Chapter 1
Recovery Technologies for Lipophilic Bioactives

C. Saldanha do Carmo, A.T. Serra and C.M.M. Duarte

Abstract The market for natural bioactive ingredients is one of the most attractive at the moment, and natural lipophilic compounds of various structures and functionalities provide excellent molecules for the production of nutraceuticals, functional foods, and food additives. The use of these products in various commercial sectors and the consumer demand for "natural", together with increasingly strict environmental regulation related to the use of organic solvents, particularly with products for human consumption, have created a great challenge for the natural ingredients industry. In order to address the need for safer and healthier foods and to offer competitive products aimed at meeting consumer expectations, many scientists and manufacturers are making genuine efforts to explore new "green sustainable processes" for obtaining bioactive ingredients. In this chapter, both conventional and new alternative techniques for the isolation of high-value lipophilic bioactives from different natural matrixes are presented. One new technology, supercritical fluid extraction (SCFE), is reviewed in greater detail. The practical issues associated with each extraction method are also discussed, as well as the potential for upscaling of the technology.

Keywords Lipophilic bioactive compounds · Conventional and Non-conventional extraction · Supercritical CO_2 extraction

C.S. do Carmo · A.T. Serra · C.M.M. Duarte (✉)
iBET, Instituto de Biologia Experimental e Tecnológica,
Apartado 12, Oeiras 2780-901, Portugal
e-mail: cduarte@itqb.unl.pt

C.S. do Carmo · A.T. Serra · C.M.M. Duarte
Instituto de Tecnologia Química e Biológica António Xavier,
Universidade Nova de Lisboa, Av. da República, Oeiras 2780-157, Portugal

Y.H. Roos and Y.D. Livney (eds.), *Engineering Foods for Bioactives Stability and Delivery*, Food Engineering Series,
DOI 10.1007/978-1-4939-6595-3_1

1.1 Introduction

Nowadays, there is increasing evidence concerning the physiological, nutritional, and medicinal benefits to human health from various natural products, as well as the possible harmful effects of the use of certain synthetic products. The market for bioactive natural ingredients is among the most attractive at the moment, and natural lipophilic compounds of various structures and functionalities provide excellent molecules for the production of active ingredients for functional foods and food additives (e.g., preservatives, flavours, or colourants).

Extracts from natural sources play an important role, and the different parts of plants such as seeds, leaves, flowers, berries, bark, and roots represent the major sources of natural extracts, although some extracts may be obtained from animal sources.

In order to address the need for safer and healthier products and to compete in meeting consumer expectations, manufacturers must adopt the most suitable procedure for producing the natural ingredients. Conventional extraction involves the use of solid–liquid techniques that typically rely on organic solvents, which present several drawbacks, including toxic residues, chemical transformation of extracts, and toxic wastes. This has led to the development of more innovative approaches for the production of the desired compounds. The selection of an extraction method is dependent on the type of compound to be extracted, and it should take into account the potential for scaling up of the technology.

The aim of this chapter is to summarize and provide an overview of the main lipophilic bioactives and their characteristics, sources, and methods of extraction from natural matrices. As supercritical carbon dioxide extraction has been shown to be an effective "green" alternative for organic solvent-based extraction of non-polar compounds, supercritical fluid technology will be discussed in greater detail.

1.2 Lipophilic Bioactives

Lipophilic bioactives constitute a wide range of substances with a variety of functions, including flavour, antimicrobial, antioxidant, and several health-promoting properties. The group of lipophilic bioactives is very diverse in terms of molecular properties (e.g., molecular weights, structures, functional groups, polarities, and charge), leading to different physicochemical and physiological properties including solubility, physical state, rheology, optical properties, chemical stability, surface activity, and bioactivity (McClements et al. 2007).

The most important classes of lipophilic bioactives are briefly presented below, including their physicochemical characteristics, bioactivity, and main sources. The chemical structures of various bioactives are schematically presented in Table 1.1.

Table 1.1 Chemical structure of some bioactives [PubChem database (Kim et al. 2015)]

Bioactives	Structures
Carotenoids	β-carotene Lycopene Lutein Zeaxanthin
PUFAs	DHA EPA
Tocopherols	α-tocopherol β-tocopherol γ-tocopherol δ-tocopherol
Squalene	Squalene
Essential oils	Thymol Carvacrol Linalool

1.2.1 Carotenoids

Carotenoids, the most significant and extensive group of pigments found in nature (Brunner 2005a), are a large group of lipophilic compounds that give a range of colours in foods from yellow to red. Furthermore, it has been strongly suggested that consuming carotenoid-rich foods reduces the risk of several types of disease, including cancer (Ziegler 1989; Gerster 1993; Astorg 1997), cardiovascular disease (Bendich 1994; Kohlmeier and Hastings 1995; Mayne 1996; Sesso et al. 2005;

Riccioni 2009), age-related macular degeneration (Seddon et al. 1994), cataracts (Jacques and Chylack 1991; Snodderly 1995; Mayne 1996; Olmedilla et al. 2003), diseases related to low immune function (Bendich 1994; Chew 1995; Meydani et al. 1995), and other degenerative diseases (Stahl and Sies 2005; Perera and Yen 2007).

Carotenoids are polyenes consisting of 3–13 conjugated double bonds and sometimes six carbon ring structures at one or both ends of the molecule (Cavalcanti et al. 2013). They can exist in *cis* and *trans* forms, but *cis* isomers are less stable than the *trans* forms due to stoichiometric conformation. Thus, the majority of natural carotenoids are in the all-*trans* configuration (McClements et al. 2007). Based on their structure, carotenoids are divided in two classes: (i) carotenes, which are pure polyene hydrocarbons containing only carbon and hydrogen atoms, and include acyclic lycopene and bicyclic β- and α-carotene; and (ii) xanthophylls, containing oxygen in the form of hydroxyl (lutein), epoxy (violaxanthin), and oxo (canthaxanthin) groups (Sajilata et al. 2008). The most abundant pigments in nature are β-carotene, lycopene, lutein, and zeaxanthin (de Paz et al. 2012).

Carotenoids are found in a large variety of natural sources: vegetables, animals, bacteria, yeast, and microalgae. About 90 % of the carotenoids in the human diet and body are β- and α-carotenes, which are commonly found in yellow-orange vegetables and fruits; α-cryptoxanthin is present in orange fruits, lutein is provided by dark green vegetables, and lycopene is obtained from tomatoes and their by-products (Rao and Rao 2007).

The carotenoids are the main dietary source of vitamin A precursors and antioxidants (de Paz et al. 2012). Although β-carotene is the main compound with pro-vitamin A activity, any carotenoid with at least one unsubstituted β ring, such as α-carotene or β-cryptoxanthin, has the added advantage of being able to be converted to vitamin A. Even though lycopene is a carotenoid with no pro-vitamin A activity, it is an important antioxidant and free radical scavenger (Pingret et al. 2013). Processed foods are frequently fortified with carotenoids such as lycopene to increase their nutritive value and/or enhance their attractiveness (Wang and Weller 2006). The major sources of lycopene are ripe tomatoes, and tomato products and by-products (skins and seeds) (Burton-Freeman and Reimers 2011).

1.2.2 Polyunsaturated Fatty Acids (PUFAs)

The polyunsaturated fatty acids (PUFAs) contain more than one double bond in their structure. PUFAs can be classified into various groups by their chemical structure: methylene-interrupted polyenes, conjugated fatty acids, and other polyunsaturated fats. The methylene-interrupted polyenes comprise the ω-3 essential fatty acids (hexadecatrienoic acid, α-linolenic acid, stearidonic acid, etc.), ω-6 fatty acids (linoleic acid, γ-linolenic acid, eicosadienoic acid, etc.), and ω-9 fatty acids (oleic acid, erucic acid, mead acid, etc.). The conjugated fatty acids have two or more conjugated double bonds, for example, linoleic acids (rumenic

acid) and linolenic acids (β-calendic acid). Pinolenic acid and podocarpic acid are examples of other PUFAs (McClements et al. 2007).

There is an extensive body of scientific literature supporting the positive effects of ω-3 fatty acids on human health. Important natural sources of ω-3 PUFA are marine organisms (fish, seafood, algae, and other marine sources) directly or indirectly fed from marine phytoplankton, the primary producer of ω-3 in the trophic chain (Rubio-Rodriguez et al. 2010), and selected seed plants and other marine sources (Sahena et al. 2009b). Consumption of ω-3 fatty acids can reduce the risk of heart disease (Hooper et al. 2006) and high blood pressure (Lungershausen et al. 1994), prevent blood clots, protect against cancer, and alleviate depression (Von Schacky et al. 1999). With regard to ω-6 fatty acids, they are often used to develop pharmaceutical drugs and for the treatment of atherosclerosis, asthma (Hodge et al. 1998; Oddy et al. 2004), arthritis (Geusens et al. 1994), vascular disease, thrombosis (Yamashita et al. 2005), immune-mediated inflammatory disorders (Fritsche 2006), and cancer (Cunnane 2003; Simopoulos 2002, 2008). Unlike ω-3, the ω-6 and ω-9 fatty acids are not classified as essential fatty acids, as they can be synthesized by the human body from unsaturated fatty acids. Among all compounds, particular attention has been focused on concentrates of EPA (*all-cis*-5,8,11,14,17-eicosapentaenoic acid) and DHA (*all-cis*-4,7,10,13,16,19-docosahexaenoic acid) due to their pharmaceutical value (Cavalcanti et al. 2013).

1.2.3 Tocopherols and Tocotrienols

Tocopherols constitute a class of chemical compounds that includes various methylated phenols. Tocopherols are extremely valuable compounds because of their vitamin bioactivity and antioxidant capacity (Lee et al. 2000). They are abundant in seeds and leaves of plants. γ-Tocopherol is present at high concentrations in seed oils (olive, sunflower, corn, and soybean) and α-tocopherol in leaf lettuce (Cavalcanti et al. 2013).

Vitamin E includes both tocopherols and tocotrienols that occur in groups of four (α, β, γ, δ) lipophilic antioxidants synthesized by photosynthetic organisms. The isomer with the highest vitamin E activity is α-tocopherol, and it has become an important additive in numerous food products. However, although it has a number of biological properties, it can cause indigestion, thus affecting its bioavailability in the intestine. Many biological functions related to tocopherol consumption have been identified, including relief of stress (Liu 2005) and premenstrual symptoms (London et al. 1983), prevention of cellular damage (Marubayashi et al. 1986), improved blood circulation (Kunisaki et al. 1998), tissue regeneration (Andıran et al. 2000), and intermittent claudication (Haeger 1974). Additionally, the antioxidant activity of tocopherols is associated with inhibition of membrane lipid peroxidation and the elimination of reactive oxygen species (Folmer et al. 2009).

1.2.4 Phytosterols

Phytosterols and phytostanols, the saturated form of phytosterols, are steroidal compounds similar to cholesterol but of plant origin. They vary only in their carbon side chains and/or presence or absence of a double bond (Ostlund 2002). Vegetable oils and vegetable oil-containing products are rich sources of phytosterols (Cavalcanti et al. 2013). Large-scale isolation of phytosterols is conducted mainly in two major raw materials, vegetable oils and tall oil (Fernandes and Cabral 2007).

