Chapter 3
Green Separation Technology in Food Processing: Supercritical-CO_2 Fluid Extraction

Xingqian Ye, Sophia Jun Xue and John Shi

Abstract One of the most important trends in the food industry today is the demand for "natural" foods and ingredients that are free from toxic-chemical additives. Supercritical-CO_2 fluid extraction is considered as one of the "green" and environmentally friendly separation technologies that have emerged as attractive alternatives to traditional methods for the concentration of bioactive compounds. The greatest advantage of supercritical-CO_2 fluid extraction is that it is rapid and highly selective with shorter extraction times than traditional methods. It is particularly favorable for the extraction of thermally labile bioactive substances that easily degrade when subjected to traditional extraction techniques. Supercritical-CO_2 fluid extraction technology is available in the form of a single stage batch process, and could be scaled up to a multistage semi-continuous batch coupled with a multi-separation process. With improved processing conditions and reduced cost, supercritical-CO_2 fluid extraction will become even more economical at low throughput. Extracts from natural sources are key elements in the manufacturing of health-promoting functional foods and ingredients. Thus, the development and use of "green" separation processes and technologies is likely to continue to be widely employed in the processing of bioactive components, especially for use as supplements for health-promoting foods.

Keywords Separation · Extraction · Bioactive compounds · Supercritical-CO_2 extraction · Health-promoting compounds

3.1 Introduction

Separation technology is used to recover high-value components from agricultural commodities, as an important operation for the production of food products such as oil and proteins. It is also especially important for the development of health-

X. Ye (✉)
School of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, China
e-mail: psu@zju.edu.cn

S. J. Xue · J. Shi
Guelph Food Research Center, Agriculture and Agri-Food Canada, Ontario, Canada

J. Jia et al. (eds.), *Advances in Food Processing Technology*,
https://doi.org/10.1007/978-981-13-6451-8_3

promoting food ingredients and high value-added food products such as antioxidants and flavors. Separation processes such as extraction, concentration, purification, and fractionation of bioactive components (phytochemicals) from agricultural materials are the main processes used to obtain high-value end products which may become wellness ingredients for use in functional foods and nutraceuticals. Many potential high-value products can be developed from natural resources by different separation technologies and processes. Carotenoids including lycopene, β-carotene, astaxanthin, and lutein make up a global market nearing $1 billion with a growth rate of approximately 3%. Therefore, efforts to utilize natural agricultural materials for the production of high value-added products, especially health-promoting foods and ingredients, are of great interest to the food and biotechnology industries.

Traditional extraction techniques employ large amounts of toxic organic solvents for removing targeted components from plant materials. In many countries, to address safety, health, and environmental issues, strict regulations regarding the use of organic solvents (i.e, hexane) are forcing the food industry to search for alternative processes. Furthermore, increasing consumer awareness of the presence and use of organic chemical solvents in food, and the desire to buy natural products, is encouraging the food and natural products industry to develop and commercialize "green" technology. Recently, there has been considerable interest from the scientific community, resulting from increasing industrial demands, in the research and development of extraction and separation technologies. The aim is to eliminate the use of organic chemicals, as these products increasingly being used in producing functional ingredients and natural products (e.g., nutraceuticals and supplements). Supercritical-CO_2 fluid extraction technology is one of the possible alternative methods for providing a "green" processing technique for food processing applications. The "green" separation technologies and processes provide detailed information on the equipment selections, system design, and methods for extraction and purification of multiple classes of phytochemicals from plant materials while retaining, or even improving their bioactivity and functionality.

3.2 "Green" Separation Process Design

All over the world, there is pressure for the industry to adopt new sustainable separation processes that do not require the use of environmentally damaging organic solvents. Separation is an interphase mass transfer process because it involves heat, mass, and phase transfers, as well as chemical reactions among the components of the plant materials. The engineering processes for separation systems include modeling, simulations, optimization control studies, and thermodynamic analysis. The principles of mass conservation and component transfer amounts are used to analyze and design industrial processes. Proper understanding of molecular properties and thermodynamics constitute powerful tools for the design of successful separation processes. Moreover, the design of a separation process is strongly dependent on the phase equilibrium scenario, which is highly sensitive to changes in operating

conditions. Thus, phase equilibrium engineering plays a key role in the synthesis and design of these processes. The applied knowledge necessary for system design is comprised of data banks, experimental data, phenomenological phase behavior, thermodynamic analyses, mathematical modeling procedures for phase equilibrium process calculations, mass and heat transfer analyses, the characteristics of the targeted components, and the effects of the processing conditions.

3.2.1 Technical Requirements for a Separation System

The design of a separation process depends on the separation to be performed and the properties of the materials used, as well as the targeted bioactive components. An important consideration, in determining how appropriate a separation technique and system are, is the purity requirements for the end products. In most organic solvent (toxic-chemical-free) separation systems, a combination of new techniques is necessary for system optimization.

Product design is related to reasonable separation and purification steps, economic feasibility, and raw material selection. "Green" separation processes are environmentally friendly processes that result in less air pollution and industrial waste (e.g., energy, greenhouse gas emission, and reduction of waste water production). The following issues may be involved in the consideration of a potential system designs:

(a) Knowledge of phase equilibrium, mass transfer rate, and solubility data are important for scaling up the extraction process and equipment.
(b) Information and experimental data on the effects of processing conditions on the physicochemical properties and degradation of the bioactivity of compounds are important for the design of a suitable extraction system and the procedure.
(c) Proper solvent selection is based on the solubility characteristics of the targeted compounds which should readily dissolve in the extraction solvent, ideally to achieve as a substance pure as possible.
(d) A pump for transporting the solvent is required for recycling. An additional pump may be required for co-solvent incorporation during extraction.
(e) An extractor, which will be responsible for charging a solid material into a high pressure and potentially high temperature zone; a separator, which may involve changes of pressure and temperature; and a sample collector, should be considered during the designing process of the system.
(f) The capacities of the heat exchanger and condenser should also be considered. For example, the heat capacity of water is very large, so considerable effort is necessary to remove the excess stored energy.
(g) For the choice of a suitable pump and extraction capacity, the dimensions of the extraction vessel and the optimization of the processing parameters are required.
(h) Economics and safety should always be considered and may be the determining factor in designing a separation system.

3.2.2 Food Quality and Separation Systems

The major issues related to product quality after separation are the effects of processing on the bioactivity of extracts and the nutritional value of the end products, as well as specific quality characteristics. To comply with food safety regulations, no toxic-chemical solvent residues are permitted in the end products, e.g., "green" food products; all nutrition and health regulations must be met. Some other important requirements include high stability of nutrients and bioactive components, low operating temperatures to reduce thermal effects, the exclusion of light to reduce light-induced UV irradiation effects, and the exclusion of oxygen to reduce the effects of oxidation. The final products must maintain uniformity, quality, and consistency, as well as a purity that can meet food or pharmaceutical grade requirements.

3.2.3 Scaling Up Technology for Industrial Production

Scaling up an innovative separation process for large-scale manufacturing is essential in order to carry out the separation in a reproducible and consistent manner for commercial purposes, and to avoid potential exposure to biological or chemical post-translational modifications which could result in poor product quality. Avoidance of enormous variations from process to process necessitates attention to detail at all stages of product development. When the technology in a food process is designed for industrial-scale production, an important area for consideration is the balance of capital and operating costs as the scale of the operation increases. The process of scale up also involves optimization with respect to increasing the efficiency of each stage, giving rise to increased demands on the accuracy of the online quality control. The process must be reliable for industrial-scale production of food or pharmaceutical grade ingredients that will be used in a wide variety of applications including food, nutraceuticals, pharmaceuticals, and cosmetic products.

3.3 Supercritical-CO_2 Fluid Extraction

The extraction of health-promoting components from plant materials has usually been accomplished by conventional extraction processes such as solid–liquid extractions employing methanol, ethanol, acetone, or hexane, and also through steam distillation or evaporation processes to remove solvents from the extracts. Currently, the demand for natural bioactive compounds is increasing due to their use by the functional food and pharmaceutical industries. Thus, there has been increasing interest in the use of "green" separation technology able to provide high quality and high bioactivity extracts while precluding any toxicity associated with the solvents. Some of the motivations for employing "green" technology as viable separation techniques are:

tightening government regulations on toxic-chemical solvent residues and pollution control, consumers' concern over the use of toxic-chemical solvents during processing, and increasing demand for higher quality products which traditional processing techniques cannot achieve.

One of the most important considerations in developing new extraction processes is safety. In this sense, a variety of processes such as supercritical-CO_2 fluid extraction, membrane-based separation, molecular distillation, and pressurized low-polarity water extraction, are generally recognized as "green" separation techniques, and are considered clean and safe processes which meet the requirements (Herrero et al. 2006; Chang et al. 2008). They have recently been developed and are regarded as emerging innovative separation technology that is able to meet food quality and safety requirements. These processes can be used to solve some of the problems associated with conventional organic solvent-oriented separation. Operation parameters and other factors related to the quality of the original plant material, including geographic origin, harvesting date, storage, and any pretreatment processes prior to extraction, also influence the separation operation and the final composition of the extracts obtained.

