant reduction of co-extracted contaminants. PLE coupled to SPE purification was reported to be much faster than the manual sample preparation and to enhance the selectivity for phenolic compounds.

Although SPE is based on the same chromatographic principles of HPLC, they are not the same technique and have important differences. The main difference can be considered to be the size and shape of the particles used; while in SPE larger and irregular particles (40 μm) are used to allow a high flow-rate through the sorbent, in HPLC much smaller (5–10 μm) and uniform particles are usually employed to maximize separation efficiency. These characteristics are reflected in the lower retention values (k) for compounds commonly obtained by HPLC (0–100) when compared to SPE (>100) and the lower separation factor (α) necessary for an effective separation in HPLC ($\alpha > 1.05$) than in SPE ($\alpha > 4$ –5 or even higher).⁵¹ Therefore, SPE can be considered adequate for the selective retention of components from a complex mixture and elimination of impurities while HPLC/SFC may be better explored for further separation of analytes retained by the solid phase, reaching high levels of purity.

SPE and HPLC column technology is still advancing and new types of materials and chemistries are constantly being developed to further improve selectivity and speed. Examples of these developments include polymeric adsorbents, molecular imprinted polymers, fused-core particles and monolith stationary phases. It can be expected that in the near future the efficiency and durability of adsorbent media that can be used for the purification of natural products will see large improvements. Each advance made in this direction will increase the potential for the integration of extraction and purification using pressurized fluid technology and adsorptive techniques.

It is also clear that SFE-SFC, SFE-SPE and PLE-SPE-HPLC are not the only possible combinations and SFE can be combined to HPLC, for example.³¹ In this case, the extraction is carried out using a supercritical fluid; the extract passes through the chromatographic column, which retains the target components, and finally, the retained components are eluted using a liquid mobile phase after the extraction. Additionally, several other techniques (such

as microwave-assisted extraction and ultrasound-assisted extraction) and different combinations have the potential to be used for the extraction and purification of natural products.^{35–41} However, it is important to highlight the cost involved when adsorptive techniques are used in a productive process. Usually adsorptive materials are expensive and have to be replaced after a few uses. Therefore, high costs are usually associated with its use and its adequacy will depend on the balance between manufacturing costs and the commercialization price of the product. In this aspect, the integration of the adsorptive purification with the extraction part of the process can reduce the impact of the use of this technique, especially when it comes to operational costs, and may represent the best alternative to fully explore its potential.

11.3.3 On-line Coupling of Extraction and Membrane Processes for Purification

Another strategy that can be explored in the field of natural products is the coupling of extraction techniques to membrane separation. This strategy can be used in a number of applications, ranging from concentrating extracts, providing additional fractionation of extracts after the extraction step, and as an alternative post-SFE separation process to avoid the recompression cost associated with phase-based separation methods for recycling the supercritical fluid. Coupling the supercritical technology to the membrane technology for supercritical fluid recovery and product purification can decrease the energy requirements and provide fractionation of the extracts.³

Briefly, membrane processes are based on the relationship between the molecular weight cut-off (MWCO) of the membrane(s) and the molecular weight of the compounds present in the extract. The compounds with molecular weight lower than the MWCO of the membrane are permeated and the compounds with molecular weight higher than MWCO of the membrane are retained (Figure 11.3). This process can be performed in different ways: by passing the entire permeate through the membrane (Figure 11.4A) or by placing the membranes along the flow of the permeate (Figure 11.4B). A singular characteristic of membrane technology is the influence of the permeate flow rate on the selectivity of the membrane; usually higher selectivity is achieved with low permeate flow rate, and increasing the permeate flow rate reduces the selectivity.⁵² The influence of the permeate flow rate in the selectivity is especially important in the case of the membrane system shown in Figure 11.4B.

The coupling of membrane separation processes to SFE is relatively straightforward (Figure 11.5), and may present several advantages over conventional methods. Besides the implicit advantage of using an on-line system and the individual benefits of SFE in the extraction stage, the use of supercritical CO₂ as extracting solvent allows working with high permeate flow rates due its low viscosity when compared to liquid solvents. However, it is important to highlight that SFE is a high pressure process while membrane separation is usually carried out at low pressure (0.1–4 MPa). Therefore, it is

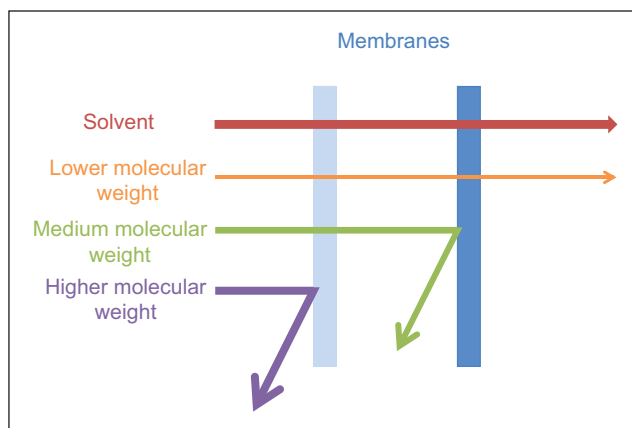


Figure 11.3 Representation of the operating principle of the membrane separation.

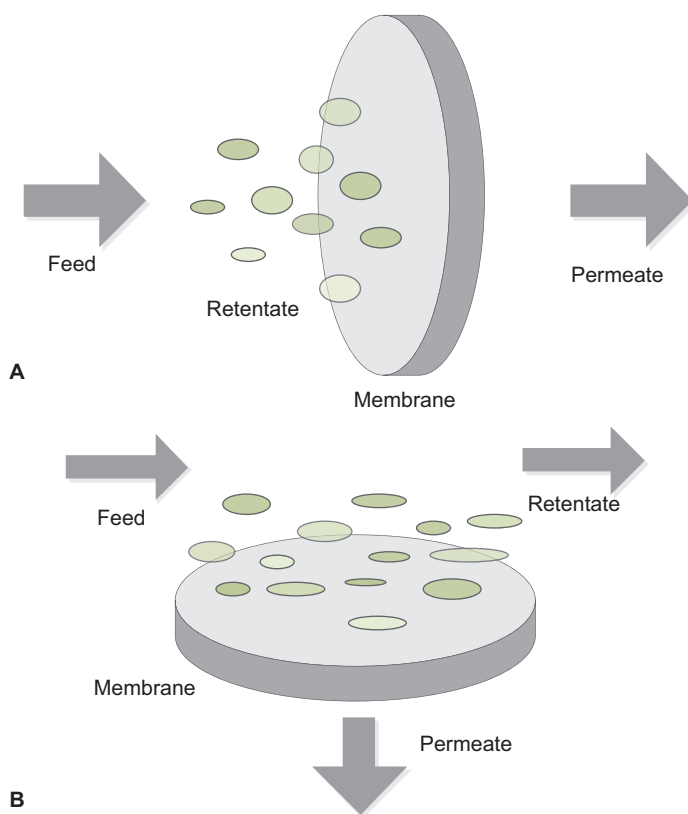


Figure 11.4 Hydrodynamic configurations of membrane separation systems: (A) dead-end mode and (B) tangential mode.

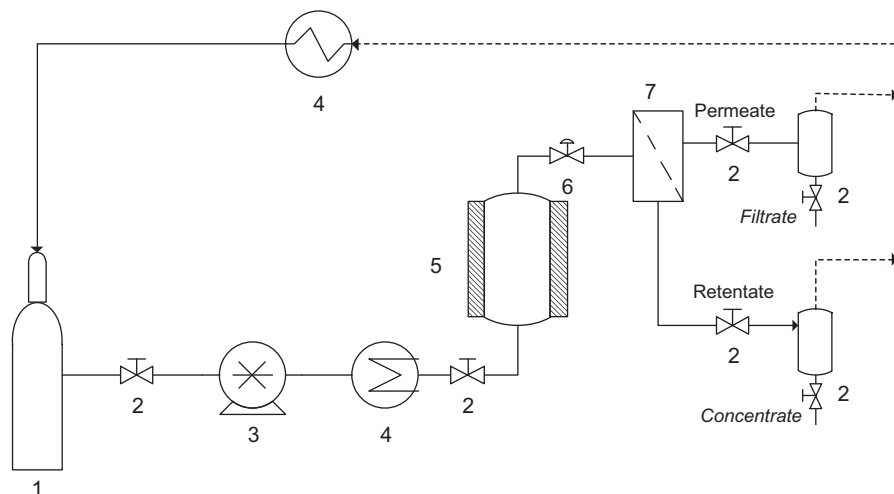


Figure 11.5 Scheme of a unit of supercritical CO₂ extraction coupled to membrane separation process. (1) CO₂ cylinder; (2) blocking valves; (3) pump; (4) heat exchanger; (5) extractor; (6) back pressure regulator valve; (7) membrane module.

necessary to reduce the pressure of the permeate (i.e. the solvent–extract mixture) after the extraction and before the membrane module, which is usually achieved by a back pressure regulator valve. These and other aspects involved in the integration of membrane separations to SFE were reviewed in detail by Sarrade *et al.*⁵³

There are several studies where membranes are used for purification of SFE extracts and recovery of CO₂ after the extraction.^{52,54–58} Different types of membranes were evaluated for the recovery of CO₂ after extraction of essential oils from natural products.^{52,54} Nanofiltration membranes were used for the separation of compounds with different molecular weights obtained by SFE of foodstuffs, such as butter and fish oil, for the recovery of lipids.⁵⁵ Nanofiltration using polymeric membranes and reverse osmosis membranes were evaluated for the concentration of polyphenols from cocoa seed extracts obtained with supercritical CO₂ and ethanol as co-solvent.⁵⁶ Furthermore, membranes can also be used for the separation between supercritical CO₂ and another solvent, such as ethanol and petroleum fractions.^{53,58} Without doubt, there is great potential in coupling these two processes for the extraction and purification of natural products and to reduce the operation costs by recycling the CO₂ used.

11.4 Integration of Pressurized Fluids to Different Technologies for Extract Stabilization

In general terms, the main goal of an extraction and purification process is to produce a highly concentrated extract rich in specific compounds or compound

classes, which can be used directly as an additive or by itself as a product. However, some compounds present in the extracts are unstable under certain conditions and may be subject to transformations or degradation after the extraction, hindering their utilization industrially. Therefore, stabilization of extracts where such compounds are present is especially important to ensure the desired activity/property when the additive/product is actually used or consumed.

In this aspect, protective techniques can be used for the stabilization of natural extracts and for the protection of sensitive compounds from moisture, oxidation, heat, light or extreme conditions during processing, in an effort to increase their shelf life and range of applications. Furthermore, these techniques can be used to mask undesirable component attributes, such as strong and unpleasant flavors, attending to sensory quality and functionality and to promote the controlled release of the active component.^{59,60}

Stabilization techniques are becoming an essential tool to increase the competitiveness of natural products and to allow their effective use by the industry, helping to increase shelf life and protecting the properties/activity of the encapsulated material.⁵⁷ In the food industry, the primary reasons for the use of encapsulation processes can be considered:^{61–64}

1. to protect unstable materials from degradation;
2. to decrease the evaporation or transfer rates of the core material to the outside environment;
3. to modify the physical characteristics of the original material to be easier to handle;
4. to mask the odor or taste of the core material;
5. to dilute the core material when small amounts are required, yet still achieving a uniform dispersion in the host material; and
6. to separate components within a mixture that would otherwise react with one another.

There are several encapsulation techniques available (Table 11.3), which can be classified according to the process of combination between coating and core material into three categories: physical, chemical and physicochemical processes.⁶⁰ Furthermore, the particles formed by these encapsulation techniques may be classified according to their size in macro ($> 5000\ \mu\text{m}$), micro ($1.0\text{--}5000\ \mu\text{m}$) and nanoparticles ($< 1.0\ \mu\text{m}$).⁶⁵

Although several of these techniques are currently being used industrially, it is noteworthy that all of them have inherent limitations. These include poor control of particle size and morphology, degradation of thermosensitive compounds and low encapsulation efficiency. These limitations are prompting the development of new techniques and several different processes are currently being used for natural products in replacement of conventional encapsulation processes.⁶⁰

Supercritical fluid technology is an alternative to conventional encapsulation techniques which allows obtaining solvent-free micro/nanoparticles and capsules with narrow size distribution.⁶⁶ Carbon dioxide is the primary fluid

Table 11.3 Encapsulation techniques classification.

<i>Category</i>	<i>Techniques</i>
based on the physical combination between coating material and core material	spray coating, spray drying, spray cooling/chilling, extrusion coating, centrifugal and rotational suspension separation, fluidized bed coating, liophilization, co-crystallization, etc.
based on the chemical combination between coating material and core material	inclusion complexation, emulsion polymerization
based on the physicochemical combination between coating material and core material	coacervation, emulsion phase separation, liposome entrapment, etc.

applied to produce composite particles using supercritical fluid methods because it enables the process to be performed at near ambient temperature in an inert atmosphere, which avoids the degradation of the sensitive compounds by heat and oxygen.⁶⁷ The supercritical state of carbon dioxide is achieved at moderate pressures and temperatures (31 °C and 7.38 MPa, respectively), which is suitable for most applications. Because of these advantages, there are several encapsulation techniques employing supercritical technology. The supercritical fluids encapsulation techniques can be distinguished from each other according to the role of the supercritical fluid in the process:⁶⁸

1. as a solvent: rapid expansion of supercritical solutions (RESS); supercritical solvent impregnation (SSI);
2. as a solute: particles from gas saturated solutions (PGSS);
3. as an anti-solvent: supercritical anti-solvent (SAS); supercritical fluid extraction of emulsions (SFEE).

One of the most researched processes is SAS, in which the solute of interest is first dissolved in a conventional solvent and then the solution is sprayed continuously into a chamber through a coaxial nozzle co-currently with the supercritical CO₂. The high pressure CO₂ acts as an anti-solvent, decreasing the solubility of the solutes in the solvent mixture. Therefore, a fast supersaturation takes place, leading to nucleation and formation of micro or nanoparticles.⁶⁹ If a coating material is also dissolved in the organic solvent, encapsulates are formed by co-precipitation with the solute.^{67,70} The SAS process has several figures of merit, including lower operating temperature than that used in conventional process such as spray drying, and lower residual solvent in the final product. Also, mean particle size, particle size distribution and morphology can be controlled by changing process parameters such as pressure and temperature.⁷¹ This process is increasingly being used to produce micro to nanometer-sized and encapsulated extracts from natural products. Examples of successful applications of this process for the encapsulation of natural products

include extracts produced from green tea, rosemary (*Rosmarinus officinalis*), Annatto (*Bixa orellana* L.) and pink shrimp (*P. brasiliensis* and *P. paulensis*) residue among others (Table 11.4).^{69–74}

In the case of off-line processes, the efficiency of the SAS process is independent of the technique used for the production of the extract. As can be seen in Table 11.4, the process can be applied to the encapsulation of extracts produced by any extraction technique and using different encapsulation agents and solvents. However, some aspects should be considered in order to explore the full potential of this technique. Besides the compatibility between the solvent of the extract and the coating agent it is important to have adequate solvent evaporation by the supercritical CO₂. Although the extraction solvent can be eliminated and the extract can be re-dissolved in another more suitable solvent, this is not practical or even logical and may increase manufacturing costs due to high energy consumption. Therefore, the logical approach is to use the same solvent for the extraction and for the encapsulation of the extract. This is an extremely important aspect for on-line processes, where the type and amount of extraction solvent will influence the encapsulation and particle formation process.

The development of coupled processes for combining bioactive compound extraction to on-line particle formation is a recent trend and only a few reports are available on that subject. There is a recent report of the development of an on-line process to obtain dried powders of extracts from natural sources in one single operation.⁷⁵ This process was defined by the authors as water extraction and particle formation on-line (WEPO). As the name implies, this process employs water as the extracting solvent. In this case, supercritical CO₂ is not suitable for the elimination of the extraction solvent due to the low solubility of water in CO₂. In general, supercritical CO₂ is used as a dispersion medium and a hot N₂ stream is used as the drying agent.⁷⁵ A similar on-line process developed by our research group, where PLE and particle formation are coupled, was also reported using organic solvents as extracting solvent instead of water.⁷⁶ Due to the similarities with the WEPO process, this process was defined as organic solvent extraction and particle formation on-line (OEPO). Differently from the WEPO process, the OEPO process allows the encapsulation of the extract immediately after its production. Indeed, the OEPO process consists of coupled PLE-SAS precipitation, PLE-SAS co-precipitation and PLE-SFEE. The results of this novel process using Brazilian ginseng roots as a natural source of bioactive compounds showed that the OEPO process as developed can be considered as a suitable and promising process to obtain, in only one step, different products (precipitated extract, co-precipitated extract or encapsulated extract in suspension) with desired particle size directly from the plant material. In order to explore the applicability of the OEPO process for the production of encapsulated extract by its co-precipitation with a carrier material, the last part of this chapter details the development of a rapid and efficient integrated process for the extraction and subsequent encapsulation of extract using PLE and SAS.

Table 11.4 Applications of SAS process to produce encapsulated extracts.

Raw material	Application	Process	Ref.
green tea (<i>Camellia sinensis</i>)	encapsulation of green tea extract	Extraction method: microwave-assisted extraction (MAE) with acetone SAS: Coating material: poly-ε-caprolactone (MW = 25 000) Pressure: 8–12 MPa Temperature: 10–34 °C Polymer concentration ratio: 4–58 (w/w) CO ₂ to solution mass flow rate ratio: 4–10	69
rosemary (<i>Rosmarinus officinalis</i>)	encapsulation of rosemary extracts	Extraction method: Soxhlet with methanol SAS: Carrier material: polycaprolactone Pressure: 20–30 MPa Temperature: 40 °C Flow rate: 20 g/min	72
annatto (<i>Bixa orellana</i> L.)	micronization / encapsulation of bixin-rich extract from annatto	Extraction method: supercritical fluid extraction with CO ₂ at 31 MPa and 60 °C SAS: Coating material: Polyethylene glycol (PEG) Pressure: 10 MPa Temperature: 40 °C Flow rate: 1 mL/min CO ₂ flow rate: 0.6 and 1.5 kg/h Ratio between bixin rich-extract and PEG: 1:2 and 1:10	73
pink shrimp (<i>P. brasiliensis</i> and <i>P. paulensis</i>) residue (waste from shrimp processing)	encapsulation of astaxanthin-rich extract from pink shrimp	Extraction method: solvent extraction (maceration) with acetone SAS: Coating material: Pluronic F127 (10 mg/mL) Pressure: 8, 10 and 12 MPa Temperature: 35, 40 and 45 °C Flow rate: 1.0, 2.0 and 3.0 mL/min Extract concentration on feed solution: 2.0, 6.0 and 12.0 mg/mL	74

11.5 Case Study – Integrated Extraction and Encapsulation of Bixin from Annatto Seeds

The red-yellow extract obtained from seeds of *Bixa orellana* L. is a mixture of several carotenoids. One of them, bixin, represents over 80% of the total carotenoids found in the outer coat of the seeds.⁷⁷ Bixin is a coloring agent widely used in the pharmaceutical, cosmetic and food industries. Bixin is also regarded as an effective antioxidant, antimutagenic and anticarcinogenic agent.⁷⁸ There are several techniques that can be used to extract bixin from annatto seeds. Among them, PLE has been reported to be highly efficient while presenting several advantages over conventional methods. On the other hand, bixin can undergo a series of degradation reactions during the production process or while stored if exposed to high temperatures, light and oxygen. One of the alternatives used to improve bixin stability is encapsulation by SAS process. If properly designed, it is possible to integrate extraction with pressurized liquids to this encapsulation technique.⁷⁹ In this context, the aim of this case study was to develop a rapid and efficient integrated process for the extraction and subsequent encapsulation of the annatto extract using PLE and SAS, respectively.

11.5.1 Materials and Methods

11.5.1.1 Plant Material

Annatto (*Bixa Orellana*) seeds, variety Piave, was obtained from the Instituto Agrônomo de Campinas – IAC (Agronomic Institute of Campinas), Department of Agriculture and Supply of the State of São Paulo, Brazil. The samples were identified and stored at -18°C until being used as raw material for the extractions.

11.5.1.2 Pressurized Liquid Extraction (PLE)

The diagram of the PLE system is shown in Figure 11.6. Several solvents were evaluated for the extraction of bixin: ethyl acetate PA ACS (Merck KGaA, K40235423, Darmstadt, Germany); chloroform PA ISO (Merck KGaA, K38554545, Darmstadt, Germany); dichloromethane PA ACS ISO (Merck KGaA, K41680250, Darmstadt, Germany); ethyl ether PA (ECIBRA, 19072, Santo Amaro, Brazil) and acetone PA ACS (ECIBRA, 18999, Santo Amaro, Brazil). Annatto seeds (4.5 g) were placed in the extraction cell (6.57 mL, Thar Designs, CL 1373, Pittsburg, USA) containing a synthesized metal filter at the bottom and upper parts. The cell containing the sample was heated at 80°C by an electrical heating jacket for 6 min to ensure that the sample reached thermal equilibrium, and then it was filled with the desired extraction solvent and pressurized. The extraction solvent was pumped by a HPLC pump (Thermo-separation Products, Model ConstaMetric 3200 P/F, San Jose, USA) into the extraction cell until the pressure of 12 MPa was reached. The pressure was

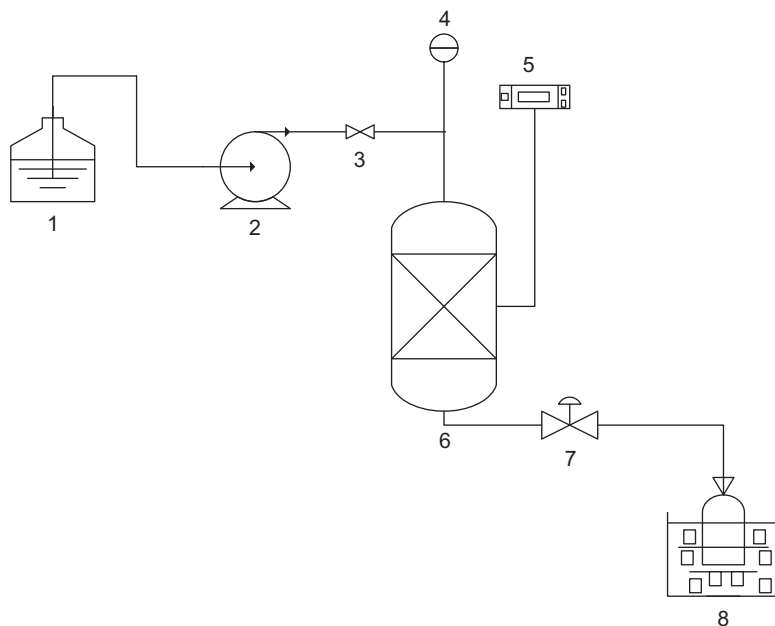


Figure 11.6 Schematic diagram of the PLE apparatus. (1) solvent reservoir; (2) HPLC pump; (3) blocking valve; (4) manometer; (5) temperature controller; (6) extractor column; (7) back pressure regulator; (8) sampling bottle.

maintained constant (12 MPa) for the duration of experimental process time (static extraction time). After the static time, the back pressure regulator (Tesco, model n° 26-1761-24-161, ELK River, USA) was adjusted to maintain the pressure and fresh solvent was pumped until 18 mL of extract was collected in an amber glass vial immersed in an ice bath to prevent bixin degradation. The solvent was later removed by vacuum rotatory evaporation (Laborota 4001 WB, Heidolph and CH-9230, Buchi, Flawil, Switzerland) at 40 °C. All the extractions were performed in duplicate.

The effect of the extraction solvent on bixin recovery was evaluated using a factorial design. Another factorial design was used to evaluate the influence of temperature (50–110 °C) and static time (6–14 min). Extraction kinetics curves using optimum conditions were built in duplicate and samples were collected in predetermined extraction times.

11.5.1.3 Off-line Encapsulation by Supercritical Anti-solvent (SAS) Process

After PLE conditions were optimized, a large amount of bixin-rich extract was produced and used as a sample for studying the encapsulation by the SAS

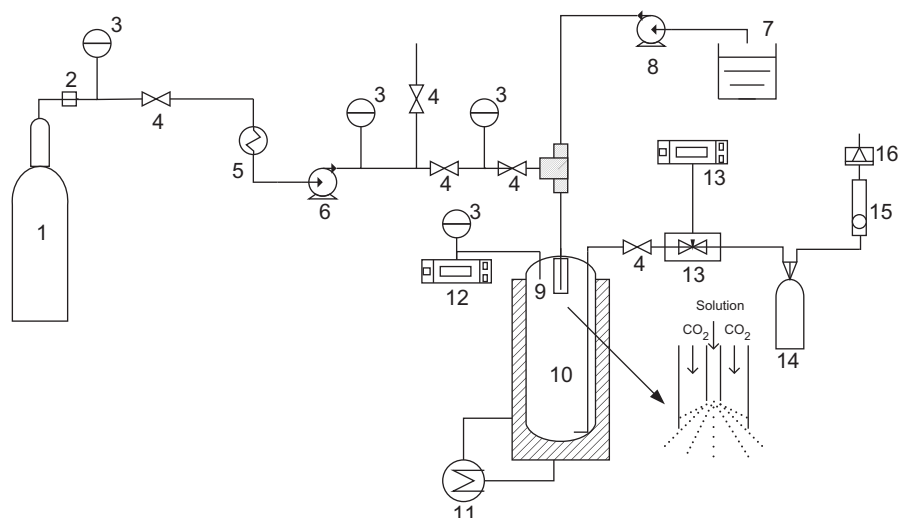


Figure 11.7 Schematic diagram of the SAS apparatus. (1) CO₂ cylinder; (2) CO₂ filter; (3) manometers; (4) blocking valves; (5) thermostatic bath; (6) CO₂ pump; (7) solution (solute/solvent) reservoir; (8) HPLC pump; (9) thermocouple; (10) precipitation vessel; (11) heating bath; (12) temperature controllers; (13) micrometric valve with a heating system; (14) glass flask; (15) glass float rotameter; (16) flow totalizer.

process. The encapsulation agent used was polyethylene glycol (PEG). A schematic diagram of the system is shown in Figure 11.7. A mixture of the PLE extract, PEG solution (mean molecular weight of 10 000 g/mol; Sigma–Aldrich, Steinheim, Germany) and dichloromethane was used as feed for the SAS process. The mass ratio between extract and PEG was 1:17.35. Carbon dioxide (99% CO₂, Gama Gases Especiais Ltd., Campinas, Brazil) was used as anti-solvent due to the very low solubility of bixin in this fluid at the assay temperatures and pressures.