The most common phytosterols in the human diet are β-sitosterol (65 %), campesterol (30 %), and stigmasterol (3 %). Among phytostanols, the most common in the human diet are sitostanol and campestanol, which together constitute about 5 % of dietary phytosterols (Cavalcanti et al. 2013). They are known to reduce low-density lipoprotein (LDL) serum cholesterol levels (Jones et al. 1997), and thus foodstuffs containing phytosterols are widely used as a dietary therapeutic option for reducing plasma cholesterol and the risk of atherosclerosis (Moghadasian and Frohlich 1999). Grain products, vegetables, fruits, and berries are not as rich in phytosterols as vegetable oils, but they can also be significant sources due to their high consumption, reaching 150–450 mg/day (Cavalcanti et al. 2013).

Phytosterols play a major role in several areas, including pharmaceuticals (production of therapeutic steroids), nutrition (anti-cholesterol additives in functional foods, anti-cancer properties), and cosmetics (due to their anti-inflammatory activity, which is an interesting property for anti-ageing products).

1.2.5 Squalene

Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) is a biosynthetic precursor to all steroids (He et al. 2001). Squalene is a symmetrical 30-carbon polyprenyl compound containing six prenyl units. Shark liver oil (*Squalus* spp.) is considered the richest source of squalene, and it is an intermediate metabolite in the synthesis of cholesterol. In humans, about 60 % of dietary squalene is absorbed and distributed in all tissues, being one of the major components in human epidermis lipids. Its major function is acting as an antioxidant, protecting the skin from lipid peroxidation (Briganti and Picardo 2003).

It is hypothesized that the lower risk of various cancers associated with high olive oil consumption could be due to the presence of squalene (Smith 2000). Research has also suggested that squalene has chemopreventive effects against colon cancer (Rao et al. 1998), and it has been recognized as having cholesterol-lowering effects (Smith et al. 1998; Kelly 1999) (Khor and Chieng 1997).

The traditional source of squalene is primarily shark (*Centrophorus squamosus*) and whale (*Physeter macrocephalus*) liver oils (Jahaniaval et al. 2000).

1.2.6 Essential Oils

Essential oils contain an important group of bioactive organic compounds that are responsible for the aroma and are involved in the defence mechanisms of many plants. EOs contain 85–99 % volatile and 1–15 % non-volatile components. The volatile constituents are a mixture of terpenes, terpenoids, and other aromatic and aliphatic constituents of low molecular weight. Terpenes are made from combinations of several five-carbon-base (C5) units called isoprenes. The main terpenes are the monoterpenes (C10) and sesquiterpenes (C15). Terpenoids are terpenes containing oxygen. Monoterpenes, formed from the coupling of two isoprene units, are the most representative molecules, constituting 90 % of the EOs. Aromatic compounds are derived from phenylpropane. Aldehydes (cinnamaldehyde), alcohols (cinnamic alcohol), phenols (eugenol), methoxy derivatives (anethole, estragole), and methylenedioxy compounds (myristicin, apiole) are examples of their aromatic components (Sánchez-González et al. 2011).

These compounds can be stored in several organs, such as flowers (orange, bergamot), leaves (lemongrass, eucalyptus, and menthe), bark (cinnamon), wood (sandalwood, rosewood), rhizomes (curcuma, ginger), fruits (star anise, fennel), and seeds (nutmeg) (Pereira and Meireles 2010).

Essential oils generally have a broad spectrum of bioactivity owing to the presence of several active ingredients that work through various modes of action. Known for their antiseptic—i.e., bactericidal (Friedman et al. 2002), virucidal (Garcia et al. 2003), and fungicidal (Ranasinghe et al. 2002)—and medicinal properties and their fragrance, they are used in embalmment, for food preservation, as antimicrobial (Burt 2004) and analgesic remedies, sedatives, anti-inflammatory (Silva et al. 2003) and spasmolytic (Sadraei et al. 2001) agents, and as a local anaesthetic (Bakkali et al. 2008). These properties have made essential oils an attractive candidate for use as ingredients in cosmetics, food, and pharmaceutical products (Pereira and Meireles 2010).

1.3 Methodologies for Extraction of Lipophilic Bioactives

Various extraction procedures have been used for the recovery of bioactive compounds from natural matrices. These include both conventional and non-conventional methods.

1.3.1 Conventional Extraction Techniques

Soxhlet extraction, hydro-distillation, and maceration with an alcohol–water mixture or hot fat are conventional techniques applied for the extraction of nutraceuticals from natural matrices using heat and/or agitation (Wang and Weller 2006; Azmir et al. 2013).

The Soxhlet extractor was first proposed in 1879 by Franz Ritter von Soxhlet, a German chemist, and it has since been widely adopted as a standard technique and the primary reference for assessing the performance of new extraction alternatives (Wang and Weller 2006; Azmir et al. 2013). With the exception of the extraction of thermolabile compounds, Soxhlet extraction is a generally well-established method, with performance exceeding that of other conventional extraction techniques. Briefly, a small amount of dry sample is placed in a thimble filter, which is then placed in a thimble holder above a distillation flask containing the solvent of interest. When the liquid reaches the overflow level, a siphon aspirates the solution passing through the thimble holder and unloads it back into the distillation flask, carrying extracted solutes into the bulk liquid. In the solvent flask, solute is separated from the solvent using distillation. The solute is left in the flask, and fresh solvent condensed by reflux after evaporation is passed back into the plant solid bed. The operation is repeated until complete extraction is achieved (Wang and Weller 2006; Azmir et al. 2013). The main criticism with this method is the length of time required to complete the process (Luque de Castro and Priego-Capote 2010).

Hydro-distillation is another traditional method for extraction of bioactive compounds and essential oils from plants. With this technique, organic solvents are not used, and it can be performed without drying the plant materials. There are three types of hydro-distillation: water distillation, water and steam distillation, and direct steam distillation (Palma et al. 2013). In water distillation, the solid matrix is sustained and packed in a still compartment and immersed in the boiling water or floating on it, depending on its density. In the water and steam distillation method, steam is also directly injected into the plant sample. Hot water and steam extract and release the bioactive compounds from the plant tissue. Indirect cooling by water condenses the vapour mixture of water and oil. The condensed mixture flows from the condenser to a separator, where oil and bioactive compounds automatically separate from the water. Lastly, in direct steam distillation, the solid matrix is supported on a perforated grid or screen inserted above the bottom of the still, but it is not in direct contact with the liquid water. The saturated steam flows up through the extracted solids, evaporating and gathering the volatile components (Palma et al. 2013), and the mixture is then condensed and separated as described above.

Hydro-distillation involves three main physicochemical processes: hydro-diffusion, hydrolysis, and decomposition by heat. This precludes its use for the extraction of thermolabile compounds, because some volatile compounds may be lost (Reverchon and Senatore 1992).

Maceration has become a popular and inexpensive way to extract essential oils and bioactive compounds, and it generally consists of several steps. First, the plant materials are ground into small particles to increase the surface area for proper mixing with the solvent. Next, an appropriate solvent, called menstruum, is added in a closed vessel. The liquid is then strained off, and the solid residue is pressed to recover as much occluded solution as possible. The strained and pressed liquids are

mixed and separated from impurities by filtration (Palma et al. 2013). In order to facilitate extraction, occasional shaking can be used, increasing the diffusion and the removal of concentrated solution from the sample surface, while carrying fresh menstruum inward for higher extraction yields (Azmir et al. 2013).

1.3.2 Non-conventional Extraction Techniques

The major drawbacks of conventional extraction procedures include long extraction time, low extraction selectivity, thermal decomposition of thermolabile compounds, and evaporation of large amounts of solvent (Azmir et al. 2013). To overcome these limitations, new and promising techniques have been explored.

1.3.2.1 Supercritical Fluid Extraction

The use of supercritical fluids (SCFs) is often highlighted as an important strategy within green chemistry to replace harmful organic solvents and to enable green sustainable technologies (Leitner and Poliakoff 2008). The critical point is characteristic of each substance and it is schematically illustrated in Fig. 1.1. The state of a substance is called supercritical when temperature and pressure exceed the critical values.

Going over the liquid-vapour line of a pure substance, the rise in temperature and pressure have different effects in each phase. When the temperature of a substance reaches its critical value, the densities of the gas and the liquid phases become identical, and the liquid is no longer distinguishable from the gas. At this point, only one phase exists and is now described as an SCF, whose properties range

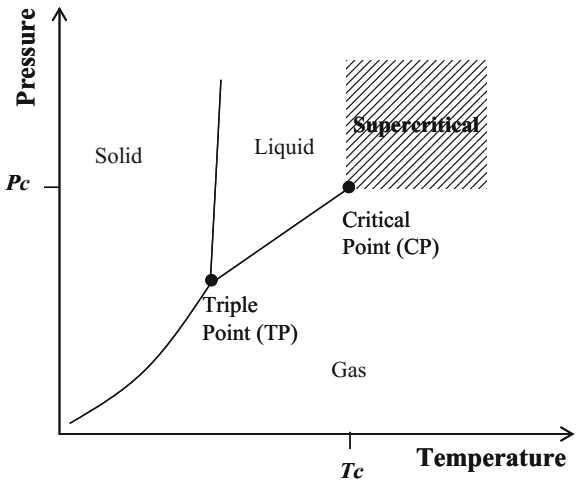

Fig. 1.1 Phase diagram for a pure component (Brunner 2005b)

Table 1.2 Orders of magnitude of main physical properties of SCFs [adapted from (Brunner 2005b)]

	Liquid	Supercritical	Gas
Density ($g.cm^3$)	1	0.1–0.5	10^{-3}
Diffusivity ($cm^2.s^{-1}$)	10^{-5}	10^{-3}	10^{-1}
Viscosity (Pa.s)	10^{-3}	10^{-4}-10^{-5}	10^{-5}

between those of gases and liquids, as shown in Table 1.2. Furthermore, SCFs are characterized by gas-like viscosities and solvating properties of a wide range of various organic solvents.

The most important feature of SCFs is that in the supercritical region (Fig. 1.1), minor variations in pressure and/or temperature lead to significant variation in density, which causes rapid changes in thermodynamic and transport properties.

Commensurate with the goal of environmentally benign processing is the use of liquefied or supercritical carbon dioxide (SC-CO_2) for non-polar to moderately polar solutes. Environmentally benign carbon dioxide in its supercritical state has demonstrated significant potential for the development of a wide range of alternative processes that totally or partially eliminate the need for some of the most commonly used organic solvents. In addition to this factor, the rapid mass transfer properties associated with the lower viscosity of SCF carbon dioxide can lead to more time-efficient production capabilities for various types of important industrial processes (King and Srinivas 2009).

The extraction of compounds from natural sources is the most widely studied application of SCFs. In fact, supercritical fluid extraction (SCFE) has significant advantages over conventional solvent methods: it is an environmentally friendly technique, it enhances extraction efficiency and selectivity, and avoids the expensive post-processing of the extracts for solvent elimination. For the above-mentioned -reasons, carbon dioxide (CO_2) is the most frequently used SCFE solvent. Extracts from supercritical processing with SC-CO_2 can be regarded as all natural, and the products allowed for food applications may have GRAS (generally recognized as safe) status. In addition to the advantages related to non-toxicity and mild operating conditions, the extraction system provides a light- and oxygen-free environment, thereby minimizing degradation and preserving their bioactivity and antioxidant properties.