3.3.1 Principles and Properties of Supercritical Fluid Extraction

A gas or liquid is normally used as the extraction solvent for supercritical fluid extraction. When a gas or liquid is compressed and heated past its critical point, it enters the "supercritical phase"; in this state the extraction medium is called a "supercritical fluid" (SCF). The critical temperature (T_c) and pressure (P_c) at which this happens are unique to each pure substance. In the supercritical state, the SCF possesses properties of both gases and liquids. For example, the liquid-like density of an SCF provides its high solvent power whereas the gas-like viscosity and diffusivity, with zero surface tension, enhance the transport properties of the solvent during extraction. Therefore, these unique properties enable the SCF to penetrate into porous solid materials more effectively than a liquid solvent, resulting in faster mass transfer, therefore providing faster and greater extraction yields.

The extraction process can easily be adjusted by altering the pressure and temperature. However, operating pressures and temperatures above the critical point would affect the properties of the SCF such as its density, viscosity, diffusivity, heat capacity, and thermal conductivity, and would enhance the ability of the SCF to penetrate and extract the target molecules from the source material. One of the main characteristics of an SCF is the possibility of modifying its density by changing the pressure and/or temperature. Since density is directly related to solubility (Raventós et al. 2002; Shi et al. 2009a, b), by altering the extraction pressure, the solvent strength of the fluid can be modified. The power of a solvent's extracting ability increases with density at a given temperature, or with temperature at a given density.

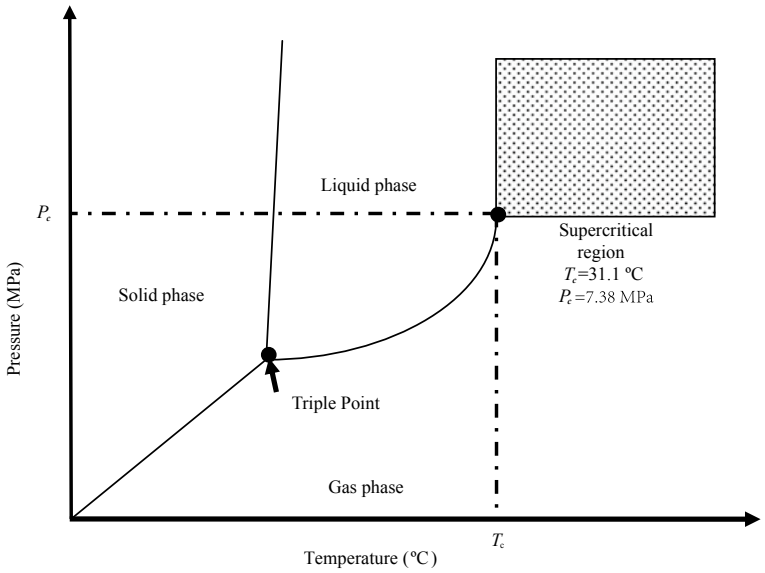

Fig. 3.1 Supercritical pressure–temperature diagram for carbon dioxide

Many solvents are candidates for SCF extraction. The most desirable SCF solvent for the extraction of natural products for food and medicine is carbon dioxide (CO_2), and this extraction process is called supercritical-CO_2 fluid extraction. The advantageous characteristics of CO_2 are that it is inert, nonflammable, noncorrosive, low cost, easily available, odorless, tasteless, and environmentally friendly, with relatively mild critical conditions of 7.38 MPa pressure (P_c) and 31.1 °C temperature (T_c) (Fig. 3.1). Its near-ambient critical temperature makes it ideal for thermolabile natural products (Mendiola et al. 2007) and because it is a gas at room temperature, once the extraction is completed, a substantial elimination of CO_2 without residues can be achieved by simply decompressing the system, yielding a solvent-free extract. However, CO_2 is not a perfect solvent for high molecular weight and polar compounds. Small amounts (ranging up to 20, molar fraction) of polar or nonpolar co-solvents called modifiers can be incorporated to increase the solubility of such compounds during supercritical-CO_2 fluid extraction.

Carbon dioxide, therefore, has favorable properties including the ease of changing selectivity by the addition of a relatively small amount of modifiers such as ethanol and other polar solvents (e.g., water). As a result, CO_2 is considered to be the most desirable SCF for extracting natural products for food and medicinal applications (Shi et al. 2007a, b, c; Kassama et al. 2008; Yi et al. 2009). Other SCFs, such as ethane, propane, butane, pentane, ethylene, ammonia, sulfur dioxide, water, and chlorodifluoromethane, are also used for supercritical fluid extraction.

In basic terms, the SCF extraction system consists of pumps for delivering the solvent and co-solvent throughout the system and for raising the pressure of the recy-

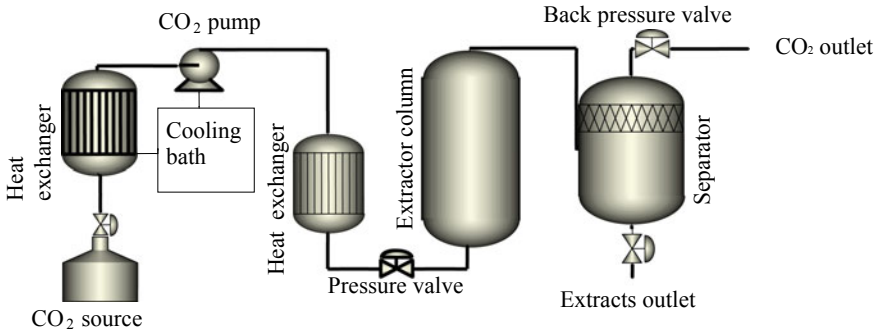

Fig. 3.2 Schematic diagram of a typical single stage supercritical fluid extraction system with CO_2

cled solvent, a high-pressure extractor, a pressure reduction valve, heat exchangers, compressors, and one or more separators in which the extract is collected and the solvent (e.g., CO_2) is depressurized and removed (Fig. 3.2). The extraction cells and the separators are commonly equipped with independent controls for temperature and pressure, thus the fractionation of the extracted compounds can be carried out by stepwise decompression. This means that different compounds can be collected in separators, depending on their differential solubility in the SCF. Additionally, it is possible to install a system to recycle the fluid employed. For batch and single stage modes, the raw materials are usually ground up and charged into a temperature controlled extractor, forming a fixed-bed (Shi and Zhou 2006). The process of SCF extraction requires intimate contact between the packed beds formed of ground solid substratum (fixed-bed of extractable material) with a supercritical-CO_2 fluid which is fed into the extractor through a high-pressure pump. During the extraction process, the solid phase comprised of the solute and the insoluble residuum (matrix) is brought into contact with the fluid phase, which is the solution of the solute in the supercritical-CO_2 fluid (solvent). The extracted material is then conveyed to a separation unit via a pressure reduction valve. At reduced temperature and pressure, the extract precipitates in the separator while the CO_2, free of extract, is recycled to the extractor.

The physicochemical properties of supercritical-CO_2 greatly influence the solvent strength and the solubility of the target compounds in the fluid. In supercritical-CO_2 fluid extraction, the physicochemical properties of supercritical-CO_2, such as the density, diffusivity, viscosity, and dielectric constant, can be controlled by varying the operating pressure, temperature, or both in combination (Tena et al. 1997). Thus, the separation process can be affected by simply changing the operating pressure and temperature to alter the solvating power of the solvent. After modifying CO_2 with a co-solvent, the extraction process can significantly enhance selectivity and separation power, and in some cases, it can even extend the solvating power to polar components.

Many challenges to the design and development of commercially viable SCF extraction processes for natural products remain. These challenges include the need for a better understanding of the phase behavior and solubility of multi-bioactive component mixtures in supercritical-CO_2 fluids. In addition, there is much need for the generation of fundamental data, including solubility, density, and interfacial tension, as well as changes in mass transfer phenomena under different operating conditions such as temperature, pressure, flow rate, and the effects of the composition and proportion of co-solvents (Lucien and Foster 2000; Marcus 2006). Solvent power is related to the density of the supercritical-CO_2 fluid, and it can be varied by changing the operating conditions, mainly temperature, pressure, and flow rate. Generally, density decreases with increasing temperature at a constant pressure, and pressure has more pronounced effects on the changes in density at a constant temperature. Shi et al. (2007a, c) observed that, with an increase in density, the solvating power of CO_2 increased lycopene extraction from tomato skins. Moreover, the region above the critical point provides the greatest density changes, and thus for even the slightest changes in pressure and temperature, the density of CO_2 will effectively change the operating variables (pressure and temperature) in this zone. In the region below the supercritical point, the density of CO_2 changes, but it does not vary substantially with the changes in pressure and temperature.

Targeted compounds have different solubilities in supercritical-CO_2 and that greatly influences the extraction efficiency and bioactivity of the extracts (Lucien and Foster 2000). The impact of solubility enhancement on selectivity has been assessed, and some opportunities for improving the selectivity of extraction have been highlighted and extensively studied (Yi et al. 2009). Generally, the elevation of the temperature leads to an increase in the solubility of the target components in supercritical-CO_2. However, this also increases concern for the stability of bioactive extracts because the bioactivity of natural extracts may degrade when subjected to high processing temperatures.