The procedure was as follows: the CO₂ from the container is cooled down to –10 °C using a thermostatic bath (Marconi, MA-184, Piracicaba, Brazil) to ensure the liquefaction of the gas being pumped by an air-driven liquid pump (Maximator GmbH, PP 111, Zorge, Germany). The liquid CO₂ is directed to the precipitation vessel (500 mL; 6.8 cm i.d.) via a coaxial nozzle. The coaxial nozzle consists of an inner 1/16-in. stainless steel tube (i.d. 177.8 mm) for the solution extract/polymer/solvent, placed inside a 1/8-in. stainless steel tube for the CO₂. Once the precipitation vessel reaches the process conditions (40 °C, 10 MPa and CO₂ flow rate of 0.3 mL/min), the solution (extract + PEG + extraction solvent) is introduced into the vessel by a high-performance liquid chromatography (HPLC) pump (Thermoseparation Products, ConstaMetric 3200 P/F, Fremont, USA) through the coaxial annular passage of the atomizer. The vessel temperature is maintained constant by a heating water bath (Marconi, MA 127BO, Piracicaba, Brazil). CO₂ flow rate is measured using a glass float

rotameter (ABB, 16/286A/2, Warminster, USA) coupled to a flow totalizer (LAO, G0,6, Osasco, Brazil).

After 20 mL of solution (bixin and PEG in dichloromethane) has been passed through the system, the HPLC pump was stopped while the flow of CO₂ was kept for an additional 10 min in order to ensure the complete removal of the solvent from the precipitation vessel. The encapsulated bixin is trapped on a filter paper fixed at the bottom of the vessel while the fluid mixture (CO₂ plus solvent) exits the vessel and flows to a collection flask (100 mL) connected to a micrometric valve. This valve is maintained at 120 °C to avoid freezing and blockage of the outlet caused by the Joule–Thompson effect of the expanding CO₂. Finally, the precipitation vessel is slowly depressurized to atmospheric pressure and the particles are collected and stored at –10 °C until subsequent analysis and characterization.

11.5.1.4 Integrated System using PLE-SAS

A schematic diagram of the PLE-SAS system used for the integrated process is shown in Figure 11.8. The on-line PLE-SAS process was carried out using the

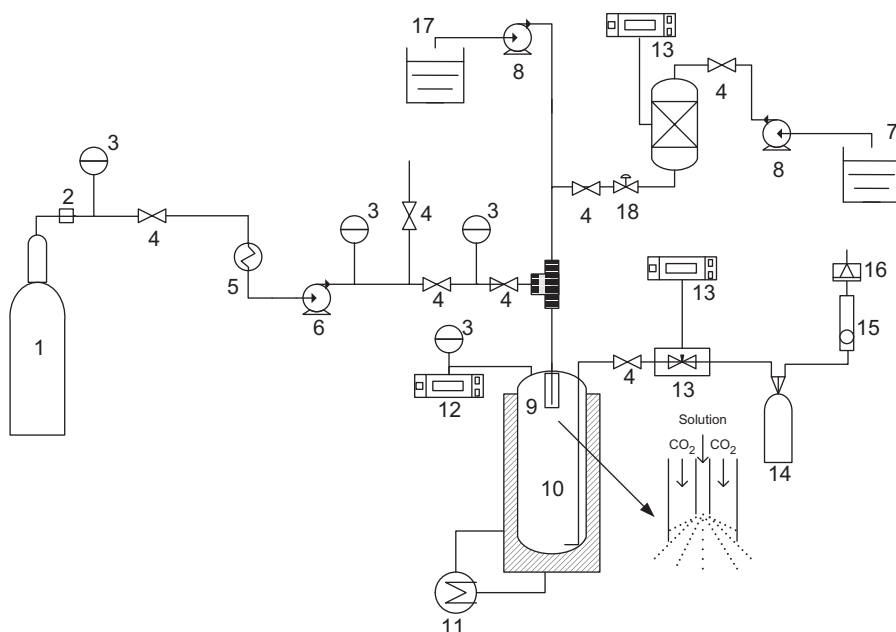


Figure 11.8 Schematic diagram of the PLE-SAS-co-precipitation apparatus. (1) CO₂ cylinder; (2) CO₂ filter; (3) manometers; (4) blocking valves; (5) thermostatic bath; (6) CO₂ pump; (7) solvent reservoir; (8) HPLC pumps; (9) thermocouple; (10) precipitation vessel; (11) heating bath; (12) temperature controllers; (13) micrometric valve with a heating system; (14) glass flask; (15) glass float rotameter; (16) flow totalizer; (17) polymer solution reservoir; (18) back pressure regulator.

experimental procedure described for each individual process and the previously optimized conditions. The solution containing the encapsulation material (PEG/dichloromethane) was introduced into the precipitation vessel, separately from the extract obtained from PLE process, by a HPLC pump (Thermoseparation Products, ConstaMetric 3200 P/F, Fremont, USA). The polymer solution, bixin extract and CO₂ were simultaneously injected into the precipitation vessel continuously until 20 mL of the bixin extract were pumped.

11.5.1.5 Extract and Capsule Characterization

Bixin Content. The bixin content of the extracts and capsules was determined according to Joint FAO/WHO Expert Committee on Food Additives Monographs.⁸⁰ The extracts or particles were diluted in acetone to adjust the concentration of bixin before the analysis. Sample absorbance was measured at 487 nm with a UV-vis spectrophotometer (FEMTO, 800 XI, São Paulo, Brazil), and the bixin content was calculated according to the Lambert-Beer law, using $E_{1cm}^{1\%} = 3090$.

Stabilization Tests. The stability of the bixin extracts and capsules obtained via SAS and PLE-SAS was evaluated under heat and light. The influence of exposure to light during storage on the stability of the extracts and capsules was evaluated at ambient temperature and 4 °C. Samples exposed to light were stored under two fluorescent lamps of 20 W in contrast to extracts stored in vials protected from light. The stability of the extracts and capsules was monitored for 32 days. Samples were periodically collected, diluted in acetone and the absorbance was measured using spectrophotometer at a wavelength of 487 nm to determine the changes in bixin content of the samples during storage, allowing determination of the half-life time ($t_{1/2}$) of the extract and capsules.

Differential Scanning Calorimetry (DSC). The phase transition of the extract, PEG, capsules and a physical mixture of extract and PEG was determined using a differential scanning calorimetry (DSC) system (Shimadzu, DSC-50, Tokyo, Japan). DSC measurement was carried out in hermetically sealed aluminum pans; 5 mg of samples were heated at a rate of 10 °C/min between 30 and 500 °C in an inert atmosphere (N₂ flow of 60 mL/min).

Dissolution Profiles. The capsules were analyzed using spectrophotometer at a wavelength of 487 nm to determine the amount of dissolved bixin every 1 min in a medium containing acetone.

Encapsulation Efficiency of Bixin. The capsules were analyzed by UV/Vis spectrophotometry recording the reading at 462 nm. The procedure used was based on the method described by Santos *et al.*⁸¹ In this method, the

absorbance is assumed to be proportional to the amount of bixin dispersed in dichloromethane. The standard curve was linear in the concentration range studied (1–8 $\mu\text{g/mL}$) with a correlation coefficient (R^2) of 0.992. The encapsulation efficiency (%) was calculated by relating the concentration of bixin initially added in the formulation of the capsules and the concentration of bixin determined in the capsules.

11.5.2 Results and Discussion

11.5.2.1 Influence of the Extraction Solvent on PLE Performance

For the initial development of the process different solvents were evaluated for the extraction of bixin from annatto seeds by PLE. Table 11.5 presents the content of bixin (g/100 g extract) in the PLE extracts obtained using solvents with different polarities.

The highest bixin yields were observed in the extracts obtained using dichloromethane (36%), and chloroform (33%) as solvents. Although relatively high amounts of bixin were also found in the extracts obtained using acetone, the results indicate that moderately polar solvents are better suited for the extraction of bixin from annatto seeds. These results are in accordance with previous reports where bixin, a polar carotenoid, showed affinity for moderately polar solvents.⁸² Dichloromethane was considered the most suitable solvent due to high extraction efficiency and due to its physicochemical characteristics. Dichloromethane is highly miscible in supercritical CO_2 , resulting in high volumetric expansion in SC-CO_2 , which can be explored in coupling of the SAS process. Although dichloromethane is considered a harmful solvent, its use is justified by two aspects. The first one is that the solvent employed in the supercritical fluid precipitation based techniques must solubilize the extract and also the carrier material used for encapsulation. The second one is that with this technique the remaining solvent concentration in the particles is much lower (<10 ppm) than the concentration limit (<600 ppm).^{83,84}

Table 11.5 Bixin content (g bixin/100 g extract, dry basis - d.b.) in the PLE extract obtained using solvents of different polarities.

<i>Solvent</i>	<i>Polarity</i>	<i>Bixin content (g/100 g)^a</i>
acetone	5.4	24.8 $\pm$ 0.9
ethyl acetate	4.3	18.1 $\pm$ 0.6
chloroform	4.1	33 $\pm$ 2
dichloromethane	3.1	36 $\pm$ 1
ethyl ether	2.8	19.4 $\pm$ 5

^a Mean $\pm$ amplitude of two determinations.

11.5.2.2 Influence of Temperature and Static Extraction Time on PLE Performance

A factorial design (Table 11.6) with two experimental factors (temperature and static time) was used to study the influence of process conditions on the response variable (bixin content). Dichloromethane was selected as solvent. Two temperatures (50 and 110 °C) and two static times (6 and 14 min) were evaluated under constant pressure (12 MPa). The operational pressure was determined based on the conditions used for the PLE process in order to allow the coupling of both processes (PLE and SAS).

The results in Table 11.6 indicate that higher bixin yields are achieved with a temperature of 80 °C or higher. On the other hand, the static time had an insignificant influence on this response variable. It is also noteworthy that bixin yield obtained using 12 MPa, 80 °C and 10 min of static time is over 3 times higher than the yields reported by Balaswamy and co-workers.⁸⁵ These authors used acetone in Soxhlet extraction and a two-solvent extraction method achieving bixin yields of 11.60% and 11.82%, respectively.

11.5.2.3 PLE Kinetic Extraction Curves

Once the suitable conditions were selected (dichloromethane, 80 °C and 10 min), extraction kinetics curves were determined using as response variables total extract yield (Figure 11.9) and bixin yield (Figure 11.10). Extraction

Table 11.6 Experimental matrix for the factorial design.

Experiment	Temperature (°C)	Static time (min)	Bixin content (g/100 g) ^a
1	50	6	24.4 ± 0.4
2	110	6	36.6 ± 0.5
3	50	14	19.4 ± 0.9
4	110	14	37 ± 1.0
5(C)	80	10	36 ± 1.0

^aMean ± amplitude of two determinations.

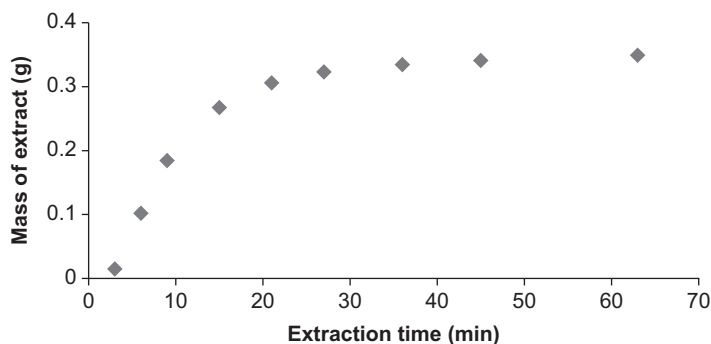


Figure 11.9 Kinetics curves for the recovery of annatto extract at 12 MPa, 80 °C and 10 min of static time.

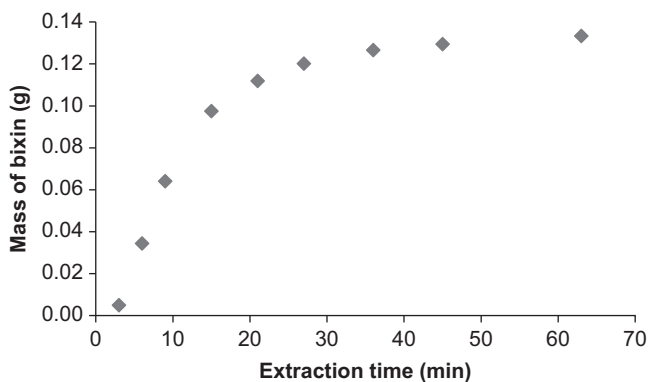


Figure 11.10 Kinetics curves for the recovery of bixin at 12 MPa, 80 °C and 10 min of static time.

kinetics curves of both response variables showed a similar trend and were characterized by two distinct phases. In the first phase, extract and bixin yields constantly increased as extraction progressed, reflecting a fast mass transfer of bixin from the raw material into the unsaturated extraction solution (solvent + extracted components). In the second phase, there is a slow diffusion of bixin and other components from the raw material into the extraction solution, which gradually decays until reaching a steady-state condition. Thus, most of the extractable components (including bixin) are readily removed from the raw material in 20 min and after this time the remaining components are slowly extracted. It can also be observed that over 50% of the extractable material was removed within 10 min. Overall, an adequate balance between extraction time and bixin yield is achieved in 18 min, which was selected as process time for the coupled PLE-SAS process.

11.5.2.4 Encapsulation of PLE Extracts by SAS

To study the encapsulation of PLE extracts by the SAS process, the on-line process was compared to the off-line process using as reference the PLE extract produced using the previously selected conditions (dichloromethane, 80 °C, 10 min of static time and 18 min of dynamic extraction time). The extraction in the on-line process was also carried out using the same conditions. To evaluate the efficiency of the encapsulation process, the degradation of bixin contained in the capsules produced by the off-line (PLE + SAS) and on-line (PLE-SAS) processes and in the reference extract was monitored during storage under different conditions for 32 days. The capsules obtained by both processes and the reference extract were stored exposed to light at ambient temperature (Figure 11.11), protected from light at ambient temperature (Figure 11.12) and protected from light under refrigeration (Figure 11.13).

Under all storage conditions, the degradation of bixin was higher in the reference extract than in the capsules produced by the on-line and off-line

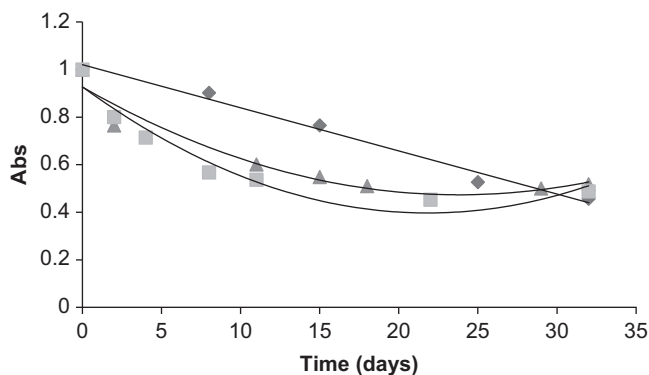


Figure 11.11 Time course of the degradation of capsules obtained by coupled process (◆) and two-step process (▲) and of the extract (■) stored at ambient temperature under illumination. Data were fitted to first-order (coupled process) and second-order (two-step process and extract) kinetics.

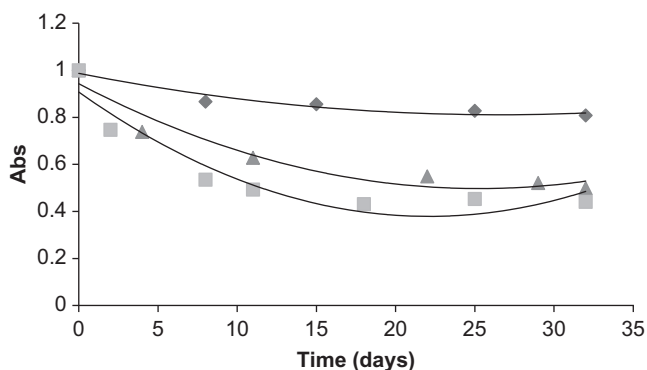


Figure 11.12 Time course of the degradation of capsules obtained by coupled process (◆) and two-step process (▲) and of the extract (■) stored at ambient temperature and in the dark. Data were fitted to second-order kinetics.

processes. However, differences between capsules and extract were highly dependent on storage conditions. As more aggressive conditions were used, greater differences between the encapsulated extracts and the reference material were observed. Another important observation was that independently of the storage condition, capsules produced by the on-line process provided better protection of the encapsulated bixin, and therefore the on-line process renders a more stable product.

The kinetic plots for the degradation of encapsulated bixin produced by the on-line process are determined by the storage temperature and exposure to light. At ambient temperature and exposed to light, encapsulated extracts by PLE-SAS presented a first-order degradation, whereas when stored under refrigeration or protected from light the kinetic data followed a second-order

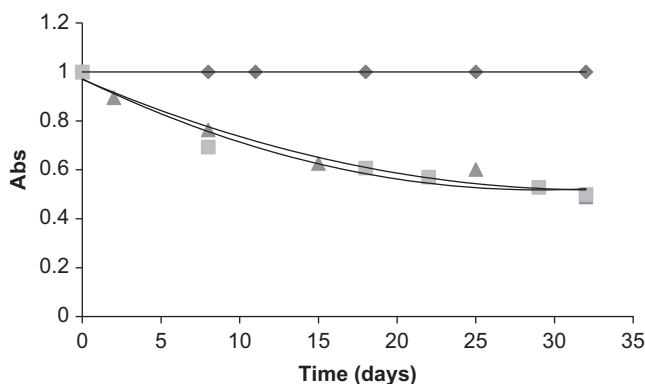


Figure 11.13 Time course of the degradation of capsules obtained by coupled process (◆) and two-step process (▲) and of the extract (■) stored under refrigeration and in the dark. Data were fitted to first-order (coupled process) and second-order (two-step process and extract) kinetics.

Table 11.7 The $t_{1/2}$ values (days) for capsules and extract under different storage conditions.

	Room temperature/light	Room temperature/dark	Refrigerated/dark
coupled process	29 ± 1	>32	>32
two-step process	17.9 ± 0.6	26 ± 1	33 ± 1
extract	12.1 ± 0.6	11.6 ± 0.5	26 ± 1

Mean $\pm$ amplitude of two determinations.

decay. In contrast, the degradation rates of encapsulated bixin obtained by the off-line process followed second-order degradation kinetics.

The higher degradation rate and the different degradation kinetics observed in the capsules formed by the off-line process when compared to the on-line process may be attributed to the little chemical interaction between the extract and polymer. The encapsulation efficiencies of both processes were similar, $19 \pm 2\%$ and $21 \pm 2\%$ for off-line and on-line processes, respectively. Therefore these differences are possibly related to the presence of bixin in the coating of the microcapsules. As the bixin present in the outer layer of the microcapsule is exposed to ambient conditions it is subjected to more aggressive conditions than the bixin in the core of the microcapsule. Similar behavior is suggested to take place in spray-dried bixin encapsulated with different edible polysaccharide preparations.⁸⁶ In this case, first-order degradation kinetics were suggested to be related to the presence of bixin outside and inside the microcapsules. In contrast, degradation of bixin in annatto oleoresin has been suggested to follow second-order kinetics.⁸⁵

As can be seen in Table 11.7, independently if carried out off-line or on-line, encapsulation of the extracts protects bixin against adverse conditions and increases its stability during storage. The $t_{1/2}$ values indicated that bixin is

highly sensitive to degradation when exposed to light and encapsulation may play an important role in the protection from this ambient factor. The observed negative effect of light on bixin stability has been reported in several other studies.^{85–88}

It is important to highlight that bixin contained in the capsules formed by the on-line process was completely stable for at least 32 days if stored protected from light. Furthermore, when the extracts encapsulated by the on-line process are exposed to more aggressive conditions (exposed to light and ambient temperature) they are stable for almost twice the time of the capsules produced by the off-line process.

11.5.2.5 DSC Analysis

DSC was used to detect possible interaction between the extract and the polymeric matrix. The DSC thermograms of bixin, PEG, its physical mixture and capsules are shown in Figure 11.14. DSC curve of PEG showed an endothermic peak corresponding to the glass transition (T_g) at 65.26 °C. The DSC analysis showed different endothermic peaks for the extract. The melting endotherm peaks related to the extract and PEG separately could also be detected in the extract/polymer physical mixtures. This indicates that the encapsulated system is represented by a physical mixture of both compounds and that a chemical interaction between the extract and the polymer did not occur. In contrast, the DSC thermograms of the capsules formed by both processes indicated the chemical interaction between extract and PEG since the melting peak of the extract was not detected. This fact suggests the effective entrapment of the extract by PEG.⁸¹

11.5.2.6 Dissolution Profiles of Capsules Formed

To evaluate the degree of chemical interaction between carrier material and solute of capsules obtained by each process, the dissolution profiles of the capsules was evaluated. The results are presented in Figure 11.15.

The dissolution profiles of capsules followed the same trend observed in the stability study, suggesting a more efficient interaction between the extract and the PEG for capsules obtained by the on-line process. The capsules obtained by the off-line process exhibited faster dissolution rates than the capsules obtained by the on-line process, indicating higher bixin concentration on the capsule surface. These results support the differences between off-line and on-line processes in terms of bixin stability. It can also be observed that dissolution curves are characterized by two phases: dissolution rate increased constantly until reaching a plateau. The capsules formed by the off-line process dissolved faster, stabilizing in approximately 13 min, while the capsules obtained by the on-line process dissolved in approximately 15 min.

Finally, the visual comparison of the capsules produced (Figure 11.16) also indicates differences between the off-line and on-line processes. While the on-line process produced a darker capsule with deep red color, the capsule

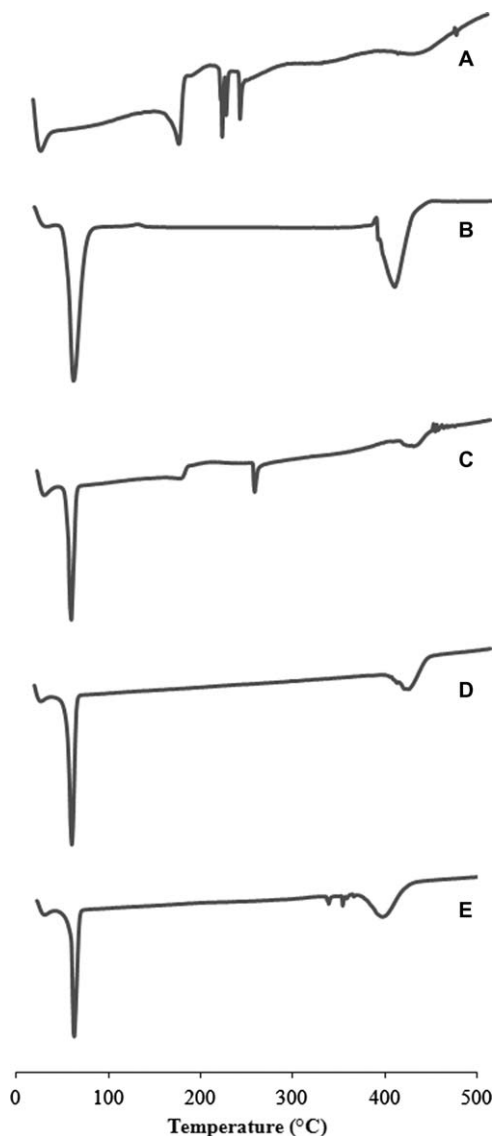


Figure 11.14 DSC thermograms of bixin (A), PEG (B), its physical mixtures (C), capsules obtained by two-step (D) and coupled process (E).

produced by the off-line process had a lighter orange coloration, indicating the degradation of bixin. The difference in coloration can be explained by the fact that bixin remains in an inert atmosphere throughout the extraction and encapsulation in the on-line process. In the off-line process, during collection of the extract and after the extraction, bixin is exposed to light and air and therefore may be subjected to degradation. Furthermore, the results showed a faster degradation of bixin from capsule obtained by the off-line process (lower

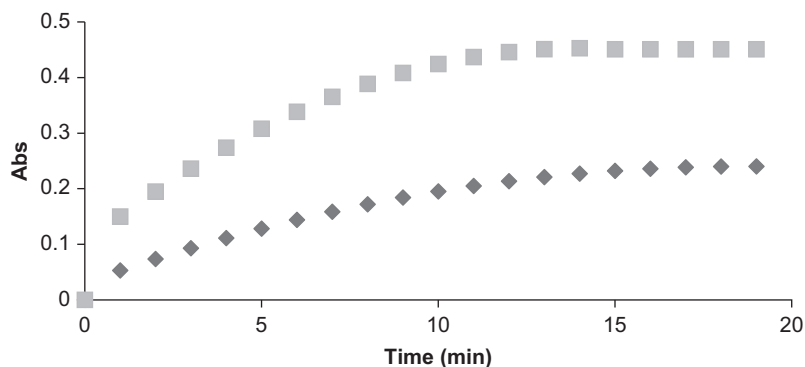


Figure 11.15 Dissolution profiles of capsules obtained by the two-step process (■) and the coupled process (◆).

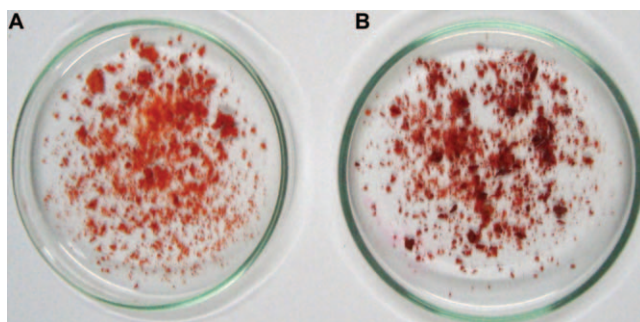


Figure 11.16 Pictures of capsules obtained by the two-step process (A) and the coupled process (B).

$t_{1/2}$) in all studied conditions. This is consistent with the results obtained from the dissolution profiles: the higher chemical interaction between extract and polymer from capsules obtained by the on-line process resulting in capsules with higher stability.

11.6 Conclusions

The evaluation of several process parameters for the extraction of bixin from annatto seed using PLE indicated that the best balance between extract and bixin yields and process time is achieved using dichloromethane at 80 °C, 10 min of static extraction time and 18 min of dynamic extraction time. The use of encapsulation processes to stabilize the PLE extracts increased bixin stability during storage when compared to the raw extract. The integration of extraction and encapsulation in one single on-line process produced capsules that are more stable and that provide better protection of the entrapped bixin than the off-line process. There are evident advantages derived from the on-line process,

indicating a great potential to be explored for the production of encapsulated bixin-rich extracts directly from annatto seeds.

It was shown in this chapter that the development of coupled processes for combining bioactive compound extraction to on-line particle formation is a recent trend and only a few reports are available on that subject. The versatility of our hyphenated-based process that uses pressurized fluids in both unit operations (extraction and particle formation) defined as organic solvent extraction and particle formation on-line (OEPO) was demonstrated. OEPO process, besides coupled PLE-SAS co-precipitation, consists of PLE-SAS precipitation and PLE-SFEE. In this processes different products (precipitated extract and encapsulated extract in suspension, respectively) with desired particle sizes can be obtained in only one step, directly from any source of bioactive compounds. In order to improve our OEPO process in terms of bioactive compound purity in the plant extract, the on-line coupling of extraction to purification with adsorptive and/or membrane separation processes before particle formation is very promising and is under study by our research group.