Typical SCFE systems for solid and liquid extraction are presented in Figs. 1.2 and 1.3, respectively. Basically, the system consists of pumps for delivering solvent and modifiers (co-solvents) throughout the system and for raising the pressure of the recycled 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. 1.2) (Shi et al. 2012). A small amount of co-solvent (ethanol, water, etc.) increases the ability of SC-CO_2 to dissolve polar compounds (Reverchon and De Marco 2006).

Column fractionation can be done with a column operating in two different modes: cross-current and counter-current. The latter is the most popular. In such a

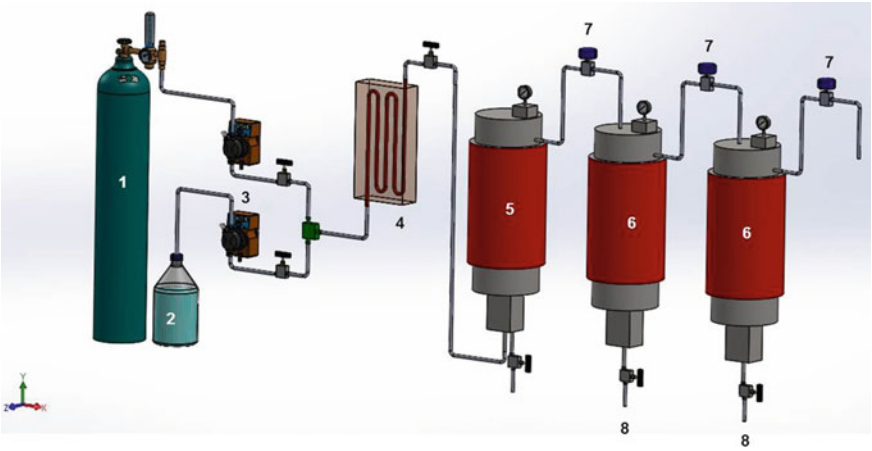

Fig. 1.2 Schematic diagram of a supercritical fluid extraction system (Solid-scFluid extraction) used to fractionate bioactive components. *1* CO_2 cylinder; *2* Co-solvent; *3* CO_2 and co-solvent pumps; *4* Heat exchanger; *5* Extraction vessel; *6* Separators; *7* Back pressure valves; *8* Extracts outlet

counter-current separation process, the components are distributed between the solvent (extract phase) and the liquid (raffinate phase) which flow in a counter-current manner through the column, reducing the amount of solvent needed and increasing throughput. Counter-current processing with SCFs extends the possibilities of separation processes like distillation, absorption and liquid–liquid extraction to the isolation and purification of components of low volatility, allowing separation of components with very similar properties (Fig. 1.3).

The industry has recently focused on "fractional separation", where the natural materials are extracted under relatively severe pressure and temperature conditions to remove all the desired components. The resulting fluid extract is then passed through a series of two, three, or four separator vessels in which the operating parameters (temperatures and pressures) in each vessel are set to selectively precipitate one specific component of interest. This can create a range of unique fractions with new application potential.

The past 20 years have seen an expansion of the "critical fluid" technology platform with respect to using or combining multiple types of unit operations and compressed fluids in both their sub- and supercritical states. For example, combining SCFE with fractionation methods using SCFs, and/or reaction chemistry in critical fluid media can produce a variety of extracts or products (King 2000).

Table 1.3 summarizes the advantages, disadvantages, parameters to control, and ease of scale-up of SCFE, in particular with carbon dioxide.

In summary, SC-CO_2 is suitable for applications in which (i) processing costs are not a limiting factor, (ii) conventional solvent extraction is restricted by environmental regulations, (iii) consumer demand or health considerations play an important role, (iv) a "natural" label on the product is required, and/or (v) the

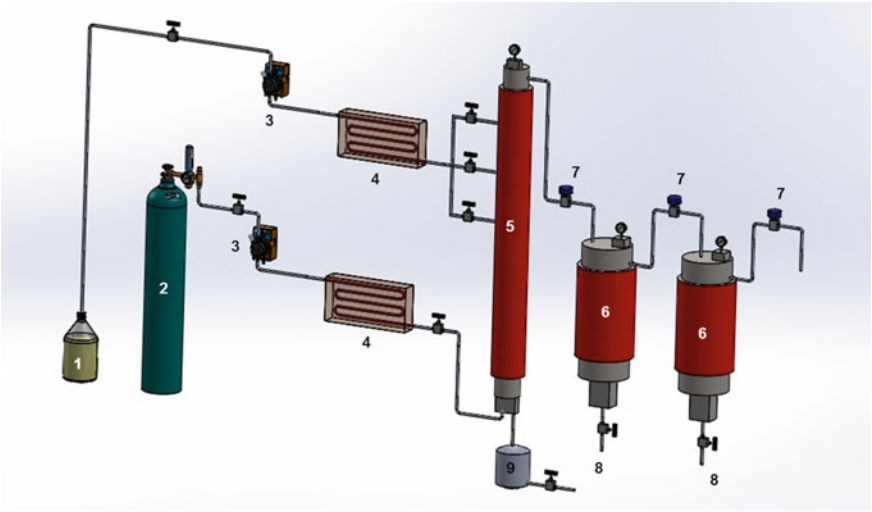

Fig. 1.3 Schematic diagram of a supercritical fluid extraction system (Liquid-scFluid extraction) used to fractionate bioactive components. *1* Feed; *2* CO_2 cylinder; *3* CO_2 and liquid pumps; *4* Heat exchangers; *5* Extraction column; *6* Separators; *7* Back pressure valves; *8* Extracts outlet; *9* Raffinate

application of traditional extraction techniques does not preserve the bioactive properties of the product. The major disadvantage of SCFE is the expensive equipment. With regard to the process itself, the drawbacks are the high pressure required and the associated hazards, which have hindered wider application.

SCFE has been commonly used for the extraction of flavours, essential oils, seed oils, antioxidants, and bioactives from several natural sources. Some of these applications have been reviewed extensively (Reverchon and De Marco 2006; Herrero et al. 2006a; Cheah et al. 2006; Díaz-Reinoso et al. 2006; Sahena et al. 2009a; Catchpole et al. 2009), and are summarized in Table 1.4.

1.3.2.2 Other Non-conventional Extraction Techniques

An overview of other non-conventional extraction techniques, including ultrasound-assisted extraction, microwave-assisted extraction (MAE), and pressurized liquid extraction (PLE), is presented in Table 1.5, along with the principles, main advantages, limitations, and information regarding the scaling up of each technology.

Table 1.3 Advantages, disadvantages, parameters to control and ease of scale-up of SCFE

Extraction methods	Advantages	Disadvantages	Parameters to control	Ease of scale-up
Supercritical fluid extraction (SCFE)	• Environmentally safe technology (Herrero et al. 2006b) • High selectivity, short times of extraction, lower pollution, and the use of non-toxic organic solvents (Wang and Weller 2006) • SFs have better transport properties than liquids because their viscosity depends on their density which, unlike liquid solvents, is adjustable by changing pressure and temperature (Sihvonen et al. 1999; Herrero et al. 2006b) • Sterilizing properties (Cheah et al. 2006)	• Low dielectric constant which suggests poor solvent power for polar materials (Wang and Weller 2006) • High pressure and the associated hazards, which have hindered a wider development (Herrero et al. 2006a) • Large number of variables to optimize (Cheah et al. 2006) • Strong dependence on matrix-analyte interactions (Cheah et al. 2006)	• *Matrix* particle size, porosity, location of the solute, moisture content, solubility • *Process* pressure and temperature (solubility), solvent flow rate (solvent-to-feed ratio)use of modifiers (co-solvents) (Mendiola et al. 2013)	Robust and economically viable process for industrial implementation (Fernandez-Ronco et al. 2013)
Supercritical carbon dioxide (SC-CO_2)	• Safe (Daintree et al. 2008) • Low cost (Daintree et al. 2008) • Ensure minimal alteration of the bioactive compounds and to preserve their curative or functional properties (Cavero et al. 2006) • Non-explosive (Wang et al. 2008; Wang and Weller 2006; Sahena et al. 2009b) • Non-toxic, inexpensive (Wang et al. 2008; Wang and Weller 2006; Sahena et al. 2009b) • Ability to solubilize lipophilic substances, and can be easily removed from the final products (Wang et al. 2008; Wang and Weller 2006; Sahena et al. 2009b) • Solvent-free extracts • This molecule is environmentally friendly and "generally recognized as safe" (GRAS) by FDA (U.S. Food and Drug Administration) and EFSA (European Food Safety Authority) • Allows the extraction of thermally labile or easily oxidized compounds (Herrero et al. 2010)	• Because of its low polarity, CO_2 is less effective in extracting highly polar compounds from their matrices (Herrero et al. 2006b) • The use of other solvent compounds is needed in order to enhance solubility and the selectivity of the process and they must be added only in small quantities (Sihvonen et al. 1999)		

Table 1.4 High-added-value compounds extracted with supercritical fluid extraction (Reverchon and De Marco 2006; Herrero et al. 2006a; Cheah et al. 2006; Díaz-Reinoso et al. 2006; Sahena et al. 2009a; Catchpole et al. 2009; Adil et al. 2007, 2008; Ibáñez et al. 2000; Nobre et al. 2009)

Bioactives	Raw materials
Essential oils	Bacuri fruit shells; Basil leaves; Black cumin; Carrot fruit; Chamomile flowers; Clove bud; Eucalyptus leaves; Ginger; Rose hip fruit; Juniper; Laurel leaves; Lemon balm; Lemon bergamot; Lemon eucalyptus; Lemon verbena; Lemongrass leaves; *Lippia alba;* Lovage leaves and roots; Marjoram leaves; Mint leaves; Oregano; Patchouli; Pennyroyal; Black pepper; Rosemary; Sage leaves; Savoury; Spiked thyme; Star anise; Sweet basil; Thyme; Tuberose concrete; Valerian root; Vetiver; Yarrow flowers
Seed oils	Black cumin seeds; Blackcurrant seed; Borage seeds; Caraway; Celery roots; Cinnamon; Coriander seeds; Daphne; Evening primrose seed; Fennel seeds; Grape seeds; Hemp seed; Rose hip seeds; Hyssop; Juniper; Kiwifruit seed; Lavender; Medlar seeds; Palm kernel oil; Peach seeds; Red pepper; Rose hip seed; Tea seed; *Vernonia* seeds
Carotenoids	Alfalfa leaf; Apricot pomace; Buriti fruit; Cardamom; Carrot; Crustaceans; Krill; Marjoram; Microalgae (Chlorella vulgaris, Nannochloropsis gaditana, Spirulina maxima, *Dunaliella salina*, *Haematococcus pluvialis*, *Phaffia rhodozyma*); Palm; Paprika; Pitanga; Rose hip; Sea buckthorn; Spirulina; Stinging nettle; Sweet potato; Tomato and tomato by-products (paste waste, skin); Watermelon
Tocopherols	*Aloe vera* leaves; Coriander seeds; Dill; *Espinheira santa*; Fresh bay; Grape seeds; Milk thistle; Olive oil industry by-products; Olive tree leaves; Palm; Paprika; Parsley; Pomegranate; Rice bran; Sacha inchi; Sesame seed, black; *Silybum marianum*; Soybean flakes; Soybean oil by-product; Spearmint; Tomato; Wheat germ
Squalene	*Espinheira santa*; Indian almond leaves; Olive oil industry by-products; Shark liver oil; Tropical almond leaves and seeds
Fatty acids	Brown seaweed; Cardamom seed; Corn bran; Cotton seed; *Cunninghamella echinulata*; Grape seeds; Ground beef; Mackerel; Northern shrimp; Oat Bran; Peanuts; Palm kernel; Pecan; Pistachio; Pupunha; *Pythium regulare*; Rapeseed; Rice bran; Rose hip seed; Safflower; Sardine oil; Saw Palmetto berries; Soybean; Sunflower; Wheat germ