Most of the investigations into solubility have been concerned with binary systems consisting of a single solute in contact with only supercritical-CO_2. In contrast, solubility data from multi-component systems have not been well established. This is important because intermolecular interactions between components can significantly alter the selectivity of the supercritical-CO_2. For example, in some studies, the addition of a co-solvent (modifier) to supercritical-CO_2 resulted in an enhancement of the solubility of the target component (Sauceau et al. 2004). Food grade solvents such as water, lipids, and ethanol can be used as co-solvents to increase solubility and enhance the extraction yield. Furthermore, not only the composition of the co-solvent, but also the proportion of co-solvent is considered to be a factor for system and process design, and for the optimization of operating conditions, as excess co-solvents may cause either negative, or negligible and noneconomic effects. Vasapollo et al. (2004) found that the presence of vegetable oil as co-solvent improved yields and contributed to the stability of lycopene. Shi et al. (2009a) investigated the effects of ethanol, water, and canola oil modifiers on the profile of lycopene extractions, and found that extraction efficiency was improved by the addition of any of the these modifiers, and that yields increased with increasing amounts of modifier

(from 5% to 10%). However, the rates of yield increase were lower when the ethanol concentration was increased from 10% to 15%.

3.3.2 Process Systems

Changes in food processing practices and new opportunities for innovative food products have spurred interest in supercritical-CO_2 fluid extraction. There are many advantages of using SCF instead of conventional organic solvents. These include achieving higher purity extracts, absence of toxic solvent residues, single-step processing, reduced operating costs, selective fractionation, faster separation, environmental friendliness, and physiological compatibility. In addition, the oxygen free operating system prevents oxidation, and the use of low temperatures minimizes thermal degradation of sensitive materials.

Due to consumer perception of the negative impacts of chemical solvent extraction, and the increasing demands for natural products from natural sources with no toxic-chemical contamination, there is world-wide pressure for industry to adopt new sustainable processes. Under such pressure, supercritical fluid-CO_2 technology has been developed and successfully used to extract essential oils, functional fatty acids, antioxidants, and other bioactive compounds, including the extraction and fractionation of carbohydrates (Glisic et al. 2007; Montañés et al. 2008, 2009; Mitra et al. 2009; Sanchez-Vicente et al. 2009; Shi et al. 2010a). It has been found to be particularly relevant in food and pharmaceutical applications that process and handle complex, thermo-sensitive, and bioactive components, which increasingly applies to production of nutraceuticals, flavorings, and other high-value items. Examples of these applications include the purification of solid materials, separations of tocopherols and antioxidants, removal of pesticide residues from herbs, production of medicines and food products, the detoxification of shellfish, the concentration of fermented broths, fruit juices, essential oils, spices, and coffee, as well as the separation of caffeine (Perrut 2000; González et al. 2002; Kassama et al. 2008; Martinez et al. 2008; Miyawaki et al. 2008; Liu et al. 2009a, b; Herrero et al. 2010).

Supercritical-CO_2 fluid extraction is governed by four key steps: extraction, expansion, separation, and solvent conditioning. These steps are accompanied by four generic primary components: extractor (high-pressure vessel), pressure and temperature control system, separator, and pressure intensifier (pump). The major process parameters are temperature, pressure, and flow rate. Most commercial operations for supercritical-CO_2 extraction of solid materials operate using a batch system (Fig. 3.2). Once the supercritical-CO_2 and the feed reach equilibrium in the extraction vessel, achieved through the manipulation of pressure and temperature to provide the ideal operating conditions, the extraction process proceeds. The mobile phase, consisting of the supercritical-CO_2 fluid and the solubilized components, is transferred to the separator where the fluid is then reduced through decrease of the pressure of the system. The extract precipitates in the separator while the supercritical-CO_2 fluid is either released to the atmosphere or recycled back to the extractor.

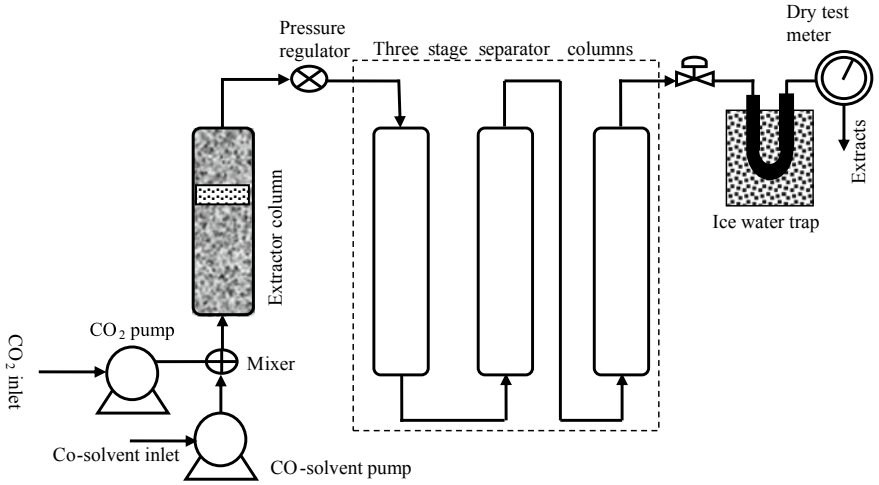

Fig. 3.3 Schematic diagram of a supercritical fluid extraction system used to fractionate bioactive components from a plant matrix using supercritical carbon dioxide

Recently, industry has focused on "fractional separation" where the natural materials are extracted under relatively severe pressure and temperature conditions to remove all of the desired components. The resulting fluid extract is then passed through a series of 2, 3, or 4 separator vessels in which the operating parameters (temperatures and pressures) are set to selectively precipitate one specific component (Fig. 3.3). This can create a range of unique fractions with new application potentials. For example, this fraction system is able to extract/fractionate a number of different carotenoids (e.g., β-carotene, lycopene), oleoresins, and colorants from tomatoes. High antioxidant activity phenolic compounds have also been extracted from rosemary leaves with supercritical-CO_2 (Chang et al. 2008; Huang et al. 2010; Shi et al. 2010b; Xiao et al. 2010). Rizvi and Bhaskar (1995) evaluated the feasibility of supercritical fluid processing of milk fat (i.e., fractionation, scale up, and economic aspects), and reported that scaling up a supercritical extraction processes was a practical approach.

In situations where highly volatile components are being extracted, a multistage configuration may have to be employed as shown in Fig. 3.4 (Kassama et al. 2008). The processes described above are semi-batch continuous processes, where the supercritical-CO_2 flows in a continuous mode while the extractable solid feed is charged into the extraction vessel in batches. In a commercial-scale processing plant, multiple extraction vessels are sequentially used to enhance the process performance and output. Semi-continuous extractor designs allow intermittent loading and unloading of solid material through lock-hopper vessels, reducing downtime and improving production efficiency. The need to improve the design to create truly continuous modes is growing. Supercritical-CO_2 fluid extraction could be cost effective under large-scale production conditions.

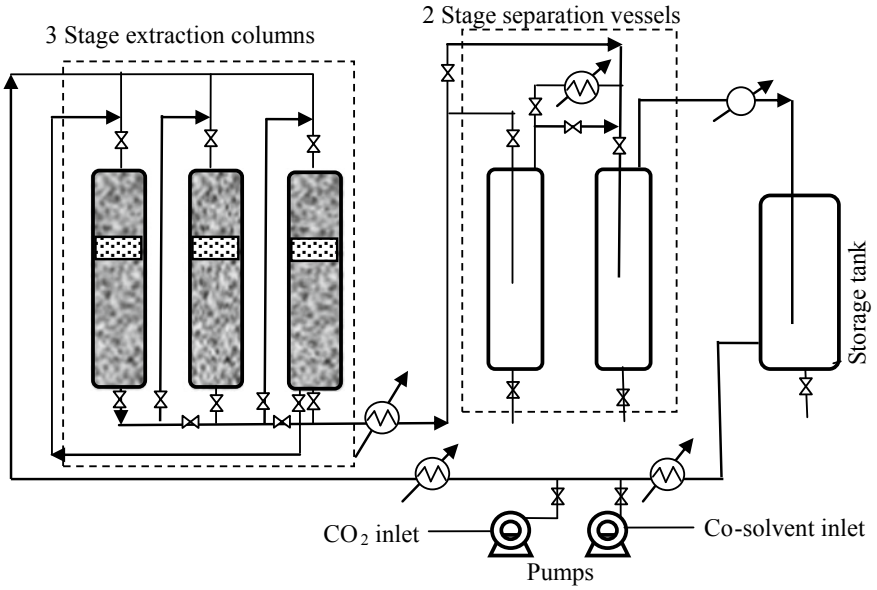

Fig. 3.4 Schematic diagram of a commercial-scale multistage supercritical fluid extraction system used to fractionate bioactive components. (The symbols "⏦" and "⧗" represent pressure valves and heat exchangers, respectively)

One of the main processing aspects that should be considered in SCF extraction is extraction optimization. The use of optimum conditions for the different variables influencing extraction could significantly enhance the recovery or extraction yield of the target compounds. With the aim of effectively optimizing these variables (extraction temperature, pressure, time, type, percentage of modifiers, sample size, etc.), different approaches have been applied. Appropriate experimental designs and statistical modeling should be used to optimize these processes as the compounds of interest will each have their own unique characteristics and are likely to require different specific temperature and pressure combinations. Understanding these specific characteristics; the physicochemical properties of the targeted bioactive compounds; the effects of the parameters influencing extraction efficiency, bioactivity, and cost; and determining the optimum parameters required to maximize the yields and bioactivity of the targeted components, can help to establish the optimal conditions for large-scale processing. One of the major advantages of superficial-CO_2 fluid extraction is that it eliminates the refining process which is otherwise required to remove undesirable compounds before consumption when conventional oil extraction techniques are used. If some valuable compounds are contaminated, they can also be lost during the refining process. By using supercritical fluid extraction, extracts can be obtained which are enriched with the particular compounds of interest, for example wheat germ oil (Eisenmenger and Dunford 2008) and rice bran oil (Soares et al. 2016).