Acknowledgements

Diego T. Santos thanks FAPESP (process 10/16485-5) for a Post-doctoral Fellowship. Maria Thereza M. S. Gomes and Renata Vardanega thank CNPq for their Doctoral and Masters Fellowships, respectively. The authors acknowledge the financial support from CNPq and FAPESP (09/17234-9; 12/10685-8).

References

1. M. A. Rostagno, M. D'Arrigo, J. A. Martínez and J. A. Martínez, *TrAC*, 2010, **29**, 553.
2. J. M. Prado, G. H. C. Prado and M. A. A. Meireles, *J. Supercrit. Fluids*, 2011, **56**, 231.
3. J. W. King and K. Srinivas, *J. Supercrit. Fluids*, 2009, **47**, 598.
4. F. Temelli, *J. Supercrit. Fluids*, 2009, **47**, 583.
5. C. G. Pereira and M. A. A. Meireles, *Food Bioprocess Tech*, 2010, **3**, 340.
6. A. Pasquel, M. A. A. Meireles, M. O. M. Marques and A. J. Petenate, *Braz. J. Chem. Eng.*, 2000, **17**, 271.
7. D. T. Santos, C. L. C. Albuquerque and M. A. A. Meireles, *Procedia Food Sci.*, 2011, **1**, 1581.
8. M. E. M. Braga and M. A. A. Meireles, *J. Food Process Eng.*, 2007, **30**, 501.
9. I. J. Seabra, M. E. M. Braga, M. T. Batista and H. C. Sousa, *J. Supercrit. Fluids*, 2010, **54**, 145.
10. I. J. Seabra, M. E. M. Braga, M. T. Batista and H. C. Sousa, *Food Bioprocess Tech.*, 2010, **3**, 674.
11. A. M. A. Dias, P. Santos, I. J. Seabra, R. N. C. Júnior, M. E. M. Braga and H. C. Sousa, *J. Supercrit. Fluids*, 2012, **61**, 62.

12. M. Ashraf-Khorassani and L. T. Taylor, *J. Agric. Food Chem.*, 2004, **52**, 2440.
13. C. Yang, Y.-R. Xu and W.-X. Yao, *J. Agric. Food Chem.*, 2002, **50**, 846.
14. T. Vatai, M. Skerget and Z. Knez, *J. Food Eng.*, 2009, **90**, 246.
15. M. Zougagh, M. Valcarcel and A. Ríos, *TrAC*, 2004, **23**, 339.
16. P. Hubert and O. G. Vitzthum, *Angew. Chem. Int. Ed.*, 1978, **17**, 710.
17. D. T. Santos and M. A. A. Meireles, *Recent Pat. Eng.*, 2011, **5**, 17.
18. P. T. V. Rosa and M. A. A. Meireles, in *Extracting Bioactive Compounds for Food Products: Theory and Application*, ed. M. A. A. Meireles, CRC Press/Taylor & Francis Group, Boca Raton, 2009, p. 272.
19. P. T. V. Rosa and M. A. A. Meireles, in *Extracting Bioactive Compounds for Food Products: Theory and Application*, ed. M. A. A. Meireles, CRC Press/Taylor & Francis Group, Boca Raton, 2009, p. 388.
20. M. A. A. Meireles, in *Supercritical Fluid Extraction of Nutraceuticals and Bioactive Compounds*, ed. J. Martinez, CRC Press/Taylor & Francis Group, Boca Raton, 2008, p. 243.
21. M. A. A. Meireles, *Electron. J. Environ., Agric. Food Chem.*, 2008, **7**, 3254.
22. P. T. V. Rosa and M. A. A. Meireles, *J. Supercrit. Fluids*, 2005, **34**, 109.
23. M. A. A. Meireles, *Curr. Opin. Solid St. M.*, 2003, **7**, 321.
24. M. O. Balaban and M. A. A. Meireles, in *The Wiley Encyclopedia of Food Science & Technology*, ed. F. J. Francis, John Wiley and Sons, New York, 1999, p. 2220.
25. E. Reverchon and I. Marco, *J. Supercrit. Fluids*, 2006, **38**, 146.
26. E. Reverchon, *J. Supercrit. Fluids*, 1997, **10**, 1.
27. H. E. Messmore, U.S. Pat. 2420185, 1943.
28. J. W. King, F. Favati and S. L. Taylor, *Sep. Sci. Technol.*, 1996, **31**, 1843.
29. K. Sato, S. S. Sasaki, Y. Goda, T. Yamada, O. Nunomura, K. Ishikawa and T. Maitani, *J. Agric. Food Chem.*, 1999, **47**, 4665.
30. S. L. Taylor and J. W. King, *J. Am. Oil Chem. Soc.*, 2002, **79**, 1133.
31. J. Pól, T. Hyötyläinen, O. Ranta-Aho and M. L. Riekkola, *J. Chromatogr. A*, 2004, **1052**, 25.
32. M. A. Rostagno, J. M. A. Araújo and D. Sandi, *Food Chem.*, 2002, **78**, 111.
33. M. Papagiannopoulos and A. Mellenthin, *J. Chromatogr. A*, 2002, **976**, 345.
34. M. Papagiannopoulos, B. Zimmermann, A. Mellenthin, M. Krappe, G. Maio and R. Galensa, *J. Chromatogr. A*, 2002, **958**, 9.
35. K. Luthje, T. Hyötyläinen, M. Rautiainen-Rama and M.-L. Riekkola, *Analyst*, 2005, **130**, 52.
36. J. You, S. Gao, H. Jin, W. Li, H. Zhang and A. Yu, *J. Chromatogr. A*, 2010, **1217**, 1875.
37. M. Kivilompolo and T. Hyötyläinen, *J. Chromatogr. A*, 2009, **1216**, 892.
38. X. Tong, X. Xiao and G. Li, *J. Chromatogr. B*, 2011, **879**, 2397.
39. L. Chen, H. Jin, L. Ding, H. Zhang, X. Wang, Z. Wang, J. Li, C. Qu, Y. Wang and H. Zhang, *J. Chromatogr. A*, 2007, **1140**, 71.
40. L. Fang, Y. Liu, H. Zhuang, W. Liu, X. Wang and L. Huang, *J. Chromatogr. B*, 2011, **879**, 3023.

41. H. Wang, L. Chen, Y. Xu, Q. Zeng, X. Zhang, Q. Zhao and L. Ding., *LWT – Food Sci. Technol.*, 2011, **44**, 1490.
42. L. Chen, L. Ding, H. Zhang, J. Li, Y. Wang, X. Wang, C. Qu and H. Zhang, *Anal. Chim. Acta*, 2006, **580**, 75.
43. M. Lohleit and K. Bachmann, *J. Chromatogr.*, 1990, **505**, 221.
44. R. W. Vannoort, J.-P. Chervet, H. Lingeman, G. J. Jong and U. A. Brinkman, *J. Chromatogr.*, 1990, **505**, 45.
45. W. M. Cross and A. Akgerman, *AIChE J.*, 1998, **44**, 1542.
46. S. L. Taylor and J. W. King, *J. Am. Oil Chem. Soc.*, 2002, **79**, 1133.
47. E. V. Petersson, J. Liu, P. J. R. Sjöberg, R. Danielsson and C. Turner, *Anal. Chim. Acta*, 2010, **663**, 27.
48. B. E. Richter, B. A. Jones, J. L. Ezzell and N. L. Porter, *Anal. Chem.*, 1997, **68**, 1033.
49. Z. Y. Ju and L. R. Howard, *J. Agric. Food Chem.*, 2003, **51**, 5207.
50. V. Camel, *Spectrochimica Acta Part B*, 2003, **58**, 1177.
51. S. C. Moidoveanu and V. David, *Sample Preparation in Chromatography*, Elsevier, Amsterdam, 2002.
52. L. A. V. Sarmento, C. B. Spricigo, J. C. C. Petrus, L. H. C. Carlson and R. A. F. Machado, *J. Membr. Sci.*, 2004, **237**, 71.
53. S. Sarrade, C. Guizard and G. M. Rios, *Sep. Purif. Technol.*, 2003, **32**, 57.
54. C. B. Spricigo, A. Bolzan, R. A. F. Machado, L. H. C. Carlson and J. C. C. Petrus, *J. Membr. Sci.*, 2001, **188**, 173.
55. S. Sarrade, M. Carles, C. Perre and P. Vignet, U.S. Pat 5,961,835, 1999.
56. L. A. V. Sarmento, R. A. F. Machado, J. C. C. Petrus, T. R. Tamanini and A. Bolzan, *J. Supercrit. Fluids*, 2008, **45**, 64.
57. S. I. Semeonova, H. Ohya, T. Higashijima and Y. Negishi, *J. Membr. Sci.*, 1992, **74**, 131.
58. T. Higashijima, H. Ohya, Y. Tsuchiya, H. Tokunaga, M. Aihara and Y. Negishi, *J. Membr. Sci.*, 1994, **93**, 165.
59. A. Fernandez and J. M. L. Torres-Giner, *Food Hydrocoll.*, 2009, **23**, 1427.
60. D. T. Santos and M. A. A. Meireles, *The Open Chem. Eng. J.*, 2010, **4**, 42.
61. F. Shahidi and X. Q. Han, *Crit. Rev. Food Technol.*, 1993, **33**, 501.
62. H. Jizomoto, E. Kanaoka, K. Sugita and K. Hirano, *Pharm. Res.*, 1993, **10**, 115.
63. C. Kibry, *J. Food Sci. Technol.*, 1991, **5**, 74.
64. K. G. H. Desai and H. J. Park, *Drying Technol.*, 2005, **23**, 1361.
65. S. M. Jafari, E. Assadpoor, Y. He and B. Bhandari, *Drying Technol.*, 2008, **26**, 816.
66. I. Kikic and M. Lora, *Ind. Eng. Chem. Res.*, 1997, **36**, 5507.
67. M. Bahrami and S. Ranjbarian, *J. Supercrit. Fluids*, 2007, **40**, 263.
68. C. Domingo, A. Vega, M. A. Fanovich, C. Elvira and P. Subra, *J. Appl. Polym. Sci.*, 2003, **90**, 3652.
69. M. V. Sosa, S. Rodríguez-Rojo, F. Mattea, M. Cismondi and M. J. Cocero, *J. Supercrit. Fluids*, 2011, **56**, 304.
70. M. J. Cocero, A. Martín, F. Mattea and S. Varona, *J. Supercrit. Fluids*, 2009, **47**, 546.

71. R. Guha, M. Vinjamur and M. Mukhopadhyay, *Ind. Eng. Res.*, 2011, **50**, 1079.
72. O. Yesil-Celiktas and E. O. Cetin-Uyanikgil, *J. Supercrit. Fluids*, 2012, **62**, 219.
73. D. T. Santos and M. A. A. Meireles, *J. Food Process Eng.*, 2013, **36**, 36.
74. N. Mezzomo, E. Paz, M. Maraschin, A. Martín, M. J. Cocero and S. R. S. Ferreira, *J. Supercrit. Fluids*, 2012, **66**, 342.
75. M. E. E. Ibanez, A. Cifuentes, I. Rodriguez-Meizoso, J. A. Mendiola, G. Reglero, J. Señorans and C. Turner, 2009, Spanish Patent no. P200900164.
76. D. T. Santos, D. F. Barbosa, K. Broccolo, M. T. M. S. Gomes, R. Vardanega and M. A. A. Meireles, *Food and Public Health*, 2012, **2**, 231.
77. H. D. Preston and M. D. Rickard, *Food Chem.*, 1980, **5**, 47.
78. L. M. G. Antunes, L. M. Pascoal, M. L. P. Bianchi and F. L. Dias, *Mutat. Res.*, 2005, **585**, 113.
79. R. W. Alves, A. A. U. Souza, S. M. A. G. U. Souza and P. Jauregi, *Sep. Purif. Technol.*, 2006, **48**, 208.
80. FAO – Food and Agriculture Organization of the United Nations, *Annatto Extracts: Chemical and Technical Assessment*, 2006.
81. D. T. Santos, A. Martín, M. A. A. Meireles and M. J. Cocero, *J. Supercrit. Fluids*, 2012, **61**, 167.
82. C. R. Cardarelli, M. T. Benassi and A. Z. Mercadante, *LWT – Food Sci. Technol.*, 2008, **41**, 1689.
83. D. T. Santos and M. A. A. Meireles, *Innovative Food Sci. Emerging Technol.*, 2011, **12**, 398.
84. L. R. Snyder, *J. Chromatogr.*, 1974, **92**, 223.
85. K. Balaswamy, P. G. Prabhakara Rao, A. Satyanarayana and D. G. Rao, *LWT – Food Sci. Technol.*, 2006, **39**, 952.
86. M. I. M. J. Barbosa, C. D. Borsarelli and A. Z. Mercadante, *Food Res. Int.*, 2005, **38**, 989.
87. M. Scotter, *Food Addit. Contam., Part A*, 2009, **26**, 1123.
88. S. M. O. Lyng, M. Passos and J. D. Fontana, *Process Biochem.*, 2005, **40**, 865.

CHAPTER 12

Economic Evaluation of Natural Product Extraction Processes

CAMILA G. PEREIRA,^{*a} JULIANA M. PRADO^b AND
M. ANGELA A. MEIRELES^b

^a Department of Chemical Engineering – UFRN (Federal University of Rio Grande do Norte), Av. Sen. Salgado Filho, 3000, CEP: 59072–970, Natal, RN, Brazil; ^b DEA/FEA (School of Food Engineering)/UNICAMP (University of Campinas), R. Monteiro Lobato, 80, Campinas, 13083-862, SP, Brazil

*Email: camila@eq.ufrn.br

12.1 Introduction

Supercritical fluid technology has been used in different areas since 1970, first in the food industry for the decaffeination of green coffee beans using CO₂,¹ and ever since, the number of applications has increased exponentially. In the last decades, supercritical fluid extraction (SFE) has been extensively studied for the selective extraction of specific components from natural products due to its unique properties and environmental compatibility. For several types of raw materials it has been demonstrated that SFE is a technically and economically feasible process to be used at the industrial scale. Nonetheless, little information about industrial costs is disclosed, and generally SFE technology is discarded by the incorrect assumption that in spite of the several advantages, the investment costs are high, raising the question of ‘why then use supercritical fluids?’

The answer of this question is simple – although the initial investment of a SFE plant is high, it has been shown that its operational costs are lower than of traditional extraction plants and therefore it is possible to redeem the

RSC Green Chemistry No. 21

Natural Product Extraction: Principles and Applications

Edited by Mauricio A. Rostagno and Juliana M. Prado

© The Royal Society of Chemistry 2013

Published by the Royal Society of Chemistry, www.rsc.org

investment in a relatively short time.²⁻⁴ In order to maximize the advantages of supercritical fluids, the use of this technology is indicated when the target compounds have high added value, when they are unstable or thermolabile and may be subject to degradation in the conventional processes, or when the final product is required to have high purity. These aspects are now clear to the researchers; however, to make this technology attractive to the industry it is necessary to have a reliable assessment of the potential benefits associated with its use in large-scale operations.

This type of preconception is common to all new technologies, including ultrasound- and microwave-assisted extraction and pressurized liquid extraction, since the investment cost of traditional technologies, namely low pressure solvent extraction and steam distillation, is low. The economic evaluation of new processes is important to produce data that can help overcome this problem and allow the modernization of the extraction processes being currently used by most industries. Considering these aspects, some methods for evaluating the economic viability of the emerging extraction technologies have been proposed. These methods are presented and their applications are discussed in the following sections.

12.2 Cost Estimation of Industrial Processes

When introducing a new process or technology in an industry, the project development requires that several studies are carried out to check the technical and economic attractiveness of the process. The economic evaluation provides the necessary support for implementing a feasible process. The accuracy of the economic analysis depends on the level of detail of the information used for the cost estimation. Furthermore, higher precision in the details of the estimated cost leads to greater confidence in the economic feasibility of the process by avoiding unnecessary loss of material and added expenses related to idle equipment. Thus, the more accurate the estimation is, the more reliable the assessment will be.

The economic analysis of an industrial process involves evaluating both the capital and the operational costs, related to the assembling and the operation of the processing plant, respectively. The precision of the analysis depends on the amount of information available. The cost estimation of a processing plant can be classified according to its level of accuracy, varying from Class 5, which consists of an order-of-magnitude estimate with 0–2% accuracy level, to Class 1, which comprises detailed estimation with 50–100% accuracy level. Usually the Class 4 and 5 estimates are made to compare alternative processes when ‘go or not to go’ decisions are involved; they require little process information. More accurate estimation procedures, of Class 2 and 3, are applied to processes considered feasible from the initial study, and require more process information to be performed. Detailed estimates (Class 1) are carried out in the final study, in order to evaluate the promising alternatives that remain from preliminary estimations, and require detailed information about the process to be performed.⁵

Table 12.1 Factors involved in cost estimation of natural products extraction.

<i>Factors</i>	<i>Costs involved</i>
raw material	– expenses related to cultivation (tillage, fertilizers, pesticides and other inputs, pruning, equipment, labor, etc.) – edaphoclimatic conditions – seasonal variability – part of the plant (seeds, leaves, bark, roots, etc.) – preparation of the raw material (drying, comminution) – transport, storage
operational conditions	– bed geometry, pump capacity, etc. – type of solvent (pure, mixtures), temperature, pressure, solvent flow rate, solvent to feed ratio, etc. – kinetic parameters and thermodynamic data
industrial requirements	– labor – plant size, amount of material to be processed per year – hours per day of production, days per year of production – cooling water, steam generation, refrigeration, electric power, etc.

There are some important economic factors that need to be addressed when the feasibility of new processes are evaluated. For the extraction of bioactive compounds from natural sources, the initial cost estimate is defined by taking into account data from three important items: the type of raw material, the operational conditions and the industrial requirements, as detailed in Table 12.1.

12.2.1 Costs Associated with the Raw Material

When it comes to natural products, special attention should be given to the costs of obtaining and preparing the raw material prior to its industrial processing. The selection of the part of the plant (leaves, flower, seeds, roots, etc.) and its preparation (drying, separation and comminution to obtain the ideal particle size, shape and porosity) are independent steps that may affect the yield and the cost of the process. It is known that, depending on the part of the plant used, there can be different amounts of a given compound.⁶ If the yield varies, the manufacturing cost will also change. Along with the choice of the part of the plant selected, the quality and yield of the extracts may be significantly influenced by cultivation practices, edaphoclimatic conditions, seasonal variability and genetics.

Once the part of the plant is selected, it must be adequately prepared to be used in the extraction step. In most cases it is necessary to dry and to comminute the raw material into specific moisture content and particle size. The inadequate preparation of the raw material can lead to low yield, poor quality and, therefore, high cost of the final product, making the process economically unfeasible. Besides, costs associated with the transportation of both the raw material and the product cannot be disregarded, as they can have a significant impact on the manufacturing cost.

The costs associated with the raw material (cost of raw material – CRM) are responsible for a large portion of the cost of manufacturing (COM) the

products.^{6,7} The high costs are related to low availability of the raw material and/or low amount of the desired compound in the raw material. The only cases where CRM do not represent a significant portion of COM usually happen when extractors of low capacity are considered, such as 5 L for SFE.⁸ The increase in the extractor capacity promotes a linear increase of the raw material needed, while the increase in the equipment costs does not follow the same proportionality, thus diluting the share of equipment cost in the COM and therefore increasing the CRM share. Another case when CRM is less important is when the raw material is a residue of the industry, with cost close to zero.⁷

12.2.2 Costs Associated with the Operational Conditions

The optimized conditions, kinetic parameters and thermodynamic data (Table 12.1) are essential to define the viability of the process. The importance of using optimized conditions is not only related to achieving higher yields; the utilities and fixed costs can also be minimized when operational conditions are optimized.

In this aspect, one important operational condition to be determined is the ideal cycle time. As extraction processes are usually carried out in batch mode, the most suitable time to end the procedure in industry is not always when the raw material is exhausted. Generally, approximately 50–90% of the total amount of extract is obtained during the constant extraction rate (CER) period, in which the maximal mass transfer rate can be achieved.⁹ Therefore, the general recommendation is to carry out the process during the CER period, when high amounts of solute are extracted in a relatively short time. After this period, diffusion becomes increasingly predominant, and the process enters in the falling-extraction rate (FER) period, when the extraction rate decreases. When the process is carried out after the CER period, up to the FER and diffusion controlled (DC) periods, the operational costs tend to increase.⁷ However, it is not always possible to extract the target compounds during the CER period; therefore, the quality and yield should be carefully balanced when optimizing the extraction conditions for industry.

An example of the effect of time on COM was presented by Albuquerque and Meireles.¹⁰ The authors evaluated the extraction of bixin with supercritical CO₂. They observed that when the process time increased 15 min after the CER time, the extract yield would increase 25%, while the COM would increase 8%; however, the specific cost of the bixin fraction would decrease around 7%, due to its content increase to 80% in the extract. They also demonstrated that the COM estimation considering the FER time would significantly increase, making the process economically unviable.

12.2.3 Costs Associated with the Industrial Requirements

Manufacturing costs are often reported as dollars per year.⁵ Based on this, the evaluation involves knowing the numbers of batches that will be performed and

how much time is necessary for each one. This analysis provides the data about the amount of material to be processed per year, the energy used per year and the labor required to do it. This information (amount of material to be processed, hours per day of production, days per year of production, etc.), is required to delineate the productivity and the costs of the industrial process. Other costs associated with cooling water, steam generation, and electric power, among others, must also be evaluated case by case.

12.3 Cost Estimation Procedures

In order to evaluate the total production costs, fixed costs must be taken into account, such as equipment, their maintenance and depreciation, insurance and property taxes. All the variable costs also need to be addressed, including the costs of raw material, utilities, labor and waste treatment.

The information about the price of the equipment is sometimes not from an up-to-date source. In this case, it is necessary to adjust the values considering the changing economic conditions. A simple relationship can be used:

$$C_2 = C_1 \left(\frac{I_2}{I_1} \right) \quad (12.1)$$

where C is the cost of the equipment; I is the cost index of the equipment selected; and the sub-indices 1 and 2 are related to the reference equipment and the desired equipment, respectively.

The Chemical Engineering Plant Cost Index (CEPCI) has been used to adjust for inflation in the value of equipment. For instance, the cost of a vessel in 1990 was US\$ 25 000. According to CEPCI, the cost index in 1990 (I_{1990}) was 358, and in 2010 it was 550 (I_{2010}). Therefore, from Equation (12.1):

$$C_{2010} = C_{1990} \left(\frac{I_{2010}}{I_{1990}} \right) = 25000 \left(\frac{550}{358} \right)$$

Thus, the estimated price of the vessel in 2010 is US\$ 38 300.84. The values of CEPCI from 1963 to 2010 are presented in Figure 12.1.

There are several methods to estimate the cost of a chemical plant. Two of them, which are especially suited to be used for supercritical technologies, are capacity factor^{11–13} and Lang factor.¹⁴

12.3.1 Cost Estimate as a Function of Equipment Capacity

As previously mentioned, the equipment cost is a decisive factor for the implementation of supercritical technology in industry. Equipment sizing should be as accurate as possible, as underestimating or overestimating the production capacity would result in unnecessary costs with raw materials and utilities. This precaution also avoids unnecessary costs with idle equipment and staff.

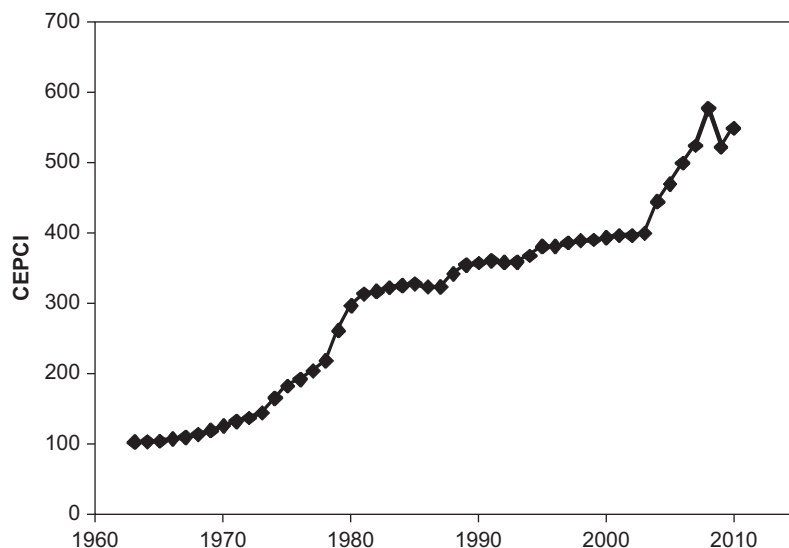


Figure 12.1 Values of CEPCI from 1963 to 2010.

Some cost estimation methodologies are based on the relationships between scaling costs and equipment size. Equation (12.2) is a correlation used when considering the purchase cost and the capacity of the units.

$$FC_2 = FC_1 \left(\frac{A_2}{A_1} \right)^n \quad (12.2)$$

where FC is the fixed cost of the equipment; A is the capacity of the equipment; n is the cost exponent; and the sub-indices 1 and 2 are related to the reference equipment and the desired equipment, respectively.

The cost exponent (n) varies from 0.26 to 1.33 depending on the class of the equipment. The value of n represents the extending capacity ratio from the plant size given.¹⁵ Several kinds of equipment have a value of n around 0.6. Because of this, a common relationship, known as the six-tenths rule (Equation 12.3), has been applied. The six-tenths rule is frequently employed to scale up/down equipment.

$$FC_2 = FC_1 \left(\frac{A_2}{A_1} \right)^{0.6} \quad (12.3)$$

Another correlation was proposed by Perrut¹³ considering a price index (PI), represented by:

$$PI = A(V_T Q)^{0.24} \quad (12.4)$$

where V_T is the total volume of the extractor; Q is the solvent flow rate; and A is a constant that depends on the linearization of values that correlate the cost with V_T and Q .

Shariaty-Niassar *et al.*¹⁶ used the PI correlation to estimate fixed costs of SFE equipment based on small-scale equipment costs, resulting in the expression:

$$FC_2 = FC_1 \left(\frac{V_2 Q_2}{V_1 Q_1} \right)^{0.24} \quad (12.5)$$

Generally, the supercritical extraction plant represents 70–85% of the total cost of investment.¹⁶

12.3.2 Lang Factor

This method considers the total cost of the process in a plant as the sum of the total purchase cost of all major components of the equipment multiplied by a constant – the Lang factor (F_L), as seen in Equation (12.6).

$$CP = F_L \sum_{i=1}^n C_i \quad (12.6)$$

where CP is the capital cost of the plant; and C_i is the purchase cost of each component.