1.4 Extraction of Lipophilic Bioactives

1.4.1 Carotenoids

Carotenoid extraction has traditionally been performed using organic solvents that are associated with the known problems of low selectivity and adverse environmental impact. For these reasons, the use of SCF has been explored for their extraction. Because carotenoids show very low or moderate solubility in SC-CO_2, depending on the molecular structure (molecular weight and polarity), suitable modifiers (co-solvents) have been employed to enhance the solubility of the target compounds. The most frequently used co-solvent is ethanol, since its presence (in traces) in final products does not compromise their use in food, nutraceutical, and pharmaceutical

Table 1.5 Conventional solvent extraction and Non-conventional extraction techniques: principle of the method, advantages, disadvantages, parameters to control, and scale-up

Extraction method	Principle	Advantages	Disadvantages	Parameters to control	Scale-up
Conventional solvent extraction (SE)	• Pre-treated raw material is exposed to different solvents (Starmans and Nijhuis 1996) • Compounds of interest and other agents are extracted (Starmans and Nijhuis 1996) • Samples are usually centrifuged and filtered to remove solid residue (Starmans and Nijhuis 1996)	• Low production cost (Szentmihalyi et al. 2002) • Ease of operation (Szentmihalyi et al. 2002)	• Toxic solvents for humans and dangerous for the environment (Li et al. 2006) • Laborious extraction conditions (Li et al. 2006) • The solvent must be removed from the final extract, especially if the product is to be used in food applications (Starmans and Nijhuis 1996) • It requires an evaporation/ concentration step for recovery, using large amounts of solvent and extended time to carry out (Szentmihalyi et al. 2002) • Possibility of occurrence of thermal degradation due to high temperatures of solvents and long extraction times (Szentmihalyi et al. 2002)	• *Matrix* Matrix composition, sample size and extraction time • *Process* temperature, type of solvent and its characteristics (polarity, volume and flow rate) (Azmir et al. 2013)	• Not found
Pressurized liquid extraction (PLE) or accelerated SE (ASE) (Priego-Capote and Delgado de la Torre 2013)	• Accelerated SE and pressurized SE using organic liquid solvents at high temperature (423 K-473 K) and pressure (10-15 MPa) (Dunford et al. 2010) • As the temperature increases, the dielectric constant of the solvent decreases, thus lowering the polarity of the solvent (Abboud and Notario, 1999)	• Rapid extraction rate of compounds (Dunford et al. 2010) • The temperature can be used to match the polarity of a solvent to that of the compounds of interest to be recovered (Dunford et al. 2010) • The high pressure helps the extraction cells to be filled faster and forces liquid into the solid matrix (Plaza et al. 2010a) • Allow a faster extraction in which less amount of solvents are used and higher yields are obtained in comparison with traditional SE (Plaza et al. 2010a) • Allows the achievement of food-grade extracts when using water or other GRAS (generally recognized as safe) solvents,	• Not suitable for thermolabile compounds, as high temperature can have harmful effects on their structure and functional activity (Ajila et al. 2010)	• *Matrix* composition, sample size and extraction time • *Process* temperature, pressure, type of solvent and its characteristics (polarity, volume and flow rate)	• Scale-up to large production units not effective (Pronyk and Mazza 2009) • Difficulties in producing a system able to operate on a continual basis (Pronyk and Mazza 2009) • Most scale-up has occurred with older pressurized fluid extraction technology of SCFE (Pronyk and Mazza 2009) • The younger technologies of PLE extraction are gaining popularity in the research community (Pronyk and Mazza 2009)

(continued)

Table 1.5 (continued)

Extraction method	Principle	Advantages	Disadvantages	Parameters to control	Scale-up
		such as ethanol (Plaza et al. 2010a)			
Microwave-Assisted Extraction (MAE)	• Extraction works by heating the moisture inside the cells and evaporating, producing a high pressure on the cell wall (Routray and Orsat 2012) • The pressure builds up inside the biomaterial, which modifies the physical properties of the biological tissues (cell wall and organelle disrupter), increasing the porosity of the biological matrix (Routray and Orsat 2012)	• Better penetration of solvent through the matrix and improved yield of the desired compounds (Routray and Orsat 2012) • Internal and external heating without a thermal gradient, in which bioactives can be extracted efficiently and safely using less energy and solvent volume (Routray and Orsat 2012) • Reduced solvent consumption (Zhang et al. 2011) • Shortened extraction times (Simsek et al. 2012; Xiao et al. 2008) • Higher yields (Simsek et al. 2012; Xiao et al. 2008) • Lower environmental pollution (Simsek et al. 2012; Xiao et al. 2008)	• Modification of the chemical structure of target compounds (modification of bioactivity) (Zhang et al. 2011) • Additional filtration or centrifugation is necessary to remove a solid residue (Zhang et al. 2011)	• *Matrix* Size and moisture • *Process* Choice of solvent composition, solvent-to-feed ratio, power applied, extraction temperature and extraction time (Destandau et al. 2013)	• Poor reproducibility, poor control over process quality, complexity in scaling up (Li et al. 2013)
Ultrasonic-Assisted Extraction (UAE)	• Sound waves with frequencies higher than 20 kHz are mechanical vibrations in a solid, liquid and gas (Wang and Weller 2006) • Sound waves must travel in a matter, involving expansion and compression cycles during travel in the medium (Wang and Weller 2006) • When the ultrasound intensity is sufficient, the expansion cycle can create cavities or microbubbles in the liquid (Leighton 2007; Soria and Villamiel 2010; Esclapez et al. 2011) • The implosion of cavitation bubbles can hit the surface of the solid matrix and disintegrate the cells, causing the release of the desired compounds	• Reduction in extraction time (Leighton 2007; Soria and Villamiel 2010; Esclapez et al. 2011) • Higher recovery of targeted compounds with lower solvent consumption and/or faster analysis and bioactivity properties (Leighton 2007; Soria and Villamiel 2010; Esclapez et al. 2011) • Reasonable extraction efficiency (Leighton 2007; Soria and Villamiel 2010; Esclapez et al. 2011). • Facilitates leaching of organic and inorganic compounds from	• UAE should be carefully used in the extraction of unstable compounds (Zhao et al. 2006) • The presence of a dispersed phase contributes to the ultrasound wave attenuation, and the active part of ultrasound inside the extractor is restricted to a zone located in the vicinity of the ultrasonic emitter (Wang and Weller 2006)	• *Matrix* Moisture content, particle size, solvent • *Process* Frequency, pressure, temperature and sonication time (Pingret et al. 2013)	• UAE is attracting growing interest, and several industrial-scale reactors have already been successfully applied in the food industry (Tao and Sun 2013)

(continued)

Table 1.5 (continued)

Extraction method	Principle	Advantages	Disadvantages	Parameters to control	Scale-up
	(Leighton 2007; Soria and Villamiel 2010; Esclapez et al. 2011)	plant matrix (Herrera and de Castro 2005) • Effective mixing (Chemat et al. 2008) • Faster energy transfer (Chemat et al. 2008) • Thermal gradients and extraction temperature are reduced (Chemat et al. 2008) • Selective extraction (Chemat et al. 2008) • Compact equipment size (Chemat et al. 2008) • Quick control of extraction process (Chemat et al. 2008) • Start-up is quick (Chemat et al. 2008) • Higher production (Chemat et al. 2008) • Process steps are eliminated (Chemat et al. 2008)			

Table 1.6 Extraction of lycopene and **β**-carotene from tomato and tomato by-products using SC-CO_2 extraction and UAE

Extracted compound(s)	Source/raw material	Extraction conditions[a]	Yield (mg/100 g)/ Recovery[b] (%)	Observations	Reference
Supercritical CO_2 extraction					
Lycopene β-Carotene	Tomato (pulp and skin)	CO_2 T = 353 K; P = 27.6 MPa	Lycopene 64.4 mg/100 g 87 % β-Carotene 34.9 mg/100 g 92 %	Extracted product contained 65 % of lycopene and 35 % of β-carotene	Cadoni et al. (1999)
Lycopene β-Carotene	Tomato paste waste	CO_2 + 5 % EtOH T = 328 K/338 K, P = 30 MPa	Lycopene 9.1 mg/100 g 54 % β-Carotene 0.53 mg/100 g 50 %	Total of 100 % recovery of supercritical extracted carotenes was not possible because of the degradation of the product at higher temperatures	Baysal et al. (2000)
Lycopene	Tomato skins	CO_2 T = 383 K; P = 40 MPa	100 %	The addition of acetone (500μL) speeded up the beginning of the extraction, leading to a relative recovery of 94 % in just 15 min	Ollanketo et al. (2001)
Lycopene	Tomato sauce by-product	CO_2 T = 359 K; P = 34.47 MPa	0.7 mg/100 g 61 %	β-Carotene, α-carotene, α-tocopherol, γ-tocopherol and δ-tocopherol were also detected in extracts	Rozzi et al. (2002)
Lycopene β-Carotene	Tomato skins	CO_2 T = 350 K; P = 30 MPa	Lycopene 31 mg/100 g 80 % β-Carotene 6.9 mg/100 g 88 %	The yield of β-carotene extracted from the raw tomato waste material was 88 %	Sabio et al. (2003)

(continued)

Table 1.6 (continued)

Extracted compound(s)	Source/raw material	Extraction conditions[a]	Yield (mg/100 g)/ Recovery[b] (%)	Observations	Reference
Lycopene	Tomato (sun dried)	CO_2 + 10 % hazelnut oil T = 339 K; P = 45 MPa	60 %	Besides the advantages of having an improvement in the yields of the lycopene extract the presence of the vegetable oil as co-solvent contribute to avoid the degradation of the lycopene	Vasapollo et al. (2004)
Lycopene	Industrial tomato by-products	CO_2 T = 353 K; P = 46 MPa	31.4 mg/100 g	Other compounds such as tocopherols and β-carotene were detected in the extracts. The content of lycopene in the final product was 90 %	Vagi et al. (2007)
Lycopene	Tomato pomace	CO_2 + 16 % EtOH T = 330 K; P = 40 MPa	28.64 mg/100 g 93 %	The optimal conditions for antioxidant capacity were 330 K and 53 MPa. IC_{50} was used as index: 0.081 mg/mL; Antioxidant activity was evaluated	Huang et al. (2008)
Lycopene	Dried tomatoes + roasted hazelnut powder	CO_2 T = 333 K; P = 40 MPa	423 mg/100 g 72.5 %	Yield of oil extracted = 80 %	Ciurlia et al. (2009)
Lycopene	Tomato skins	CO_2 + 10 % EtOH + 10 % olive oil T = 348 K; P = 35 MPa	73.3 %	The addition of olive oil increases the solubility of lycopene	Shi et al. (2009)
Lycopene	Tomato industrial waste	CO_2 T = 333 K; P = 30 MPa	93 %	The recovery of trans-lycopene was faster and higher for the sample with lower content	Nobre et al. (2009)

(continued)

Table 1.6 (continued)

