CITUS ESSENTIAL OILS

Flavor and Fragrance

Edited by
Masayoshi Sawamura
Kochi University, Japan

WILEY

A JOHN WILEY & SONS, INC., PUBLICATION

CITRUS ESSENTIAL OILS

CITRUS ESSENTIAL OILS
Flavor and Fragrance

Edited by
Masayoshi Sawamura
Kochi University, Japan

WILEY

A JOHN WILEY & SONS, INC., PUBLICATION

Copyright © 2010 by John Wiley & Sons, Inc. All rights reserved.

Published by John Wiley & Sons, Inc., Hoboken, New Jersey.
Published simultaneously in Canada.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, scanning, or otherwise, except as permitted under Section 107 or 108 of the 1976 United States Copyright Act, without either the prior written permission of the Publisher, or authorization through payment of the appropriate per-copy fee to the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, (978) 750-8400, fax (978) 750-4470, or on the web at www.copyright.com. Requests to the Publisher for permission should be addressed to the Permissions Department, John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, (201) 748-6011, fax (201) 748-6008, or online at http://www.wiley.com/go/permission.

Limit of Liability/Disclaimer of Warranty: While the publisher and author have used their best efforts in preparing this book, they make no representations or warranties with respect to the accuracy or completeness of the contents of this book and specifically disclaim any implied warranties of merchantability or fitness for a particular purpose. No warranty may be created or extended by sales representatives or written sales materials. The advice and strategies contained herein may not be suitable for your situation. You should consult with a professional where appropriate. Neither the publisher nor author shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

For general information on our other products and services or for technical support, please contact our Customer Care Department within the United States at (800) 762-2974, outside the United States at (317) 572-3993 or fax (317) 572-4002.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic formats. For more information about Wiley products, visit our web site at www.wiley.com.

Library of Congress Cataloging-in-Publication Data:

Citrus essential oils : flavor and fragrance / edited by Masayoshi Sawamura.
p. cm.
ISBN 978-0-470-37218-0 (cloth)
1. Citrus oils. I. Sawamura, Masayoshi.
TP959.C54C58 2010
661′.806—dc22

2009045874

Printed in Singapore.

10 9 8 7 6 5 4 3 2 1

CONTENTS

PREFACE

Masayoshi Sawamura

Today, citrus fruit is widely grown from temperate to tropical zones in the northern and southern hemispheres. Citrus fruit has been first ranked in the annual amount of world fruit production since 1992, meaning that it is the favorite type of fruit worldwide. It has pleasant taste and aroma, and is also an important source of vitamin C. Essential oil of citrus fruit is intensively accumulated in oil glands of the peel, accounting for approximately 1% of fruit weight on average. Citrus essential oil is also the most popular of natural essential oils. The oil is commercially used for food flavorings, toiletry products, cosmetics, perfumes, and so forth. Recently, citrus essential oil has been known not only for its aromatic functions, but also for its physiological properties, such as chemoprevention against cancer and aromatherapy effects. There are more than 10,000 varieties of citrus fruit. However, many citrus varieties are not familiar to us. Each aroma is unique and quite different even among those varieties. There has been lack of information on the overall characteristics of the *Citrus* genus. Citrus fruit is an important aromatic resource and its value will increase in the future. There is much useful information to be gleaned about the creative aromatic character and functional properties. This book provides an overview of citrus essential oils from various aspects covering basic and methodological application and recent topics of citrus essential oils research.

We hope that this book will provide inspiration and creative ideas for flavor and fragrance professionals and researchers. The book is intended for professionals as well as advanced students active in the food sciences, flavor chemistry, and aromatherapy. We believe our readers would benefit from a book that describes concepts, analytical methods, and diverse properties.

I owe a debt of gratitude to all the authors of this volume, who are leading specialists in their fields.

CONTRIBUTORS

Prof. Farid Chemat Université d'Avignon et des Pays de Vaucluse, UMR A 408 INRA-UPAV, 84000 Avignon, France

Hyang-Sook Choi, Ph.D. Asst. Prof., Department of Food Nutrition and Culinary Art, Kyungin Women's College, 101 Gyesangil Kyeyanggu, Inchon 407-740, Korea

Dr. Hisakatsu Iwabuchi San-Ei Gen F.F.I., Inc., 1-1-11 Sanwa-cho, Toyonaka, Osaka 561-8588, Japan

Dr. Takehiro Kashiwagi Assoc. Prof., Major of Food Science, Faculty of Agriculture, Kochi University, B-200 Monobe, Nankoku, Kochi 783-8502, Japan

Dr. Chizu Kumagai Palliative Care Unit, Municipal Ida Hospital, City of Kawasaki 2-27-1 Ida, Nakahara-ku, Kawasaki, Kanagawa, Japan 211-0035

Nguyen Thi Lan-Phi, Ph.D. Faculty of Chemical Engineering, Ho Chi Minh City University of Technology (HCMUT), 268 Ly Thuong Kiet Street, District 10, Ho Chi Minh City, Vietnam

Nguyen Thi Minh-Tu, Ph.D. Institute of Biological and Food Technology, Hanoi University of Technology, 1 Dai Co, Viet Road, Hanoi, Vietnam

Simon Muhoho Njoroge, Ph.D. Assoc. Prof., Department of Food Science and Technology, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

Prof. Marco Poiana Dipartimento di Biotecnologie per il Monitoraggio Agroalimentare ed Ambientale, Facoltà di Agraria, Università Degli Studi di Reggio Calabria, 89124, Reggio Calabria, Italy

Dr. Atsushi Satake Research and Development Center, Nagaoka Perfumary Co. Ltd., 1-3-30, Itsukaichi, Ibaraki, Osaka 567-0005, Japan

Prof. Masayoshi Sawamura Tosa Food Business Creator Project Team, Kochi University, B-200 Monobe, Nankoku, Kochi 783-8502, Japan

Masaki Shimada, M.Eng. San-Ei Gen F.F.I., Inc., 1-1-11 Sanwa-cho, Toyonaka, Osaka 561-8588, Japan

U-Sun Son, M.Agr. Flavor Part/Scent Berry Perfume House, LG Household & Health Care Ltd., Seoul National University, San 56-1, Silim-dong, Gwanak-gu, Seoul 151-742, Korea