Supercritical fluid extraction has attracted a great deal of interest in recent times as a "green" processing technology and is increasingly being used in food, natural products, and pharmaceutical applications. It is of particular interest due to its environmental benefits. Furthermore, it provides flexibility as the conditions can be optimized and various modifiers can be added to obtain selective fractionation of target compounds.

3.4 Applications in Food Industry

One of the most important trends in the food industry today is the demand for all-natural food ingredients that are free of chemical additives. Natural antioxidants for food are made from derivatives of plant byproducts. A major advancement in supercritical-CO_2 fluid extraction technology was made by its application to the decaffeination of coffee, tea, and other bioactive components (essential oils from spices) used as ingredients in food. Likewise, supercritical-CO_2 fluid extraction is used to extract flavor, fragrance, and high-value compounds used as ingredients in food, pharmaceutical, and nutraceutical products.

Large-scale supercritical-CO_2 fluid extraction has become practical for the extraction of high-value products from natural materials. The solvating power of supercritical-CO_2 fluid is sensitive to temperature and pressure changes. Thus the extraction parameters may be optimized to provide the highest possible extraction yields of the target components with maximum bioactivity (Kassama et al. 2008; Shi et al. 2009c; Yi et al. 2009). Although a temperature rise in the extraction process can increase the solubility of components in supercritical-CO_2 fluids, any negative effects on thermally labile target components should be considered. The intensity and duration of heat processing can affect the health-promoting properties of bioactive components. Therefore, ideally the extraction time and temperature should be minimized which also leads to a more economically viable process. Excessively high flow rates may reduce the contact time between the solute and the solvent and restrict the fluid flow in the sample if it becomes compacted. The optimal flow rate appears to vary with the targeted molecule and relatively high flow rates may have a negative effect on the extraction of some components. Under consistent flow rates and operating temperatures, increasing pressure can significantly improve extraction yields. Most supercritical-CO_2 applications extract natural products or bioactive compounds at pressures between 20 and 50 MPa, and at temperatures between 40 and 80 °C. Because of the poor solubility of some bioactive substances in supercritical-CO_2, food and/or pharmaceutical grade modifiers are sometimes added, in proportions between 3% and 20%, to help with extracting more polar compounds from plant materials.

Large--scale supercritical-CO_2 fluid extraction has become a reality for the extraction of high-value products from natural materials. The solvating power of supercritical-CO_2 fluids is sensitive to temperature and pressure changes; thus, the extraction parameters may be optimized to provide the highest possible extraction

yields with maximum antioxidant activity (Kassama et al. 2008; Shi et al. 2009c; Yi et al. 2009). With this innovative technology, a process could be designed to extract natural nutrients without the fear of organic solvent residues. A compendium of process parameters used for different product applications is listed in Table 3.1.

3.4.1 Extraction of Bioactive Compounds

Most separation procedures involve physical and chemical processes such as centrifugation, filtration, membrane separation, precipitation, chromatography, solvent extraction, crystallization, evaporation, molecular distillation, and supercritical-CO_2 fluid extraction. To overcome the detrimental effects of conventional extraction techniques, a rapid separation process is needed to avoid significant loss of quality or stability of the natural components. Most bioactive components used as functional food additives are used in concentrated form and consequently, appropriate extraction procedures are required when removing them from their original matrices. Some compounds are thermolabile, volatile, and prone to degradation when they are subjected to intensive heat in their concentrated form.

Supercritical extraction with CO_2 is the most viable method for food applications. Baysal et al. (2000) extracted lycopene and β-carotene from tomatoes using supercritical-CO_2 fluid. The processing conditions used were pressures of 20, 25, and 30 MPa; temperatures of 35, 45, 55, and 65 °C; resident times of 1, 2, and 3 h; and CO_2 flow rates of 2, 4, and 8 kg h^{-1}. The best conditions for lycopene extraction were 2 h at a flow rate of 4 kg h^{-1}, pressure of 30 MPa, temperature of 55 °C, and with the addition of 5% co-solvent (ethanol). They noted that, too much ethanol decreased the homogeneity of the extraction mixture, and reduced the separation efficiency.

Yi et al. (2009) investigated the effects of supercritical-CO_2 fluid extraction parameters on the antioxidant activities of lycopene extracts from tomato skins, and found that the activity of lycopene extracts differed with the yield. For each unit of lycopene extract, the antioxidant activity level was constant below 70 °C, but then gradually decreased above 70 °C due to isomerization of the lycopene which occurs as a result of the higher temperature. The ratio of all-*trans*-lycopene to the *cis*-isomers changed from 1.70 to 1.32 when the operating temperature increased from 40 to 100 °C. No significant effects of pressure or flow rate on the antioxidant activity were observed.

Shi et al. (2009a, b) found that the optimized conditions for lycopene extraction to achieve higher yields with maximum anti-oxidative properties were 70 °C and 30 MPa, with a flow rate of 1.5 mL min^{-1} using an I-L separation cell. Optimum process parameters of 56 °C and 26 MPa with an extraction time of 4 h were reported for the extraction of passion fruit seed oil with a good yield of 25.8%; yields of 89.4% and 72%, were achieved for high unsaturated fatty acids and linoleic acid, respectively (Liu et al. 2009a). Aromatic plants are mostly used as raw materials from which to extract natural antioxidants. Yépez et al. (2002) obtained high yields of odorless and flavorless extracts with high antioxidant activity from coriander (*Coriandram stivum*) under moderate conditions of 45 °C and 17.7 MPa. Ribeiro et al. (2001)

Table 3.1 Supercritical extraction process parameters for some selected bioactive components from agricultural material by supercritical-CO_2 fluid extraction

Product extracted	Raw material	Raw material Pretreatment	Component concentration (%)	Temp. (°C)	Pressure (MPa)	CO_2 flow rate (L r^{-1})	Time (h)	MC (%)	Co-solvent	Recovery (%)	Source
Wheat germ oil	Wheat germ	Milled, powder (20 μm diameter)	10.2	35–50	13–41	0.12	2	4.3–11.5		98.7	Ge et al. (2002)
Essential oil piperine	Black pepper	Dried ground	1.5 5.7	40	9–15	2.55		9		18	Perakis et al. (2005)
Essential oil	Eucaliptus leaves	Air drying		50	9	1.1	2.5	9.5		2.4	Porta et al. (1999)
Essential oils gingeroles	Ginger rhizomes	Dried ground	30	40	30	138	2	8.8		8.4	Catchpole et al. (2003)
Triglycerides carotenoid	Palm oil fiber	Oven dried		45–55	20–30	1.45	2.25	5		7	Franca and Meirele (2000)

(continued)

Table 3.1 (continued)

Product extracted	Raw material	Raw material Pretreatment	Component concentration (%)	Temp. (°C)	Pressure (MPa)	CO_2 flow rate (L r^{-1})	Time (h)	MC (%)	Co-solvent	Recovery (%)	Source
Essential oil Capsaicine alkaloids	Chili pepper	Dried ground	2	40	30		2	8.8			Catchpole et al. (2003)
Essential oil	Coriander	Dried grounds (0.4 mm)	96	50	15	3	3			0.61	Anitescu et al. (1997)
Antioxidants	Sweet Thai Tamarinds	Dried ground (mesh 40–70)		35–80	10–30	0.3	5		Ethanol		Luengthanaphol et al. (2004)
Essential oil	Grape seed	Wash dry ground	80	35–75	20–47		0.75	10	2% Ethanol	77	Lee et al. (2000)

found that high antioxidant activity in extracts from lemon balm (*Melissa officinalis* L.), was achieved by optimizing the extraction conditions at 10 MPa and 35 °C for 4 h. Hadolin et al. (2001) found that extraction conditions of 60 °C and 20 MPa produced the most concentrated vitamin E-rich oil from *Silybum marianum*, with a relatively high extraction yield. Perretti et al. (2007) demonstrated the fractionation of fish oil containing a high fraction enriched in ω-3 fatty acids and with a suitable EPA/DHA ratio. These results highlight the possibility of modifying the fatty acid ethyl ester composition of extracts by optimizing the extraction conditions in terms of pressure, temperature, and flow rate.

Tsuda et al. (1995) and Luengthanaphol et al. (2004) compared supercritical-CO_2 fluid extraction to other extraction methods, in terms of their effects on the bioactivity of the extracted compounds. The studies illustrated the superiority of antioxidants extracted with supercritical-CO_2 fluid and modifiers, although some disparity occurred which could have been caused by the varieties used. Macias-Sanchez et al. (2005) extracted carotenoids and chlorophyll from *Nannochloropsis gaditana* and achieved the highest yield at 20 MPa and 60 °C. Wang et al. (2005) also reported that the antioxidant activity of *Bupleurum kaoi* fractionated with supercritical-CO_2 fluid gave the highest yield of phenol and the strongest antioxidant capacities.

Extraction and fractionation of carbohydrates by supercritical fluid extraction have been also been recently reported. The extractions involve the use of CO_2 with a relatively low amount of polar modifier to effectively fractionate the carbohydrate extract formed by lactose and lactulose. Montañés et al. (2008) used a full factorial experimental design to evaluate the effects of extraction pressure, temperature, proportion of modifier, and flow rate. They later reported the optimum conditions for processing to be 100 bar, 100 °C, and 0.2 mL min^{-1} with 4% modifier (Montañés et al. 2009). Supercritical fluid extraction has also been widely employed for the extraction of aromatic compounds. The application of mild pressures and temperatures (100 bar and 40 °C) allowed the highest concentration of aromatic compounds to be extracted from sugar cane (Gracia et al. 2007).