Extracted compound(s)	Source/raw material	Extraction conditions[a]	Yield (mg/100 g)/ Recovery[b] (%)	Observations	Reference
Lycopene	Tomato peel by-product + tomato seed	CO_2 T = 363 K; P = 40 MPa	56 %	44 % of lycopene remained in both the pipeline and the solid matrix. The presence of fatty acid content in the tomato seed oil improved recovery of lycopene from 18–56 %	Machmudah et al. (2012)
Lycopene	Tomato juice	CO_2 T = 313/353 K; P = 35 MPa	76.7 %	Effect of temperature and pressure on extraction efficiency. Identification and quantification of lycopene on the active fractions, yield and antioxidant capacity	Egydio et al. (2010)
Lycopene	Tomato skins	CO_2 T = 373 K; P = 40 MPa	3.125 mg/100 g	Increased bioavailability due to the generation of cis-isomers; antioxidant activity was evaluated	Yi et al. (2009)
Trans-Lycopene	Tomato skins	CO_2 + 14 % EtOH T = 335 K; P = 45 MPa	33 %	Increased yield was observed at higher temperatures	Kassama et al. (2008)
Lycopene	Tomato skins	CO_2 T = 373 K; P = 40 MPa	118 mg/100 g 94 %	Lycopene was extracted from tomato skin with negligible degradation under optimal conditions	Topal et al. (2006)
Ultrasound-assisted extraction					
Lycopene	Tomato peel	1:1 Petroleum ether and acetone, 328 K, 5–25 min, 460 W/cm^2	60 mg/100 g	Sonication significantly enhanced biocatalytic extraction of tomato-peel lycopene Antioxidant activity was evaluated. Extracted lycopene retained structural integrity and DPPH scavenging potency	Konwarh et al. (2012)

(continued)

Table 1.6 (continued)

Extracted compound(s)	Source/raw material	Extraction conditions[a]	Yield (mg/100 g)/ Recovery[b] (%)	Observations	Reference
Lycopene	Tomato	*n*-Hexane, acetone, EtOH; 45.6 min, 321 K, 74.4:1 (v/w)	76 %	UAE enhanced the extraction yield of lycopene, and at the same time minimized the degradation and isomerization of lycopene	Eh and Teoh (2012)
Lycopene	Tomato paste	Ethyl acetate, 29.1 min, 369 K, solvent to tomato paste 8.0:1 (v/w)	89.4 %	The percentage of lycopene recovered was higher when using ultrasonic- and microwave-assisted extraction (UMAE)	Lianfu and Zelong (2008)

EtOH Ethanol; *MeOH* Methanol; *nd* not determined
[a]selected optimal conditions for maximum recovery
[b]when compared with conventional extractions techniques

Table 1.7 Extraction of carotenoids from selected microorganisms and marine organisms by SC-CO_2, UAE, MAE and PLE

Extracted compounds	Source/raw material	Extraction conditions[a]	Yield (mg/100 g)/ Recovery[b] (%)	Reference
Supercritical CO_2 extraction				
Astaxanthin	*Phaffia rhodozyma*	CO_2 + EtOH (15 %) T = 313 K; P = 50 MPa	90 %	Lim et al. (2002)
Astaxanthin	*Haematococcus pluvialis*	CO_2 + 10 % olive oil T = 343 K; 40 MPa	51 %	Krichnavaruk et al. (2008)
Total carotenoids	*Chlorella vulgaris*	CO_2 T = 313/328 K; P = 20–35 MPa	≈50 mg/100 g	Mendes et al. (2003)
Astaxanthin	*Krill*	CO_2 T = 313–353 K; P = 24.4 MPa	nd	Yamaguchi et al. (1986)
Total carotenoids (mainly astaxanthin)	*Crustaceans*	CO_2 + EtOH (0-20 %) T = 313–333 K; P = 20–35 MPa	50 %	López et al. (2004)
β-Carotene	*Scenedesmus almeriensis*	CO_2 T = 333 K; P = 40 MPa	53 %	Macias-Sanchez et al. (2010)
Lutein	*Chlorella vulgaris*	CO_2 + EtOH T = 313 K; P = 40 MPa	70 %	Ruen-ngam et al. (2012)
Carotenoids (canthaxanthin and astaxanthin)	*Chlorella vulgaris*	CO_2 + EtOH T = 313 K; P = 30 MPa	246 mg/100 g 100 %	Palavra et al. (2011)
Astaxanthin	*Monoraphidium* sp. GK12	CO_2 + EtOH T = 303 K; P = 20 MPa	90 %	Fujii (2012)
Ultrasound assisted extraction				
β-Carotene	*Spirulina platensis*	*n*-Heptane T = 303 K;167 W/cm^2; 8 min	47 %	Dey and Rathod (2013)

(continued)

Table 1.7 (continued)

Extracted compounds	Source/raw material	Extraction conditions[a]	Yield (mg/100 g)/ Recovery[b] (%)	Reference
Microwave assisted extraction				
β-carotene Fucoxanthin	*Dunaliella tertiolecta* and *Cylindrotheca closterium*	Acetone T = 329 K; 50 W; 3–5 min	Hot soaking: 9.31 ± 0.44 mg/g	Pasquet et al. (2011)
Pressurized liquid extraction				
Carotenoids (astaxanthin, lutein)	*Haematococcus pluvialis*	EtOH T = 473 K; P = 10.34 MPa; 20 min	nd	Jaime et al. (2010)
Carotenoids (β-carotene, lutein, violaxanthin and neoxanthin)	*Phormidium*	EtOH T = 423 K; P = 10 MPa; 20 min	nd	Rodriguez-Meizoso et al. (2010)
Fucoxanthin	*Eisenia bicyclis*	90 % EtOH + 10 % water T = 383 K; P = 10 MPa; 5 min	42 mg/100 g	Shang et al. (2011)
Astaxanthin Lutein	*Haematococcus pluvialis and Dunaliella salina*	Acetone T = 313 K; P = 10 MPa two 5-min extraction cycles	Total astaxanthin: 950 mg/100 g Lutein 90 mg/100 g	Denery et al. (2004)
Zeaxanthin	*Chlorella elipsoidea*	EtOH T = 388 K P = 10 MPa; 23.3 min	428 mg/100 g	Koo et al. (2012)

EtOH Ethanol; *nd* not determined

[a]selected optimal conditions for maximum recovery

[b]when compared with conventional extractions techniques

applications (Reverchon and De Marco 2006). Vegetables oils have also been used as potential modifiers for the extraction of carotenoids (Longo et al. 2012).

SCFE of lycopene, a carotenoid with relevant antioxidant activity and protective effects against coronary heart disease and cancer, was performed using pure CO_2 with the modifiers (co-solvents) ethanol, olive oil, and hazelnut oil (Ciurlia et al. 2009; Perretti et al. 2013; Vasapollo et al. 2004; Shi et al. 2009; Baysal et al. 2000), and high recovery yields were obtained. Ultrasound-assisted extraction with organic solvents (petroleum ether, acetone, *n*-hexane, ethanol, and ethyl acetate) has also been explored (Konwarh et al. 2012; Eh and Teoh 2012; Lianfu and Zelong 2008). Table 1.6 summarizes studies involving lycopene extraction from tomato and tomato by-products.

In other works, astaxanthin, β-carotene, and other carotenoids have been extracted by SC-CO, MAE, ultrasonic-assisted extraction (UAE), and PLE from microorganisms and small marine animals that produce high levels of carotenoids. These studies are summarized in Table 1.7, and extraction yields, when available, are presented.

1.4.2 Fatty Acids

Several researchers have investigated SC-CO_2 extraction of oils rich in ω-3 fatty acids, and have demonstrated that this technology has a negligible environmental impact, and is thus also a potential tool for functional foods and nutraceuticals applications. For example, Cheung et al. (1998a) compared the yields of ω-3 fatty acids extracted from brown seaweed (*Sargassum hemiphyllum*) using SC-CO_2 (P = 24.1–37.9 MPa; T = 313/323 K) versus Soxhlet extraction with chloroform/methanol as control. Extraction conditions of 37.9 MPa/313 K and 37.9 MPa/323 K gave the highest lipid yield, which was comparable to that of the Soxhlet method (Fig. 1.4), and the highest concentrations of total and individual ω-3 fatty acids, which were significantly higher than with solvent extraction (Fig. 1.5).

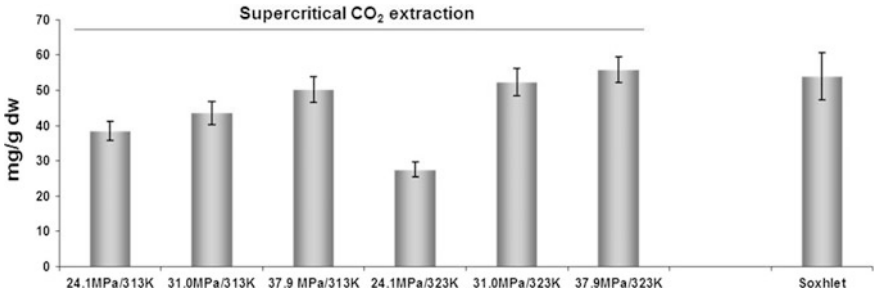

Fig. 1.4 Original lipid extracted (mg/g of dry weight) from *S. hemiphyllum* using SC-CO_2 and Soxhlet solvent extraction (Cheung et al. 1998b)

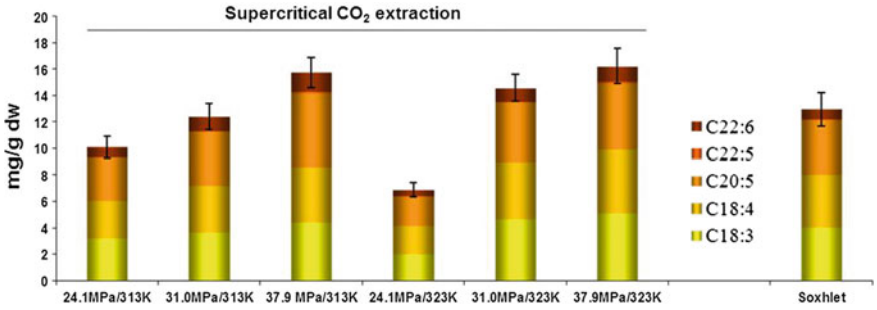

Fig. 1.5 Extraction yield of ω-3 fatty acids (mg/g of dry weight) from *S. hemiphyllum* using CO_2 and Soxhlet solvent extraction (Cheung et al. 1998b)

Amiguet et al. (2012) recently investigated the potential of northern shrimp by-products as a source of omega-3 polyunsaturated fatty acids (ω-3 PUFAs). Supercritical CO_2 extraction (SCFE) of shrimp by-products, at 35 MPa and 313 K, generated a deep red oil, rich in ω-3 PUFAs, specifically 7.8 ± 0.06 % eicosapentaenoic acid (EPA) and 8.0 ± 0.07 % docosahexaenoic acid DHA).

The SCFE of the omega-3-rich oil contained in hake by-products of the fish industry were studied by Rubio-Rodriguez et al. (2008) and the results are presented in Fig. 1.6. Extraction conditions of 25 MPa/313 K gave the highest lipid yield, which was comparable to that of the Soxhlet method (Fig. 1.6), and the highest concentrations of total fatty acids (FA), monounsaturated FA (MUFAs), polyunsaturated FA (PUFAs), EPA + DHA, ω-3, and ω-6 fatty acids, which were significantly higher than with the solvent extraction.