Hee-Sun Song, Ph.D. Assoc. Prof., Department of Food and Nutrition, Gwangju Health College, 688-3, Shinchang-dong, Gwangsan-gu, GwangJu, 506-701, Korea

Nguyen Thi Thao, Ph.D. Institute of Biological and Food Technology, Hanoi University of Technology, 1 Dai Co, Viet Road, Hanoi, Vietnam

Prof. C. Hanny Wijaya Department of Food Science and Technology, Faculty of Agricultural Technology, Bogor Agricultural University, Kampus IPB Damaga, Bogor 16002, Indonesia

Xiaolin Yu, M.Agr. Assoc. Prof., Department of Food Science, South China Agricultural University, Wushan, Guangzhou 510642, China

Dr. Chiyoki Yukawa San-Ei Gen F.F.I., Inc., 1-1-11 Sanwa-cho, Toyonaka, Osaka 561-8588, Japan

1

INTRODUCTION AND OVERVIEW

Masayoshi Sawamura

Kochi University
Kochi, Japan

There are a great number of *Citrus* varieties widely distributed in the world. It is said that the *Citrus* genus originated near Assam in India about 30 or 40 million years ago (Iwamasa, 1976). The *Citrus* fruits that spread to the West migrated to the Middle East and the Mediterranean, crossed the Atlantic Ocean, and finally reached America via the West Indies. Others, spreading to the East, migrated to Thailand, Malaysia, China and other Southeast Asian countries. Nowadays, most citrus fruits are grown extensively in the temperate and tropical zones of the northern and southern hemispheres.

Citrus can be propagated and new varieties can be produced by asexual nuclear or chance seedlings, by crossing, and by mutation. In addition to these natural forms of propagation, many new artificially crossed cultivars have been created by *Citrus* breeders. The classification of this expanding family is complex and is becoming confused. The best-known taxonomies of genus *Citrus* are those of Swingle (1943) and Tanaka (1969a,b). These two taxonomies differ greatly in the number of species admitted: Swingle identified 16 species, Tanaka 159. Although the basic concept underlying the two taxonomies is different, assignment is almost the same.

Citrus Essential Oils: Flavor and Fragrance, Edited by Masayoshi Sawamura

The four major fruit types commercially produced worldwide are citrus fruit, bananas, apples and grapes, followed by pears, peaches, and plums. Citrus fruit finally replaced grapes as the world's most-produced fruit in 1991. The recent production volume of major fruits is shown in Figure 1.1. The production of citrus

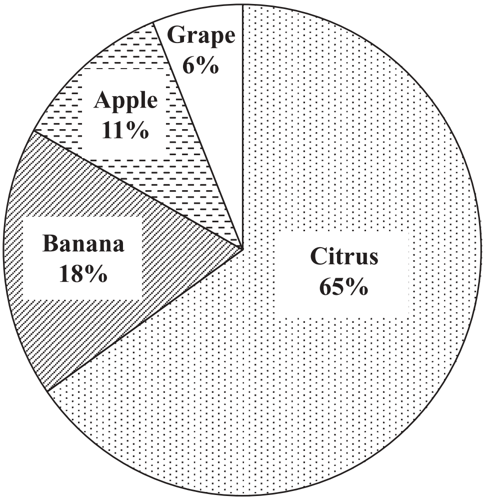

Figure 1.1. Worldwide production of major fruits.

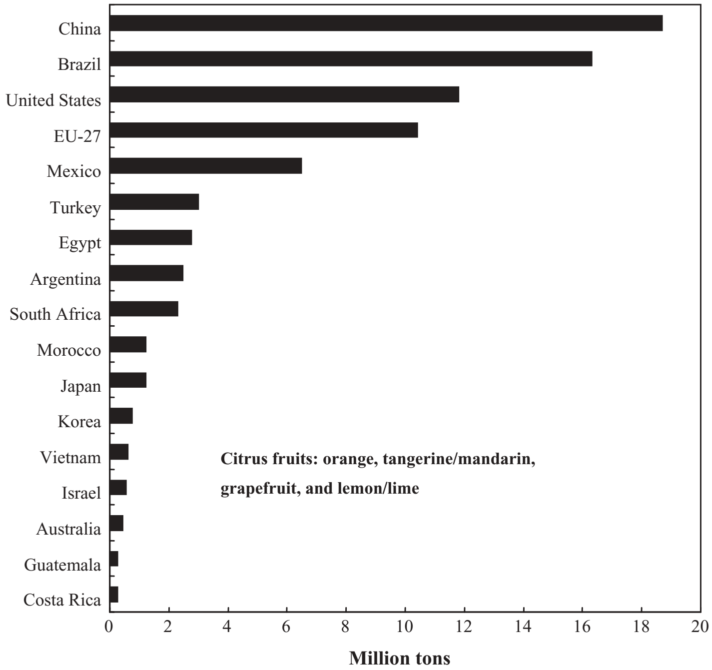

Figure 1.2. Major citrus fruits production in 2007.

fruit accounts for more than 65% of fruits produced. The total world production of citrus fruit in 2008 was about 79.6 million tons; major citrus-producing countries are shown in Figure 1.2. The greatest producer is China, followed by Brazil, the United States, the EU, Mexico, and Turkey. Citrus essential oils have also long been the most popular source of perfume and fragrance essences. There are four reasons why citrus fruit is the most popular fruit in the world: (1) good sour and sweet tastes; (2) pleasant, refreshing aroma; (3) good source of vitamin C; and (4) extensive growing areas worldwide. There are two categories of citrus fruit in terms of food chemistry: sweet citrus fruit, with a sugar/acid ratio of approximately 10, and sour citrus fruit, with a ratio of less than 1. Sweet citrus fruit such as orange, grapefruit, Satsuma mandarin, and pummelo are popular varieties. Sour citrus fruit, on the other hand, such as lemon, lime, bergamot, and yuzu, are less produced, but they are popular in culinary materials such as fruit juice vinegar, and their essential oils are also frequently used in flavoring, cosmetics, and perfume.