The Lang factor is an experimental measure. Besides the equipment costs, it also considers other items required for the installation, such as insulation, pipes, etc. According to the type of processing, the Lang factor presents different values: for solids the factor is 3.10; for solids-fluids, 3.63; and for fluids, 4.74. Lang's approach was simple, using a factor that varies only with the type of process. Many methods of equipment factoring have been proposed. Nevertheless, the Lang factor is frequently used to refer generically to all of them. This method is not very precise; however, it gives an approximate order of magnitude for the purpose of comparing different processes.

The Lang factor can also be determined in terms of individual factors f_i , related to items that have the same physical nature (installation, isolation, pipe, building, structures, etc.); and f_j , related to additional charges associated with the installation of the equipment (engineering services, overheads, etc.), using the following expression:¹⁷

$$F_L = \left(1 + \sum_{i=1} f_i \right) \left(1 + \sum_{j=1} f_j \right) \quad (12.7)$$

12.3.3 Manufacturing Cost Estimation

The manufacturing cost can be determined as the sum of the direct manufacturing cost (DMC) or prime cost, the fixed (indirect) manufacturing cost (FMC), and general expenses (GE). The estimation can be performed using the expression:

$$COM = DMC + FMC + GE \quad (12.8)$$

12.3.3.1 Direct Manufacturing Cost (DMC)

The direct manufacturing cost is composed of the expenses that vary with the production rate, i.e. this type of cost increases when the production rate is high and decreases with low production. The costs of raw material (CRM), operational labor (COL), utilities (CUT) and waste treatment (CWT) are included in this class.

The raw materials used in extraction processes of natural products usually consist of the plant material containing the bioactive compounds and the solvents. The costs to obtain, to transport and to prepare the raw materials prior to extraction must also be taken into account in the CRM. Special attention must be paid to the recycling of solvents. To be effective, this step needs to be properly designed so that the solvent does not carry contaminants from one batch to the next. Although recycling brings advantages by reducing the cost associated with the solvent, if it is not properly conducted the contaminants can decrease the quality of the product and reduce the yield because of the decreased solubility.

The operational labor (COL) represents the costs related to the workers directly responsible for the processing. When using classical technologies for extraction at industrial level, usually the labor required is high. This happens because these technologies are simple and have low levels of automation, which is an option often selected to decrease the investment costs. As for modern processes, they usually require automated systems, which imply higher investment but also a reduced number of direct workers.

To estimate the COL, it is necessary to identify the hourly wage of an operator. Turton *et al.*⁵ employ Equation (12.9) to define the number of operators in an industrial unit.

$$N_{OL} = (6.29 + 31.7P^2 + 0.23N_{np})^{0.5} \quad (12.9)$$

where N_{OL} is the number of operators per shift; P is the number of processing steps directly involving the industrial unit; and N_{np} is the number of steps that do not directly involve the processing unit, such as compression, heating, mixing, etc.

The cost of utilities (CUT) includes electricity, cooling water and steam. The higher the pressure and the temperature used in the process, the higher the utilities cost. In extraction processes the CUT includes: the heating of the extraction vessel and of the solvent to the desired temperature; the energy to be used in the pump and other electrical devices; and the cooling of the water needed in the condenser, separator, etc.

The last cost that needs to be considered is waste treatment (CWT). In cases where the solvent is recycled, there is little quantity of residue that can be released to the atmosphere (in supercritical processes) or remain in the exhausted plant material. The extraction of natural products is usually carried out with GRAS (generally recognized as safe) solvents; therefore the small quantity of solvent lost in the process is non-toxic to the environment or to the operators. Furthermore, the raw material residue is organic, and thus can be

disposed directly in the soil or can sometimes be reused by other industries. For these reasons, the CWT is usually disregarded in processes of extraction of natural products.

12.3.3.2 Fixed (Indirect) Manufacturing Cost (FMC)

The fixed costs are related to depreciation, taxes and insurance. These costs do not depend directly on the production rate, and must be considered even if the process is stopped. The taxes depend on local legislation. The insurance is associated with the costs to protect the company and represents 1–3% of FCI.^{5,17}

The depreciation cost is related to the physical plant. It is the cost or expense arising from natural wear or obsolescence of fixed assets (property and equipment) of the company used in the production. Depreciation of fixed assets directly related to the production must be allocated as costs, while assets that are not used directly in the production should have their depreciation recorded as expenses. Usually in economic evaluations the depreciation time of the equipment is stipulated as 10–15 years, representing a cost of 10–15% of the fixed capital investment (FCI).^{5,17}

12.3.3.3 General Expenses (GE)

General expenses are overheads of the plant needed to maintain the business and consists of administrative cost (salaries and costs related to them, other administration costs), sales expenses (marketing and sales costs) and research and development, among others. These costs are not directly associated with the manufacturing process, but are indirectly related to the costs of FCI and COL.

12.4 Manufacturing Cost of Vegetable Extracts

When developing products, the economic evaluation allows for determining the balance between yield and cost, while taking the product's quality into account. Due to the preconception of investors towards the high cost of supercritical extraction processes, researchers started working on demonstrating the economic feasibility of this technology. More recently, other modern extraction processes have been evaluated for their economic feasibility. Next these studies will be presented.

12.4.1 Supercritical Extraction Process

Due to the high investment costs of supercritical extraction processes, this technology has received special attention in terms of economic evaluation. Tables 12.2, 12.3 and 12.4 summarize some reports found in the recent literature.

Table 12.2 Operational and yield data of supercritical processes required for cost estimations.

Raw material	Temperature (K)	Pressure (MPa)	Feed (kg raw material/batch)	Solvent flow rate (kg/s)	Bed density (kg/m ³)	Time (min)	Extract yield (%)	Ref.
Anise (<i>Pimpinella anisum</i>)	303	10	0.304	6.12×10^{-5}	760	100	7.9	9
Annatto (<i>Bixa orellana</i> L.) seeds	1) 333 2) 300	1) 31 2) 20	0.0203	17.3×10^{-5}	656	1) 250 2) 40	1) 1.89 2) 2.12	10
Beans (<i>Phaseolus vulgaris</i>)	323	35	n.i.	a) CO ₂ b) CO ₂ + ethanol (10 %, v/v)	n.i.	a) 40 b) 77	a) 0.4 b) 0.5	18
Brazilian ginseng (<i>Pfaffia glomerata</i>) roots	303	20	0.020	7×10^{-5} (CO ₂) + 10 % (v/v, ethanol)	n.i.	360	0.53	19
Buriti (<i>Mauritia flexuosa</i>) fruits	313	20–30	0.100	$2.8\text{--}4.3 \times 10^{-4}$	590	55–210	7.5–15.7	7
Clove (<i>Eugenia caryophyllus</i>) buds	288	10	0.180–0.200	1.60×10^{-5}	520	70–120	12.9–14.1	20
Fennel (<i>Foeniculum vulgare</i>) leaves	303	25	0.176	8.33×10^{-5}	440	80	12.5	9
Flame vine (<i>Pyrostegia venusta</i>) leaves	323	35	n.i.	a) CO ₂ b) CO ₂ + ethanol (10 %, v/v)	n.i.	a) 40 b) 77	a) 0.6 b) 1.5	18
Ginger (<i>Zingiber officinalis</i>) rhizome	313	20	0.080	5.6×10^{-5}	340	150	2.7	20
Grape (<i>Vitis vinifera</i>) seeds	313	35	4.677	2.14×10^{-3}	908	300	13.42	8
<i>Heteropterys aphrodisiaca</i> roots	323	35	n.i.	a) CO ₂ b) CO ₂ + ethanol (10 %, v/v)	n.i.	a) 40 b) 77	a) 0.8 b) 2.5	18
Ice-cream-bean (<i>Inga edulis</i>) leaves	323	35	n.i.	a) CO ₂ b) CO ₂ + ethanol (10 %, v/v)	n.i.	a) 40 b) 77	a) 1.5 b) 2.7	18
Jatoba (<i>Hymenaea courbaril</i>) bark	323	35	n.i.	a) CO ₂ b) CO ₂ + ethanol (10 %, v/v)	n.i.	a) 40 b) 77	a) 1.3 b) 2.4	18

Table 12.2 (Continued)

Raw material	Temperature (K)	Pressure (MPa)	Feed (kg raw material/batch)	Solvent flow rate (kg/s)	Bed density (kg/m ³)	Time (min)	Extract yield (%)	Ref.
Lemon verbena (<i>Aloysia triphylla</i>) leaves	318	35	0.005	7.3×10^{-5}	360	120	1.49	21
Mango (<i>Mangifera indica</i>) leaves	318	25	0.005	3.96×10^{-5}	360	120	3.04	21
Marigold (<i>Calendula officinalis</i> L)	313	20	0.040	2.16×10^{-5}	304	900–1146	n.i.	22
Palm (<i>Elaeis guineensis</i>) pressed fiber	318–328	20–30	n.i.	3.30×10^{-5}	177	24–140	2.71–7.01	7
Peach (<i>Prunus persica</i>)	313	20	0.003	Eq 1: 1.60×10^{-4} Eq 2: 5.50×10^{-5}	741	30–360	n.i.	22
Pupunha (<i>Guiljelma speciosa</i>) fruits	318–323	25–30	n.i.	$3.10\text{--}4.80 \times 10^{-4}$	671	24–100	6.6–13	7
Rosemary (<i>Rosmarinus officinalis</i>) leaves	313	30	0.143	8.33×10^{-5}	358	100	5.0	9
Spearmint (<i>Mentha spicata</i> L.)	323	20–30	0.015	Eq 1: 8.33×10^{-5} Eq 2: 1.50×10^{-5}	150	180	n.i.	22
Sugarcane residue (filter cake)	333	35	1.339	1.84×10^{-3}	260	30–180	2.88	23
Sweet basil (<i>Ocimum basilicum</i>)	a) 303–323 b) 303 c) 303	10–30	0.0025	$5\text{--}8 \times 10^{-5}$ (CO ₂) + a) 1 % (w/w, water) b) 10 % (w/w, water) c) 20 % (w/w, water)	135	60	a) 1.1–2.0 b) 7–11 c) 14–24	24
Tabernaemontana catharinensis branches	a) 318 b) 328	a) 35 b) 30	a) 0.085 b) n.i.	a) 3.5×10^{-5} (CO ₂) + 5 % (v/v, ethanol) b) 6.1×10^{-5} (CO ₂) + 10 % (v/v, ethanol)	a) 310 b) 322	90	a) 1.6 b) 1.04	25
Vetiver (<i>vetiveria zizanoides</i>) roots	313	20	0.003	4.7×10^{-5}	829	40	1.28–1.41	26

Eq = equipment; n.i. = not indicated.

Table 12.3 Economic data required for the cost estimations.

Raw material	Class	FCI	COL	CRM	CWT	CUT	N	Ref.
anise (<i>Pimpinella anisum</i>)	4–5	2 × 0.4 m ³ (US\$ 2,000,000)	US\$ 3.00/h	raw material (US\$ 468.25/ton) + pre-processing (US\$ 30.00/ton) + CO ₂ (US\$ 0.15/kg)	0	separator (US\$ 3.18 × 10 ⁻³ /MJ) + condenser (US\$ 20.00 × 10 ⁻³ /MJ) + pump (US\$ 16.80 × 10 ⁻³ /MJ) + heat exchanger (US\$ 3.18 × 10 ⁻³ /MJ)	5940	9
annatto (Bixa orellana L.) seeds	2–3	a) 2 × 0.005 m ³ (US\$ 200,000) b) 2 × 0.1 m ³ (US\$ 450,000–750,000) c) 2 × 0.5 m ³ (US\$ 1,150,000–2,000,000)	US\$ 6.00/h	raw material (US\$ 2,000/ton) + CO ₂ (US\$ 0.15/kg)	0	electricity (0.092 US\$/kWh)	n.i.	10
beans (<i>Phaseolus vulgaris</i>)	2–3	2 × 0.3 m ³	n.i.	n.i.	0	n.i.	n.i.	18
Brazilian ginseng (<i>Pfaffia glomerata</i>) roots	2–3	2 × 0.4 m ³ (US\$ 1,750,000)	US\$ 4.00/h	raw material (US\$ 4,710/ton) + pre-processing (US\$ 40.00/ton) + CO ₂ (US\$ 0.15/kg) + ethanol (US\$ 0.65/kg)	0	steam, cold water, electricity	n.i.	19
buriti (<i>Mauritia flexuosa</i>) fruits	4–5	2 × 0.4 m ³ (US\$ 2,000,000)	US\$ 3.00/h	raw material (US\$ 846.45/ton) + pre-processing (US\$ 30.00/ton) + CO ₂ (US\$ 0.10/kg)	0	saturated steam (US\$ 1.33 × 10 ⁻² /Mcal) + cold water (US\$ 8.37 × 10 ⁻² /Mcal) + electricity (US\$ 7.03 × 10 ⁻² /Mcal)	n.i.	7

Table 12.3 (Continued)

Raw material	Class	FCI	COL	CRM	CWT	CUT	N	Ref.
clove (<i>Eugenia caryophyllus</i>) buds	4–5	$2 \times 0.4 \text{ m}^3$ (US\$ 2,000,000)	US\$ 3.00/h	raw material (US\$ 505/ton) + pre-processing (US\$ 30.00/ton) + CO ₂ (US\$ 0.10/kg)	0	saturated steam (US\$ 1.33×10^{-2} /Mcal) + cold water (US\$ 8.37×10^{-2} /Mcal) + electricity (US\$ 7.03×10^{-2} /Mcal)	n.i.	20
fennel (<i>Foeniculum vulgare</i>) leaves	4–5	$2 \times 0.4 \text{ m}^3$ (US\$ 2,000,000)	US\$ 3.00/h	raw material (US\$ 159.29) + pre-processing (US\$ 30.00/ton) + CO ₂ (US\$ 0.15/kg)	0	separator (US\$ 3.18×10^{-3} /MJ) + condenser (US\$ 20.00×10^{-3} /MJ) + pump (US\$ 16.80×10^{-3} /MJ) + heat exchanger (US\$ 3.18×10^{-3} /MJ)	5940	9
flame vine (<i>Pyrostegia venusta</i>) leaves	2–3	$2 \times 0.3 \text{ m}^3$	n.i.	n.i.	0	n.i.	n.i.	18
ginger (<i>Zingiber officinalis</i>) rhizome	4–5	$2 \times 0.4 \text{ m}^3$ (US\$ 2,000,000)	US\$ 3.00/h	raw material (US\$ 495/ton) + pre-processing (US\$ 30.00/ton) + CO ₂ (US\$ 0.10/kg)	0	saturated steam (US\$ 1.33×10^{-2} /Mcal) + cold water (US\$ 8.37×10^{-2} /Mcal) + electricity (US\$ 7.03×10^{-2} /Mcal)	n.i.	20
grape (<i>Vitis vinifera</i>) seeds	2–3	a) $2 \times 0.005 \text{ m}^3$ (US\$ 100,000) b) $2 \times 0.05 \text{ m}^3$ (US\$ 300,000) c) $2 \times 0.5 \text{ m}^3$ (US\$ 1,150,000)	US\$ 4.00/h	raw material (US\$ 0–2.70/ton) + pre-processing (US\$ 40.00/ton) + CO ₂ (US\$ 0.15/kg)	0	electricity (US\$ 0.092/kW) + cooling water (US\$ 0.19/ton) + steam (US\$ 4.20/ton)	n.i.	8

<i>Heteropterys aphrodisiaca</i> roots	2–3	$2 \times 0.3 \text{ m}^3$	n.i.	n.i.	0	n.i.	n.i.	18
ice-cream-bean (<i>Inga edulis</i>) leaves	2–3	$2 \times 0.3 \text{ m}^3$	n.i.	n.i.	0	n.i.	n.i.	18
jatoba (<i>Hymenaea courbaril</i>) bark	2–3	$2 \times 0.3 \text{ m}^3$	n.i.	n.i.	0	n.i.	n.i.	18
lemon verbena (<i>Aloysia triphylla</i>) leaves	4–5	$2 \times 0.4 \text{ m}^3$ (US\$ 2,000,000)	US\$ 3.00/h	raw material (US\$ 34.53/ton) + pre-processing (US\$ 30.00/ton) + CO ₂ (US\$ 0.15/kg)	0	separator (US\$ 2.39×10^{-3} /MJ) + condenser (US\$ 19.12×10^{-3} /MJ) + pump (US\$ 16.73×10^{-3} /MJ) + heat exchanger (US\$ 2.39×10^{-3} /MJ)	3960	21
mango (<i>Mangifera indica</i>) leaves	4–5	$2 \times 0.4 \text{ m}^3$ (US\$ 2,000,000)	US\$ 3.00/h	raw material (US\$ 128.65/ton) + pre-processing (US\$ 30.00/ton) + CO ₂ (US\$ 0.15/kg)	0	separator (US\$ 2.39×10^{-3} /MJ) + condenser (US\$ 19.12×10^{-3} /MJ) + pump (US\$ 16.73×10^{-3} /MJ) + heat exchanger (US\$ 2.39×10^{-3} /MJ)	3960	21
marigold (<i>Calendula officinalis</i> L)	4–5	a) $2 \times 0.4 \text{ m}^3$ (US\$ 2,000,000) b) $3 \times 0.3 \text{ m}^3$ (US\$ 1,800,000)	US\$ 3.00/h	raw material (US\$ 875/ton) + pre-processing (US\$ 30.00/ton) + CO ₂ (US\$ 0.15/kg)	0	electricity (US\$ 70.3×10^{-3} /Mcal) + water refrigeration (US\$ 83.7×10^{-3} /Mcal) + saturated steam (US\$ 13.33×10^{-3} /Mcal)	n.i.	22
palm (<i>Elaeis guineensis</i>) pressed fiber	4–5	$2 \times 0.4 \text{ m}^3$ (US\$ 2,000,000)	US\$ 3.00/h	raw material (zero) + pre-processing (US\$ 30.00/ton) + CO ₂ (US\$ 0.10/kg)	0	saturated steam (US\$ 1.33×10^{-2} /Mcal) + cold water (US\$ 8.37×10^{-2} /Mcal) + electricity (US\$ 7.03×10^{-2} /Mcal)	7920	7

Table 12.3 (Continued)

Raw material	Class	FCI	COL	CRM	CWT	CUT	N	Ref.
peach (<i>Prunus persica</i>)	4–5	a) $2 \times 0.4 \text{ m}^3$ (US\$ 2,000,000) b) $3 \times 0.3 \text{ m}^3$ (US\$ 1,800,000)	US\$ 3.00/h	raw material (zero) + CO ₂ (US\$ 0.15/kg)	0	electricity (US\$ 70.3×10^{-3} /Mcal) + water refrigeration (US\$ 83.7×10^{-3} /Mcal) + saturated steam (US\$ 13.33×10^{-3} /Mcal)	n.i.	22
pupunha (<i>Guilielma speciosa</i>) fruits	4–5	$2 \times 0.4 \text{ m}^3$ (US\$ 2,000,000)	US\$ 3.00/h	raw material (US\$ 746.55/ton) + pre- processing (US\$ 30.00/ton) + CO ₂ (US\$ 0.10/kg)	0	saturated steam (US\$ 1.33×10^{-2} /Mcal) + cold water (US\$ 8.37×10^{-2} /Mcal) + electricity (US\$ 7.03×10^{-2} /Mcal)	7920	7
rosemary (<i>Rosmarinus officinalis</i>) leaves	4–5	$2 \times 0.4 \text{ m}^3$ (US\$ 2,000,000)	US\$ 3.00/h	raw material (US\$ 283.19/ton) + pre- processing (US\$ 30.00/ton) + CO ₂ (US\$ 0.15/kg)	0	separator (US\$ 3.18×10^{-3} /MJ) + condenser (US\$ 20.00×10^{-3} /MJ) + pump (US\$ 16.80×10^{-3} /MJ) + heat exchanger (US\$ 3.18×10^{-3} /MJ)	4752	9
spearmint (<i>Mentha spicata</i> L.)	4–5	a) $2 \times 0.4 \text{ m}^3$ (US\$ 2,000,000) b) $3 \times 0.3 \text{ m}^3$ (US\$ 1,800,000)	US\$ 3.00/h	raw material (US\$ 300/ton) + pre- processing (US\$ 30.00/ton) + CO ₂ (US\$ 0.15/kg)	0	electricity (US\$ 70.3×10^{-3} /Mcal) + water refrigeration (US\$ 83.7×10^{-3} /Mcal) + saturated steam (US\$ 13.33×10^{-3} /Mcal)	n.i.	22

sugar cane residue (filter cake)	2–3	a) $2 \times 0.005 \text{ m}^3$ (US\$ 100,000) b) $2 \times 0.05 \text{ m}^3$ (US\$ 300,000) c) $2 \times 0.5 \text{ m}^3$ (US\$ 1,150,000)	US\$ 4.00/h	raw material (zero) + pre-processing (US\$ 40.00/ton) + CO ₂ (US\$ 0.15/kg)	0	electricity (US\$ 0.092/kW) + cooling water (US\$ 0.19/ton) + steam (US\$ 4.20/ton)	n.i.	23
sweet basil (<i>Ocimum basilicum</i>)	4–5	$2 \times 0.4 \text{ m}^3$ (US\$ 2,000,000)	US\$ 3.00/h	raw material (US\$ 6,900/ton) + CO ₂ (US\$ 0.15/kg) + water (US\$ 5.32/kg)	0	electricity (US\$ 16.8/GJ) + cooling water (US\$ 19.99/kJ) + saturated steam (US\$ 3.18/kJ)	7920	24
<i>Tabernaemontana catharinensis</i> branches	4–5	$2 \times 0.4 \text{ m}^3$ (US\$ 2,000,000)	US\$ 3.00/h	raw material (US\$ 83/ton) + pre-processing (US\$ 30.00/ton) + CO ₂ (US\$ 0.15/kg) + ethanol (US\$ 0.221/kg)	0	separator (US\$ 3.18×10^{-3} /MJ) + condenser (US\$ 20.00×10^{-3} /MJ) + pump (US\$ 16.80×10^{-3} /MJ) + heat exchanger (US\$ 3.18×10^{-3} /MJ)	5280	25
vetiver (<i>vetiveria zizanoides</i>) roots	4–5	$2 \times 0.4 \text{ m}^3$ (US\$ 2,000,000)	US\$ 3.00/h	raw material (US\$ 66.67/ton) + pre-processing (US\$ 30.00/ton) + CO ₂ (US\$ 0.15/kg)	0	US\$ 12,759.20/ton (condensation of CO ₂ + flash separation = 89.55% of the CUT)	n.i.	26

FCI = fixed cost of investment; COL = operational labor (per worker); CRM = raw material cost; CWT = waste treatment cost; CUT = utilities cost; N = number of extraction batches/year considering 330 days, working 24 h/day; n.i. = not indicated.

Table 12.4 Results of the economic evaluation.

Raw material	FCI (%)	CRM (%)	COL (%)	CWT (%)	CUT (%)	COM (US\$/kg)		Ref.
						SFE	Conventional process	
anise (<i>Pimpinella anisum</i>)	36.97	54.67	7.31	0	1.05	14.34 (extract) 14.34–28.68 (essential oil)	51.31 (essential oil)	9
annatto (<i>Bixa orellana</i> L.)	a1) 76 b1) 70 c1) 62	a1) 3 b1) 14 c1) 22	a1) 20 b1), c1) COL + CUT < 10	0	a1) 1 2) n.i.	a1) 1,781.62 b1) 382.00 c1) 258.54	n.i.	10
	a2) 14	a2) 33 b2) 78 c2) 89	2) n.i.			a1) 292.50 c2) 124.58 c2) 109.27		
	n.i.	n.i.	n.i.	n.i.	n.i.	a) 938 b) 7,000	n.i.	18
beans (<i>Phaseolus vulgaris</i>)	n.i.	n.i.	n.i.	n.i.	n.i.	2,766	n.i.	19
Brazilian ginseng (<i>Pfaffia glomerata</i>)	12	75	5	0	7			
buriti (<i>Mauritia flexuosa</i>) fruits	2.9–26.9	62.3–94.0	0.6–5.3	0	1.0–5.9	22.56–125.55 (oil) 2,550–5,380 (carotenoids)	15.00 (oil)	7
clove (<i>Eugenia caryophyllus</i>) buds	36.75	55.67	7.25	0	0.33	9.15	40.00	20
fennel (<i>Foeniculum vulgare</i>) leaves	60.26	25.5	11.92	0	2.32	7.72 (extract) 7.72–15.44 (essential oil)	24.40 (essential oil)	9
flame vine (<i>Pyrostegia venusta</i>) leaves	n.i.	n.i.	n.i.	n.i.	n.i.	a) 1,773 b) 22,000	n.i.	18
ginger (<i>Zingiber officinalis</i>) rhizome	60.59	25.65	11.98	0	1.78	98.80	100.00	20

grape (<i>Vitis vinifera</i>) seeds	a) 28.98–29.20 b) 35.65–36.76 c) 45.85–50.97	a) 0.40–1.28 b) 1.68–5.23 c) 2.57–17.54	a) 60.14–60.57 b) 49.32–50.83 c) 24.82–27.58	0	a) 0.59–0.74 b) 2.40–3.10 c) 8.06–11.22	a) 179.91–290.17 b) 43.02–70.85 c) 11.88–21.13	4.85 (hexane extracted oil) 40.00–80.00 (pressed oil)	8
<i>Heteropterys aphrodisiaca</i> roots	n.i.	n.i.	n.i.	n.i.	n.i.	a) 4,170 b) 30,000	n.i.	18
ice-cream-bean (<i>Inga edulis</i>) leaves	n.i.	n.i.	n.i.	n.i.	n.i.	a) 3,004 b) 47,000	n.i.	18
jatoba (<i>Hymenaea courbaril</i>) bark	n.i.	n.i.	n.i.	n.i.	n.i.	a) 16,130 b) 48,000	n.i.	18
lemon verbena (<i>Aloysia triplylla</i>) leaves	74.71	7.29	14.77	0	3.23	95.79	182.01	21
mango (<i>Mangifera indica</i>) leaves	70.61	13.85	13.96	0	1.58	151.01	n.i.	21
marigold (<i>Calendula officinalis</i> L)	a) 94.25–94.77 b) 94.61–95.05	a) 3.28–2.67 b) 2.75–2.23	a) 2.07–2.08 b) 2.31–2.32	0	a) 0.40–0.47 b) 0.33–0.39	a) 611.12–785.85 b) 730.93–728.27	283.00–583.70	22
palm (<i>Elaeis guineensis</i>) pressed fiber	77.1–77.8	1.9–2.7	15.2–15.3	0	5.0–5.6	19.46–62.82 (oil) 3,560–17,220 (carotenoids)	1.74 (oil)	7
peach (<i>Prunus persica</i>)	a) 46.45–52.90 b) 49.61–55.65	a) 24.52–14.04 b) 21.82–12.30	a) 9.19–10.47 b) 10.91–12.24	0	a) 19.84–22.59 b) 17.66–19.81	a) 5.22–30.08 b) 4.64–26.37	40.00–150.00 (oil)	22
pupunha (<i>Guiljelma speciosa</i>) fruits	8.9–20	49.9–78.7	1.8–4.0	0	10.7–26.2	17.15–22.39	n.i.	7
rosemary (<i>Rosmarinus officinalis</i>) leaves	59.00	26.87	11.67	0	2.46	30.29 (extract) 30.29–60.57 (essential oil)	76.50 (essential oil)	9
spearmint (<i>Mentha spicata</i> L.)	a) 91.24–91.14 b) 91.74–91.66	a) 4.45–4.44 b) 4.09	a) 2.01–2.00 b) 2.24	0	a) 2.30–2.41 b) 1.93–2.02	a) 276.29–241.26 b) 328.86–288.22	574.60–1,647.00	22

Table 12.4 (Continued)

Raw material	FCI (%)	CRM (%)	COL (%)	CWT (%)	CUT (%)	COM (US\$/kg)		Ref.
						SFE	Conventional process	
sugar cane residue (filter cake)	a) 29.17–29.20	a) 0.25–0.46	a) 60.56–60.62	0	a) 0.73–0.84	a) 1,250.00–1,731.04	310–1,090 (tablets with 3 % policosanol)	23
	b) 36.66–36.82	b) 1.05–1.93	b) 50.75–50.96		b) 3.06–3.52	b) 301.79–424.33		
	c) 50.57–51.35	c) 3.84–6.93	c) 27.39–27.82		c) 11.00–12.82	c) 83.39–116.55 (extracts with 3–6 % policosanol)		
sweet basil (<i>Ocimum basilicum</i>)	12.5	80	2.5	0	4.0–6.3	a) 572.82–1,049.58 b) 107.37–152.45 c) 47.96–85.83	n.i.	24
<i>Tabernaemontana catharinensis</i> branches	a) 72.86 b) 71.23	a) 11.56 b) 12.37	a) 14.41 b) 14.08	0	a) 1.16 b) 2.32	a) 79.35 (extract) 440.31 (alkaloids) b) 121.79	n.i.	25
vetiver (<i>vetiveria zizanoides</i>) roots	49.71	39.18	9.83	0	1.29	9.70 (National variety) 24.26 (Bourbon variety)	151.79 (essential oil)	26

FCI = fixed cost of investment; COL = operational labor; CRM = raw material cost; CWT = waste treatment cost; CUT = utilities cost; COM = manufacturing cost; SFE = supercritical fluid extraction; n.i. = not indicated; the indications a) and b) are related to the specifications presented in Table 13.2.