High-value seed oils are those that contain one or more PUFAs with desirable bioactivity. The most commonly extracted oils are those rich in γ-linoleic acid

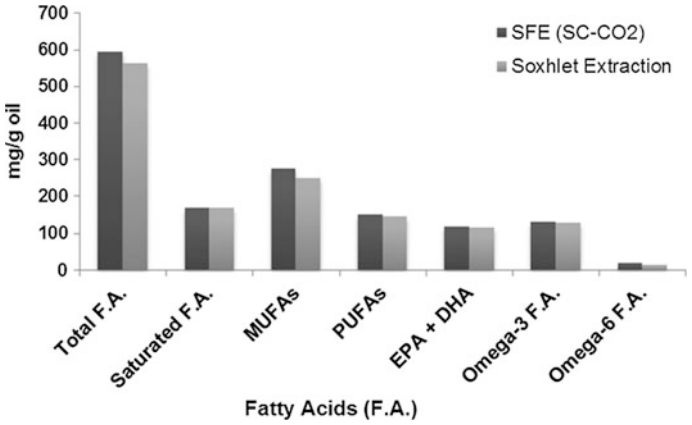

Fig. 1.6 Fatty acid profile of hake oil extracted with SC-CO_2 and with hexane as determined by AOAC method (Rubio-Rodríguez et al. 2008)

(GLA or C18:3 ω-3) and α-linoleic acid (ALA or C18:3 ω-6). They are traditionally produced by the extraction of hexane from ground seeds. Despite the efficiency of this extraction method, however, solvent elimination after extraction and possible thermal degradation of the oil represent major problems. Therefore, several authors have proposed the substitution of the traditional process with SC-CO_2 extraction of oil from seeds. In fact, the extraction of these oils by SC-CO_2 has reached the commercial stage, and the most commonly studied seeds are evening primrose, borage, blackcurrant, kiwifruit, hemp, and rose hip (Catchpole et al. 2009).

Wang et al. (2011) recently studied the fatty acid composition of tea (*Camellia sinensis* L.*)* seed oil extracted using optimized SC-CO_2. The objectives of this study were to employ SC-CO_2 to extract oil from tea seed and to determine the fatty acid composition of the extracted oil and its antioxidant activity. The authors then compared the extracts obtained by SC-CO_2 and by Soxhlet extraction. The total yield of tea seed oil by Soxhlet extraction was 25.3 ± 1.0 %, significantly lower than that (29.2 ± 0.6 %) using SC-CO_2 under optimal conditions.

The fatty acid (FA) profiles of tea seed oil extracted by SC-CO_2 and Soxhlet extraction (SE) were analysed by GC, and the results were similar (Fig. 1.7).

The tea seed oil extracted by SC-CO_2 showed much stronger DPPH radical scavenging ability than the oil extracted by Soxhlet.

Table 1.8 presents additional studies that explored the use of SC-CO_2 technology to recover fatty acids from plants, fish, algae, and fruits. non-conventional extraction techniques, namely MAE, UAE, and PLE, with hexane, ethanol, water, and acetyl lactate have been revealed as alternative technologies for isolating fatty acids and fatty acid methyl esters (FAME) from natural sources (Table 1.8).

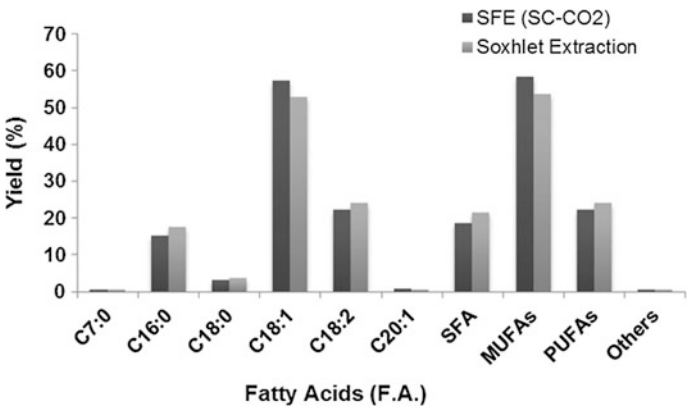

Fig. 1.7 Comparison of main fatty acid content of tea seed oil by different methods [adapted from (Wang et al. 2011)]

Table 1.8 Extraction of Fatty-acids by SC-CO_2, UAE, MAE and PLE

Extracted compounds	Source/raw material	Processing conditions[a]	Yield (mg/100 g)/ Recovery[b](%)	Reference
Supercritical CO_2 extraction[c]				
γ-Linolenic	*Arthrospira platensis*	CO_2 + EtOH T = 313 K; P = 30 MPa	35.3 %	Golmakani et al. (2012)
Fatty acids (PUFAs)	*Boletus edulis* (mushrooms)	CO_2 T = 313 K; P = 23.8 MPa	41–45 %	Vidovic et al. (2011)
Fatty acids (ω-3 PUFA, DHA, EPA)	Corn and fish oils	CO_2 T = 313; P = 16 MPa	nd	Weber et al. (2008)
Fatty acids (unsaturated)	Peach kernels	CO_2 T = 323 K; P = 30 MPa	91.5 %[d]	Mezzomo et al. (2010)
DHA	*Schizochytrium limacinum*	CO_2 + EtOH T = 313 K; P = 35 MPa	9.3 g/100 g	Tang et al. (2011)
Fatty acids (unsaturated)	Brazilian red-spotted shrimp waste	CO_2 + 15 % EtOH T = 323 K; P = 30 MPa	95.3 %	Sanchez-Camargo et al. (2012)
PUFAs	Brazilian red-spotted shrimp waste	CO_2 T = 323 K; P = 30 MPa	61.36 %	Sanchez-Camargo et al. (2011)
PUFAs	Fish by-product	CO_2 T = 333 K; P = 50 MPa	nd	Fiori et al. (2012)
ω-3 and ω-6 PUFAs	Fish oil	CO_2 T = 306/313 K; P = 20 MPa	nd	Lopes et al. (2012)
ω-3 and ω-6 PUFAs	Northern shrimp by-products	CO_2 T = 313 K; P = 35 MPa	nd	Amiguet et al. (2012)
PUFAs	Sea urchin gonad	CO_2 T = 323 K; P = 28 MPa	17.9 g/100 g	Zhu et al. (2010)
ω-3 and ω-6 PUFAs	Shellfish by-products	CO_2 T = 323 K; P = 28 MPa	17.8 g/100 g	Zhou et al. (2012)
Total fatty acids	Broccoli leaves	CO_2 T = 333 K; P = 30 MPa	1 g/100 g	Arnaiz et al. (2011)
PUFAs	Hemp seeds	CO_2 T = 313 K; P = 30 MPa	18 g/100 g	Da Porto et al. (2012)
PUFAs	Pomegranate seed oil	CO_2 T = 308 K; P = 15–30 MPa	nd[d]	Liu et al. (2012)
PUFAs	Sesame	CO_2 + EtOH T = 308–338 K; P = 20 MPa	Palmitic- 3.0 g/100 g; Stearic- 1.8 g/100 g; Oleic- 10.6 g/100 g; Linoleic- 10.9 g/100 g	Carvalho et al. (2012)

(continued)

Table 1.8 (continued)

Extracted compounds	Source/raw material	Processing conditions[a]	Yield (mg/100 g)/ Recovery[b](%)	Reference
Ultrasound-assisted extraction				
Fatty acids	Algae: *Nannochloropsis oculata*	Solvent-free, 1000 W, 30 min., 5 % dry weight;	EPA-62 mg/100 g	Adam et al. (2012)
Microwave-Assisted Extraction				
Fatty acids	Fish wastes from mackerel fillet and cod liver	Acetate:cyclohexane, 1:1 v/v, 75 W, 30 min, 2450 MHz	5.6 g/100 g mackerel and 62.6 g/100 g cod liver	Batista et al. (2001)
Pressurized liquid extraction				
Fatty acids	Algae: *Himanthalia elongata* and *Synechocystis* sp.	Hexane, EtOH and water, 323–473 K, 20 min	nd[d]	Plaza et al. (2010b)
Fatty acids	*Arthrospira platensis* (Spirulina)	EtOH:ethyl lactate (50:50), 453 K, 20 MPa, 15 min	68.3 %	Golmakani et al. (2012)

EtOH Ethanol; *nd* not determined
[a]selected optimal conditions for maximum recovery
[b]when compared with conventional extractions techniques
[c]studies performed between 2008 and 2013
[d]Antioxidant activity of extracts was evaluated

1.4.3 Tocopherols and Tocotrienols

The extraction of vitamin E from natural sources has attracted increasing interest due to the high antioxidant activity associated with this family of compounds. Moreover, natural vitamin E has been demonstrated to be more effective than a synthetic form (Friedrich 1987). Several natural matrices have been used as sources of vitamin E and tocopherols, and SCFE has been highlighted as one of the most promising methodologies for recovery of these compounds.

Wheat germ is an important source of vitamin E, and some studies have confirmed that SCFE (with CO_2) can achieve extraction yields for tocopherols similar to traditional hexane extraction (Molero and Martinez de la Ossa, 2000). In particular, Ge et al. (2002a) demonstrated that the extract derived from SCFE using the best operating conditions (P = 15 MPa; T = 213 K; CO_2 flow rate 1.5 L/min) presented higher quality (free fatty acids, 12.4 %; tocopherol content, 416.7 mg tocopherol/g wheat germ oil). Similarly another study performed with wheat germ showed higher extracted amounts of vitamin E and its isomers than those obtained using traditional methods (with *n*-hexane or chloroform/methanol mixtures) (Fig. 1.8). Thus, the extraction process using CO_2 may be economically competitive with conventional processes, as it shortens the oil refinement steps significantly and avoids the solvent distillation stage, both of which are very costly in terms of energy consumption.

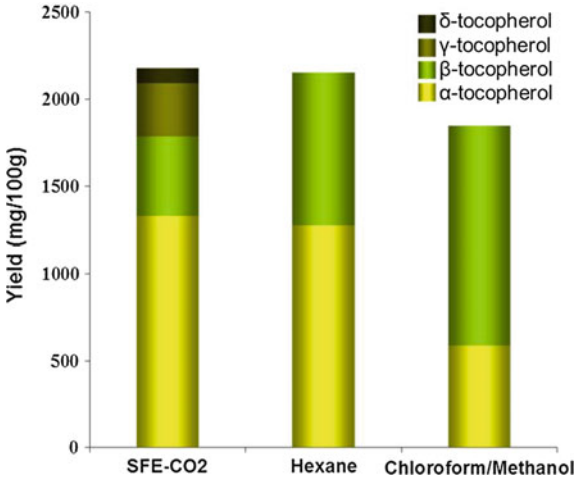

Fig. 1.8 Yield comparison of vitamin E and its isomers extracted by SCFE-CO_2 (P = 27.6 MPa; T = 313 K) with those by conventional solvent extraction (Ge et al. 2002b)

Table 1.9 summarizes published works regarding the extraction of tocopherols through SC-CO_2 and other non-conventional extraction techniques, namely MAE, UAE, and PLE.

1.4.4 Phytosterols

Plant-derived sterols in tissues and oil seeds can be isolated by solvent extraction with hexane, chloroform, diethyl ether, and acetone. However, these extraction procedures require large amounts of organic solvents, which are often expensive and potentially harmful. Extraction with supercritical carbon dioxide fluid constitutes an alternative that is advantageous, at least in terms of environmental impact (Lu et al. 2007).