The aim of flavor or aroma research is to determine a fruit's composition, aroma characteristics, functionality, and industrial or commercial value. The concept of flavor research is outlined in Figure 1.3. First, essential oil is extracted from raw material such as citrus fruits. Then, aroma samples are analyzed using modern instruments, organoleptic procedures, and/or mechanical sensors. The resulting information can give a detailed understanding of the fruit and can be used in further studies of aroma or flavor.

The *Citrus* genus is said to have more than 10,000 varieties and to be produced more than any other kind of fruit in the world. Citrus essential oils account for the largest proportion of commercial natural flavors and fragrances. Essential oils from citrus peel are natural flavoring materials of commercial importance. They have been used in beverages, confectioneries, pharmaceuticals, cosmetics, and perfumes. The quality, freshness, and uniqueness of citrus oils are major factors contributing to their value and application. Citrus fruits, with their unique and attractive individual aromas, are popularly accepted worldwide, and citrus essential oils are a large and important aroma resource. Quantitative data concerning the volatile components of a number of citrus essential oils (Shaw, 1979; Sawamura, 2000) and their wide commercial use are presented in this book.

The twenty-first century has been referred to as "the era of fragrance." We live in an atmosphere greatly enriched by aromas and fragrances. Everyday items such as fresh and cooked foods, perfumes, cosmetic and toiletry goods, medicines, and insecticides contain natural or artificial fragrances. Aroma commonly gives us a strong impact in trace amounts. The characteristic odor of an individual substance is composed of roughly a thousand compounds. It is almost impossible to blend every compound of an odor in the exact proportion required to reconstruct the original. It has been determined, however, that there are usually one or two or a few key compounds that can accurately simulate the original odor. One goal of flavor research is to elucidate key aroma compounds by means of a combination of instrumental and organoleptic analyses. Many flavor researchers have tried to find the key aroma compounds of various kinds of citrus. *Gas*

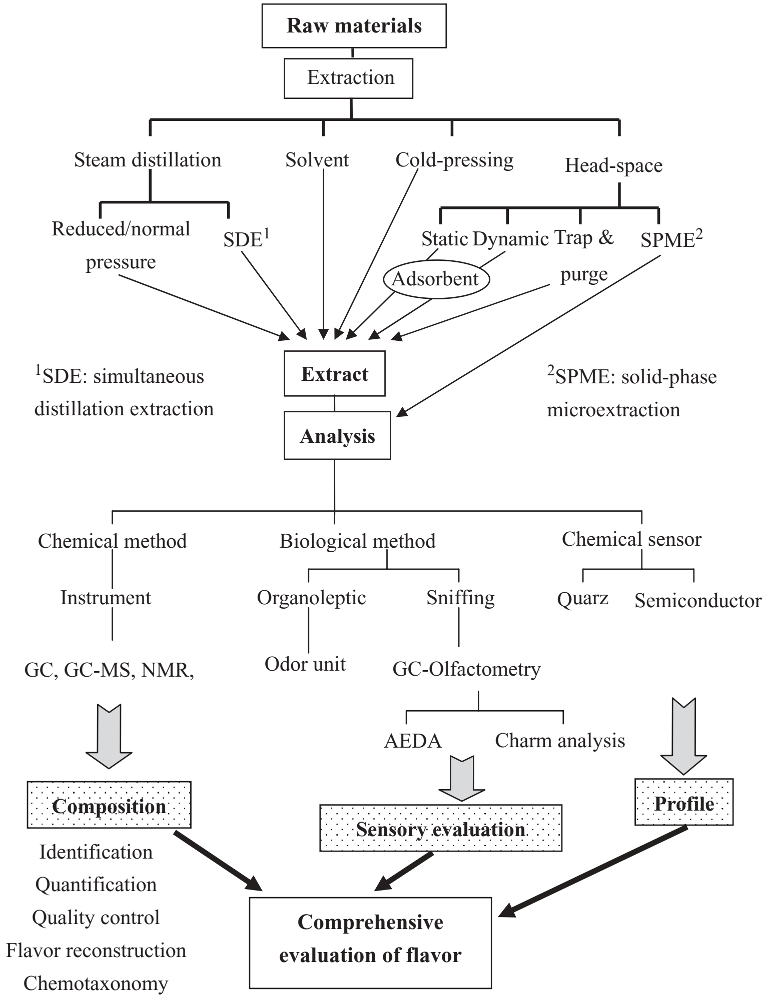

Figure 1.3. Flowchart of flavor research.

chromatography–olfactomery (GC-O) is a superior method for such studies (Acree, 1993).

Aroma is one of the functional properties of food because aroma compounds stimulate us physically and physiologically (this is referred to as *organoleptic effects*). A great number of aroma compounds have been identified in a variety of foods to date. Such studies have been a major theme in flavor research. Aroma compounds have a number of properties other than odor production, including antibiotic, deodorant, and blood vessel stimulation. *Aromatherapeutic effect* also falls into this category. Antioxidants have been investigated most intensively as constituents preventing diseases associated with oxidative damage, and decreasing lipid oxidation during the processing and storage of seafood (Pisano, 1986).

Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate (PG) are used as food additives to inhibit the actions of toxic and carcinogenic substances (Chang et al., 1977). Natural antioxidants from natural foods such as herbs (Boyd et al., 1993; Pizzocaro et al., 1994), vegetables (Tsushida et al., 1994; Vinson et al., 1998), fruits (Nogata et al., 1996), oilseeds (Medina et al., 1999), spices (Shahidi et al., 1994), green tea (Amarowicz and Shahidi, 1996), and cereals (Hendelman et al., 1999) have been studied, and some of them, such as ascorbate and tocopherols, are currently used in a variety of food products. In recent reports much attention has been given to citrus components, since they present various pharmaceutical activities including anticarcinogenicity, antimutagenicity, antioxidative activity, antiaging, and radical-scavenging (Nogata et al., 1996; Rapisarda et al., 1999; Choi et al., 2000; Sawamura et al., 1999, 2005). Grapefruit oil and lemon extracts have been suggested as effective natural antioxidative compounds (Tokoro, 1997). It has been recently discovered that some foods or foodstuffs serve to inhibit the formation of carcinogens. Essential oils containing terpenoids are well known to have some physiological and pharmaceutical effects, and it is known that citrus essential oils have antimicrobiological (Griffin et al., 1999) and chemopreventive properties (Crowell, 1997; Gould, 1997; Kawaii et al., 1999). The major component of citrus essential oils is *terpenes*, whose basic structure is isoprenoid (C_5H_8). The most typical terpenes are limonene, citrus-like odor; γ-terpinene, waxy; terpinolene, green; α-pinene and β-pinene, pine-like. These compounds have been reported to inhibit the growth of cancer cells. One carcinogen, nitrosodimethylamine, which is formed with dimethylamine and nitrite in an acidic condition, has been noted in this regard. Dimethylamine and nitrite are commonly present in meats and vegetables, respectively. It was suggested that some foods or foodstuffs might contain cancer-inhibitory and -preventive compounds as well as cancer-inducing substances (Sawamura et al., 1999, 2005).