3.4.2 Fractionation of Flavors and Fragrances

The extraction of flavor compounds and fragrances by supercritical-CO_2 is paramount in the food industry. Mother Nature is a splendid synthesizer of flavors and fragrances in natural products. The fact that it is cleaner and safer makes supercritical technology an ideal candidate for extracting such valuable and heat sensitive products in contrast to toxic organic solvents. The high value-added natural products are good for use in soft drinks; one example is ginger extract which gives the pungency and flavor to ginger ale (Fasoli et al. 2012).

Bhattacharjee et al. (2003) compared Likens-Nickerson extraction and supercritical-CO_2 fluid extraction methods on Basmati rice. They reported that supercritical fluid extraction was superior and that the extract of the flavor components was purer and bore the closest resemblance to the original Basmati flavor (Liken-

Nickerson method). A desired fragrance is isolated from concentrates extracted from flowers using a several-stage process. This process consists of initial solvent extraction, usually with an organic solvent (hexane), which yields an intermediate product called concrete (Reverchon and Poletto 1996). This product contains fragrances and other components such as paraffin, fatty acids, fatty acids methyl ester, *di*-, and *tri*-terpenic compounds, and pigments. The post processing of the concrete can be done using supercritical-CO_2 fluid extraction. Reverchon (1997) used single step supercritical-CO_2 fluid extraction, at a pressure of 8 MPa and temperature of 40 °C, followed by a two-stage fractional separation procedure, with the first separator at 9 MPa and −5 °C, and the second at 1.5 MPa and 10 °C. These conditions allowed highly efficient fractionation. The first stage was used to remove cuticular waxes. Under these optimum conditions the extracted volatile rose oil contained 50% 2-phenylethanol. When a co-solvent (ethanol) was mixed with supercritical-CO_2 fluid, a yield of 50%–60% was obtained (Sastry and Mukhopadhyay 1994). Jasmine fragrance extracted at 12 MPa and 40 °C gave superior results compared to other solvents. Sastry and Mukhopadhyay (1994) experienced an increase in yield from 45% to 53% with the use of co-solvents. Similarly, supercritical-CO_2 fluid has been used effectively to extract fragrances from orange, marigold, sandalwood, and vetiver.

3.4.3 Cholesterol Free Food Products

Cholesterol is an inevitable substance required for the daily maintenance of the human body. Lipoproteins are vehicles that transport cholesterol to various bodily tissues to be used, stored, or excreted. High density lipoprotein (HDL) termed "good cholesterol" transports cholesterol back to the liver, where endogenous metabolism prevents cholesterol buildup and reduces the risk of heart disease. Low-density lipoprotein (LDL) termed "bad cholesterol" causes fat buildup in the arteries, increasing the risk of coronary heart disease (atherosclerosis). The indiscriminate consumption of saturated fats in our diet may raise the total LDL above 100 mg/dL and decrease HDL below 35 mg/dL, thus increasing the risk of heart disease. The recommended daily intake of cholesterol is about 300 mg (James and Ralph 2000). The correlation between serum cholesterol level and mortality rate associated to cardiovascular disease has been reported in many studies (Griffin 1999).

Pork meat has cholesterol content about 30–450 mg per 100 g, for poultry this is 70 mg per 100 g, fish is 35–70 mg per 100 g, and beef is 65–331 mg per 100 g. One common source of cholesterol is from the consumption of fried fast-food products (French fries, onion ring, chicken nuggets, etc.). The fast-food industry uses hydrogenated fats for their deep fat frying processes because of its stability and high economic turnover. Hydrogenated fat is a potential source of *trans*-fatty acids which are taken up by food during cooking and ultimately ingested by the consumer. *Trans*-fats have been shown to increase LDL cholesterol levels and reduce HDL cholesterol levels, thus raising the risk of heart disease. Public health initiatives such as the National Cholesterol Education programs have raised consumer awareness,

resulting in increasing advocation for healthy foods with low cholesterol. Thus, the food industry is under tremendous pressure to address this consumer concern.

Supercritical-CO_2 fluid extraction has the potential to revolutionize the oil/fat industry. Many researchers (Dunford and Temelli 1995) reported the feasibility of supercritical fluid extraction of lipids from food without compromising their organoleptic quality. Chao et al. (1993) used a similar extractor configuration to the one discussed earlier with three stage separation to remove cholesterol. They applied pressure at 17, 11, and 4 MPa for each stage sequentially, and were able to achieve higher selectivity for cholesterol at the lower pressures. The results also showed that the fractions collected from the separator at 4 MPa contained cholesterol concentrations of approximately 272–433 mg per 100 g of lipid. Furthermore, Chao et al. (1993) used an operating pressure between 10 and 30 MPa, and temperature range of 30–50 °C to reduce the cholesterol level in ground beef.

Hardardottir and Kinsella (1988) also explored the removal of lipids and cholesterol from fish muscle with supercritical-CO_2 fluid. They removed between 80% and 99% of the cholesterol using fluid pressures of 14–35 MPa and temperatures of 40–50 °C. Although the authors noted limited effect on the lipid/cholesterol yield with increased extraction pressure and temperature, increasing the extraction time from 3 to 9 h significantly ($P < 0.05$) increased the yield. Yeh et al. (1991) used eight operating conditions (pressures from 10.3 to 3.8 MPa, temperatures from 40 to 55 °C, and CO_2 density from 342.3 to 723.4 g L^{-1}) to optimize their process, and observed that at 10.3 MPa and 55 °C the cholesterol level was reduced from 2867 mg per 100 g to 14.1 mg per 100 g. Supercritical-CO_2 fluid technology was used to fractionate milk fat, which is an excellent raw material with specific functionalities used in many products (Rizvi and Bhaskar 1995). Extracting cholesterol from anhydrous milk fat with supercritical-CO_2 fluid, used in conjunction with adsorbents (silica gel) to maximized yield was demonstrated by Huber et al. (1996).

3.4.4 Separation of Spices and Essential Oils

Spices have strongly flavored or aromatic components that can be used in small quantities in food as a preservative or flavoring ingredient. Chilli (*capsicium species*), ginger (*Zingiber officinalis*), and pepper (*piper nigrum* L.) are classic pungent flavorings while ginger and chili have additional nutraceutical values. These products have high economic value in their concentrated forms. The extraction of spices is usually carried out in two stages (the first stage separates the pungent oleoresins and the second stage separates the essential oil fractions. Essentials oils are typically volatile terpenes and esters). Essential oils are concentrated from pure plant extracts and have long been revered for their therapeutic applications; they are derivatives from flowers, leaves, stems, berries, rinds, resins, or roots of plants (Sanchez-Vicente et al. 2009; Liu et al. 2009a). These are very important ingredients and food additives of high value.

Catchpole et al. (2003) performed a detailed study on the extraction of spices and essential oils using supercritical CO_2, propane, and dimethylether fluids. They reported ginger to be the easiest of all spices in terms of optimized yield relative to pressure and temperature, while capsaicin in chili could be extracted at moderate pressure and temperature especially with the use of modifiers. The chili oil fraction contains fatty oil and carotenoids, and it is speculated that the fatty oil acts as modifier for the capsaicin (Peusch et al. 1997). Perakis et al. (2005) extracted black pepper oil with much duress, because its viscous characteristics meant that higher pressure and moderate to high temperatures were required. The use of supercritical propane for extracting spices was reported by Illes et al. (2000). They found propane could adequately extract fatty oils, tocopherols, and carotenoid but was inadequate for capsaicins; on the other hand, CO_2 was adequate for capsaicinoids, fatty oils, and tocopherols but not for carotenoids.

Catchpole et al. (2003) have conducted the supercritical extraction of ginger with three extraction fluids (CO_2, propane, and dimethyl ether). Propane gave the lowest yields while dimethyl ether gave the highest. They reported dimethyl ether to have mutual solubility with water. Ginger contains a high amount of volatiles and CO_2 extraction offers the advantage of dividing the extract into oleoresins and essential oil fractions by using a two-stage separation procedure with sequential pressure reduction.

Similarly, if propane or dimethyl ether is used, considerable heating is required which ultimately results in thermal degradation, and a larger energy requirement in the form of cooling, depressurization, and boiling to recover the essential oils. In the case of ginger, the oxygenated fraction was much greater than the steam distilled oils and the gingerol in the oleoresin was extracted without decomposition. Oleoresins and piperine from peppers were extracted with insignificant losses, although a longer processing time was required. Similar trends were observed for chili and pepper. The extracts contained carotenoid pigments, and those obtained with supercritical-CO_2 fluid were bright red with pink residues while those from propane and dimethyl ether were dark red. The extract obtained from chili with supercritical-CO_2 fluid was a yellow viscous pastry semisolid, while those extracted with dimethyl ether were yellow/black and liquid at room temperature with a high quantity of water diluting the essential oil and piperine content. Nguyen (1991) described the extraction of antioxidants from Labiatae herbs (rosemary, sage, oregano, and thymus) with supercritical-CO_2 fluid at pressures in the vicinity of 50 MPa and temperatures ranging from 80 to 100 °C. The extracted oleoresin was precipitated into two fractions at various levels of pressure and temperature. The first fraction consisted of a green-brown, oil-soluble, heat-stable, resin containing less than 2% essential oil and exhibited remarkable antioxidant properties. The second fraction was the essential oil containing more than 95 mL steam distilled oil per 100 grams.