For instance, King and Dunford (2002a) described a two-step supercritical fluid fractionation (SFF) method for obtaining phytosterol-enriched triglyceride fractions from rice bran and soybean oil deodorizer distillates (DD). The method comprised an extraction at 13.6 MPa and 318 K as a first step in order to remove the free fatty acids (not desirable in edible oils), while in a second step, the sterol ester-enriched triglyceride fractions were collected at 20.4 MPa and 353 K. With this method it was possible to obtain oil fractions with ~20 and 30 % (w/w) sterol content from rice bran oil and soybean oil DD, respectively, with relatively low free fatty acid content (Fig. 1.9) (King and Dunford 2002b).

The two-step SFF method demonstrated improvement over conventional methods used to obtain phytosterol-enriched products.

Huang et al. (2007) extracted β–sitosterol, stigmasterol, and ergosterol from *Anoectochilus* roxburghii. Under optimal conditions, i.e., pressure of 25 MPa, temperature of 318 K, and using ethanol as modifier, the concentrations of

Table 1.9 Extraction of tocopherols and tocotrienols by SC-CO_2, UAE, MAE and PLE

Extracted compounds	Source/raw-material	Processing conditions[a]	Yield (mg/g)/ Recovery[b] (%)	Reference
Supercritical CO_2 extraction				
Tocopherols	Sea buckthorn *(Hippophae rhamnoides)*	CO_2 T = 308 K; P = 40 MPa	77.2 %[c]	Kagliwal et al. (2011)
Tocopherols	Pomegranate seed oil	CO_2 T = 323 K; P = 45 MPa	0.43 mg/g[c]	Liu et al. (2012)
α-Tocopherol β-Tocopherol	Wheat bran	CO_2 T = 333 K; P = 30 MPa	0.03 mg/g 0.02 mg/g	Kwon et al. (2010)
α-Tocopherol γ-Tocopherol	Okra seed	CO_2 + EtOH T = 323 K; P = 45 MPa	0.15 mg/g 0.41 mg/g	Andrass et al. (2005)
α-Tocopherol β-Tocopherol γ-Tocopherol δ-Tocopherol	Wheat germ	CO_2 T = 313 K; P = 27 MPa	1.33 mg/g 0.46 mg/g 0.30 mg/g 0.09 mg/g	Ge et al. (2002a)
Pressurized liquid extraction				
Tocopherols	Palm oil	MeOH, acetonitrile, T = 323 K; P = 11 MPa 5 min	0.25 mg/g	Delgado-Zamarreno et al. (2009b)
Tocopherols	*Piper gaudichaudianum* Kunth	EtOH and petroleum ether T = 303 K; ethanol: 30 min; petroleum ether: 180 min	EtOH: 0.09 mg/g Petroleum ether: 0.02 mg/g	Peres et al. (2006)
Microwave-assisted extraction				
Tocopherols	Rice bran oil	Isopropanol and hexane, T = 393 K; solvent-to-rice bran ratio of 3:1 (w/w)	Isopropanol: 0.023 mg/g Hexane: 0.029 mg/g[c]	Zigoneanu et al. (2008)
Tocopherols	Wheat bran	MeOH T = 333–393 K; 500 W; 20 min	0.0195 mg/g[c]	Oufnac et al. (2007)
Ultrasound-assisted extraction				
Tocopherols	*Piper gaudichaudianum* Kunth	EtOH and petroleum ether T = 303 K; EtOH: 30 min; petroleum ether: 180 min	EtOH: 0.01 mg/g Petroleum ether: 0.0013 mg/g	Peres et al. (2006)

EtOH Ethanol; *MeOH* Methanol; *nd* not determined

[a]selected optimal conditions for maximum recovery

[b]when compared with conventional extractions techniques

[c]Antioxidant activity of extracts was evaluated

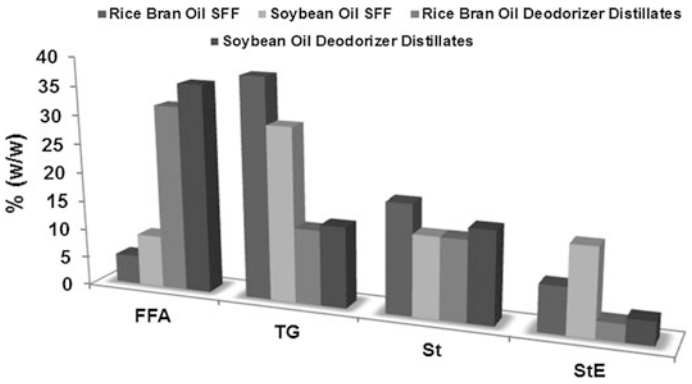

Fig. 1.9 Comparison of sterol and lipid composition of final products obtained using a two-step SFF method (rice bran oil SFF and soybean oil SFF) with rice bran oils DD and soybean oil DD as feeds of SFF (King and Dunford, 2002a)

β-sitosterol, stigmasterol, and ergosterol in the extract were found to be 2.89, 3.56, and 2.96 (g/g %), respectively (Fig. 1.10).

The concentrations of β-sitosterol, stigmasterol, and ergosterol in the extract were 4.7, 4.0 and 4.0 times higher, respectively, when SCFE was used compared with Soxhlet extraction. SCFE produced higher yields of sterols than did Soxhlet extraction.

Table 1.10 presents published works involving the extraction of phytosterols through SC-CO_2, MAE, UAE, and PLE.

1.4.5 Squalene

The importance of squalene in the cosmetic and pharmaceutical industries has driven the development of new technologies, such as counter-current SCFE, for the

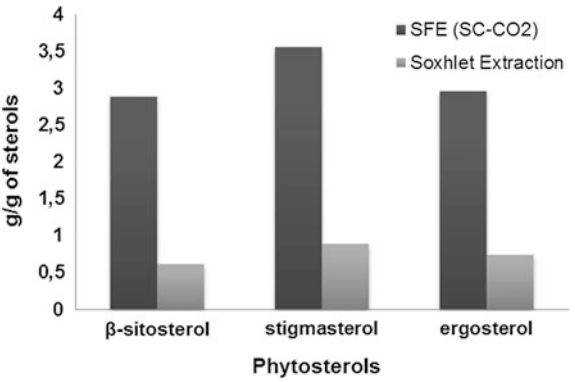

Fig. 1.10 Comparison of concentration of sterols obtained by SCFE and Soxhlet extraction [adapted from (Huang et al. 2007)]

Table 1.10 Extraction of phytosterols by SC-CO_2, UAE, MAE and PLE

Extracted compounds	Source/raw material	Processing conditions[a]	Yield (mg/g)/ recovery[b](%)	Reference
Supercritical CO_2 extraction				
Phytosterols	*Kalahari melon* seed	CO_2 T = 313 K; P = 30 MPa	0.064 mg/g	Nyam et al. (2011)
Sitosterols	Tomato by-products	CO_2 T = 353 K; P = 38 MPa	0.06 mg/g	Vagi et al. (2007)
β-Sitosterol Stigmasterol	*A. roxburghii*	CO_2 + ethanol T = 318 K; P = 25 MPa	1.06 mg/g 1.19 mg/g	Huang et al. (2007)
β-Sitosterol	Roasted loquat seed	CO_2 T = 353 K; P = 30 MPa	≈75 %	Kawahito et al. (2008)
Phytosterols	Sea buckthorn (*Hippophae rhamnoides* L.)	CO_2 T = 323 K; P = 51.7 MPa	nd	Li et al. (2007)
Campesterol Stigmasterol β-Sitosterol β-Amyrin	Lotus bee pollen (*Nelumbo nucifera* Gaertn)	CO_2 T = 323 K; P = 38.2 MPa	0.49 mg/g	Xu et al. (2011b)
Phytosterols	Roselle seed (*Hibiscus sabdariffa* L.)	CO_2 + ethanol T = 313 K; P = 40 MPa	0.040 mg/g	Nyam et al. (2010)
β-Sitosterol	Sea buckthorn seed (*Hippophae rhamnoides* L)	CO_2 T = 313 K; P = 15 MPa	0.31 mg/g	Sajfrtova et al. (2010)
Pressurized liquid extraction				
β-Sitosterol	Seeds and nuts	MeOH, 50°C; 11 MPa and two 5 min cycles	Almond- 1.16 mg/g Pistachio-1.85 mg/g	Delgado-Zamarreno et al. (2009a)
Fucosterol	*Himanthalia elongate*	EtOH, 100°C, 10 MPa, 20 min	nd[c]	Santoyo et al. (2011)
Stigmasterol β-Sitosterol	*Piper gaudichaudianum* Kunth	EtOH and petroleum ether, 303 K, EtOH: 30 min; petroleum ether: 180 min.	Stigmasterol: EtOH-0.06 mg/g Petroleum ether-0.05 mg/g β-Sitosterol: EtOH-0.02 mg/g Petroleum ether-0.01 mg/g	Peres et al. (2006)
Ultrasound-assisted extraction				
Stigmasterol β-Sitosterol	*Piper gaudichaudianum* Kunth	EtOH and petroleum ether, 303 K, ethanol: 30 min; petroleum ether: 180 min.	Stigmasterol: EtOH-0.005 mg/g Petroleum ether-0.004 mg/g β-Sitosterol: EtOH-0.010 mg/g Petroleum ether-0.0009 mg/g	Peres et al. (2006)
Microwave-assisted extraction				
Fucosterol	Marine algae	Ethanolic potassium hydroxide, 30:1 (liquid:solid ratio), 343 K, 300 W, 30 min	1.15 mg/g	Xiao et al. (2013)

[a]selected optimal conditions for maximum recovery
EtOH Ethanol; *MeOH* Methanol; *nd* not determined
[b]when compared with conventional extractions techniques
[c]Antioxidant activity of extracts was evaluated

Table 1.11 Extraction of squalene from animal and vegetable sources

Source	Squalene concentration (%w/w)		Reference
	Raw material (%)	SCF extract (%)	
Shark liver oil	55	92–99	Catchpole et al. (1997)
Olive oil by-product	52	90	Vasquez et al. (2007)
Palm fatty acid distillate (PFAD)	50	95	Al-Darmaki et al. (2012)

purification of squalene from various sources. The primary source of squalene is shark liver oil, but it can also be found in olive oil by-products.

The recovery of squalene from shark liver oil by SCFE has been reported in the literature (Catchpole et al. 2000a, b). This raw material also contains many triacylglycerols, alkoxyglycerols, sterol esters, and pristine squalene. Therefore, extraction process conditions are needed that allow the highest solubility of only squalene, in order to maximize the purity and yield of the extract. This is also the case when olive oil DD containing free fatty acids and methyl and ethyl esters are used as raw materials.

Al-Darmaki et al. (2012) recently studied the extraction and recovery of squalene using SC-CO_2 as solvent from palm fatty acid distillate (PFAD). The process was carried out on a counter-current glass bead-packed column. The operating conditions investigated were pressure and temperature, which varied from 10 to 20 MPa and from 313 to 353 K, respectively (Al-Darmaki et al. 2012).

Table 1.11 presents three studies related to successful isolation of squalene-rich extracts from shark liver oil and olive oil by-products.