Aromatherapy, a medical treatment intended to stimulate or calm the mind, is an applied therapy using the functional properties of essential oils. A variety of vegetable essential oils have been widely used in aromatherapy. Essential oils are extracted from the flowers, leaves, stems, roots, and fruits of various plants and purified for commercial use. Aromatherapy originated in Europe in the eighteenth century and has grown popular in many countries recently, but the most famous essential oil products are still produced in Europe. There are currently seven kinds of commercial citrus essential oils used in aromatherapy: orange, mandarin, lemon, lime, bergamot, grapefruit, and neroli. Yuzu (*Citrus junos* Sieb. ex Tanaka), a typical Japanese sour citrus fruit, has attracted the interest of aroma therapists over the past ten years. Recently, the effect of yuzu essential oil on the autonomic nervous system has been studied (Sawamura et al., 2009). It is expected that yuzu essential oil will soon be adopted for use in aromatherapy. One obstacle to such application for aromatherapy is that the composition of essential oils changes readily. It has been pointed out that the composition of yuzu (Njoroge et al., 1996) and lemon essential oils (Sawamura et al., 2004) can change considerably under different storage conditions. It is important to carefully consider quality change in commercial essential oil products intended for therapeutic use. This book presents a few attractive studies of functional citrus properties, including aromatherapy.

Recently, food safety or reliability has been a prominent concern, along with food quality. All Japanese foods and products, for example, are controlled under standards such as the European Union regulations regarding food safety and traceability, Japanese Agricultural Standard (JAS), and other regulations governing responsibility to consumers and manufacturers in the United States, Canada, Australia, and England. However, there are few methods for discriminating as to the quality and characteristics of crops from various producing districts. One of the most reliable methods is isotope analysis of food constituents by mass spectrometry. In nature, the isotopes of each element are distributed in a fixed ratio. Plants on the earth first convert solar energy into biochemical energy; the food chain begins with plants. Higher plants fix CO_2 by the Calvin-Benson cycle to biosynthesize various organic compounds for their constituents. It is known that the enzyme ribulose-1,5-diphosphate carboxylase differentiates a small mass difference between $^{12}CO_2$ and $^{13}CO_2$, when it fixes CO_2 in the atmosphere (O'Leary, 1981). This function is referred to as the *isotope effect*. It is thought that the isotope effect could occur in every enzyme involved in biosynthetic and metabolic pathways. Thus, we can see that this effect should also be applicable to the essential oils comprising terpene compounds. Every species, variety, or strain of a plant has some substantially distinct characteristics. Even among the same cultivars, different growing conditions such as annual atmosphere and moisture, or soil and fertilizers, can bring about small but appreciable differences in composition. Several researchers (Faber et al., 1995; Faulharber et al., 1997; Sawamura et al., 2001; Thao et al., 2007) have tried to distinguish these isotope differences of biological constituents. Some sections of this book discuss methods of verifying the genuineness of food.

Finally, fundamental and academic information about citrus essential oils have contributed to commercial development. In the references section on this chapter, several experts present the reader with attractive topics regarding the wide utilization of citrus essential oils.

REFERENCES

Acree, T.E. (1993). Bioassays for flavor. In: Acree, T.E., Teranishi, R. (eds.), Flavor science. Washington, DC: American Chemical Society, pp. 1–18.

Amarowicz, R., Shahidi, F. (1996). A rapid chromatographic method for separation of individual catechins from green tea. *Food Research International* **29**: 71–76.

Boyd, L.C., Green, D.P., Giesbrecht, F.B., King, M.F. (1993). Inhibition of oxidative rancidity in frozen cooked fish flakes by *tert*-butylhydroquinone and rosemary extract. *Journal of the Science of Food and Agriculture* **61**: 87–93.

Chang, S.S., Ostric-Matijasevic, B., Hsieh, O.A., Chang, C.L. (1977). Natural antioxidants from rosemary and sage. *Journal of Food Science* **42**: 1102–1106.

Choi, H.S., Song, H.S., Ukeda, H., Sawamura, M. (2000). Radical-scavenging activities of citrus essential oils and their components: Detection using 1,1-diphenyl-2-picrylhydrazyl. *Journal of Agricultural and Food Chemistry* **48**: 4156–4161.

Crowell, P.L. (1997). Monoterpenes in breast cancer chemoprevention. *Breast Cancer Research Treatment* **46**: 191–197.

Faber, B., Krause, B., Dietrich, A., Mosandl, A. (1995). Gas chromatography–isotope ratio mass spectrometry in the analysis of peppermint oil and its importance in the authenticity control. *Journal of Essential Oil Research* **7**: 123–131.

Faulharber, S., Hener, U., Mosandl, A. (1997). GC-IRMS analysis of mandarin essential oils. 2. $d^{13}C_{PDB}$ values of characteristic flavor components. *Journal of Agricultural and Food Chemistry* **45**: 4719–4725.

Gould, M.N. (1997). Cancer chemoprevention and therapy by monoterpenes. *Environmental Health Perspectives* **105**: 977–979.

Griffin, S.G., Wyllie, S.G., Markham, J.L., Leach, D.N. (1999). The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. *Flavour Fragrance Journal* **14**: 322–332.

Hendelman, G.J., Cao, G., Walter, M.F., Nightingale, Z.D., Paul, G.L., Prior, R.L., Blumberg, J.B. (1999). Antioxidant capacity of oat (*Avena sativa* L.) extracts. 1. Inhibition of low-density lipoprotein oxidation and oxygen radical absorbance capacity. *Journal of Agricultural and Food Chemistry* **47**: 4888–4893.