The use of supercritical-CO_2 fluid for the production of essential oils or oleoresins from spices is possible while a suitable combination of pressure and temperature is selected. The oils extracted with supercritical technology were found to be more valuable than those extracted using other techniques due to their high quality in terms of chemical composition and percentage of sesquiterpene compounds. Supercritical-

CO_2 fluid extraction and hydrodistillation extraction methods were used to extract essential oil from juniper (*Juniperus communis* L.) (Pourmortazavi et al. 2004). Oils obtained by supercritical-CO_2 fluid and hydrodistillation showed significant differences ($P < 0.05$); the former was more selective and particularly efficient for the isolation of α-thoujone and limonene. Anitescu et al. (1997) did a comparative analysis of coriander oil with supercritical-CO_2 and steam distillation. They concluded that oils obtained by supercritical extraction possessed a far better aroma than either the commercial or hydrodistillation extracted oils.

3.4.5 Decaffeination of Coffee and Tea

Caffeine (1,3,7-trimethylxanthine) is a bioactive plant component commonly found in popular beverages such as teas (*Camellia sinensis*), coffees (*Coffee Arabica, canephora, liberica*) (since 1820s), and soft drinks (Ashihara and Crozier 2001). Caffeine is a secondary metabolite, a product of nucleic acid catabolism, and belongs to the group of compounds known as purine alkaloids. Excessive ingestion of caffeine may cause certain health problems such as palpitations, gastrointestinal disturbance, anxiety, tremors, increased blood pressure, dizziness, and insomnia (Ogita et al. 2002).

Caffeine provides aroma and flavor coupled with stimulant. Coffee beans contain approximately 2%–3% caffeine, while tea leaves contain approximately 5%, depending on the variety and species (Jameel 2003). Decaffeinated coffee must contain less than 0.1% caffeine by dry weight, as specified by European Economic Commission (EEC) regulations. Therefore, the decaffeination of coffee and tea presents a significant challenge to both producers and processors. The demand for decaffeinated coffee on the world market is high, it accounts for more than 20% of coffee sales in the USA, with demand growing by 50% among the adult population (Jameel 2003).

Research in genetic engineering to produce transgenic tea and coffee plants deficient in caffeine is in progress (Uefuji et al. 2003). However, the consumption of genetically modified products is still contentious globally, and supercritical-CO_2 fluid extraction technology gives the best option for combating these issues. The decaffeination of coffee and tea is the first known commercial operation using supercritical-CO_2 fluid extraction technology in the food industry.

In the past, methylene chloride was used for the decaffeination of coffee with one cycle of production lasting between 24 and 36 h while the end products usually contained toxic residues, thus posing more harm than the caffeine. Due to its suspected carcinogenic effect, the FDA placed regulations against methylene chloride. However, the decaffeination process with supercritical-CO_2 fluid can be accomplished on green coffee beans, roasted coffee beans, or tea leaves without deleterious effects on the flavor even after 10 h of processing, and many patents already exist for such processes.

The process requires charging the extraction vessel containing the coffee beans with CO_2 at a pressure of 7–22 Mpa and temperature of 31 °C. The caffeine is

dissolved in the supercritical-CO_2 fluid stream, which subsequently enters a washing tower or alternatively activated carbon scrubbers. Distillation, recrystallization, or reverse osmosis is used in some instances to entrain the caffeine. This method can strip coffee of its caffeine content (0.7%–3%) by 71%–97% (Caragay and Little 1981). The caffeine recovered is sold for medicinal purpose and for use in soft drinks. Peker et al. (1992) reported that soaking raw coffee beans in water prior to processing could enhance the rate of decaffeination.

3.4.6 Fish Oil Concentration

Fish oils are characterized by a high percentage of unsaturated straight-chain fatty acids ranging from C_{14} to C_{22} with one to six double bonds. They contain essential fatty acids (EFA) and polyunsaturated fatty acids, grouped into omega-6 and omega-3 EFAs. The main sources of omega-3 are flaxseed, walnut, marine plankton, and fish. This review focuses on omega-3 oils derived from fish. Eicosapentaenoic acid (EPA) and decosahexaenoic acid (DHA) are predominant in fish oil, and have been reported to contribute to the prevention of atherosclerosis, heart attacks, depression, and cancer if consumed in sufficient quantities (Chow 2000). Fish oil derivatives in the form of omega-3 oils are in high demand as food additives. For example, Ocean Nutrition's ME-3TM Omega-3 powder is currently used in several breads. In the US Wegman's Food Markets, Rochester, New York (NY) launched breads fortified with MEG omega-3 fats, two slices of which offer 80–90 mg of omega-3 (Ohr 2005). Encapsulated omega-3 fatty acids are available for fortified bakery products.

Fish oils are processed as fatty acids, or as methyl or ethyl esters which are more stable than the free acid form (Espinosa et al. 2002). Fatty acids are highly soluble in CO_2 and as a result supercritical-CO_2 fluid extraction is a preferred method of fractionation. With this technology, it is possible to separate heat sensitive compounds (omega-3 fatty acids) and avoid toxic solvent residues in the final product. The isolation and fractionation of omega-3 PUFA (polyunsaturated fatty acid) from fish, fish oil, and esters using supercritical-CO_2 fluid have been studied by several researchers (Letisse et al. 2006; Rubio-Rodríguez et al. 2008; Amiguet et al. 2012).

Amiguet et al. (2012) extracted omega-3 PUFAs rich oil from byproducts by supercritical CO_2 extraction at 35 MPa and 40 °C, and produced 137 mg of oil per gram of dried byproducts with 7.8% ± 0.06% EPA and 8.0% ± 0.07% of DHA. Eisenbach (1984) fractionated the ethyl esters from cold fish oil using supercritical-CO_2 fluid at a pressure of 15 MPa and an extraction temperature of 50 °C. Alkio et al. (2000) produced EPA and DHA with 50% and 90% purity, respectively, from transesterified tuna oil using carbon dioxide. Temelli et al. (1995) obtained the highest yield of omega-3 fatty acids at 35 MPa and 35 °C without denaturing the protein during supercritical-CO_2 fluid extraction. A higher concentration of omega-3 was achieved with supercritical-CO_2 fluid. At 25 MPa pressure, and temperature from 40 to 80 °C, no significant effect on the yield was observed in oil extraction from krill (Yamaguchi et al. 1986). Hardardottir and Kinsella (1988) did not observe any

change from the recovery of fatty acids in rainbow trout at operating pressure ranging from 13 to 35 MPa and temperature from 40 to 50 °C.

3.5 Summary

One of the most important trends in the food industry today is the demand for "natural" foods and ingredients that are free from toxic-chemical additives. The growing interest in natural food has raised the demand for natural health-promoting products of non-synthetic origin. The demand for ultra-pure and high value-added bioactive compounds is redirecting the focus of the food and pharmaceutical industries into seeking the development "green" technologies for their products. Extracts from natural sources are key elements in the manufacturing of health-promoting functional foods and ingredients. Improving the efficacy of "green" separation processes and technologies is critical to the use of bioactive components in health-promoting functional foods and in nutritional supplements. High-value functional substances can be obtained from biological materials by various purification and separation techniques from plant materials and byproducts. The challenge in the separation processes is to meet food regulation guidelines while conducting the separation effectively and economically. Public health, environmental, and safety issues are all major concerns in the use of organic solvents in food processing. Emerging "green" processing technologies, such as supercritical-CO_2 fluid extraction, have been widely used in different fields, including the extraction of essential oils, food ingredients, natural products, pharmaceutical and cosmetic products, and by-product recovery, as well as for food toxicology and eco-toxicology studies.

Supercritical-CO_2 fluid extraction is considered to be a "green" and environmentally friendly separation technology which has emerged as an attractive alternative to traditional methods for the concentration of bioactive compounds. A supercritical-CO_2 fluid extraction process offers the unique advantage of adding value to agricultural material by extracting the bioactive compounds from agricultural raw materials and byproducts for functional food development. The separation problems encountered in the production of soluble materials have a number of aspects which influence the nature of the extraction technique chosen. One of the greatest advantages of supercritical-CO_2 fluid extraction is its rapidity, with shorter extraction times than traditional methods. Supercritical-CO_2 fluid extraction is particularly favorable for the extraction of thermally labile bioactive substances and the process can be easily controlled by adjusting the temperature and pressure.

Supercritical-CO_2 fluid extraction also offers the advantage of adding value to agricultural waste by extracting antioxidants and flavonoids, such as lycopene from tomato skin, essential oils, flavors, and fragrances, which are then used for the fortification of foods and other applications. Supercritical-CO_2 fluid extraction can be utilized to provide healthy snack foods. De-fatting and de-cholesterol treatment with supercritical-CO_2 fluid extraction has been demonstrated to be applicable to food products. Although most of the tests were conducted on dehydrated products,

research has shown successful application of supercritical-CO_2 fluid on high moisture products where extraction could be accomplished without compromising the organoleptic characteristics.

Supercritical-CO_2 fluid extraction is available in the form of single stage batch process and could be augmented to allow multistage semi-continuous batch processing coupled with multi-separation. Although significant accomplishments have been obtained in the last couple of years that should not warrant complacency. Since batch modes render supercritical-CO_2 fluid extraction technology cumbersome for certain industrial applications, the need to improve the design to allow for continuous modes is growing. Supercritical-CO_2 extraction is only cost effective for large-scale production, which makes it ideal for the decaffeination of coffee, tea, and hops. With improvements in processing conditions and reduced cost, supercritical-CO_2 fluid extraction will become increasingly economical at low throughput. Extracts from natural sources are key elements in the manufacturing of health-promoting functional foods and ingredients. Thus, the development and use of "green" separation processes and technology is likely to continue to be widely employed in the processing of bioactive components, especially for use as supplements for health-promoting foods.