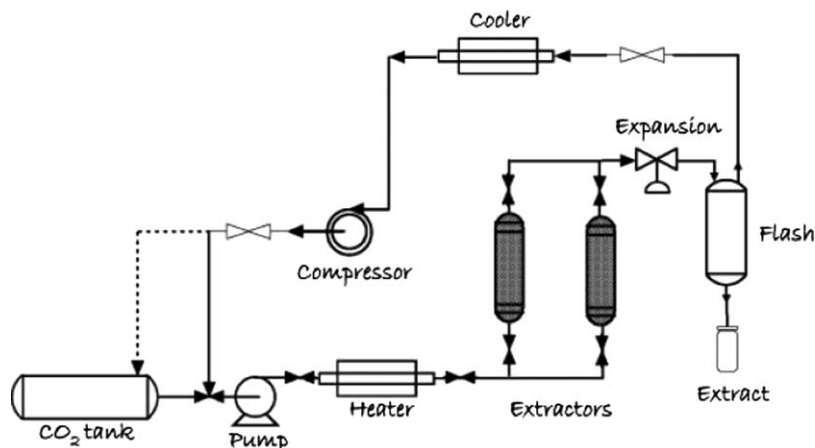


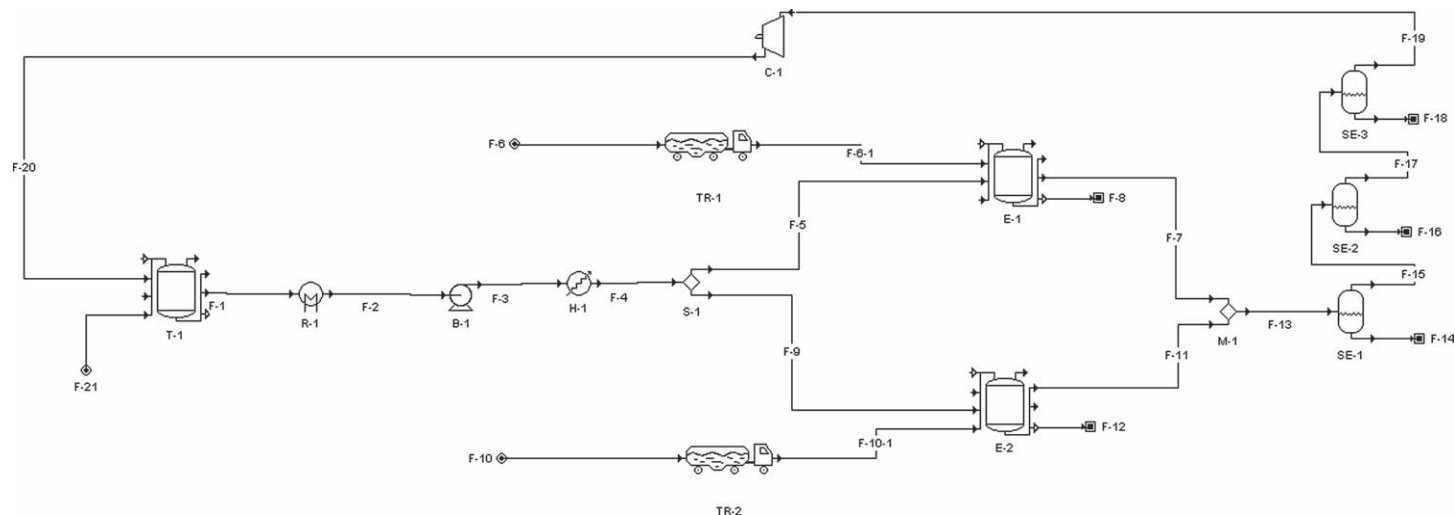
Figure 12.2 Flow diagram of SFE unit used to estimate the manufacturing cost of natural products using the methodology of Rosa and Meireles.²⁰ Reprinted from *Flavour and Fragrance Journal*, **22**, C. G. Pereira, M. A. A. Meireles, Economic analysis of rosemary, fennel and anise essential oils obtained by supercritical fluid extraction, pp. 407–413, 2007, with permission from John Wiley & Sons.

The COM can be estimated using Equation (12.8) by attributing different values to each factor.^{5,17} A simple manufacturing cost estimation method, class 4–5, was proposed by Rosa and Meireles (Figure 12.2),²⁰ based on the method described by Turton *et al.*⁵ These authors consider that the COM can be calculated using the following expression:

$$\text{COM} = 0.304 \text{ FCI} + 2.73 \text{ COL} + 1.23(\text{CRM} + \text{CWT} + \text{CUT}) \quad (12.10)$$

Rosa and Meireles²⁰ applied this procedure to estimate the manufacturing cost to obtain extracts from clove and ginger by SFE, demonstrating the economic feasibility of the supercritical technology to process natural products. Several other researchers have reached the same conclusion using this methodology.^{7,9,21,22,24–26} Later, Prado *et al.*²⁷ developed a class 2–3 methodology to estimate the COM of SFE processes using the commercial simulator SuperPro Designer (Figure 12.3). The COM estimated by the simulator is more accurate, and also showed the economic feasibility of SFE in Brazil for several raw materials.^{8,10,18,19,23} For grape seed extract the return time was shown to be as low as 1.5 years.⁸

Before performing the economic evaluation, some information is required, such as operational conditions (temperature, pressure, solvent flow rate), kinetics and yield data, which can be obtained from laboratory experiments (Table 12.2). The overall extraction curves are important information because they show the period of constant mass transfer rate. The estimated cost tends to be minimal towards the end of the CER period (Figure 12.4). Because of this, usually low COM is obtained when the CER period is short and the yield is high during this period. Departing from the experimental laboratory-scale data



Streams

F-1/F-2/F-3/F-4/F-5/F-9 – CO₂ feeding
 F-6/F-6-1/F-10/F-10-1 – raw material feeding
 F-7/F-11/F-13 – CO₂ + extract exit
 F-8/F-12 – solid residue exit + CO₂ loss
 F-14/F-16/F-18 – product exits
 F-15/F-17 – extract and CO₂ fractionation
 F-19/F-20 – CO₂ recycling
 F-21 – replacement of CO₂ lost

Equipment

B-1 – CO₂ pump
 C-1 – compressor
 E-1/E-2 – extractors
 H-1 – heat exchanger for CO₂ heating
 M-1/S-1 – stream mixer and separator, respectively
 R-1 – heat exchanger for CO₂ cooling
 SE-1/SE-2/SE-3 – separators
 T-1 – CO₂ tank
 TR-1/TR-2 – raw material pre-processing

Figure 12.3 Flow diagram of SFE unit used to estimate the cost of manufacturing SFE extracts in the SuperPro Designer simulator. Reprinted from *Journal of Food Engineering*, **109**, J. M. Prado, I. Dalmolin, N. D. D. Carareto, R. C. Basso, A. J. A. Meirelles, J. V. Oliveira, E. A. C. Batista, M. A. A. Meireles, Supercritical fluid extraction of grape seed: process scale-up, extract chemical composition and economic evaluation, pp. 249–257, 2012, with permission from Elsevier.

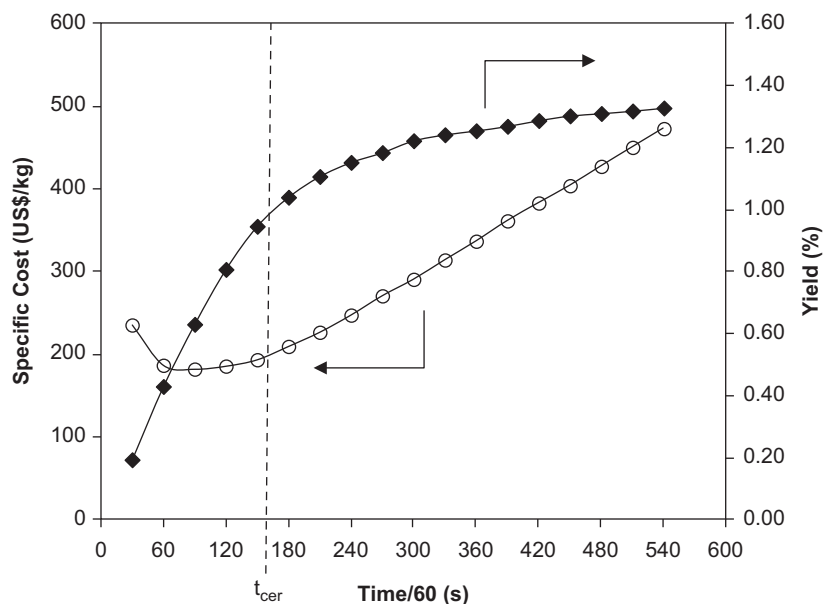


Figure 12.4 Overall extraction curve (◆) and specific cost (○) for SFE of alkaloids from *T. catharinensis* at 318 K and 35 MPa using 5% (v/v) of ethanol. Data from Pereira *et al.*²⁵

and using a scale-up criterion it is possible to estimate the COM of the process at the industrial level. For SFE, the scale-up criterion more often applied is the assumption that both the yield and the extraction time of the industrial process are similar to the laboratory scale if the solvent to feed ratio is kept constant, as demonstrated by Prado *et al.*²⁸

A summary of industrial information and economic data needed for SFE economic evaluation is presented in Table 12.3. It can be noticed that SFE capital costs have been decreasing due to competition amongst manufacturers.²⁹ Moreover, when a large quantity of raw material is processed, as in the coffee and tea decaffeination industries, or when the raw material is a residue with cost close to zero, SFE operational costs are below US\$ 3.00/kg raw material.^{7,8,22,23,30}

The information in Tables 12.2 and 12.3 were used to estimate the cost of manufacturing vegetable extracts of several species by SFE (Table 12.4). It can be observed that the COM is related to the type of raw material and its yield. The cost that has more effect on the COM is frequently the CRM, especially when dealing with high added value raw materials, which is often the case in the natural products industry. For instance, the CRM fraction represented 62–94% of the COM of buriti oil obtained by SFE.⁷ In this case, the high cost of the raw material was attributed to the fact that buriti is an indigenous species to Amazonia that comes from sustainable harvesting of local populations. The prices of other species similar to buriti and also rich in carotenoids were

considered in the estimation of the COM of buriti oil in this study, such as babassu (*Orbignya speciosa*), carnauba palm (*Copernicia cerifera*) and assai palm (*Euterpe oleracea*). It was reported that the high COM of buriti oil obtained by SFE was due to the raw material cost, instead of equipment and utilities. On the other hand, when working with residues, which imply low CRM, FCI and COL gain more importance. CUT share is always low in SFE processes, usually below 1%.

In Table 12.4 it can be noticed that the COM for SFE process is often lower than the COM of conventional processes. However, this is not the case for products of low added value, such as vegetable oils. One more exception was marigold; in this case the SFE process was considered economically unfeasible by the authors.²² Another aspect that needs to be considered is the use of co-solvents. It is known that the application of co-solvents is sometimes necessary to extract specific classes of compounds, mainly when the supercritical solvent is CO₂ and the target compound is polar. When this situation exists, the final product must be evaluated not only in terms of composition, bioactivity and yield, but also in terms of the new costs involved (co-solvent cost, extra pump, solvent removal, utilities, etc.). Within this context, some works have reported that using co-solvents increases the yield of the process and therefore reduces the COM of the product.^{19,24} On the other hand, other works have demonstrated that despite increasing the yield, sometimes using co-solvents is not economically attractive.^{18,25}

12.4.2 Other Extraction Processes

Economic evaluation using simulation software is becoming a valuable tool to determine the feasibility of SFE processes. In addition, this same methodology was successfully used to simulate other novel extraction processes, including ultrasound-assisted extraction and pressurized liquid extraction and to compare them to classical extraction methods.^{31–33}

Table 12.5 shows the cost of manufacturing jabuticaba extracts by different methods, including classical, as agitated bed extraction (ABE) and Soxhlet (SOX), novel, as ultrasound-assisted extraction (UAE) and pressurized liquid

Table 12.5 Cost of manufacturing jabuticaba extracts in a 0.3 m³ vessel by different methods. Adapted from Santos *et al.*^{31,33} and Veggi *et al.*³²

Method	Time (min)	Yield (%)	COM (US\$/kg)
ABE	120	9.01	401.21
UAE + ABE	130	10.08	422.18
UAE	120	11.93	387.20
SOX	480	9.92	778.42
AC-SOX	480	9.5	1001.00
PLE	9 (static) + 12 (dynamic)	13.01	15.53

ABE=agitated bed extraction; AC-SOX=acidified Soxhlet; COM=cost of manufacturing; PLE=pressurized liquid extraction; SOX=Soxhlet; UAE=ultrasound-assisted extraction.

extraction (PLE), as well as their combination. Due to the long processing time associated with the high temperature, Soxhlet proved to be the most expensive method. ABE and UAE presented similar COM, and their combination did not improve yield; therefore, the COM increased when ABE + UAE was used. PLE was the method that presented the lowest COM, because it was fast and presented high yield.

12.5 Case Study

12.5.1 Introduction

Clove (*Eugenia caryophyllus*) is a plant adapted to Brazilian cultivation, and the country has become a major producer of it. Its flower buds are rich in volatile oil, and eugenol is its main compound. Eugenol is a phenolic compound used in the pharmaceutical industry for its antiseptic, anti-inflammatory, bactericidal and anesthetic effects.³⁴ The oil also has fungicidal, antiviral, antitumor and insecticide properties, and in the food industry it is used as a flavoring, anti-microbial and antioxidant agent.^{35–39} The objective of the present work was to carry out an economic evaluation of SFE of clove based on the Brazilian reality.

12.5.2 Materials and Methods

The commercial simulator SuperPro Designer v6.0 was used to estimate the cost of manufacturing (COM) of clove oil obtained by SFE. The methodology developed by Prado *et al.*²⁷ was adapted. A SFE unit, including extraction, separation and solvent recycling steps, equipped with two extractors working semi-continuously and three separators in series was built with tools available from the simulator databanks (Figure 12.3). Plant design was based on the equipment used for determining experimental data.²⁸ Three scales were evaluated: extractor volumes of 5 L, 50 L and 500 L (Table 12.6). Experimental data of clove SFE at 313 K/15 MPa using a 5 L extractor were obtained from Prado *et al.* (Table 12.7).²⁸ Scale-up criterion to 50 L and 500 L consisted in keeping S/F (solvent to feed ratio) constant.²⁸

It was considered the industrial unit will run 24 h per day with three daily shifts, for 330 days, which represents a total of 7920 h of operation per year; 30 days will be destined for plant maintenance.²⁰ The number of operators needed per shift varies according to the capacity of the plant (Table 12.6). Labor charges and labor not directly associated with production were estimated by the simulator.

The raw material cost is related to plant material and CO₂ lost during the process. CO₂ loss is mainly due to depressurization of the extractor at the end of each batch.²⁹ Clove costs varied between US\$ 2.54/kg and US\$ 3.51/kg within a one-year period;⁴⁰ the COM was estimated using the highest raw material cost, that is, the worst case scenario. Pre-processing costs involve drying and comminution of raw material.

Table 12.6 Economic parameters used for COM estimation.

Industrial units ^a	
2 extractors of 5 L	US\$ 100,000.00
2 extractors of 50 L	US\$ 300,000.00
2 extractors of 500 L	US\$ 1,150,000.00
Depreciation rate	10 %/year
Labor ^a	US\$ 4.00/h
2 extractors of 5 L	1 operator
2 extractors of 50 L	2 operators
2 extractors of 500 L	3 operators
Raw materials	
Clove	US\$ 3.51/kg ^b
Pre-processing	US\$ 40.00/ton
CO ₂ (2 % loss)	US\$ 0.15/kg
Utilities ^a	
Electricity	US\$ 0.092/kWh
Cooling water	US\$ 0.19/ton
Steam	US\$ 4.20/ton

^aPrado *et al.*²⁷^bSEAGRI⁴⁰**Table 12.7** Experimental data used to estimate the cost of manufacturing clove extracts (data from Prado *et al.*²⁸); temperature of 313 K, pressure of 15 MPa.

<i>S/F</i> (kg CO ₂ /kg clove)	$\underline{Q} = 1.45 \times 10^{-3} \text{ kg CO}_2/\text{s}$		$\underline{Q} = 3.00 \times 10^{-3} \text{ kg CO}_2/\text{s}$	
	<i>Time</i> (min)	<i>Yield</i> (% , d.b.)	<i>Time</i> (min)	<i>Yield</i> (% , d.b.)
1.12	40	6.44	16	7.33
1.96	70	9.61	28	10.97
2.80	100	11.81	40	13.06
3.65	130	13.36	52	14.19

Utility costs comprise producing heat exchange agents and the electricity used in the process. Utilities needed for the operation of each piece of equipment were estimated by the simulator energy balance. Cost of waste treatment may be neglected, since the residue of SFE process is the dry exhausted clove, which may be incorporated to the soil as fertilizer. The CO₂ lost during system depressurization needs no treatment since in small quantities it is not toxic.³⁰

Transportation costs still have to be added to the COM that is estimated, as these will depend on the location of the industry.

12.5.3 Results and Discussion

Figure 12.5 presents the COM of clove oil obtained by SFE according to experimental data (Table 12.7).²⁸ The dotted line represents the selling price of clove oil obtained by SFE in the international market. The price of clove volatile oil, obtained by steam distillation, varies between US\$ 26.00/kg and

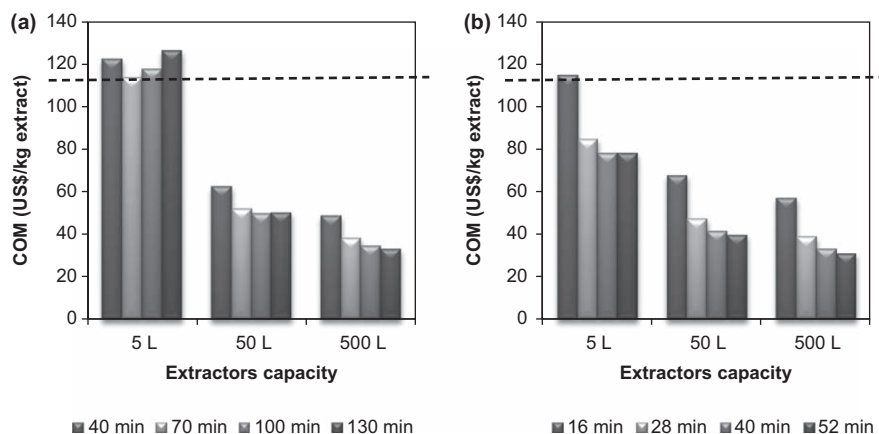


Figure 12.5 Manufacturing cost (COM) of clove oil obtained by SFE; dotted line represents its selling price in the international market. Experimental conditions: temperature of 313 K, pressure of 15 MPa, CO₂ flow rate of 1.45×10^{-3} kg/s (a) and 3.00×10^{-3} kg/s (b).

US\$ 86.00/kg,⁴¹ depending on the raw material origin and on the physico-chemical characteristics of the product.

As can be seen, the COM reduces as plant scale increases. Considering that the SFE plant would be processing only clove, a 5 L plant is economically unfeasible, but 50 L and 500 L plants would operate with COM far below the product's selling price. Moreover, comparing Figures 12.5a and 12.5b, as the solvent flow rate increases with consequent cycle time decrease for the same S/F, the processes economic viability improves. Therefore, SFE is economically feasible, after the process is appropriately optimized. It is still worth remembering that the raw material cost used for COM estimations was the highest found in market, that is, the COM can be further reduced with raw material cost decrease.

Rosa and Meireles²⁰ estimated clove oil COM as US\$ 9.15/kg for a plant operating with two extractors of 400 L. However, they used lower clove cost (US\$ 0.50/kg) since they considered the raw material would be purchased directly from the producer. In our study, it was the price of the distribution market that was considered. Moreover, Rosa and Meireles²⁰ used a class 4–5 methodology, while the methodology used in the present work can be considered as class 2–3.

Other economic parameters evaluated are presented in Tables 12.8 and 12.9. With scale increase the raw material cost share increases, diluting the other costs. The fixed cost of investment is not the main one, and for the largest plant, it is below US\$ 2.00/kg of extract. It is also interesting to notice that for both 50 L and 500 L scales the COM and return time are viable. Other authors²⁷ noticed a similar trend, with substantial decrease of COM for scale increase up to 100 L, and subsequent lower COM reduction with scale-up. Therefore, due to the lowest investment cost needed for the 50 L plant (US\$ 300 000.00) when

Table 12.8 Economic evaluation of clove oil production by SFE. Experimental conditions: temperature of 313 K, pressure of 15 MPa, CO₂ flow rate of 1.45×10^{-3} kg/s.

Time (min)	Productivity (kg/year)	Operation cost (US\$/year)	COM (US\$/kg)	CRM (%)	COL (%)	FCI (%)	CQC (%)	CUT (%)	Return time (years)
5 L									
40	1523	186000.00	122.13	35.86	39.16	18.86	5.87	0.25	—
70	1492	169000.00	113.27	29.25	43.18	20.80	6.48	0.29	18.45
100	1356	159000.00	117.26	24.70	45.95	22.14	6.89	0.32	35.89
130	1206	152000.00	126.04	21.38	47.97	23.12	7.20	0.34	—
50 L									
40	15228	945000.00	62.06	70.62	15.42	11.14	2.31	0.50	1.18
70	14914	771000.00	51.70	63.98	18.89	13.65	2.83	0.64	0.98
100	13563	669000.00	49.33	58.49	21.76	15.73	3.26	0.76	1.01
130	12065	603000.00	49.98	53.86	24.17	17.47	3.63	0.86	1.12
500 L									
40	152281	7374000.00	48.42	90.49	2.96	5.47	0.44	0.64	0.43
70	149134	5639000.00	37.81	87.51	3.88	7.16	0.58	0.88	0.36
100	135628	4621000.00	34.07	84.72	4.73	8.73	0.71	1.10	0.36
130	120649	3952000.00	32.76	82.12	5.53	10.21	0.83	1.32	0.38

COM = cost of manufacturing; CRM = cost of raw material; COL = cost of labor; FCI = fixed cost of investment; CQC = cost of quality control; CUT = cost of utilities.

Table 12.9 Economic evaluation of clove oil production by SFE. Experimental conditions: temperature of 313 K, pressure of 15 MPa, CO₂ flow rate of 3.00×10^{-3} kg/s.

Time (min)	Productivity (kg/year)	Operation cost (US\$/year)	COM (US\$/kg)	CRM (%)	COL (%)	FCI (%)	CQC (%)	CUT (%)	Return time (years)
5 L									
16	1951	223000.00	114.30	46.32	32.69	15.75	4.90	0.34	30.22
28	2440	206000.00	84.43	41.86	35.32	17.02	5.30	0.50	3.47
40	2495	194000.00	77.76	38.22	37.53	18.08	5.63	0.56	2.85
52	2375	185000.00	77.89	35.14	39.35	18.96	5.90	0.64	2.95
50 L									
16	19515	1313000.00	67.28	78.64	11.10	8.02	1.67	0.57	1.10
28	24396	1146000.00	46.97	75.30	12.71	9.18	1.91	0.90	0.61
40	24949	1026000.00	41.12	72.34	14.21	10.27	2.13	1.05	0.53
52	23750	935000.00	39.37	69.57	15.58	11.26	2.34	1.26	0.54
500 L									
16	195137	11054000.00	56.65	93.40	1.98	3.65	0.30	0.68	0.44
28	243969	9391000.00	38.49	91.93	2.33	4.30	0.35	1.10	0.25
40	249481	8182000.00	32.80	90.67	2.67	4.93	0.40	1.32	0.22
52	237502	7279000.00	30.65	89.38	3.00	5.54	0.45	1.62	0.22

COM = cost of manufacturing; CRM = cost of raw material; COL = cost of labor; FCI = fixed cost of investment; CQC = cost of quality control; CUT = cost of utilities.

compared to the 500 L plant (US\$ 1 150 000.00), a 50 L plant may be a good alternative for a first investment on SFE in Brazil.

More information that can be obtained from cost analysis is the best batch time. To evaluate the optimized cycle time, the yield, chemical composition and cost data should be considered. The extract quality has been confirmed up to S/F = 5.11.²⁸ By the economic evaluation, S/F of 3.65 (130 min for CO₂ flow rate of 1.45×10^{-3} kg/s and 52 min for CO₂ flow rate of 3.00×10^{-3} kg/s) is the batch time presenting lower COM. Therefore, it can be concluded that for clove SFE at 313 K/15 MPa, 52 min and S/F of 3.65 present the best relation between cost, yield and product quality. It is still worth remembering that due to the different colors of the extracts obtained in each separator,²⁸ the selling price of the products may vary.