Iwamasa, M. (1976). Citrus varieties. Shizuoka: Shizuoka Prefectural Citrus Agricultural Cooperative Association, p. 30.

Kawaii, S., Tomono, Y., Katase, E., Ogawa, K., Yano, M. (1999). Quantitation of flavonoid constituents in *Citrus* fruits. *Journal of Agricultural and Food Chemistry* **47**: 3565–3571.

Medina, I.M., Satué-Gracia, T., German, J.B., Frankel, E.N. (1999). Comparison of natural polyphenol antioxidants from extra virgin olive oil with synthetic antioxidants in tuna lipids during thermal oxidation. *Journal of Agricultural and Food Chemistry* **47**: 4873–4879.

Njoroge, S.M., Ukeda, H., Sawamura, M. (1996). Changes in the volatile composition of yuzu (*Citrus junos* Tanaka) cold-pressed oil during storage. *Journal of Agricultural and Food Chemistry* **44**: 550–556.

Nogata, Y., Yoza, K., Kusumoto, K., Kohyama, N., Sekiya, K., Ohta, H. (1996). Screening for inhibitory activity of citrus fruit extracts against platelet cyclooxigenase and lipoxigenase. *Journal of Agricultural and Food Chemistry* **44**: 725–729.

O'Leary, M.H. (1981). Carbon isotope fractionation in plants. *Phytochemistry* **20**: 553–567.

Pisano, R.C. (1986). The future of natural essential oils in the flavor and fragrance industry. *Perfumer and Flavorist* **11**: 35–41.

Pizzocaro, F., Senesi, E., Babbini, G. (1994). Protective effect of rosemary and sage on frozen beef hamburgers. *Industrie Alimentari* **33**: 289–294.

Rapisarda, P., Tomaino, A., Lo Cascio, C., Bonina, F., Saijo, A., De Pasquale, A. (1999). Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *Journal of Agricultural and Food Chemistry* **47**: 4718–4723.

Sawamura, M., Song, H.S., Ozaki, K., Ishikawa, J., Ukeda, H. (1999). Inhibitory effects of citrus essential oils and their components on the formation of *N*-nitrosodimethylamine. *Journal of Agricultural and Food Chemistry* **47**: 4868–4872.

Sawamura, M. (2000). Volatile components of essential oils of the *Citrus* genus. In: Pandalai, S.G. (ed.), Recent research developments in agricultural and food chemistry, Vol. 4. Trivandrum, India: Research Signpost, pp. 131–164.

Sawamura, M., Ito, T., Une, A., Ukeda, H., Yamasaki, Y. (2001). Isotope ratio by HRGC-MS of *Citrus junos* Tanaka (yuzu) essential oils: *m/z* 137/136 of terpene hydrocarbons. *Bioscience, Biotechnology, and Biochemistry* **65**: 2622–2629.

Sawamura, M., Son, U.S., Choi, H.S., Lee Kim, M.S., Lan Phi, N.T., Fears, M., Kumagai, C. (2004). Compositional changes in commercial lemon essential oil for aromatherapy. *International Journal of Aromatherapy* **14**: 27–36.

Sawamura, M., Wu, Y., Fujiwara, C., Urushibata, M. (2005). Inhibitory effects of yuzu essential oil on the formation of *N*-nitrosodimethylamine in vegetables. *Journal of Agricultural and Food Chemistry* **53**: 4281–4287.

Sawamura, M., Fukata, J., Kumagai, C., Lan Phi, N.T., Mizushima, N., Hori, N., Wada, M., Kamano, S. (2009). Functional activities of Japanese yuzu essential oil. *Japanese Journal of Aromatherapy* **9**: 1–11.

Shahidi, F., Wanasundara, U.N., Amarowicz, R. (1994). Natural antioxidants from low-pungency mustard flour. *Food Research International* **27**: 489–493.

Shaw, P.E. (1979). Review of quantitative analyses of citrus essential oils. *Journal of Agricultural and Food Chemistry* **27**: 246–257.

Swingle, W.T. (1943). The botany of *Citrus* and its wild relatives. In: Webber, H.J., Batchelor, L.D. (eds.), The citrus industry, Vol. 1. Berkeley, FL: University of California Press, pp. 190–430.

Tanaka, T. (1969a). Taxonomic problem of citrus fruits in the orient. *Bulletin of University of Osaka Prefecture* **B 21**: 133–138.

Tanaka, T. (1969b). Misunderstanding with regard to citrus classification and nomenclature. *Bulletin of University of Osaka Prefecture* **B 21**: 139–145.

Thao, N.T., Kashiwagi, T., Sawamura, M. (2007). Characterization by GC-MS of Vietnamese citrus species and hybrids based on the isotope ratio of monoterpene hydrocarbons. *Bioscience, Biotechnology, and Biochemistry* **71**: 2155–2161.

Tokoro, K. (1997). Antioxidants in grapefruit oil. *Aromatopia* **24**: 42–44.

Tsushida, T., Suzuki, M., Kurogi, M. (1994). Evaluation of antioxidant activity of vegetable extracts and determination of some active compounds. *Nippon Shokuhin Kogyo Gakkaishi* **41**: 611–618.

Vinson, J.A., Hao, Y., Su, X., Zubik, L. (1998). Phenol antioxidant quantity and quality in foods: Vegetables. *Journal of Agricultural and Food Chemistry* **46**: 3630–3634.

2

TECHNIQUES FOR OIL EXTRACTION

Farid Chemat

Université d'Avignon
Avignon, France

Essential oils have been used probably since the discovery of fire. Egyptians and Phoenicians, Jews, Arabs, Indians, Chinese, Greeks, Romans, and even Mayans and Aztecs all possessed a fragrance culture of great refinement. The Egyptian art of perfumery was preeminent in the civilized antique world, hence the use of aromatic fumigation in religious ceremonies and in mummification. The advent of Christianity and the fall of the Roman Empire caused the art and science of perfumery to move into the Arabic world, where it reached an unequalled level of refinement. During the Middle Ages, the Crusaders introduced the art of alchemy to Europe (Guenther, 1948). The process used by alchemists was the distillation technique using the alambic to produce *spirit* or *Quinta essentia*, precisely what we know today as *essential oils* (French, 1651). Essential oils are also known as *volatile oils* in contrast to fatty vegetable, animal, and mineral oils. Thus, a drop of essential oil on a piece of cloth or paper disappears within a few minutes or a few days at most, depending on the temperature, which is not the case for fatty oils.