References

Alkio, M., C. Gonzalez, M. Jantii, et al. 2000. Purification of polyunsaturated fatty acid ester from tuna oil with supercritical fluid chromatography. *Journal of the American Oil Chemists' Society* 77: 315–321.

Amiguet, V.T., K.L. Kramp, J. Maoa, et al. 2012. Supercritical carbon dioxide extraction of polyunsaturated fatty acids from Northern shrimp (*Pandalus borealis* Kreyer) processing by-products. *Food Chemistry* 853–858.

Anitescu, G., C. Doneanu, and V. Radulescu. 1997. Isolation of Coriander oil: comparison between steam distillation and supercritical CO_2 extraction. *Flavour and Fragrance Journal* 12: 173–176.

Ashihara, H., and A. Crozier. 2001. Caffeine: A well-known but little mentioned compound in plant science. *Trends in Plant Science* 6: 407–413.

Baysal, T., S. Ersus, and D.A.J. Starmans. 2000. Supercritical CO_2 extraction of β-carotene and lycopene from tomato paste waste. *Journal of Agriculture and Food Chemistry* 48: 5507–5511.

Bhattacharjee, P., T.V. Ranganathan, R.S. Singhal, et al. 2003. Comparative aroma profiles using supercritical carbon dioxide and Likens-Nickerson extraction from a commercial brand of basmati rice. *Journal of the Science of Food and Agriculture* 83: 880–883.

Caragay, A.B., and A.D. Little. 1981. Supercritical fluids for extraction of flavours and fragrances from natural products. *Perfumer and Flavorsito* 6: 43–54.

Catchpole, O.J., J.B. Grey, N.B. Perry, et al. 2003. Extraction of chilli, pepper and ginger with near critical CO_2, propane and dimethyle ether: Analysis of the extracts by quantitative nuclear magnetic resonance. *Journal of Agriculture and Chemistry* 51: 4853–4860.

Chang, C.H., C.C. Chyau, C.L. Hsieh, et al. 2008. Relevance of phenolic diterpene constituents to antioxidant activity of supercritical CO_2 extract from the leaves of rosemary. *Natural Product Research* 22: 76–90.

Chao, R.R., S.J. Mulvaney, and H. Hanah. 1993. Effects of extraction and fractionation pressures on supercritical extraction of cholesterol from beef tallow. *Journal of the American Oil Chemists' Society* 70: 139–143.

Chow, C.K. 2000. *Fatty acids in foods and their health implications*, 2nd ed. (revised and expanded). New York: Marcel Dekker Inc.

Dunford, N.T., and F. Temelli. 1995. Extraction and fractionation of canola phospholipids with supercritical carbon dioxide and ethanol mixture. *Journal of the American Oil Chemists' Society* 70: 1009–1015.

Eisenmenger, M., and N.T. Dunford. 2008. Bioactive components of commercial and supercritical carbon dioxide processed wheat germ oil. *Journal of the American Oil Chemists' Society* 85: 55–61.

Eisenbach, W. 1984. Supercritical extraction. *Physical Chemistry Chemical Physics* 88: 882.

Espinosa, S., S. Diaz, and E.A. Brignole. 2002. Thermodynamic modeling and process optimization of supercritical fluid fractionation of fish oil fatty acid ethyl esters. *Industrial and Engineering Chemistry Research* 41: 1516–1527.

Fasoli, E., A. D'Amato, A. Citterio, et al. 2012. Ginger rogers? No, ginger ale and its invisible proteome. *Journal of Proteomics* 75: 1960–1965.

Franca, L.F., and M.A.A. Meireles. 2000. Extraction of oil from pressed palm oil (Elaes guineensis) fibers using supercritical CO_2. *Ciência e Tecnologia de Alimentos* 17: 1–12.

Ge, Y.Q., H. Yan, B.D. Hui, et al. 2002. Extraction of natural vitamin E from wheat germ by supercritical carbon dioxide. *Journal of Agricultural and Food Chemistry* 50: 686–689.

Glisic, S.B., D.R. Misic, D. Stamenic, et al. 2007. Supercritical carbon dioxide extraction of carrot fruit essential oil: Chemical composition and antimicrobial activity. *Food Chemistry* 105 (1): 346–352.

González, J.C., O.I. Fontal, R. Mercedes, et al. 2002. Basis for a new procedure to eliminate diarrheic shellfish toxins from a contaminated Matrix. *Journal of Agriculture and Food Chemistry* 50 (2): 400–405.

Gracia, I., J.F. Rodriguez, M.T. Garcia, et al. 2007. Isolation of aroma compounds from sugar cane spirits by supercritical CO_2. *Journal of Supercritical Fluids* 43: 37–42.

Griffin, B. 1999. Lipoprotein atherogenicity: An overview of current mechanism. *Proceedings of the Nutrition Society* 58: 163–169.

Hadolin, M., M. Skerget, Z. Knez, et al. 2001. High pressure extraction of vitamin E-rich oil from *Silybum marianum*. *Food Chemistry* 74: 355–364.

Hardardottir, I., and J.E. Kinsella. 1988. Extraction of lipid and cholesterol from fish muscle with supercritical fluids. *Journal of Food Science* 53: 1656–1661.

Herrero, M., A. Cifuentes, and E. Ibáñz. 2006. Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review. *Food Chemistry* 98: 136–148.

Herrero, M., J.A. Mendiola, A. Cifuentes, et al. 2010. Supercritical fluid extraction: Recent advances and applications. *Journal of Chromatograph A* 1217: 2495–2511.

Huang, W., Q.C. Deng, B.J. Xie, et al. 2010. Purification and characterization of an antioxidant protein from ginkgo biloba seeds. *Food Research International* 43 (1): 86–94.

Huber, W., A. Molero, C. Pereyra, et al. 1996. Dynamic supercritical CO_2 extraction of cholesterol from anhydrous milk fat. *International Journal of Food Science* 31: 143–151.

Illes, V., H.G. Daood, S. Perneczki, et al. 2000. Extraction of coriander seed oil by CO_2 and propane at super- and subcritical conditions. *Journal of Supercritical Fluids* 17: 177–186.

Jameel, S. 2003. Genetically decaffeinated coffee. *Journal of Biosciences* 28: 529–531.

James, W.P.T., and A. Ralph. 2000. Policy and a prudent diet. In *Human Nutrition and Dietetics*, ed. J.S. Garrow, W.T.P. James, and A. Ralph. Great Britain: Churchill Livingstone, Edinburgh.

Kassama, L.S., J. Shi, and G.S. Mittal. 2008. Optimization of supercritical fluid extraction of cycopene from tomato skin with central composite rotatable design model. *Separation and Purification Technology* 60 (3): 278–284.

Lee, A., D.I. Thurnham, and M. Chopra. 2000. Consumption of tomato products with olive oil but not sunflower oil increases the antioxidant activity of plasma. *Free Radical Biology and Medicine* 29: 1051–1055.

Letisse, M., M. Rozieres, A. Hiol, et al. 2006. Enrichment of EPA and DHA from sardine by supercritical fluid extraction without organic modifier. I. Optimization of extraction conditions. *Journal of Supercritical Fluids* 38: 27–36.

Liu, G.M., X.A. Xu, Q.F. Hao, et al. 2009a. Supercritical CO_2 extraction optimization of pomegranate (*Punica granatum* L.) seed oil using response surface methodology. *LWT-Food Science and Technology* 42: 1491–1495.

Liu, S.C., F. Yang, C.H. Zhang, et al. 2009b. Optimization of process parameters for supercritical carbon dioxide extraction of *Passiflora* seed oil by response surface methodology. *Journal of Supercritical Fluids* 48 (1): 9–14.

Lucien, F.P., and N.R. Foster. 2000. Solubilities of solid mixtures in supercritical carbon dioxide. *Journal of Supercritical Fluids* 17: 111–134.

Luengthanaphol, S., D. Mongkholkhajornsilp, S. Douglas, et al. 2004. Extraction of antioxidants from sweet Thai tamarind seed coat-preliminary experiments. *Journal of Food Engineering* 63: 247–252.

Macias-Sanchez, M.D., C. Mantell, M. Rodriguez, et al. 2005. Supercritical fluid extraction of carotenoids and chlorophyll from *Nannochloropsis gaditana*. *Journal of Food Engineering* 66: 247–251.

Marcus, Y. 2006. Are solubility parameters relevant to supercritical fluids? *Journal of Supercritical Fluids* 38: 7–12.

Martinez, M.L., M.A. Mattea, and D.M. Maestri. 2008. Pressing and supercritical carbon dioxide extraction of walnut oil. *Journal of Food Engineering* 88: 399–404.

Mendiola, J.A., M. Herrero, A. Cifuentes, et al. 2007. Use of compressed fluids for sample preparation: Food applications. *Journal of Chromatography A* 1152 (1–2): 234–246.

Mitra, P., H.S. Ramaswamy, and K.S. Chang. 2009. Pumpkin (*Cucurbita maxima*) seed oil extraction using supercritical carbon dioxide and physicochemical properties of the oil. *Journal of Food Engineering* 9: 208–213.