12.6 Conclusion

Taking new processes to industry requires not only technical feasibility, but also economic attractiveness. In the field of natural products, the use of supercritical fluid extraction has been looked at to evaluate the economics of this process. Literature shows that supercritical technology can be economically feasible to recover different classes of compounds from several types of raw materials. In this chapter, some methods to estimate the manufacturing cost of extracts by different techniques were presented. They have been applied to show that the supercritical technology can be economically feasible for the production of extracts from natural products at a lower cost than using conventional separation techniques. These same cost estimate methodologies were shown to be applicable to other modern extraction processes.

Acknowledgement

J. M. Prado is grateful for financial support from São Paulo Research Foundation (FAPESP, process 2010/08684-8).

References

1. L. T. Taylor, *Supercritical Fluid Extraction*, John Wiley & Sons Inc., Canada, 1996.
2. R. N. Patel, S. Bandyopadhyay and A. Ganesh, *J. Chromatogr. A*, 2006, **1124**, 130.
3. R. N. Cavalcanti, P. C. Veggi and M. A. A. Meireles, *Proc. Food Sci.*, 2011, **1**, 1672.
4. L. Fiori, *Chem. Eng. Process*, 2010, **48**, 866.
5. R. C. Turton, W. B. Bailie, J. A. Whiting and J. A. Shaeiwitz, *Analysis, Synthesis, and Design of Chemical Process*, Prentice Hall, PTR, Upper Saddle River, NJ, 1998.
6. C. G. Pereira and M. A. A. Meireles, *Food Bioprocess Technol.*, 2010, **3**, 340.

7. J. M. Prado, A. R. Assis, M. R. Maróstica Jr. and M. A. A. Meireles, *J. Food Proc. Eng.*, 2010, **33**, 348.
8. J. M. Prado, I. Dalmolin, N. D. D. Carareto, R. C. Basso, A. J. A. Meirelles, J. V. Oliveira, E. A. C. Batista and M. A. A. Meireles, *J. Food Eng.*, 2012, **109**, 249.
9. C. G. Pereira and M. A. A. Meireles, *Flavour Frag. J.*, 2007, **22**, 407.
10. C. L. C. Albuquerque and M. A. A. Meireles, *J. Supercrit Fluids*, 2012, **66**, 86.
11. C. A. Passey, in *Supercritical Fluid Processing of Food and Biomaterials*, ed. S. S. H. Rizvi, Blackie, London, UK, 1994, p. 223.
12. J. M. del Valle, J. C. Fuente and D. A. Cardarelli, *J. Food Eng.*, 2005, **67**, 35.
13. M. Perrut, *Ind. Eng. Chem. Res.*, 2000, **39**, 4531.
14. A. L. Yver, L. M. Bonnaillie, W. Yee, A. McAloon and P. M. Tomasula, *Int. J. Mol. Sci.*, 2012, **13**, 240.
15. R. H. Perry and D. W. Green, *Perry's Chemical Engineers Handbook*, 7th edn., McGraw-Hill, New York, NY, 1997.
16. M. Shariaty-Niassar, B. Aminzadeh, P. Azadi and S. Soltanali, *Chem. Ind. Chem. Eng. Q.*, 2009, **15**, 143.
17. C. A. G. Perlingeiro, *Engenharia de Processos: Análise, Simulação, Otimização e Síntese de Processos Químicos*, Blucher, São Paulo, Brazil, 2005.
18. P. C. Veggi, D. T. Santos and M. A. A. Meireles, *Proc. Food Sci.*, 2011, **1**, 1717.
19. P. F. Leal, M. B. Kfoury, F. C. Alexandre, F. H. R. Fagundes, J. M. Prado, M. H. Toyama and M. A. A. Meireles, *J. Supercrit. Fluids*, 2010, **54**, 38.
20. P. T. V. Rosa and M. A. A. Meireles, *J. Food Eng.*, 2005, **67**, 235.
21. C. G. Pereira and M. A. A. Meireles, *J. Food Proc. Eng.*, 2007, **30**, 150.
22. N. Mezzomo, J. Martínez and S. R. S. Ferreira, *J. Food Eng.*, 2011, **103**, 473.
23. J. M. Prado and M. A. A. Meireles, in *Biorefinery Co-products: Phytochemicals, Primary Metabolites and Value-added Biomass Processing*, ed. C. Bergeron, D. J. Carrier and S. Ramaswamy, John Wiley & Sons, Hoboken, NJ, 2012, p. 133.
24. P. F. Leal, N. B. Maia, Q. A. C. Carmello, R. R. Catharino, M. N. Eberlin and M. A. A. Meireles, *Food Bioproc. Technol.*, 2008, **1**, 326.
25. C. G. Pereira, P. T. V. Rosa and M. A. A. Meireles, *J. Supercrit Fluids*, 2007, **40**, 232.
26. C. G. Pereira, I. P. Gualtieri, N. B. Maia and M. A. Meireles, *J. Agric. Sci. Technol.*, 2008, **35**, 44.
27. I. M. Prado, C. L. C. Albuquerque, R. N. Cavalcanti, M. A. A. Meireles, in *9th International Symposium on Supercritical Fluids*, Arcachon, France, 2009.
28. J. M. Prado, G. H. C. Prado and M. A. A. Meireles, *J. Supercrit. Fluids*, 2011, **56**, 231.
29. M. Perrut, in *Iberoamerican Conference on Supercritical Fluids*, Iguassu Falls, Brazil, 2007.

30. G. Brunner, *J. Food Eng.*, 2005, **67**, 21.
31. D. T. Santos, P. C. Veggi and M. A. A. Meireles, *J. Food Eng.*, 2010, **101**, 23.
32. P. C. Veggi, D. T. Santos and M. A. A. Meireles, *Proc. Food Sci.*, 2011, 1725.
33. D. T. Santos, P. C. Veggi and M. A. A. Meireles, *J. Food Eng.*, 2012, **108**, 444.
34. A. Alqareer, A. Alyahya and L. Andersson, *J. Dent.*, 2006, **34**, 747.
35. K. Lee and T. Shibamoto, *Food Chem.*, 2001, **74**, 443.
36. K. V. Menon and S. R. Garg, *Food Microbiol.*, 2001, **18**, 647.
37. İ. Gülçin, S. Güngör, S. Beydemir, M. Elmastas and Ö. I. Küfrevioğlu, *Food Chem.*, 2004, **87**, 393.
38. B. M. Naveena, M. Muthukumar, A. R. Sem, Y. Babji and T. R. K. Murthy, *Meat Sci.*, 2006, **74**, 409.
39. K. Chaieb, H. Hajlaoui, T. Zmantar, A. B. Kahla-Nakbi, M. Rouabhia, K. Mahdouani and A. Bakhrouf, *Phytother. Res.*, 2007, **21**, 501.
40. SEAGRI, available from: www.seagri.ba.gov.br, 2010.
41. Liberty Natural, available from: www.libertynatural.com, 2010.

Subject Index

References to figures are given in *italic* type. References to tables are given in **bold** type.

ABE *see* agitated bed extraction
Abelmoschus moschatus, 25
 ABS *see* aqueous biphasic systems
 absorbed, distributed, metabolized
 and excreted (ADME), 237
 accelerated liquid extraction,
 157–90
 accelerated solvent extraction
 (ASE), 157–8
 applications for isolation of
 natural products, 176–7,
 178–81, 182–7
 lipids, 177, **181**, 182–3
 polar compounds, 177, 184–7
 antioxidants, 184–5
 essential oils, 184–6
 nutraceuticals and
 drugs, 184, 186–7
 volatile compounds, 177,
 183–4
 benefits and limitations for
 isolation of natural
 products, 190
 comparison with other extraction
 techniques, 172–6
 coupling to other steps of the
 analytical process, 165–7, *166*
 dynamic accelerated solvent
 extraction (*see* dynamic
 accelerated solvent extraction)
 integration of pressurized
 fluid-based technologies, 416

parameters affecting
 performance, 167–72
 extraction time, 167, 171–2
 particle size, 167, 171
 pressure, 167, 169
 sample composition, 167, 171
 solvent-to-feed ratio, 170–1
 solvent type, 167, 169–70
 temperature, 167–9, *168*
 recent trends and
 perspectives, 257–8
 acetic acid, 317
 acetone, 80–1, 122, 169–70, 182, 255,
 260, 262, 401
 Class 3 solvents, 286
 extracted with, 341, 430
 acetonitrile, 80, 169–70, 268, 272,
 326
 acid/bases, 60, 261
 acid salts, 263
 acyclic lycopene, 6
 ADA *see* American Dietetic
 Association
 adlay seeds, 257
 ADME *see* absorbed, distributed,
 metabolized and excreted
 adsorption, 234
 AEOE *see* aqueous enzymatic oil
 extraction
 aerosol solvent extraction system
 (ASES), 295
 agarwood, 26

- agitated bed extraction (ABE), **464**, 464–5
- aglicones, 13, 14
- aglycone, 147–8, 356
- Agrimonia eupatoria*, 187
- alcohols, 21, 23, 197, 272
- aromatic, 265
- aldehydes, 21, 23
- alfalfa, 6, 13
- algae, 213, 216, 248–9, 256, 275
- green, 182
- alginates, 264
- alimentary oil, 271
- alkaloids, 3, 21, 105, 212, 294, 317–18, 328, 332
- case study, isolation of, 347, 347–8
- recent trends and perspectives for the extraction of, 232, 239, 241, 244, 263–4, 266, 270–1, 275
- alkyl carbonic acid, 258, 273
- alkylresorcinols, 260
- almond, 105, 256, 260
- oil, 256–7
- aloin A, 130
- alumina, 325
- aluminum oxide, 325
- Amaranthaceae plants, 17
- Ambrosia artemisiifolia*, 3
- American Dietetic Association (ADA), 36
- American ginseng, 187 (*see also* Asian ginseng; Brazilian ginseng; ginseng)
- amides, 318
- amidine, 274
- amidocyanogen, 318
- amino acids, 37, 186, 232, 239, 270
- aminopropyl, 325
- amplitude of wave, 97–8
- amyris, 26
- analgesics, 275
- Andrews, Thomas, 197
- anethole, 22, 23
- Angelica*, 183
- Angelica sinensis*, 187
- Aniba rosaeodora*, 26
- anise, 25, 83, 84–5
- oil, 25
- annatto, 6–8, 423
- seeds, 400, 425–37
- Anthemis tinctoria*, 3
- antheraxanthin, 8
- anthocyanidins, 13, 14
- anthocyanins, 5, 6, 13–16, 37–8, 42, 173, 185
- case study, isolation of, 352, 353, 354
- chemical structure, 14
- recent trends and perspectives for the extraction of, 258–60, 263, 273
- anthocyanins, 173
- anthraquinones, 125, 256
- anticarcinogenic agent, 425
- antifungals, 244, 275
- anti-inflammatories, 244, 275
- antimicrobials, 244, 275, 465
- antimutagenic agent, 425
- antioxidant(s), 3, 103–4, 184–6, 244, 275, 425, 465
- compounds, 212, 216
- natural, 305
- antiproliferatives, 244
- Apiaceae, 26
- apigenin, 175, 345
- 7-glucoside, 184
- turinoside, 175
- apiole, 22
- apocarotenic acid, 8
- apocarotenoid, 7
- apple peel, 185
- Applied Separations, 391
- apricot, 27, 105, 140, 256
- aqueous biphasic systems (ABS), 269–71
- aqueous enzymatic oil extraction (AEOE), 256
- Arecaceae, 35
- argon, 255
- aristolochic acid, 187
- aromas, 244 (*see also* fragrances)
- aromatherapy, 20, 29

- aromatic salts, 265
Arthospyra platensis, 220, 273
 arthritis, 42
 ascorbic acid, 4, 14, 17, 128–30
 ASE *see* accelerated solvent
 extraction
 ASES *see* aerosol solvent extraction
 system
 Asian ginseng, 40 (*see also* American
 ginseng; Brazilian ginseng; ginseng)
 astaxanthin, 130, 182, 213, 216
 atherosclerosis, 20, 36, 39, 42
 azeotropic distillation, 136, 339
- babassu, 464
Bacillus cereus, 137
 bacteria, 248–9, 275
 barks
 essential oil sources, 26
 basic compounds, 263
 Basidiomycota, 16
 basil, 27, 92, 93, 124, 137, 173
 beer, 259
 beets, 270
 red, 17
 sugar, 264
 benzene, 80, 286
 benzoic acid, 4
 benzopyran derivatives, 6
 benzyl acetate, 29
 berberine, 266
 acid, 187
 bergamot oil, 29–30
 betacyanins, 16, 17, 37
 betalains, 5, 5–6, 16–17, 37, 270
 betalamic acid, 16, 17
 betanidin, 16
Beta vulgaris, 17
 betaxanthins, 16, 17, 37
 betulin, 258
 birch, 26, 258
 bisabolene, 25
 α -bisabolol, 25
 bisdemethoxycurcumin, 18
Bixa orellana, 6, 423, 425–37
 bixin, 6–7, 400, 425–37, **430**, 445
- blackcurrant, 16
 bleaching, 31
 BMC *see* minimum bactericidal
 concentration
 borapetosides, 342, 345
 bornyl acetate, 31
 boswellic acid, 265
 brandy, 104
 Branson, 95
 Brazilian ginseng, 40, 423 (*see also*
 American ginseng; Asian ginseng;
 ginseng)
 Brazil wood, 3
 Buchi, 163, 292
 buriti
 fruits, 35
 oil, 35, 463–4
 butane, 262, 286
 butter, 420
 butyridenephthalide, 187
- cabbage, red, 16
 cactus fruits, 17
 cade, 26
 caffeic acid, 42
 caffeine, 81, 205, 294
 Cagniard de la Tour, Charles, 197
Calamintha nepeta, 137
C. albiflora, 8
 β -calendic acid, 38
 calf brain, 182
 campestanol, 39
 camphene, 31
 camphor, 27
Cananga odorata, 30
 cancer, 3, 20, 42, 186
 chemoprevention, 216
Candida utilis, 17
 canola oil, 209
 canthaxanthin, 6, 216
 capillary supercritical fluid
 chromatography (cSFC), 339
 capsaicin, 8, 42, 104
 capsaicinoids, 8, 80
 capsaicinoids, 104
 capsanthin, 8

- Capsicum*, 80, 414
 capsicuman, 264
Capsicum annuum, 8
Capsicum frutescens, 104
 case study, 391–6, **393–5**
 capsolutein, 8
 capsorubin, 8
 caraway seeds, 260
 carbohydrates, 36–8, 65, 239–40, 275, 325
 carbon-based compounds, 232
 carbon bisulfide, 197
 carbon dioxide, 199, 199–200, 202, 208, 222–3, 262, 271–3, 286, 293
 carbon dioxide-expanded liquids (CXL), 220, 222, 224
 carbon disulfide, 81
 carbon disulphide, 169
 carbonic acid, 258, 272–3
 carbon nanotubes, 321
 carbon powder, 138
 carbon tetrachloride, 80
 carbonyl iron powder, 254
 carboxylic acids, 325
 cardamom, 124, 127
 cardiovascular disease, 3, 20, 186, 305
 prevention by soybeans, 45, 45
 carnauba palm, 464
 carnosic acid, 104, 123
 carotenes, 6, 35, 37, 81, 123
 α -carotene, 6, 41
 β -carotene, 6, 8–10, 82, 130, 212, 216, 266, 303, 304
 case study: formulation as
 natural colorant, 305, 307–11, 309–10
 coloring agents, 9–10
 nutraceuticals, 41
 solvent-free ultrasound-assisted extraction, 106–8, 106–8
 carotenoids, 3, 5, 5–11, 35, 37, 80–1, 182, 425, 463
 chemical structure, 7
 conventional extraction, 105–6
 natural colorants, 305
 nutraceuticals, 41–2
 post-extraction processes, 303, 305
 solvent-free ultrasound-assisted extraction, 105–8
 supercritical fluid extraction, 205, 209, 212–13, 216–17, 223
 ultrasound-assisted extraction, 89, 104
 uses, 105–6
 carrot, 6, 9–10, 105–6
 black, 16
 carvacrol, 28, 260
 carvone, 104
 Caryophyllales, 16
 caryophyllene, 25
 β -caryophyllene, 29
 cascarilla, 26
 cassia, 26
 cataracts, 41–2, 305
 (+)-catechin, 43, 82, 401
 cavitation bubbles, 92, 92, 97–9, 101–2
 cavity, 119
 monomode, 119, 119
 multimode, 119, 119
 cayenne peppers, 80, 128
C. camphora, 27
 CCC *see* counter-current chromatography
 CCD *see* Central Composite Design
 CC-SFE *see* counter-current supercritical fluid extraction
 cedar, 26
 leaf, 24
Cedrela toona, 8
 cellobiohydrolases, 263
 cellulases, 263
 cellulose, 245, 263, 272
 Central Composite Design (CCD), 107
 centrifugal extraction, 83, 234
 centrifugal partition chromatography (CPC), 342
 CEPICI *see* Chemical Engineering Plant Cost Index
 CER *see* constant extraction rate period

- Chaenomeles sinensis*, 122, 127
 chalcones, 42
Chamaemelum nobile, 29
 chamomile, 3, 25, 29–30
 German, 29–30
 Roman, 29–30
 chavicol, 22, 23
 Chemical Engineering Plant Cost
 Index (CEPCI), 446
 cherry, 16, 260
 Chinese herbal medicine, 182, 187,
 341, 347–8
 Chinese herbs, 130, 254–5
Chlorella protothecoides, 270
Chlorella vulgaris, 216
 chlorinated solvents, 197
 chloroform, 80–1, 122, 317, 326, 401,
 430
 chlorogenic acid (GCA), 266
 chlorophylls, 3, 5, 5–6, 11–13
 chemical structure, 11
 limitations of use as coloring
 agents, 12
 chlorophyllins, 12
 cholesterol, 20, 39, 294
 chromatographic techniques, vii, 66,
 234, 323–39, **333–6**, **338**, **340**
 chrysantenone, 137
 1,8-cineole, 27
 cinnamaldehyde, 22, 23, 26
 cinnamon, 26
 cinnamyl alcohol, 23
cis-clerodane-type
 furanoditerpenoids, 341–3, 344
 citral, 265
 citronella, 24
 citronellal, 186
 citronellol, 23–4, 24
 citrus
 oils, 24, 30
 peel, 6, 30, 140
Citrus sinensis, 124
Cladiella krempfi, 341, 343–4
Clematis chinensis, 340–1, 342–3
 cloud point concentration (CPC),
 266
 clove, 20, 25, 29, 75, 260
 case study, 465–7, **466**, 467, **468**,
 469
 clover, 187
C. lutens, 8
 CMC *see* critical micellar
 concentrations
 cobalt, 232
 cocaine, 128
 cocoa
 beans, 32
 butter, 32, 267, 296
 seed, 420
 coffee, 81, 463 (*see also* green coffee
 beans)
 COL *see* costs of operational labor
 cold expression, 30
 colorants, 104, 200, 244, 275
 inorganic, 4
 natural (*see* natural colorants)
 synthetic, 4 (*see also* synthetic
 dyes)
 coloring agents, 3–18 (*see also under*
 cosmetics; pharmaceutical)
 color production
 mechanism of, 4
 column liquid chromatography, 321
 COM *see* cost of manufacturing
 compression phases, 91, 91–2
 coniferyl, 168
 constant extraction rate period
 (CER), 64, 65, 203, 445
 α -copaene, 186
 copaiba oil, 28
Copaifera, 28
Copernicia cerifera, 464
 coriander, 20
 oil, 20
Coriandrum sativum, 20
 corilagin, 255
 corn, 33, 267
 germ oil, 33–4
 cornmint, 24
 cosmetics
 accelerated liquid extraction, 163,
 172, 174, 185

- applications of natural products, v, 12–13, 83, 357, 372
- coloring agents, 3–4, 6, 9, 305, 425
- flavors and fragrances, 19–20, 26, 28–30, 35–6
- formulations, 298, **299**, 300
- extracts of natural products, 1, 2, 314, 356–7
- recent trends and perspectives for extraction of natural products, 231–2, 234, 237, 241, 244, 250, 265, 275
- ultrasound-assisted extraction, 90, 96, 103–5, 109
- co-solvents, 208 (*see also* modifiers)
- cost of manufacturing (COM), 444–5, 465–6, **466**
- cost of raw material (CRM), 444–5, 449
- costs of operational labor (COL), 449
- costs of utilities (CUT), 449
- costs of waste treatment (CWT), 449
- coumarins, 3, 123, 318, 332
- counter-current chromatography (CCC), 315, 329–30
- counter-current supercritical fluid extraction (CC-SFE), 201
- CPC *see* centrifugal partition chromatography; cloud point concentration
- cranberry, 16
- (–)-crebanine, 348
- cress seed, 264
- critical micellar concentrations (CMC), 265
- CRM *see* cost of raw material
- crocin, 8–9
- crocetin, 8
- Crocus sativus*, 3, 8
- crustaceans, 213
- β-cryptoxanthin, 8, 41
- crystallization, 234
 - from a solution, 291
- cSFC *see* capillary supercritical fluid chromatography
- C. speciosus*, 266
- Cuminum cyminum*, 124, 138
- cupuassu butter, 32
- Curcuma longa*, 17–18
- curcumin, 18, 18, 173
 - purified, 18
- curcuminoids, 5, 5–6, 17–18, 259, 265
- Curie, Pierre, 198
- CUT *see* costs of utilities
- cuttlefish bag, 3
- CWT *see* costs of waste treatment
- CXL *see* carbon dioxide expanded liquids
- cyanobacteria, 216, 220–3, 256
- cyanopropyl silica, 182
- Cymbopogon citrates*, 186
- Cymbopogon flexuosus*, 265
- cymyl compounds, 28
- cyperene, 186
- cyperone
 - α-cyperone, 186
 - β-cyperone, 186
- Cyperus rotundus*, 186
- cystitis, 42
- Dactylopius coccus*, 2
- Dactylopius coccus Costa*, 5
- DAG *see* diacyl glycerols
- daidzein, 45
- Daucus carota*, 9
- DC *see* diffusion-controlled period
- DCR *see* diffusion-controlled rate period
- demethoxycurcumin, 18
- dextran, 269, 325
- DHA *see* docosahexaenoic acid
- diabetes, 3
- diacyl glycerols (DAG), 104
- dichloroethane
 - 1,1-dichloroethane, 80, 286
 - 1,2-dichloroethane, 80, 286
- dichloromethane, 80–1, 169, 183, 186, 262, 326, 427–31
- dietary supplements, 357
- diethylamine, 212

diethyl ether, 169
 differential scanning calorimetry (DSC), 429, 435
 diffusion
 scale-up of extraction processes, limited by, 384–7
 diffusion-controlled period (DC), 64, 65, 67, 445
 diffusion-controlled rate period (DCR), 203
 dihydrocapsaicin, 8, 104
 dillapiole, 22
 dimethylallyl pyrophosphate, 22
 dimethyl ether (DME), 224
 dimethyl sulfoxide (DMSO), 272, 286
 Dionex, 158, 162, 166
 dioscin, 265
 diosmetin, 175
 dioxane, 122
 1,4-dioxane, 169
 dipole rotation, 115, 115
 direct manufacturing cost (DMC), 448–50
 diseases
 prevention of, 3, 20
 diterpenoids, 341
 DMC *see* direct manufacturing cost
 DME *see* dimethyl ether
 DMSO *see* dimethyl sulfoxide
 docosahexaenoic acid (DHA), 213
 drugs, 1, 289
 natural products extracted by ASE, 184, 186–7
 DSC *see* differential scanning calorimetry
 dynamic accelerated solvent extraction (dynamic ASE), 160–1, 163–5 (*see also* accelerated solvent extraction)
 laboratory-designed devices, 161, 164–5
 steps in process, 160, 163–4
 dynamic ASE *see* dynamic accelerated solvent extraction
 dysmenorrhea, 344

economic evaluation of natural product extraction processes, 80, 442–69
 case study, 465–7, **466**, 467, **468**, 469
 materials and methods, 465–6, **466**
 results and discussion, 466–7, 467, **468**, 469
 cost estimation of industrial processes, 443–4, **444**
 costs associated with industrial requirements, **444**, 445–6
 costs associated with operational conditions, **444**, 445
 costs associated with raw material, **444**, 444–5
 cost estimation
 procedures, 446–50
 cost estimate as a function of equipment capacity, 446–8
 Lang factor, 446, 448
 manufacturing cost estimation, 448–50
 direct manufacturing cost, 448–50
 fixed (indirect) manufacturing cost, 450, 458
 general expenses, 448, 450
 manufacturing cost of vegetable extracts, 450, **451–60**, 461–5
 other extraction processes, **464**, 464–5
 supercritical extraction process, 450, **451–2**, 461, 462, 463–4
 edible fats, *see* fats, edible
 edible oils, *see* oils, edible
 effervescent atomization, 293
 eggplant, 352, 353, 354
 egg yolk, 182
 eicosadienoic acid, 38
 eicosapentaenoic acid (EPA), 213
Elaeis guineensis, 10, 35
 Elan Nanosystems, 290