The traditional way of isolating volatile compounds as essential oils from *Citrus* is performed mostly by cold pressing the *Citrus* peels. Steam distillation is also used to recover citrus essential oils but principally for production of

Citrus Essential Oils: Flavor and Fragrance, Edited by Masayoshi Sawamura

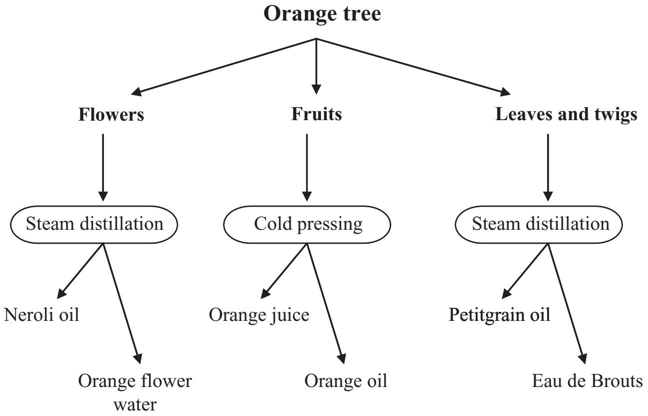

Figure 2.1. Complete valorization of citrus trees.

limonene used as green solvent. Not only are citrus fruits completely used but all of the citrus tree could be valorized, for example, by steam distillation of orange flowers to get neroli oil and the by-product orange flower water. With increasing energy prices, it was not economical to perform steam distillation of citrus leaves and twigs to get petitgrain oil and the by-product eau de brouts (Figure 2.1). Citrus essential oils have been applied in many products such as foods, beverages, cosmetics, and medicines, as flavoring agents as well as for aromatherapy. They are also used for their germicidal, antioxidant, and anticarcinogenic properties (Mukhopadhyay, 2000).

Essential oils and aromas are complex mixtures of volatile substances generally present at low concentrations. Before such substances can be used or analyzed, they have to be extracted from the plant matrix. Different methods can be used for this purpose (e.g., hydrodistillation, steam distillation, cold pressing, solvent extraction, and simultaneous distillation-extraction). Losses of some volatile compounds, low extraction efficiency, degradation of unsaturated or ester compounds through thermal or hydrolytic effects, and toxic solvent residue in the extract may be encountered using these extraction methods. Citrus essential oils are a mixture of volatile compounds and consist mainly of monoterpene hydrocarbons that possess high levels of unsaturation and are generally unstable due to many factors such as light, heat, oxidation, and hydration. These shortcomings have led to the consideration of the use of new "green" techniques in essential oil extraction, which typically use less solvent and energy, such as microwave extraction, supercritical fluid extraction, ultrasound extraction, and subcritical water extraction. Alternatives to conventional extraction procedures may increase production efficiency and contribute to environmental preservation by reducing the use of solvents and fossil energy and the generation of hazardous substances.

This chapter presents a complete picture of current knowledge on conventional and innovative techniques of extraction of citrus essential oils.

2.1 STEAM AND HYDRODISTILLATION

Farid Chemat

According to ISO and AFNOR standards, essential oils are defined as products obtained from raw plant material that must be isolated by physical means only. The physical methods used are distillation (steam, steam/water, and water), expression (also known as cold pressing for citrus peel oils), or dry distillation of natural materials. Following distillation, the essential oil is physically separated from the water phase (Figure 2.2) (Essentielle, 2000).

The traditional way of isolating volatile compounds as essential oils from plant material is distillation. During distillation, fragrant plants exposed to boiling water or steam release their essential oils through evaporation. Recovery of the essential oil is facilitated by distillation of two immiscible liquids, namely, water and essential oil, based on the principle that, at the boiling temperature, the combined vapor pressure equals the ambient pressure. Thus the essential oil ingredients, for which boiling points normally range from 100 to 300°C, are evaporated at a temperature close to that of water. The essential oil–laden steam rises and enters narrow tubing that is cooled by an outside source. As steam

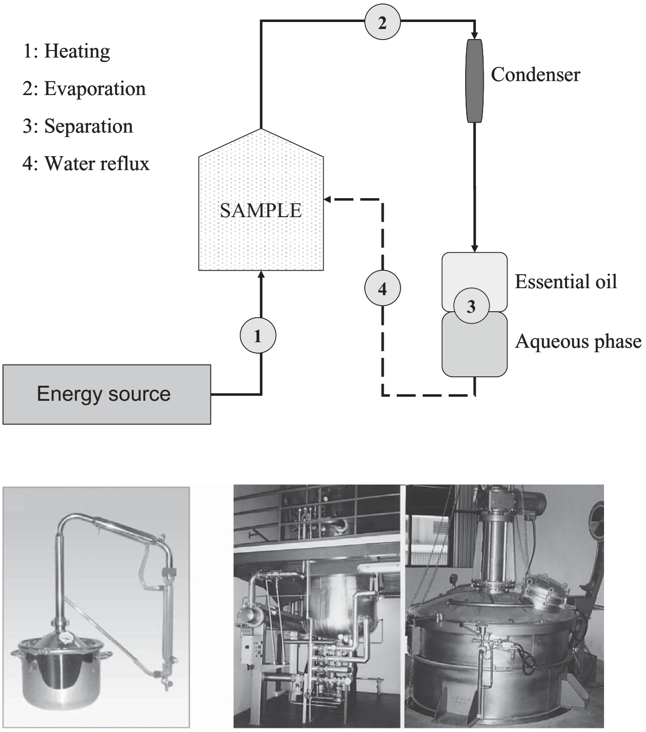

Figure 2.2. Laboratory and industrial conventional recovery of essential oils.

and essential oil vapors are condensed, both are collected and separated in a vessel traditionally called the "Florentine flask" (Guenther, 1948). The essential oil, being lighter than water, floats at the top while water goes to the bottom and can be easily separated. The amount of essential oil produced depends on four main criteria: the length of distillation time, the temperature, the operating pressure and, most importantly, the type and quality of the plant material. Typically, the yield of essential oils from plants is between 0.005 and 10% (Naves, 1974).