Miyawaki, T., A. Kawashima, and K. Honda. 2008. Development of supercritical carbon dioxide extraction with a solid phase trap for dioxins in soils and sediments. *Chemosphere* 70 (4): 648–655.

Montañés, F., N. Corzo, A. Olano, et al. 2008. Selective fractionation of carbohydrate complex mixtures by supercritical extraction with CO_2 and different co-solvents. *Journal of Supercritical Fluids* 45 (2): 189–194.

Montañés, F., A. Olano, G. Regiero, et al. 2009. Supercritical technology as an alternative to fractionate prebiotic galactooligosaccharides. *Separation and Purification Technology* 66 (2): 383–389.

Nguyen, U. 1991. Process for extracting antioxidants from labiatae herb, US Patent No. 5017397.

Ogita, S., H. Uefuji, Y.E. Choi, et al. 2002. Genetic modification of coffee plants. *Journal of Plant Biotechnology* 4: 91–94.

Ohr, L.M. 2005. Nutraceuticals and functional foods. *Food Technology* 59: 63–65.

Peker, H., M.P. Srinivasan, J.M. Smith, et al. 1992. Caffeine extraction rates from coffee beans with supercritical carbon dioxide. *AIChE Journal* 38: 761–770.

Perakis, C., V. Louli, and K. Magoulas. 2005. Supercritical fluid extraction of black pepper oil. *Journal of Food Engineering* 71: 386–393.

Perretti, G., A. Motori, E. Bravi, et al. 2007. Supercritical carbon dioxide fractionation of fish oil fatty acid ethyl esters. *Journal of Supercritical Fluids* 40: 349–353.

Perrut, M. 2000. Supercritical fluid applications: industrial developments and economic issues. *Industry and Engineering Chemistry Research* 39 (12): 4531–4535.

Peusch, M., E. Müller-Seitz, M. Petz, et al. 1997. Extraction of capsaicinoids from chillies (*Capsicum frutescens* L.) and paprika (*Capsicum annuum* L.) using supercritical fluids and organic solvents. *Zeitschrift für Lebensmitteluntersuchung und -Forschung A* 204 (5): 351–355.

Porta, D.G., S. Porcedda, B. Marongiu, et al. 1999. Isolation of eucalyptus oil by supercritical fluid extraction. *Flavour and Fragrance Journal* 14: 214–218.

Pourmortazavi, S.M., P. Baghaee, and M.A. Mirhosseini. 2004. Extraction of volatile compounds from *Juniperus communis* L. leaves with supercritical fluid carbon dioxide: Comparison with hydrodistillation. *Flavour and Fragrance Journal* 19: 417–420.

Raventós, M., S. Duarte, and R. Alarcón. 2002. Application and possibilities of supercritical CO_2 extraction in food processing industry: An overview. *Food Science and Technology International* 8: 269–284.

Reverchon, E. 1997. Supercritical fluid extraction and fractionation of essential oils and related products. *Journal of Supercritical Fluids* 10: 1–37.

Reverchon, E., and M. Poletto. 1996. Mathematical modelling of supercritical CO_2 fractionation of flower concretes. *Chemical Engineering Science* 51: 3741–3753.

Ribeiro, M.A., M.G. Bernardo-Gil, and M.M. Esquivel. 2001. *Melissa officinalis* L.: Study of antioxidant activity in supercritical residues. *Journal of Supercritical Fluids* 21: 51–60.

Rizvi, S.S.H., and A.R. Bhaskar. 1995. Supercritical fluid processing of milk fat: Fractionation, scale-up, and economics. *Food Technologies* 49 (2): 90–97.

Rubio-Rodríguez, N., S. de-Diego-Rupérez, S. Beltrán, et al. 2008. Supercritical fluid extraction of the omega-3 rich oil contained in hake (*Merluccius capensis–Merluccius paradoxus*) by-products: Study of the influence of process parameters on the extraction yield and oil quality. *Journal of Supercritical Fluids* 47: 215–226.

Sanchez-Vicente, Y., A. Cabañs, J.A.R. Renuncio, et al. 2009. Supercritical fluid extraction of peach (*Prunus persica*) seed oil using carbon dioxide and ethanol. *Journal of Supercritical Fluids* 49: 167–173.

Sastry, S.V.G., M. Mukhopadhyay, 1994. Substrate hindrance in supercritical extraction of fragrance from jasmine flowers. In *3rd International symposium supercritical fluids*, France.

Sauceau, M., J.J. Letourneau, B. Freiss, et al. 2004. Solubility of eflucimibe in supercritical carbon dioxide with or without a co-solvent. *Journal of Supercritical Fluids* 31 (2): 133–140.

Shi, J., and X.Q. Zhou. 2006. Solubility property of bioactive components on recovery yield in separation process by supercritical fluid. In *Functional Food Ingredients and Nutraceuticals: Processing Technology*, ed. J. Shi, 45–74. USA: CRC Press.

Shi, J., Y. Kakuda, X.Q. Zhou, et al. 2007a. Correlation of mass transfer coefficient in the extraction of plant oil in a fixed bed for supercritical CO_2. *Journal of Food Engineering* 78: 33–40.

Shi, J., G. Mittal, E. Kim, et al. 2007b. Solubility of carotenoids in supercritical CO_2. *Food Review International* 23: 341–371.

Shi, J., X.Q. Zhou, and L. Kassama. 2007c. Correlation of mass transfer coefficient in separation process with supercritical CO_2. *Drying Technology International* 25: 335–339.

Shi, J., M. Khatri, S.J. Xue, et al. 2009a. Solubility of lycopene in supercritical CO_2 fluid as affected by temperature and pressure. *Separation and Purification Technology* 66: 322–328.

Shi, J., C. Yi, S.J. Xue, et al. 2009b. Effects of modifier on the profile of lycopene extracted from tomato skins by supercritical CO_2. *Journal of Food Engineering* 93 (4): 431–436.

Shi, J., C. Yi, X.Q. Ye, et al. 2009c. Effects of supercritical CO_2 fluid parameters on chemical composition and yield of carotenoids extracted from pumpkin. *LWT-Food Science and Technology* 43: 39–44.

Shi, J., S.J. Xue, Y.M. Jiang, et al. 2010a. Supercritical-fluid extraction of lycopene from tomatoes. In *Separation, extraction and concentration processes in the food, beverage and nutraceutical industries*, ed. S. Rizvi, 619–639. UK: Woodhead Publishing Limited.

Shi, J., C. Yi, X.Q. Ye, et al. 2010b. Effects of supercritical CO_2 fluid parameters on chemical composition and yield of carotenoids extracted from pumpkin. *LWT-Food Science and Technology* 43 (1): 39–44.

Soares, J.F., V.D. Pra, M. Souza, et al. 2016. Extraction of rice bran oil using supercritical CO_2 and compressed liquefied petroleum gas. *Journal of Food Engineering* 170: 58–63.

Temelli, F., E. LeBlanc, and L. Fu. 1995. Supercritical CO_2 extraction of oil from Atlantic Mackerel (*Scomber scombrus*) and protein functionality. *Journal of Food Science* 60: 703–706.

Tena, M.T., M. Valcarcel, P. Hidalogo, et al. 1997. Supercritical fluid extraction of natural antioxidants from rosemary: Comparison with liquid solvent sonication. *Analytical Chemistry* 69 (3): 521–526.

Tsuda, T., K. Mizuno, K. Oshima, et al. 1995. Supercritical carbon dioxide extraction if antioxidative components from tamarind (*Tamarindus indica* L.) seed coat. *Journal of \ Agriculture and Food Chemistry* 42: 2671–2674.

Uefuji, H., S. Ogita, Y. Yamaguchi, et al. 2003. Molecular cloning and functional characterization of three distinct *N*-Methyltransferases involved in the caffeine biosynthetic pathway in coffee plants. *Plant Physiology* 132: 372–380.

Vasapollo, G., L. Longo, L. Rescio, et al. 2004. Innovative supercritical CO_2 extraction of lycopene from tomato in the presence of vegetable oil as co-solvent. *Journal of Supercritical Fluids* 29: 87–96.

Wang, B.J., C.T. Liu, C.Y. Tseng, et al. 2005. Antioxidant activity of *Bulpeurum kaoi* Liu (Chao et Chuang) fractions fractionated by supercritical CO_2. *LWT-Food Science and Technology* 38: 281–287.

Xiao, J., B.Q. Tian, B.J. Xie, et al. 2010. Supercritical fluid extraction and identification of isoquinoline alkaloids from leaves of *Nelumbo nucifera* Gaertn. *European Food Research and Technology* 231 (3): 407–414.

Yamaguchi, K., M. Murakami, H. Nakano, et al. 1986. Supercritical carbon dioxide extraction of oil from Antarctic krill. *Journal of Agricultural and Food Chemistry* 34: 904–907.

Yeh, A., J.H. Liang, and L.S. Hwang. 1991. Separation of fatty acids esters from cholesterol in esterified natural and synthetic mixtures by supercritical carbon dioxide. *Journal of the American Oil Chemist Society* 68: 224–229.

Yépez, B., M. Espinosa, S. López, et al. 2002. Producing antioxidant fractions from herbaceous matrices by supercritical fluid extraction. *Fluid Phase Equilibria* 197: 879–884.

Yi, C., J. Shi, S.J. Xue, et al. 2009. Effects of supercritical fluid extraction parameters on lycopene yield and antioxidant activity. *Food Chemistry* 113 (4): 1088–1094.