- elderberry, 16, 258–9
 elemicin, 22
 ellagic acid, 42
Elletaria cardamomum, 124
 emitter, 93–4, 99–100
 emodin, 130
 encapsulation techniques, 421–3, **422**,
424, 432–5
 enfleurage, 29
 enocianina, 16
 enocyanin, 16
 enuresis, 348
 enzymes, 261, 263
 EPA *see* eicosapentaenoic acid;
 United States Environmental
 Protection Agency
 (–)-epicatechin, 43, 82, 401
 gallate, 82
 (–)-epigallocatechin, 82
 gallate, 82
Epimedium, 117, 118
 ER *see* extract reservoir
Erigeron breviscapus, 124
 erythrose-4-phosphate, 21
Escherichia coli, 137
 essential oils, 19–31, 212, 217, 223,
 239–41, 244–5, 254, 275, 405
 natural products extracted by
 ASE, 184–6
 sources, 25–31
 esters, 23, 76, 318
 estradiol hormones, 43
 estragole, 22, 23
 ethane, 224, 271
 ethanol, 68, 71, 80–1, 104, 122, 124,
 400–1, 416
 accelerated liquid extraction, 169,
 174, 182, 187
 Class 3 solvents, 286
 modifier, 208, 212–13, 216–17,
 221, 260, 420
 recent trends and perspectives for
 extraction of natural
 products, 262–3, 267, 272–3
 supercritical fluid extraction, 200,
 222–3
 ether, 197
 ethyl
 acetate, 80–1, 133, 217, 262,
 416
 isolation and purification of
 natural products, 317, 326,
 345
 post-extraction processes, 286,
 305, 307
 ether, 81
 lactate, 81, 271
 ethylene glycol, 265
Eucalyptus, 20
 eucalyptus oil, 28
Eugenia caryophyllata, 29
Eugenia caryophyllus, 465
 eugenol, 22, 23, 25–7, 465
Euterpe oleracea, 464
 evaporation, 234
 of solvents, 287, 300–1
Evernia prunastrii, 21
 exhaustive extraction methods,
 66–7, 69
 extraction bed geometry
 secondary scale-up criteria, 371–2,
 375–6
 extraction of natural products,
 vi–vii, 46
 analytical, 59, 66, 85
 conventional techniques, 67–78,
 172
 soaking, 67–9, 85
 Soxhlet, 69–73, 70, 83, 85
 water and/or steam
 distillation, 73–8, 74,
 83–5, 85
 exhaustive *vs.* non-exhaustive
 methods, 66–7
 extraction efficiency, 67, 171,
 212
 industrial production, 59,
 66, 85
 isolation and purification of
 natural products (*see* isolation
 and purification of natural
 products)

extraction of natural products

(continued)

main variables, 78–82

preparation of the solid, 78–9, 85

solvent, 79–82, 85

solvent to feed ratio, 82, 85

temperature, 82, 85

time, 82, 85

preparative separations, 59–60, 85

principles and

fundamentals, 59–66

recent trends and

perspectives, 231–6, 274–5

extraction methods, 250–61, 275

extraction solvents and solvent

mixtures, 261–75

aqueous biphasic

systems, 269–71

extraction solvent

modification with

additives, 263–7

ionic liquids, 268–9

solvent mixtures and non-

conventional highly

hydrophobic organic

solvents, 267

tunable solvents, 271–4

raw materials, 244–9

target extracts/compounds,

236–7, **238**, 239–44

semi-preparative

separations, 59–60, 85

extract reservoir (ER), 166

Extrelut particles, 159

falling extraction rate period

(FER), 64, 65, 67, 203, 445

farnesal, 25

farnesol, 24, 25

fats, 36

fats, edible, 1, 31–6

commercial applications, 32–6

biodiesel feed stock, 36

liquid oils, 33–6

buriti oil, 35

corn germ oil, 33–4

grape seed oil, 34–5

jojoba oil, 36

olive husk oil, 34

palm oil, 35

rice bran oil, 35

safflower oil, 33–4

soybean oil, 33–4

sunflower oil, 33

wheat germ oil, 36

shortening products, 32

spread products, 32–3

cocoa butter, 32

cupuassu butter, 32

margarine, 33

processing, 31

sources of, 31

fatty acids, 31, 35, 71, 182, 186, 217,

232, 305

free (FFA), 259

 ω -3 fatty acids, 38, 213FBE *see* fluidized-bed extractionFC *see* flash chromatographyFDA *see* United States Food and

Drug Administration

fenchone, 23–4

fennel, 24, 83, 84, 186

FER *see* falling extraction rate period

ferromagnetic materials, 197

ferulic acid, 42, 187

FFA *see* free fatty acids

Fick's

law, 64, 374

second law, 68

fish, 213, 248–9, 275

oil, 420

fixed (indirect) manufacturing cost

(FMC), 448, 450

flash-boiling atomization, 293

flash chromatography (FC), 327

flavones, 42–3, 123

flavonoids, 3, 5, 13, 37, 42–3, 68, 80,

216, 401

case studies

isolation and purification of,

354, 354–6, 356

isolation of, 344–6, 345

- isolation and purification of, 318, 320, 332
- microwave-assisted
 - extraction, 126–7, 140
- recent trends and perspectives for
 - extraction of, 256, 263, 266
- flavonols, 42–3, 82, 173
- flavors, 1, 3, 8, 19–31, 103–4, 200, 273, 357, 465
- flaxseed, 105
 - oil, 256
- Florisol, 159
- flowers
 - essential oil sources, 28–30
- fluidized-bed extraction (FBE), 73
- Fluid Management System (FMS), 163
- FMAE *see* focused microwave-assisted extraction
- FMSD *see* focused microwave-assisted steam distillation
- FMASE *see* focused microwave-assisted Soxhlet extraction
- FMC *see* fixed (indirect)
 - manufacturing cost
- FMS *see* Fluid Management System
- focused microwave-assisted
 - extraction (FMAE), 120
- focused microwave-assisted Soxhlet extraction (FMASE), 71, 72, 130–2, 131
- focused microwave-assisted steam distillation (FMSD), 77–8, 78
- Foeniculum vulgare*, 83, 186
- Foeniculum vulgaris*, 187
- Folch extraction, 172
- Folin–Ciocalteu test, 187
- food (*see also* fruits and vegetables)
 - additives, v, 112
 - applications of natural products, v, viii, 6–9, 20, 83, 163
 - agricultural and food by-products, 216–17, **218–19**, 220
 - formulations, 298, **299**, 300
 - engineering, viii
 - extracts of natural products, 1, 2, 3–4, 163, 169, 236
 - functional, 1–3, 36–45
 - preservatives, 20, 184
 - processing, 89
 - recent trends and perspectives for
 - extraction of natural products, 231, 241, 244, 250, 262, 264–5
 - science, viii
 - supplements, 3, 232, 237
- formic acid, 352
- formononetin, 126
- Foundation for Innovation in Medicine, 186
- fractionation/separation method, 259
- fragrances, 1, 3, 19–31, 103–4, 163, 273 (*see also* aromas)
- freeze-drying, 287, 288
- frequency of wave, 97–9
- friction
 - secondary scale-up criteria, 370
- fruits and vegetables, 103–4 (*see also* food)
- fungi, 248–9, 256, 275
- fungicides, 243–4, 465
- galactomannans, 264
- galactosidases, 263
- galbanum, 23
- gallic acid, 22, 42, 190, 266
- (-)-gallo catechin gallate, 82
- Ganoderma atrum*, 124–6
- Garcinia mangostana*, 212
- Gardenia jasminoides*, 8
- GAS *see* gas antisolvent
- gas antisolvent (GAS), 224
- gas chromatography (GC), 414
- gas chromatography–mass spectrometry (GC–MS), 166
- gas-expanded liquids (GXL), 222–4, 272–3
- gas–liquid chromatography (GLC), 339
- gas-to-product (GTP) ratio, 296
- GBE *see* *Ginkgo biloba* extracts

- GC *see* gas chromatography
 GCA *see* chlorogenic acid
 GC–MS *see* gas chromatography–mass spectrometry
 GCP *see* Good Clinical Practice
 GE *see* general expenses
 gelucires, 270–1
 general expenses (GE), 448, 450
 generally recognized as safe (GRAS), 200, 208, 307, 449
 genistein, 45
 gensenoids, 170
 geraniin, 255
 geraniol, 23–4, 24, 186
 geranium, 24
Geranium sibiricum, 255
 germacrone, 25
 Gibbs free energy, 60
 ginger, 27, 245, 257
 gingerols, 27, 187
Ginkgo biloba extracts (GBE), 320
Ginkgo biloba, 263, 320, 401
 ginseng, 40–1 (*see also* American ginseng; Asian ginseng; Brazilian ginseng)
 ginsenosides, 40, 41, 187
 GIOTTI, 96
 glace fruits, 170
 GLC *see* gas–liquid chromatography
 GLP *see* Good Laboratory Practice
 glucose, 21, 60
 D-glucose, 42
 glucosidases, 263
 glucoside, 42
 esters, 267
 glucosinolates, 3
 glutaric acid, 289
 glycerol, 265, 271
 glyceryl esters, 31
 glycitein, 45
 glycosidases, 15
 glycosides, 28, 140, 317
Glycyrrhizae radix, 128
 glycyrrhizin, 187
 glycyrrhizic acid, 128
 G. Mariana and C. Spa (GMC), 96
 GMC *see* G. Mariana and C. Spa
 GMP *see* Good Manufacturing Practice
 Good Clinical Practice (GCP), 234
 Good Laboratory Practice (GLP), 234
 Good Manufacturing Practice (GMP), 234, 262
 Goto model, 261
 grapefruit, 25, 30
 grape(s), 140, 185, 260
 canes, 68
 extracts, 16
 pomace, 174
 red, 173, 258
 seed oil, 34–5
 seeds, 259, 401, 461
 graphite, 131
 powder, 138
 GRAS *see* generally recognized as safe
 grass, 22
 green algae, *see* algae: green
 green coffee beans, 205, 442 (*see also* coffee)
 green extraction, 128, 172
 without solvent, 135–40, 144
 green solvents, 224, 272–3
 green tea, 423 (*see also* tea)
 extracts, 297
 leaves, 257
 GTP ratio *see* gas-to-product ratio
 guaiac, 26
 guar, 264
 guava, 128, 217, 254
 gums, 264
 gut flora, 38
 GXL *see* gas-expanded liquids

Haematococcus pluvialis, 205
 hasteloid, 165
 hazelnut, 68, 217, 260
 HBA *see* hydroxybenzoic acid
 HCA *see* hydroxycinnamic acid

- heat transfer
 secondary scale-up criteria, 367
Helianthus annuus, 33
Hemerocallis disticha, 212
hemes, 5, 6
hemicellulose, 263
hemiterpenoids, 23, 24
hemoglobin, 3
heptane, 81
herbs and spices, 104
hexadecatrienoic acid, 38
hexane, 70–1, 80–1, 105, 116, 167, 169, 262, 267, 274
 Class 2 solvents, 286
 n-hexane, 71, 132, 169, 175, 184, 267, 317, 326
HF *see* hydrogen fluoride
HHPE *see* high hydro-static pressure extraction
Hielscher, 95
high hydro-static pressure extraction (HHPE), 257
high-performance liquid chromatography (HPLC), 325, 414, 416–17, 427
high-pressure emulsion techniques, 301–3, 302, 307–8
high-pressure homogenization, 290–1
high-pressure liquid extraction, 257–8
high-pressure solvent extraction (HPSE), 157
high-speed counter-current chromatography (HSCCC), 329–30, 330, 332, 333–6, 337
high-throughput screening (HTS), 237
Hippophaë rhamnoides, 114, 122, 144
homocapsaicin, 104
homodihydrocapsaicin, 104
hop(s), 23, 258–9, 405
 extracts, 29
HPLC *see* high-performance liquid chromatography
HPSE *see* high-pressure solvent extraction
HSCCC *see* high-speed counter-current chromatography
HTS *see* high-throughput screening
humulene, 29
 α -humulene, 25, 29
Humulus lupulus, 405
hydrocolloids, 264
hydro-distillation, 74, 76, 253, 256
hydrogen fluoride (HF), 320
hydrosol, 74
hydrotropes, 265
hydroxybenzoic acid (HBA), 42
hydroxy carboxylic acid, 42
hydroxycinnamic acid (HCA), 37, 42, 43
hydroxytyrosol, 184
Hylocereus, 17
Hylocereus ptyrhizus, 17
hyoscyamine, 264

IL *see* ionic liquids
Illicium anisatum, 137
Illicium verum, 25, 138
ILMAE *see* ionic liquid microwave-assisted extraction
imidazolium, 268
Indigofera tinctoria, 3
indole, 29, 37
industrial applications, vii–viii (*see also* scale-up of extraction processes)
inflammation, 3
Inonotus obliquus, 133, 256
insecticides, 465
instantaneous controlled pressure-drop process, 259
integration of pressurized fluid-based technologies, 399–438
 case study: integrated extraction and encapsulation of bixin from annatto seeds, 425–37, **430**
 materials and methods, 425–30
 extract and capsule characterization, 429–30

- integration of pressurized fluid-based technologies (*continued*)
 - integrated system using PLE–SAS, 428, 428–9
 - off-line encapsulation by supercritical anti-solvent process, 426–8, 427
 - plant material, 425
 - pressurized liquid extraction, 425–6, 426
 - results and discussion, 430–7
 - dissolution profiles of capsules formed, 435–7, 437
 - DSC analysis, 435
 - encapsulation of PLE extracts by SAS, 432–5
 - influence of extraction solvent on PLE performance, 430, **430**
 - influence of temperature and static extraction time on PLE performance, 431, **431**
 - PLE kinetic extraction curve, 431–2, 431–2
 - integration of pressurized fluids to different technologies for extract stabilization, 420–3, **422, 424**
 - on-line fractionation/ purification, 404–6, **407–13**, 414–20
 - on-line coupling of extraction and membrane processes for purification, 418, 419, 420
 - on-line extraction and adsorptive purification processes, 406, **407–13**, 414–18
 - on-line separators: fractionation by changes in temperature and pressure, 404–6
 - sequential extraction using different process conditions or techniques, 400–1, **402–3**, 404
- ionic conduction, 115
- ionic liquid microwave-assisted extraction (ILMAE), 123
- ionic liquids (IL), 123, 170, 224, 254
 - extraction solvents and solvent mixtures, 262, 268–9
- β -ionone, 9
- iron, 232
- iron carbonyl powder, 124, 138
- Isatis indigotica*, 105
- isoflavones, 38, 42–3, 65, 68, 186–7, 216, 256
 - case study, isolation of, 348, 351–2
- isoflavonoids, 37, 45
 - case study: isolation and purification of, 354, 354–6, 356
- isolation and purification of natural products, vii, 314–57, 316
 - case studies, 340–56
 - pre-isolation or enrichment, 315–22
 - adsorption enrichment, 318
 - membrane separation, 318–20, **321**
 - solid phase extraction, 321–2, **322, 322**
 - solvent partitioning, 316–18, 317
 - purification, 323–39, **329, 333–6, 338, 340**
 - chromatographic techniques, 323–39, **333–6, 338, 340**
 - crystallization, 339, **340**
- isomenthone, 137
- isooctane, 81
- isopentenyl pyrophosphate, 22–3
- isoprene, 23, 24, 25
 - (2-methylbutadiene), 22
- isoprenoid derivatives, 6
- isopropanol, 71, 272
- isopsoralen, 348, 351
- isorhamnetin, 140, 146, 150
 - 3-*O*-glucoside, 147
 - 3-*O*-rutinoside, 147
 - 7-*O*-rhamnoside, 146, 150
- isovaleric acid, 31

- jabuticaba, 258, **464**, 464–5
 jambu, 246
 jasmine, 28–9
Jatropha curcas, 256
 jatropha seeds, 256
 jatrorrhizine, 266
 jojoba oil, 36

 kaempferol, 140, 217, 345
 Kalamon fruit, 81
 kaurenoic acid, 28
 kavalactones, 182
 kermes lice, 3
 ketocarotenoids, 8
 khusimol, 25
 khusimone, 25
Klebsiella pneumoniae, 137

 labile compounds, 130
 lactic acid
 L-lactic acid, 270
 Lang factor, 446, 448
 lanolin, 267
 LAS *see* liquid anti-solvent
 Lauraceae, 26
Lavandula stoechas, 75
Lavandula viridis, 75
 lavender, 24–5, 29, 135
 LCA *see* life-cycle analysis
 LC-DAD *see* liquid chromatography
 coupled to diode array detection
 LC-MD *see* liquid chromatography
 coupled to mass detection
 LDL *see* low density lipoprotein
 leaching
 kinetics, 168, 170
 process, 167–71, 190
 leaves
 essential oil sources, 27–8
 lecithin, 294, 309
 Leguminosae, 28
 lemon, 30
 lemongrass, 24, 186
 lichens, 123
 licorice, 128
 life-cycle analysis (LCA), 223

 lignans, 3, 37, 42–3, 239
 lignin, 168, 263, 272–3
 lignocellulosic materials, 168, 189
 ligustilide, 187
 lime, 30
 limonene, 23–4, 30, 104, 176, 186,
 260, 271
 d-limonene, 71
 limonin, 265
 linalool, 27, 29–30
 linalyl acetate, 29–30
 linoleic acid, 36, 38
 linolenic acid
 α -linolenic acid, 38
 γ -linolenic acid, 38, 222–3, 273
 lipids, 21, 22, 65, 103, 213, 224, 317
 lipidic natural products extracted
 by ASE, 177, **181**, 182–3
 recent trends and perspectives for
 the extraction of, 239–41, 244,
 260, 275
 liposomes, 298
Lippia alba, 134
 liquid anti-solvent (LAS), 291
 liquid chromatography, vi, 167, 416
 liquid chromatography coupled to
 diode array detection (LC-
 DAD), 167, 187
 liquid chromatography coupled to
 mass detection (LC-MD), 167
 liquid-liquid extraction (LLE), 66,
 197, 234, 264, 321
 LLE *see* liquid-liquid extraction
l-menthol, 24
 longan fruit pericarp, 257
 low density lipoprotein (LDL), 36, 39
 low-pressure liquid column
 chromatography (LPLC), 323,
 325–7
 LPLC *see* low-pressure liquid column
 chromatography
 lutein, 6, 41, 182, 212, 216, 270, 305
 esters, 257
 luteolin, 175, 345
 -7-glucoside, 184
 glucoside, 175

- lycopene, 7, 10–11, 41–2, 133, 217, 256, 260, 305, 310
Lycopersicum esculentum, 10
 maceration, 29, 107, 107, 148, 149, 150, 172–3
 macroporous resins, 318, **319**, 355
 macular degeneration, 42, 305
 MAE *see* microwave-assisted extraction
 MAG *see* monacyl glycerols
 magnetron tube, 119
 MAHD *see* microwave hydro-distillation
 maize, 33
 malagueta, 391
 malic acid
 L-malic acid, 270
 malto-oligosaccharides, 328
 MAM *see* microwave absorption medium
 mammalian cells, 248
 margarine, 33
 marigold, 257
 marine sources
 applications of SFE for extracts of natural products, 213, **214–15**, 216
 raw material for extracts of natural products, 248–9, 275
 MASD *see* microwave-accelerated steam distillation
 maslinic acid, 81
 massaia, 26
Matricaria recutita, 29, 187
Mauritia flexuosa, 35
Medicago sativa, 13
 medical devices
 applications of natural products, 3
 medicines
 applications of natural products (*see under* pharmaceutical)
 medium-pressure liquid column chromatography (MPLC), 325
 MEKC *see* micellar electrokinetic chromatography
 membrane separation, 234, 315, 318–20, **321**, 418–20
 menstruation, irregular, 344
Mentha arvensis, 24
Mentha crispa, 124
Mentha piperita, 24, 138
Mentha pulegium, 140
Mentha spicata, 140
 menthol, 23
 mint, 138 (*see also* mint)
 menthone, 137
 metaphosphoric acid, 129
 methanol, 80–1, 104, 200, 326, 401
 accelerated liquid extraction, 167, 170, 174
 Class 2 solvents, 286
 microwave-assisted extraction, 122, 124
 modifier, 208, 212, 216, 260
 recent trends and perspectives for extraction of natural products, 262, 267–8, 273
 methoxy derivatives, 21
 methyl
 acetate, 262
 chavicol, 22, 27
 cinnamate, 22
 ethyl ketone, 80
 eugenols, 22
 jasmonate, 21
 tertiary butyl ether (MtBE), 337
 zizanoate, 25
 methylene dioxy compounds, 21
 mevalonic acid, 22–3
 MHG *see* microwave hydro-diffusion and gravity
 micellar electrokinetic chromatography (MEKC), 266
 micelle-mediated separation (MMS), 265–6
 micelles, 265, 298, 299
 microalgae, 71, 205, 213, 216, 220, 248–9, 256, 266, 275
 microwave absorption medium (MAM), 137–8

- microwave-accelerated steam distillation (MASD), 134
- microwave-assisted extraction (MAE), 75, 113–51, **121**
 - advantages of, 150–1
 - comparison with other extraction methods, 172, 174–5, 189–90
 - costs and limitations, 151
 - economic evaluation, 443
 - microwave heating applied to plant matrices, 117–18
 - microwave heating
 - principle, 114–17, 116
 - microwave
 - instrumentation, 118–21
 - oven design, 119, 119–20
 - reactor design, 120–1
 - closed systems, 120–1
 - open systems, 120
 - parameter influence on, 121–8, 151
 - extraction time, 121, 126, 128
 - microwave power, 121, 126–8
 - nature of matrix, 127–8
 - matrix moisture, 127
 - matrix size, 127–8
 - pressure, 125–6
 - solvent composition, 121–4, 128
 - solvent to feed ratio, 121, 124–5, 128
 - temperature, 121, 125–6, 128
 - recent trends and perspectives, 253–5
 - trends and applications, 128–40, **141–3**, 144
 - case study of pressurized solvent-free microwave extraction, 144–8, 145, 149, 150
 - advantages of PSFME, 150
 - comparison with other extraction methods, 148, 149, 150
 - influence of the number of cycles, 145–7, 146
 - proposed mechanism of PSFME, 147–8, **148**, 148
 - extraction methods improved by microwave heating, 130–5
 - focused microwave-assisted Soxhlet extraction, 130–2, 131
 - microwave hydro-distillation, 133, 133–4
 - microwave steam distillation, 134–5, 135
 - ultrasonic microwave-assisted extraction, 132, 132–3
 - extraction of sensitive compounds, 128–30
 - nitrogen-protected microwave-assisted extraction, 128–9
 - vacuum microwave-assisted extraction, 129, 129–30
 - green extraction without solvent, 135–40, 144
 - microwave hydro-diffusion and gravity, 139, 139–40, 144
 - solvent-free microwave extraction, 136, 136–8
 - vacuum microwave hydro-distillation, 138
 - microwave-assisted Soxhlet extraction, 255–6
 - microwave heating supercritical fluid extraction (MSFE), 260
 - microwave hydro-diffusion and gravity (MHG), 139, 139–40, 144, 254
 - microwave hydro-distillation (MWHd or MAHD), 133, 133–4
 - microwave-integrated Soxhlet (MIS), 131
 - microwave ovens for extraction, 119, 119–20
 - microwave(s), vii, 114–17
 - electromagnetic spectrum, 114, 114
 - power, 121, 126–8

- microwave steam diffusion (MSDf), 135
- microwave steam distillation (MSD), 134–5, 135
- milling, 290, 379
- minerals, 36, 186, 232
- minimum bactericidal concentration (BMC), 137
- mint, 24 (*see also* menthol: mint)
garden, 124
- MIS *see* microwave-integrated Soxhlet
- M. laevigata*, 266
- MMS *see* micelle-mediated separation
- modifiers, 208–9, 221–2 (*see also* co-solvents)
- molecular weight cut-off (MWCO), 418
- monacyl glycerols (MAG), 104
- Monarda fistulosa*, 24
- monoterpenes, 23, 27, 37, 74, 137
- monoterpenoids, 23–4
- MPLC *see* medium-pressure liquid column chromatography
- MSD *see* microwave steam distillation
- MSDf *see* microwave steam diffusion
- MSFE *see* microwave heating
supercritical fluid extraction
- MtBE *see* methyl tertiary butyl ether
- mulberry leaves, 267
- murex shellfish, 3
- MWCO *see* molecular weight cut-off
- MWHD *see* microwave hydro-distillation
- myrcene, 23, 29
- myricetin, 130, 140
- Myristicaceae, 25–6
- Myristica fragrans*, 20, 25
- myristicin, 22
- Myrtaceae, 29
- Myrtus comunis*, 212
- Nannochloropsis oculata*, 216
- NanoCrystals, 290
- naphthalenes, 239
- natural colorants, 1, 3–4, 6–8, 270, 298
- natural product(s)
applications, v, vii, 46 (*see also* under cosmetics; food; medical devices; medicines; pharmaceutical)
definition, v
extraction (*see* extraction of natural products)
- n*-butanol, 317, 337, 345
- neral, 186
- nerolidol, 25
- nettles, 13
- nicotine, 271
- Nigella sativa*, 183
- nitrogen, 255, 273
- nitrogen-based compounds, 232
- nitrogen-protected microwave-assisted extraction (NPMAE), 128–9
- nitrous oxide, 262, 286
- n*-octenyl succinate (OSA)
starch, 307–8
- non-chromatographic techniques, vii
- non-exhaustive extraction
methods, 66–7
- nootkatone, 25
- noridihydrocapsaicin, 104
- nor-patchoulanol, 25
- nor-tetrapatchoulol, 25
- NPMAE *see* nitrogen-protected microwave-assisted extraction
- nutmeg, 20, 25–6
- nutraceuticals, 1–3, 8, 36–45
- natural products extracted by ASE, 184, 186–7
- nutrition, viii
- Nyctasthes arbortristes*, 8
- OAHD *see* Ohmic-assisted hydro-distillation
- oakmoss, 21
- oak wood, 183
- Ocimum basilicum*, 124, 137, 173
- octacosanol, 36

- OEC *see* overall extraction curve
 OEPO *see* organic solvent extraction
 and particle formation on-line
 Ohmic-assisted hydro-distillation (OAHD), 76, 77
 oil-in-water (O/W) emulsion, 300, 302, 304–5
 oils, edible, 1, 3, 31–6
 commercial applications, 32–6
 biodiesel feed stock, 36
 liquid oils, 33–6
 buriti oil, 35
 corn germ oil, 33–4
 grape seed oil, 34–5
 jojoba oil, 36
 olive husk oil, 34
 palm oil, 35
 rice bran oil, 35
 safflower oil, 33–4
 soybean oil, 33–4
 sunflower oil, 33
 wheat germ oil, 36
 shortening products, 32
 spread products, 32–3
 cocoa butter, 32
 cupuassu butter, 32
 margarine, 33
 processing, 31
 sources of, 31
Olea europaea, 184
 oleaginous seeds, 104–5, 256
 oleanolic acid, 81, 122
 oleic acid, 34, 71
 oleoresin, 28, 246
 oleuropein, 175, 184
 oligoethylene glycol monoalkyl ether, 266
 oligopeptides, 239
 olive, 81
 leaves, 126, 171
 oil, 34, 132, 164, 184, 205
 pomace, 164, 171
 Tunisian leaves, 175
 onion, 140, 255
Opuntia, 17
Opuntia ficus-indica cv. *Gialla*, 17
Opuntia ficus-indica cv. *Rossa*, 17
 orange, 30
 peels, 124, 135, 138, 257, 271
Orbignya speciosa, 464
 oregano, 27–8, 124, 137, 260
 organic acids, 4, 37
 organic solvent extraction and
 particle formation on-line
 (OEPO), 423, 438
 organic solvents, 262, 267, 286, 400, 416
 organosulfides, 37
Origanum onites, 20, 75
Origanum vulgare, 124, 137
Orthosiphon stamineus, 258
 OSA *see* *n*-octenyl succinate starch
 osteoporosis, 4, 186, 348
 overall extraction curve (OEC), 203
 O/W emulsion *see* oil-in-water
 emulsion
 ox liver, 182

 packed-column supercritical fluid
 chromatography (pSFC), 339
Paeonia suffruticosa, 344–6, 345–6
 palm, 464
 leaves, 222
 oil, 6, 10, 35, 266
 palmitine, 266
 palmitic acid, 71, 132
 Panax, 40
Panax ginseng, 40, 187
Panax quinquefolius, 40
 paprika, 6, 8, 42, 122–3
 oleoresin, 8
 particles from gas-saturated
 solutions (PGSS), 294, 296, 303, 305, 422
 drying process, 297, 297, 309
 particle size distribution (PSD), 289
 passiflora seed oil, 207
 patchouli, 25
 alcohol, 25
 oil, 25
P. brasiliensis, 423
 PC *see* principal components