Historically, there have been three types of distillation: water distillation, water/steam distillation, and steam distillation. Water distillation is sometimes referred to as *indirect* steam distillation. In this method, plant material is soaked in water and heated until it boils. The resulting steam from boiling water carries the volatile oils with it. Cooling and condensation subsequently separate the oil from the water. Apart from its slowness, the disadvantage of this technique is that both materials and scent deteriorate from constant heat exposure. In the water/steam method, the leafy plant material is placed on a grill above the hot water, and the steam passes through the plant material. The leaves must be carefully distributed on the grill to allow for even steaming and thorough extraction. "Direct" steam distillation is the most common method for essential oil extraction. In this process, no water is placed inside the distillation tank itself. Instead, steam is directed into the tank from an outside source. The essential oils are released from the plant material when the steam bursts the sacs containing the oil molecules. From this stage, the process of condensation and separation is standard.

In addition to those mentioned previously, there are numerous other improved methods of producing natural fragrance materials and essential oils, including turbodistillation (Figure 2.3), hydrodiffusion, vacuum-distillation, continuous-distillation, dry-distillation, and molecular-distillation (Koedam, 1987).

All these conventional extraction techniques have important drawbacks, such as low yields, formation of by-products, and limited stability. For steam-distillation and hydrodistillation methods, the steam is percolated through the flask with plants from the bottom and the oil evaporates. The emerging mixture of vaporized water and oil moves through a coil usually cooled with running water, where the steam is condensed.

The mixture of condensed water and essential oil is collected and separated by decantation or, in rare cases, by centrifugation. The elevated temperatures and prolonged extraction time can cause chemical modifications of the essential oil components and often a loss of the most volatile molecules. Manufacturers use dry distillation to extract high-boiling-point oils from wood. In this process, heat is applied usually as a direct flame to the vessel containing plant material. The high temperatures release essential oils by evaporation. The vapor is piped away and condensed to give a mixture of liquid oils. Some of the components in the plant material get degraded (pyrolyze) at the high temperatures used and add burned, smoky notes to the odor of the oil.

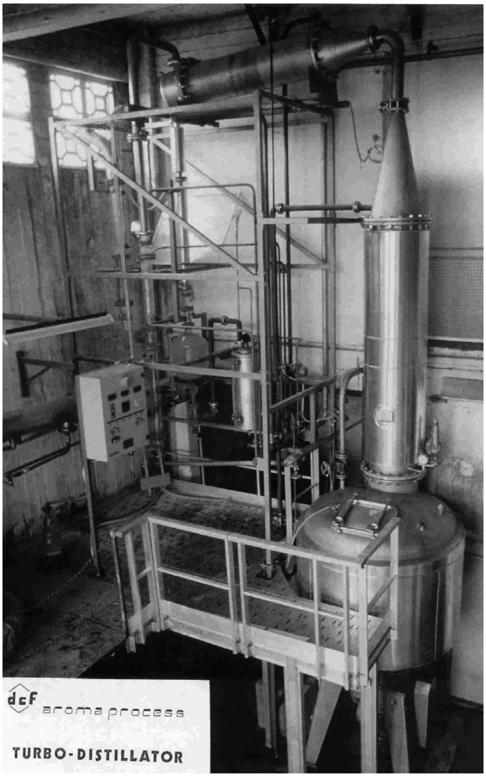

Figure 2.3. Turbodistillation of citrus essential oils.

With the increasing energy prices and the drive to reduce CO_2 emissions, chemical and food industries are challenged to find new technologies in order to reduce energy consumption, to meet legal requirements on emissions, product/process safety, and control, and for cost reduction and increased quality as well as functionality. Extraction and distillation technologies are the promising innovation themes that could contribute to sustainable growth of chemical and food industries. For example, existing extraction technologies have considerable technological and scientific bottlenecks to overcome—often requiring up to 50% of investments in a new plant and more than 70% of total process energy used in food, fine chemicals, and pharmaceutical industries. These shortcomings have led to the consideration of the use of new "green" techniques in separation, which typically use less solvent and energy, such as microwave extraction, supercritical fluid extraction, and ultrasound extraction. Separation under extreme or nonclassical conditions is currently a dynamically developing area in applied research and industry.

2.2 SOLVENT- AND WATER-FREE MICROWAVE EXTRACTION

Farid Chemat

Microwave energy is well known to have a significant effect on the rate of various processes in the chemical and food industries. Much attention has been given to the application of microwave dielectric heating for the extraction of natural products that typically need hours or days to reach completion with conventional methods. Using microwaves, fully reproducible extractions can now be completed in seconds or minutes with high reproducibility, reducing the consumption of solvent, simplifying manipulation and workup, giving higher purity of the final product, eliminating post-treatment of waste water, and consuming only a fraction of the energy normally needed for a conventional extraction method such as steam distillation or solvent extraction. Several classes of compounds such as essential oils, aromas, pigments, antioxidants, and other organic compounds have been extracted efficiently from a variety of matrices (mainly animal tissues, food, and plant materials) (Paré and Bélanger, 1997).

Microwave extraction is a research area that has an impact in several fields of modern chemistry. All the reported applications have shown that microwave-assisted extraction is an alternative to conventional techniques for such matrices. The main benefits are decrease of extraction time and solvents used. The advantages of using microwave energy, which is a noncontact heat source, for the extraction of essential oils from plant materials include more effective heating, faster energy transfer, reduced thermal gradients, selective heating, reduced equipment size, faster response to process heating control, faster startup, increased production, and elimination of process steps. Extraction processes performed under the action of microwave radiation are believed to be affected in part by polarization, and volumetric and selective heating (Metaxas and Meredith, 1983).