- PCA *see* precipitation from a
 compressed anti-solvent; principal
 component analysis
- p*-cymene, 28, 71
- peach almond oil, 381, 385
- peanuts, 256
- pectin, 257, 263–4
- pectinases, 263
- PEG *see* polyethylene glycol
- Penicillium decumbens*, 263
- pepper, 27, 104, 123
 green, 128, 254
 red, 8
 case study, 391–6, **393–5**
 sweet, 264
 yellow, 128
- peppermint, 24, 138
 oil, 28
- peptides, 37, 325
- perfluoroalkoxy (PFA), 120
- perfumery
 extracts of natural products,
 1, 2, 3
 perfumes, 1, 19–20, 25–6, 28–30
- period of wave cycle, 97
- peroxidases, 15
- Pertusaria pseudocorallina*, 123
- pervaporation, 234
- pesticides, 243–4, 275
- petroleum, 420
 ether, 81, 123, 317, 341
- PFA *see* perfluoroalkoxy
- Pfaffia, 40
- Pfaffia glomerata*, 40
- Pfaffia iresinoides*, 40
- Pfaffia paniculata*, 40
- pfaffic acid, 41
- PGSS *see* particles from gas-saturated
 solutions
- pH, extraction, 189
- pharmaceutical
 applications of natural products, v,
 viii, 38, 80, 185, 318, 357, 372,
 465
 coloring agents, 4, 6, 8–9, 12,
 305, 425
 flavors and fragrances, 19–20,
 26–7
 formulations, 298, **299**, 300
 extracts of natural products, 1, 2,
 34, 83, 169, 200, 234, 235
 isolation and purification of
 natural products, 314, 318,
 356–7
 post-extraction processes, 286–9,
 291, 295, 300
 recent trends and perspectives for
 extraction of natural
 products, 241, 244, 250, 262,
 264–5, 275
 ultrasound-assisted extraction, 90,
 96, 104–5, 109
- phenolases, 15
- phenolic acids, 3, 37–8, 42–3, 185
- phenolic compounds, 37, 42–3, 44,
 81, 145, 216–17, 223
 accelerated liquid extraction, 171,
 173, 184, 186, 189–90
 recent trends and perspectives for
 extraction of, 232, 241, 254–5,
 257, 260
- phenolics, 37–8, 42–3, 45, 68, 80, 318,
 332
 recent trends and perspectives
 for extraction of, 239–40, 244,
 275
- phenolic terpenes, 20
- phenols, 21, 42, 184–5, 189, 268
- phenylalanine, 22, 42
- phenylpropane, 21, 27
- phenylpropanoids, 22, 23, 43
- pheophorbides, 12
- pheophytins, 11–12
- phosphatidylcholine, 37
- phosphoenolpyruvate, 21
- phospholipids, 182–3, 294, 298
- phosphorus, 232
- photosynthesis, 11, 21
- PHSE *see* pressurized hot solvent
 extraction
- phthalides, 187
- phytic acid, 43

- phytochemical(s), 3, 38, 43, 46, 58, 103
 composition, viii
 profile, 79
 phytoestrogens, 37, 43
 phytol, 11–12
 phytosterols, 39
 phytosterols, 3, 37, 39, 43
 pH-zone-refining counter-current chromatography, 332, 337, **338**
 picrocrocin, 8–9
Pimpinella anisum, 25, 83
 pinene
 α -pinene, 30–1, 71
 β -pinene, 30–1
 pinolenic acid, 37–8
Pinus densiflora, 257
Pinus pinaster, 258–9
 Piperaceae, 26
 piperine, 123, 265
 piperitone, 137
Piper nigrum, 123
 β -pirene, 176
 pistachio, 258
 plankton, 248
 plants
 applications of SFE for extracts of natural products, 209, **210–11**, 212–13
 raw material for extracts of natural products, 245–8, 275
 PLE *see* pressurized liquid extraction
 plums, 140
 PMAE *see* pressurized microwave-assisted extraction
 podocarpic acid, 38
 polar compounds, 373, 400
 natural products extracted by ASE, 177, 184–7
 polyacrylamide, 325
 polyethylene glycol (PEG), 123, 269, 271–2, 291, 296, 309–10, 427–8
 polyglycolized glycerides, 270–1
 polyketides, 21, 239
 polylactic acid, 300
 polylactic-*co*-glycolic acid, 300
 polyphenoloxidase (PPO), 17
 polyphenols, 3, 42–3, 81, 212, 216–17, 267, 401, 420
 polypropylene glycol (PPG), 271
 polypyrroles, 239
 polysaccharides, 133, 256, 264–5, 325
 polystyrene, 318, 325
 polytetrafluoroethylene, 131
 polyunsaturated fatty acids (PUFA), 37–8, 213
 ω -3, 213, 223
 polyvinylidene fluoride (PVDF), 320
 polyvinyl pyrrolidone (PVP), 320
 pomegranate, 208
 post-extraction processes, 285–311
 case study: formulation of β -carotene as natural colorant, 305, 307–11, 309–10
 formulations, 298–305, **299**, **306**
 high-pressure emulsion techniques, 301–3, 302
 solvent evaporation method, 300–1
 spray-drying technique, 301
 supercritical fluid processes, 303–5
 particle size reduction, 289–97, **306**
 bottom-up methods, 291–7
 crystallization from a solution, 291
 drying processes with enhanced atomization, 293
 micronization processes with supercritical fluids, 293–7
 spray-drying, 291–2, 292
 top-down methods, 290–1
 high-pressure homogenization, 290–1
 milling, 290
 purification of extracts and elimination of solvents, 286–8
 evaporation of solvents, 287
 freeze-drying, 287, 288
 reverse osmosis, 287–8, 288

- potassium, 232
- potato, purple sweet, 16
- P. paulensis*, 423
- PPG *see* polypropylene glycol
- PPO *see* polyphenoloxidase
- precipitation from a compressed anti-solvent (PCA), 295
- preferential pathways, 375
- prenylflavonoids, 258
- preparation of solid
main variable in extraction, 78–9, 85
- preparative high-performance liquid chromatography (prep-HPLC), 315, 323, 327–9, **329**, 348, 351–2
- prep-HPLC *see* preparative high-performance liquid chromatography
- pressing, 148, 149, 150
- pressure
parameter affecting performance in ASE, 167, 169
parameter affecting solubility in SFE, 205–8
parameter effect in SFE case study, 222–2
parameter influence on MAE, 125–6
primary scale-up criteria, 367
- pressurized hot solvent extraction (PHSE), 157
- pressurized liquid extraction (PLE), 75, 148, 149, 157, 177, 223, 373
economic evaluation, 443, **464**, 464–5
integration of pressurized fluid-based technologies, 416–17, 425–6, 428–33, 437–8
recent trends and perspectives, 253, 257–8, 266
- pressurized liquids, vii
- pressurized microwave-assisted extraction (PMAE), 120
- pressurized solvent extraction (PSE), 416
- pressurized solvent-free microwave extraction (PSFME), 144–8, 145, 149, 150
- principal component analysis (PCA), 207–8
- principal components (PC), 207
- proanthocyanidins, 82, 167
- procyanidin, 43, 258, 401
- propagation velocity, 98
- propane, 262, 272, 286
- propanol
1-propanol, 80
2-propanol, 80, 169
- propyl acetate, 80, 262
- propylene glycol, 265
- prostaglandins, 21
- protease inhibitors, 43
- protein-based compounds, 232
- protein inhibitors, 37
- protein-polyphenol complexes, 65
- proteins, 37, 65, 186, 245, 263
- PSD *see* particle size distribution
- PSE *see* pressurized solvent extraction
- pSFC *see* packed-column supercritical fluid chromatography
- PSFME *see* pressurized solvent-free microwave extraction
- Psidium guajava*, 123
- Psoralea corylifolia*, 348, 352
- psoralen, 348, 351
- PUFA *see* polyunsaturated fatty acids
- purification and isolation of natural products *see* isolation and purification of natural products
- purity, vi, 59–60
- PVDF *see* polyvinylidene fluoride
- PVP *see* polyvinyl pyrrolidone
- pyridine, 286
- Queen Elisabeth of Hungary, 19
- quercetin, 130, 140, 146, 150, 266
- quercitrin, 266
- quinones, 239
- quintessential oil, 19

- radish, 16
- Radix astragali*, 126
- Radix glycyrrhizae*, 187
- ragweed, 3
- Raoult's law equation, 373
- rape seeds, 105
- rapid expansion of supercritical
 - solution into aqueous solution (RESSAS), 295
- rapid expansion of supercritical solutions (RESS), 293–5, 294, 422
- rarefaction phases, 91, 91, 98
- raspberry, 16
- refractive index (RI), 328
- resins
 - essential oil sources, 28
- response surface methodology (RSM), 207, 220
- RESS *see* rapid expansion of supercritical solutions
- RESSAS *see* rapid expansion of supercritical solution into aqueous solution
- RESS non-solvent (RESS-NS), 295
- RESS-NS *see* RESS non-solvent
- resveratrol, 68, 130, 217
- REUS, 95, 106
- reverse osmosis, 287–8, 288
- reversible ionic liquids (RevIL), 273
- RevIL *see* reversible ionic liquids
- rhizomes, 17–18
 - essential oil sources, 27
- rhubarb, 256
- RI *see* refractive index
- rice bran, 105
 - oil, 35
- roots
 - essential oil sources, 31
- rose, 24, 28
 - hips, 82
- rosemary, 19, 25, 27–8, 104, 174, 185, 267, 423
 - leaves, 140, 296
- rosewood, 26
- rosmarinic acid, 104, 123
- Rosmarinus officinalis*, 28, 123, 137, 185, 187, 423
- RSM *see* response surface methodology
- rumenic acid, 38
- rutin, 266
- Saccocalyx satureioides*, 75
- safflomin A, 130
- safflower
 - oil, 33–4
- saffron, 6, 8–9
 - flower, 3
- safranal, 8–9
- safrole, 23
- sage, 173, 267
 - garden, 138
- Saint John's wort, 166
- Salvia officinalis*, 138, 173
- Salvia triloba*, 266
- sandalwood, 26
- Santalum*, 26
- saponins, 37, 40, 43, 124–6, 182, 208, 318
 - case study, isolation of, 340–1, 342–3
- SAS *see* supercritical anti solvent process
- scale-up of extraction
 - processes, 363–97
 - case study: supercritical CO₂ extraction from red pepper, 391–6, **393–5**
 - experimental procedures, 391–3
 - extractions, 391–2, **393**
 - materials, 391
 - mathematical model, 392–3
 - results and discussion, 393–6, **394–5**
- factors involved, 372–6
 - extraction bed geometry, 375–6
 - solubility, 373–4
 - solvent flow rate, 374
 - substrate properties, 374–5

- scale-up of extraction processes
(*continued*)
fundamental aspects of scale-up
operations, 354–72
definition of scale-up, 364–5
in extraction
processes, 365–6
scale-up criteria, 366, 366–72
primary, 367
secondary, 367–72
state of the art, 376–91
configurations of industrial
units, 388–90
operation modes, 388–9, 389
working principles, 389–90,
390
examples of scale-up criteria in
extraction processes, 380–7
processes limited by
diffusion, 384–7
processes limited by
solubility, 381–4
models of the extraction
process, 376–80
empirical models, 377
models with theoretical
basis, 377–80
scale-up correlations, 387–8, **388**
some published works, 390–1,
391
scanning electron micrograph
(SEM), 134
SC-CO₂ *see* supercritical carbon
dioxide
SCD *see* simplex centroid design
SCF *see* supercritical fluids
Schefflera heptaphylla, 134
Schisandra chinensis, 123
Schizochytrium limacinum, 216
scopolamine, 264
scutellarin, 124
SD *see* steam distillation
Sdf *see* steam diffusion
SDS *see* sodium dodecyl sulfate
sea buckthorn, 114, 140
berries, 144–8, 147, **148**, 150
sea urchin, 213
seaweed, 263
brown, 13
sedimentation, 234
SEDS *see* supercritical enhanced
dispersion of solutions
SEE *see* supercritical extraction of
emulsions
seeds
essential oil sources, 25–6
 β -selinene, 186
SEM *see* scanning electron
micrograph
Semen sojæ praeparatum, 348, 351–2,
353
Sephadex, 325, 341, 343
sepiolite, 205
sesquiterpenes, 25, 74, 137
sesquiterpenoids, 23, 24, 25
S/F *see* solvent-to-feed ratio
SFC *see* supercritical fluid
chromatography
SFE *see* supercritical fluid extraction
SFEE *see* supercritical fluid extraction
of emulsions
SFME *see* solvent-free microwave
extraction
shikimates, 21
shikimic acid derivatives, 21–2
SHLE *see* superheated liquid
extraction
shrimp, pink, 423
SI *see* supercritical impregnation
silica, 164
gel, 325, 341
silicic acid, 182
simplex centroid design (SCD), 207
sitostanol, 39
S. japonica, 264
SLE *see* solid–liquid extraction
Smilax china, 123
soaking, 67–9, 85
soap industry, 292
sodium
chloride, 271
citrate, 266

- dodecyl sulfate (SDS), 266
- phosphate, 271
- sulfate, 159
- solerone, 183
- solid-liquid extraction (SLE), 172–3, 184, 190, 197, 254, 257
- solid phase extraction (SPE), 166–7, 321–2, **322**, 322, 406, 416–17
- solid-phase microwave extraction (SPME), 173
- solubility, 3, 60, 233, 260, 289
 - determined by pressure and temperature in SFE, 205–8
 - factor involved in scale-up of extraction processes, 373–4, 381–4
- solvent
 - extraction solvents and solvent mixtures, 261–75
 - extraction solvent modification with additives, 263–7
 - solvent mixtures and non-conventional highly hydrophobic organic solvents, 267
 - tunable solvents, 271–4
 - main variable in extraction, 79–82, 85
 - parameter effect in SFE case study, 222–3
 - supercritical, used in SFE, 199–200, **200**
- solvent composition
 - parameter influence on MAE, 121–4, 128
- solvent distribution
 - secondary scale-up criteria, 368, 368–9
- solvent evaporation method, 300–1
- solvent extraction method, 29
- solvent flow rate, 209
 - secondary scale-up criteria, 370–1, 374
- solvent-free microwave extraction (SFME), 123–4, 136, 136–8
- solvent recycling, 390
- solvent-to-feed ratio (S/F), 245
 - main variable in extraction, 82, 85
 - parameter affecting performance in ASE, 170–1
 - parameter affecting solvent flow rate in SFE, 209
 - parameter influence on MAE, 121, 124–5, 128
- solvent toxicity, 286
- solvent type
 - medium parameter in UAE, 101
 - parameter affecting performance in ASE, 167, 169–70
- solvent velocity
 - primary scale-up criteria, 367
- sonochemistry, 90, 98
- sonotrode, 94
- Sophora japonica*, 354, 354–6, 356
- sorbent-based extraction, 66
- sorptive extraction, 253, 275
- Sovová model, 261, 379, 392
- Soxhlet extraction, 66, 69–73, 70, 83, 85, 172–3, 253, 256, 464–5
- soybeans, 43, 68, 105, 182, 186, 222, 254, 268
 - germ, 256
 - lecithin, 309
 - oil, 33–4, 273–4
- Spatholobus suberectus*, 256
- SPE *see* solid phase extraction
- spearmint, 256
- spectroscopic techniques, 66
- sphingolipids, 37
- spilanthol, 246
- Spirulina*, 220–3, 273
- SPME *see* solid-phase microwave extraction
- sponges, 248
- spray-drying, 291–2, 292, 301
- SSE *see* supercritical solvent extraction
- SSI *see* supercritical solvent impregnation
- stabilization techniques, vii, 420–3
- stanols, 39
- Staphylococcus aureus*, 137
- star anise, 25

- starch, 245
 static-accelerated solvent extraction
 (static ASE), 158–63, 160 (*see also*
 accelerated solvent extraction)
 commercial and laboratory-
 designed devices, 161–2, 161–3
 steps in process, 158–9, 160, 161
 static ASE *see* static-accelerated
 solvent extraction
 steam diffusion (SDf), 135
 steam distillation (SD), 20, 25–30,
 73–8, 74–7, 135, 173, 185, 443
 case study, 83–5, 85
 direct, 74
 dry, 74
 stearidonic acid, 38
Stephania kwangsiensis, 347, 347–8,
 349, 351
 (–)-stephanine, 348
 steroids, 239, 275, 341
 sterols, 39, 105, 182–4
 stilbenes, 3, 42–3
 stirring inside the extractor
 secondary scale-up criteria, 368
 subcritical solvent extraction
 (SSE), 157
 succinic acid, 270
 sugars, 3, 13, 15, 232
 sulfides, 3
 sulfones, 274
 sulfur, 232
 sunflower, 33
 oil, 33, 106–7, 267
 seeds, 105
 supercritical anti solvent process
 (SAS), 294–5, 303–4, 309–10
 integration of pressurized fluid-
 based technologies, 422–3, **424**,
 425–9, 432–5, 438
 supercritical carbon dioxide (SC-
 CO₂), 200, 212, 224, 258, 293, 400,
 420
 case study, extraction from red
 pepper, 391–6, **393–5**
 supercritical enhanced dispersion of
 solutions (SEDS), 295
 supercritical extraction of emulsions
 (SEE), 303–4, 310–11
 supercritical fluid chromatography
 (SFC), 315, 337, 339, 414
 supercritical fluid extraction
 (SFE), 29, 75, 165, 172, 176–7,
 196–224, 373
 applications, 209, **210–11**, 212–13,
 214–15, 216–17, **218–19**, 220
 agricultural and food by-
 products, 216–17, **218–19**,
 220
 marine products, 213, **214–15**,
 216
 plants, 209, **210–11**, 212–13
 case study, 220–3, 391–6
 effect of extraction time, 220
 effect of pressure, temperature
 and modifier, 221–2
 effect of solvent, 222–3
 economic evaluation, 442, 450,
 451–60, 461, 462, 463–7, **468**,
 469
 fundamentals of, 197–200
 physical properties of
 supercritical fluids, 197–9,
 198, 198
 supercritical solvents, 199–200,
 200
 instrumentation, 201, 201–3
 integration of pressurized fluid-
 based technologies, 404–6, 414,
 416–17
 parameters affecting the extraction
 process, 203–9
 raw material (particle size,
 porosity, location of the
 solute, moisture
 content), 204–5
 solubility (pressure and
 temperature), 205–8
 solvent flow rate (solvent-to-
 feed ratio), 209
 use of modifiers, 208–9
 recent trends and
 perspectives, 258–61

- supercritical fluid extraction of
 emulsions (SFEE), 422, 438
 supercritical fluids (SCF), vii, 60, 197, 258
 micronization processes, 293–7
 physical properties of, 197–9, **198**, 198
 processes in formulations, 303–5
 supercritical impregnation (SI), 303, 303–4
 supercritical solvent impregnation (SSI), 422
 supercritical water extraction (SWE), 75
 superheated liquid extraction (SHLE), 158, 176, 183, 185
 case study, 187–90, **188**, 189
 comparison of SHLE with MAE and UAE, 189–90
 influence of extraction pH, 189
 optimisation of main variables, 187–8, **188**
 surfactants, 261, 265–7
 SWE *see* supercritical water extraction
 sweet potato, purple, *see* potato, purple sweet
 synthetic dyes (*see also* coloring agents)
 advantages, 4
 safety concerns, 4–5
 syringaldehyde, 185, 273
 syringol, 273
 syringyl, 168
Syzygium aromaticum, 20

 TAG *see* triacyl glycerols
 tannins, 3, 42–3, 185, 239, 241, 244, 267, 275, 325
 tara seed, 258
t-butyl amidine, 274
 tea, 79, 81–2, 254, 463 (*see also* green tea)
 Earl Grey, 29

 temperature
 main variable in extraction, 61, 82, 85
 medium parameter in UAE, 101, 107
 parameter affecting performance in ASE, 167–9, 168
 parameter affecting solubility in SFE, 205–8
 parameter effect in SFE case study, 221–2
 parameter influence on MAE, 121, 125–6, 128
 primary scale-up criteria, 367
 terpenes, 3, 21, 24, 25, 29, 37, 71, 74–5, 267
 terpenoids, 21–5, 38, 182–4, 232, 245, 317, 320, 341, 401
 γ -terpinene, 30
 tetrahydrofuran (THF), 122, 272
 tetrapyrrole derivatives, 6
 textiles
 extracts of natural products, 1, 2, 3
 Thar Technologies, 392
 theophylline, 294
 thermodynamics, 64, 233, 250, 260, 364
 THF *see* tetrahydrofuran
 thin-layer chromatography (TLC), 414
 thioesters, 23
 thrombosis, 20
Thymbra spicata, 186
 thyme, 75, 124, 134, 173, 260, 267
 oils, 28
 thymol, 28, 260
Thymus vulgaris, 124, 134, 173
 time
 effect in SFE case study, 220
 main variable in extraction, 82, 85, 107
 parameter affecting performance in ASE, 167, 171–2
 parameter influence on MAE, 121, 126, 128
Tinospora crispa, 341–3, 344–5

- TLC *see* thin-layer chromatography
- tobacco, 184
- tocopherols, 35–7, 39–40, 40, 217
 α -tocopherol, 39–40, 130, 220, 266
 δ -tocopherol, 130
- tocotrienols, 39–40
- toluene, 80
- tomato, 6, 10, 133, 217, 260
 paste, 133, 256
- total phenolic content (TPC), 145
- toxicity, 80, 261, 302
- TPC *see* total phenolic content
- treemoss, 21
- triacyl glycerols (TAG), 105, 213
- 1,1,1-trichloroethane, 80
- Trifolium*, 187
- triglycerides, 20, 31
- triterpenic acids, 81, 122
- triticale bran, 260
- tritoniopsins A–D, 341, 344
- turmeric, 18, 173, 245–6, 259, 270
 oleoresin, 18
 powder, 18
- turmerone, 259
- tyrosine, 22, 42
- tyrosol esters, 37
- UAE *see* ultrasound-assisted extraction
- UASE *see* ultrasound-assisted Soxhlet extraction
- UASFE *see* ultrasound-assisted supercritical fluid extraction
- ultra-high-pressure extraction (UPE), 257
- ultrasonic bath systems, 94, 94, 96
- ultrasonic extraction reactor, 93–4, 99–100, 106
- ultrasonic intensity, 97, 99
- ultrasonic microwave-assisted extraction (UMAE), 132, 132–3, 255
- ultrasonic probe systems, 94–5, 95–6, 99, 100
- ultrasound, vii, 90–3
 diagnostic (high frequency), 90
 power (low frequency), 90, 97–9, 107
- ultrasound-assisted extraction (UAE), 89–109
 applications in food, 102–5, 103
 fruits and vegetables, 103–4
 herbs and spices, 104
 oleaginous seeds, 104–5
 comparison with other extraction methods, 172, 175–6, 189–90
 costs and investment in industrial ultrasound, 108–9
 economic evaluation, 443, 464, 464–5
 instrumentation, 93–6
 industrial scale, 95–6, 109
 laboratory scale, 94–5, 109
 matrix parameters, 102
 medium parameters, 100–2
 presence of dissolved gases, 101–2
 solvent type, 101
 temperature, 101, 107
 physical parameters, 96–100, 97
 amplitude, 97–8
 frequency, 97–9
 period of wave cycle, 97
 propagation velocity, 98
 shape and size of ultrasonic reactors, 99–100, 100
 ultrasonic intensity, 97, 99
 ultrasound power, 97–9, 107
 wavelength, 97
 recent trends and perspectives, 255–7
 ultrasound principles, 90–3
 ultrasound-assisted Soxhlet extraction (UASE), 71–3, 72
 ultrasound-assisted supercritical fluid extraction (UASFE), 256–7, 260
 ultrasound transducer, 93, 106
 piezoelectric, 93
 ultraviolet/visible (UV–vis), 328
 UMAE *see* ultrasonic microwave-assisted extraction

- United States Environmental Protection Agency (EPA), 69, 176
- United States Food and Drug Administration (FDA), 80, 286
- UPE *see* ultra-high-pressure extraction
- ursolic acid, 122
- Urtica dioica*, 13
- UV-spectrophotometer, 107
- UV-vis *see* ultraviolet-visible
- UV/vis spectrophotometry, 429
- vacuum liquid chromatography (VLC), 327
- vacuum microwave-assisted extraction (VMAE), 129, 129–30, 254
- vacuum microwave hydro-diffusion and gravity (VMHG), 140, 255
- vacuum microwave hydro-distillation (VMHD), 138
- valerian, 207
- Valeriana officinalis*, 207
- valerian oil, 31
- vanilla, 183
- vanillin, 22, 23, 185, 273
- vanillylamides, 42
- vapor pressure, 60
- vegetable
- extracts
 - manufacturing cost, 450, **451–60**, 461, 463–4
 - oil, 70, 133
 - modifier, 209, 217, 260
- velocity effects
- secondary scale-up
 - criteria, 369–70
- verbascoside, 184
- Verbascum phlomides*, 8
- vetiver, 25
- vetivone
 - α -vetivone, 25
 - β -vetivone, 25
- Vian, Abert, 254
- Vibracell, 95
- vine shoots, 187–90, **188**, 189
- violaxanthin, 6, 8
- viscosity, 80, 268, 296, 364
- vitamin(s), 36, 287
 - A, 105, 305
 - C, 130, 254
 - E, 37, 39–40, 182, 220–2, 254
 - liposoluble, 182
 - supplements, 1
- Vitis vinifera*, 16, 185
- VLC *see* vacuum liquid chromatography
- VMAE *see* vacuum microwave-assisted extraction
- VMHD *see* vacuum microwave hydro-distillation
- VMHG *see* vacuum microwave hydro-diffusion and gravity
- volatile compounds
 - natural products extracted by
 - ASE, 177, 183–4
- von Soxhlet, Franz, 69
- vulgaxanthine I and II, 16
- walnut-tree
 - leaves, 175
- water, 80–1, 255, 262–3, 268, 272, 326, 416
 - accelerated liquid extraction, 167, 169–70, 173–4
 - modifier, 208, 212
 - supercritical fluid extraction, 197, 200, 224
- water distillation, 30, 73–8, 85
- water extraction and particle formation on-line (WEPO), 423
- water-in-oil-in-water (W/O/W) emulsion, 300
- wave guide, 119
- wavelength, 97
- waxes, 405
- WEPO *see* water extraction and particle formation on-line
- wheat germ oil, 36
- wine, 104

- winery industry, 163, 184
- wood(s), 171
 - essential oil sources, 26–7
- W/O/W emulsion *see* water-in-oil-in-water emulsion
- xanthan, 264
- xanthophylls, 6, 37, 81
- Xylopia aromatic*, 134
- xylosidades, 263
- yerba mate, 43
- Ylang-Ylang essential oil, 30
- Zanthoxylum bungeanum*, 124, 138
- zeacarotene, 10
- zeaxanthin, 8, 212, 216, 305
- Zingiberaceae, 17
- Zingiber officinale*, 138, 187
- zizanal, 25
- Ziziphus jujube*, 182