Microwave-assisted solvent extraction (MASE) was first used in the extraction of several compounds from food products (citrus, aromatic plants, cereals, etc.). Numerous classes of compounds such as aromas, antioxidants, colors, biophenols, and other secondary and primary metabolites have been extracted efficiently, in terms of rapidity and reproducibility, from a variety of matrices. The technique was patented in 1990 as Microwave Assisted Process (MAP) (Paré and Bélanger, 1990). Typically, plant material is immersed in a nonabsorbing microwave solvent such as hexane and irradiated by microwave energy. When the oil glands of the plant are subjected to severe thermal stresses and localized high pressures, as in the case of microwave heating, the pressure buildup within the glands exceeds their capacity for expansion, and causes their rupture more rapidly than in conventional extraction. Volatile oil dissolves in the organic solvent before being separated by liquid–liquid extraction. In all cases, yields and composition of microwave extracts are comparable to those obtained by a classical solvent extraction such as Soxhlet extraction but have been achieved with reduced extraction time (Paré and Bélanger, 1997).

Vacuum Microwave Hydro Distillation (VMHD) was elaborated and patented by Archimex in 1994 (Mompon and Mengal, 1994). This technique is based on selective heating by microwaves combined with application of sequential vacuum. The plant material is placed in a microwave cavity with water to refresh the dry material. The plant material is afterward exposed to microwave radiation to free the natural extract. Reducing the pressure to between 100 and 200 mbar allowed the evaporation of the azeotropic water-volatile oil mixture from the biological matrix. The procedure is repeated in a stepwise fashion in order to extract all the volatile oil from the plant. Up to 30 kilograms per hour of material can be treated (Figure 2.4).

Solvent Free Microwave Extraction (SFME) is a recent method of extraction patented in 2004 and specifically aimed at obtaining essential oil from plant material (Chemat et al., 2004). SFME apparatus is an original combination of microwave heating and distillation at atmospheric pressure (Figure 2.5). SFME was conceived for laboratory-scale applications in the extraction of essential oils from different kinds of aromatic plants and fruits. Based on a relatively simple principle, this method involves placing 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. Thus, this process frees essential oil that is evaporated by the in-situ water of the plant material. A cooling system outside the microwave oven condenses the distillate continuously. The water excess is refluxed to the extraction vessel in order to restore the in-situ water to the plant material. The effect of SFME on the kinetic of extraction is illustrated in Figure 2.6. Microwaving offered advantages such as less extraction time, less solvent consumption, high efficiency, and high yield and reproducibility; organoleptic properties of essential oil were also improved. Essential oils obtained by SFME from fresh orange peels were colorless with fresh, light, and sweet citrusy odor as compared to the pale-yellow oil with pungent smell extracted with traditional methods. Higher abundance of oxygenated compounds in essential oil is related to the rapid heating of polar substances by microwaves, and only in-situ water was used, which prevented the decomposition of principal oxygenated constituents by thermal and hydrolytic reactions. The SFME method also offers a reduced environmental burden as it rejects less CO_2 in atmosphere (200 g CO_2 per gram of essential oil compared to the traditional method, which was rejecting 3600 g CO_2 per gram of essential oil) (Ferhat et al., 2006, 2007; Lucchesi et al., 2004a, 2004b).

Microwave Hydrodiffusion and Gravity (MHG) (Chemat et al., 2008a) is a new and green technique for the extraction of essential oils discovered at the Laboratory of Green Extractions (Université d'Avignon–France) in 2008 (Figure 2.7). This green extraction technique is an original "upside-down" microwave alembic combining microwave heating and earth gravity at atmospheric pressure. MHG was conceived for laboratory and industrial-scale applications for the extraction of essential oils from different kinds of aromatic plants. Based on a relatively simple principle, this method involves placing plant material in a microwave reactor, without adding any solvent or water. The internal heating of the

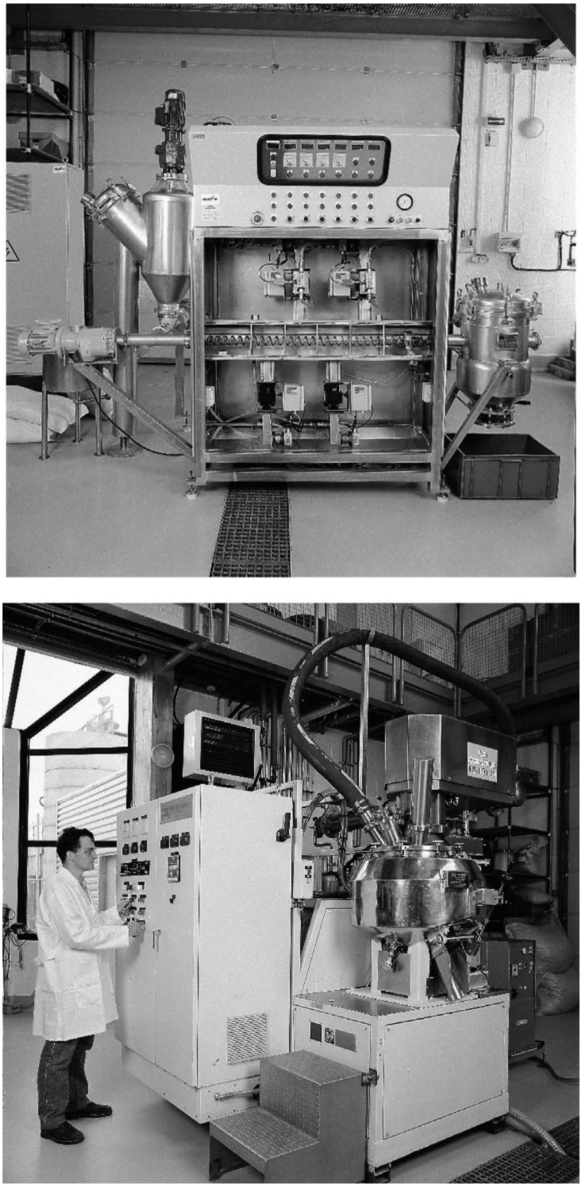

Figure 2.4. MASE (100 kg/h) and VMHD (100 liters).

in-situ water within the plant material distends the plant cells and leads to the rupture of glands and oleiferous receptacles. The heating action of microwaves thus frees essential oil and in-situ water, which are transferred from the inside to the outside of the plant material. This physical phenomenon, known as *hydro-diffusion*, allows the extract (water and essential oil), diffused outside the plant