1.4.6 Essential Oils

Essential oils have become a target for the recovery of natural bioactive substances. According to Fornari et al. (2012), since 2000, nearly 4000 articles have been published in which "essential oil" or "volatile oil" appeared as keyword, and around 3000 of these also included the word "bioactive" or "bioactivity" in the article text (Fornari et al. 2012). Moreover, essential oils and their extraction have been reviewed extensively (Moyler 1993; Chen and Spiro 1994; Kerrola 1995; Reverchon 1997; Burt 2004; Lucchesi et al. 2004; Edris 2007; Mustafa and Turner 2011; Mason et al. 2011; Xu et al. 2011a; Sovová 2012; Abad et al. 2012; Fornari et al. 2012; Kokolakis and Golfinopoulos 2013; Capuzzo et al. 2013). For this reason, an overview of selected publications regarding the extraction of essential oils using different methodologies is presented instead of a resume table.

In industrial practice, essential oils are obtained by steam distillation; in laboratory practice, steam distillation and hydro-distillation are used. However, processing temperature is a drawback in the extraction of thermally labile compounds (Pereira and Meireles 2010). In order to overcome this, SCFs have been used

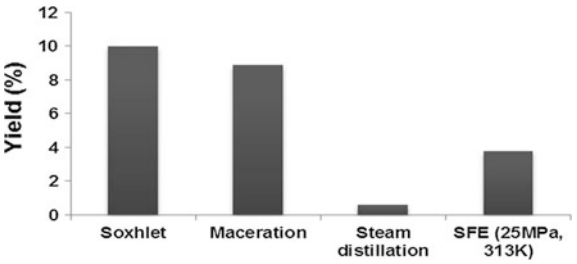

Fig. 1.11 Extraction Yield (%) of chamomile flower head with SCFE and conventional methods [adapted from (Kotnik et al. 2007)]

successfully for the recovery of these substances, and in some cases, comparisons with conventional methods have been performed.

Chamomile flower heads, which are widely used in the cosmetic, pharmaceutical, and food industries due to their biological activity (anti-spasmodic, anti-inflammatory, and antimicrobial properties), were extracted using SC-CO_2 technology (Kotnik et al. 2007). Various pressures (10, 15, and 25 MPa) and temperatures (303 and 313 K) were assessed, with the highest extraction yield achieved at 25 MPa and 313 K. The results were further compared with those obtained using Soxhlet extraction, steam distillation, and maceration (Fig. 1.11), and SC-CO_2 was found to offer considerable advantages over the others. Although the yield was lower, the extract obtained with SC-CO_2 had much higher content of the active compounds matricine and bisabol (Fig. 1.12). In addition, using SC-CO_2, the matricine content was able to be increased almost threefold by using two-step separation procedures. It is also important to note that the process was successfully implemented at a pilot scale.

The same was observed in a study conducted by Moura et al. (2012), which evaluated SCFE of essential oils using ethanol and isopropyl alcohol as co-solvents. The global yield obtained with conventional techniques, including Soxhlet extraction, low-pressure solvent extraction (LPSE), ultrasound, and hydro-distillation, were compared with the global yield of SCFE (Moura et al. 2012). Essential oils were detected in extracts from all extraction methods used in this study (Fig. 1.13). Although higher global yields were obtained with conventional extraction methods, SCFE resulted in better recovery of essential oils.

In another work, three methods—hydro-distillation, PLE, and SCFE—were optimized and compared for the extraction of volatile compounds from *Cyperus*

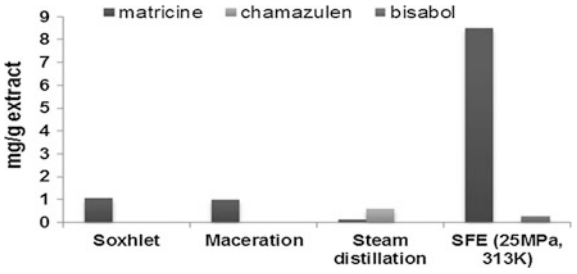

Fig. 1.12 Content of active components in chamomile extracts obtained by SCF extraction at 25 MPa, 313 K, with SCFE and conventional methods [adapted from (Kotnik et al. 2007)]

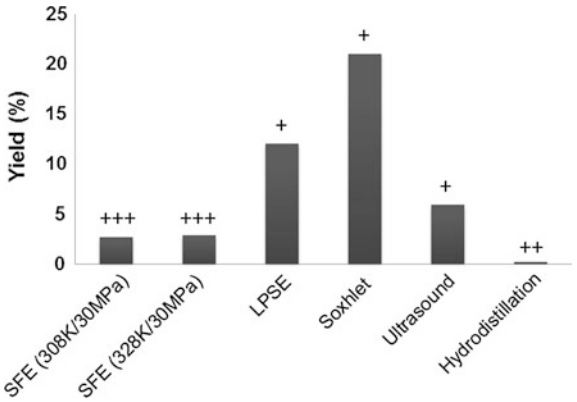

Fig. 1.13 Global yield and relative intensity of essential oils in extracts of *P. guajava* leaves from different extraction processes [adapted from (Moura et al. 2012)]

rotundus. The study showed the highest extraction efficiency with PLE, while SCFE had the best selectivity for the extraction of β-cyperone and α-cyperone (Tam et al. 2007).

The extraction of lavender essential oil was investigated using hydro-distillation, SC-CO_2, and hexane extraction techniques, and results were compared in terms of yield, chemical composition, and antimicrobial and antioxidant activity. SC-CO_2 produced a yield of 6.7 % (dry weight), which was comparable to that of solvent extraction (7.6 %) but significantly higher than that of hydro-distillation (4.6 %). The chemical composition of the oils showed considerable variation among extraction methods. Hexane extraction produced oils with the presence of waxes, colour pigments, and albuminous materials with semi-solid consistency, while hydro-distillation extracts showed evidence of thermal degradation. The SC-CO_2 extract had an aroma most closely resembling the starting material, showed negligible thermal degradation, and exhibited significantly higher antioxidant activity than the hydro-distillation and hexane extracts. Antimicrobial activity was higher in oils produced by SC-CO_2 and hydro-distillation than by hexane extraction. The results of this study demonstrate that SC-CO_2 is a promising technique for the extraction of lavender essential oil (Danh et al. 2013).

In a study by Okoh et al. (2010), *Rosmarinus officinalis* essential oil extracts obtained by hydro-distillation and microwave extraction were compared in terms of yield and antibacterial activity. Briefly, the total yield of the volatile fractions obtained through hydro-distillation and microwave extraction were 0.31 and 0.39 %, respectively. With regard to the minimum inhibitory concentration (MIC) of oils obtained by the two methods, results showed greater activity against microorganisms in the oil obtained by microwave extraction than that obtained by hydro-distillation. This observation was explained by the fact that the microwave-extracted oil contained more oxygenated compounds, and that this class of compounds has been proven to possess strong antibacterial and antifungal activity. This result shows that the composition of essential oils may vary depending on the method of extraction used (Okoh et al. 2010).

In a study by Herzi et al. (2013), the extraction of two species of *Eucalyptus* by SC-CO_2 and hydro-distillation were compared in terms of yield, chemical composition, and antioxidant activity. Superior yields were obtained using SC-CO_2. The antioxidant activity of the extracts was also assessed using the two methods, and promising radical scavenging activity was observed in the SC-CO_2 extracts. In this work, SC-CO_2 demonstrated important advantages compared to hydro-distillation, including faster extraction, improved yield, and extracts with high antioxidant quality (Herzi et al. 2013).

1.5 Process Optimization for Targeting Bioactivity

Over the last few years, special focus has been given to the design and optimization of extraction processes for the recovery of mixtures of bioactive molecules with potential health benefits, rather than the isolation of pure and single compounds. In this field, the recovery of anticancer-bioactive-rich fractions from natural sources has attracted the attention of the scientific community in their efforts to develop new natural chemotherapeutic agents. For this propose, high-pressure processes, including SCFE, were the most common recovery technologies applied. For example, in the work performed by Serra et al. (2010) a high-pressure fractionation process was optimized to isolate compounds with antiproliferative effects against colon cancer, including perillyl alcohol from sweet cherries ("Saco" variety). The methodology employed comprised a first step with SC-CO_2 followed by a second step using mixtures of CO_2 and ethanol. The effect of SC-CO_2 pre-treatment and the influence of the ethanol concentration in the solvent mixture composition was studied in relation to extract yield, phenolic content, and antiproliferative effects in human colon cancer cells. The product derived from CO_2:ethanol (90:10 v/v) extraction exhibited the highest antiproliferative activity, likely due to the presence of perillyl alcohol and high polyphenol content in the resulting extract (Fig. 1.14).

This methodology was later applied to other sweet cherry varieties, as well as to plum and peach pomaces, in order to evaluate its effectiveness on the isolation of bioactive fractions with anticancer properties from these natural sources (Silva 2013). Results showed that all extracts inhibited colon cancer cell growth and exhibited similar terpenes profiles (Fig. 1.15), evidencing the selectivity of this extraction methodology.

In a study performed by Vicente et al. (2013) SCFs technology was explored for producing rosemary extracts of different composition and their anticancer effects in human liver carcinoma cells. Several extraction conditions employed produced different extract composition in terms of carnosic acid and volatile compounds, namely 1,8 cineole, camphor, borneol, verbenone, and bornyl acetate. Results showed that the conditions P = 15 MPa, 5 % (w/w) of ethanol as co-solvent and t = 180 min obtained a fraction with an effective composition of carnosic acid and volatile compounds demonstrating the highest antiproliferative potential in cancer cells (Fig. 1.16). Among compounds, carnosic acid was shown to have a crucial

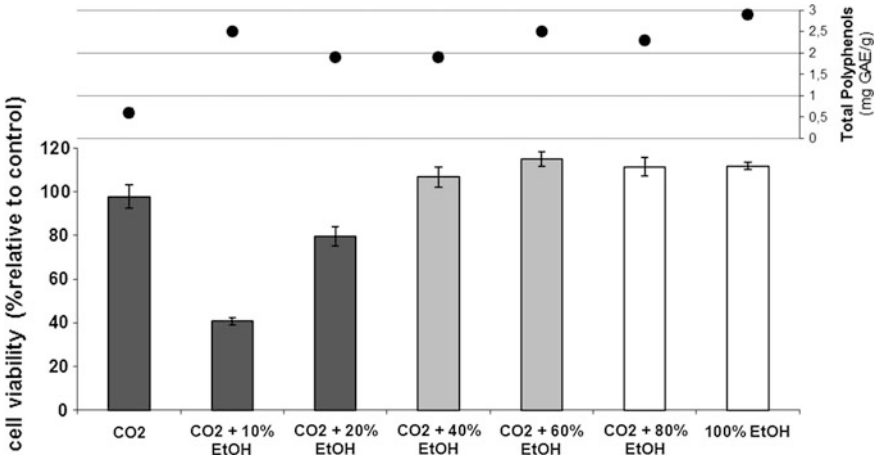

Fig. 1.14 Antiproliferative effect in HT29 colon cancer cell line of different cherry extracts produced by high-pressure technology (P = 250 bar, T = 323 K) (results obtained after 96 h of treatment with 0.5 mg/mL of extract). Comparison of bioactive effect with phenolic content (*filled circle*) and presence of perillyl alcohol (*dark filled bar*-well-defined zone detected; *light filled bar*-less-defined zone detected; *unfilled bar*-none detected) [adapted from Serra et al. (2010)]

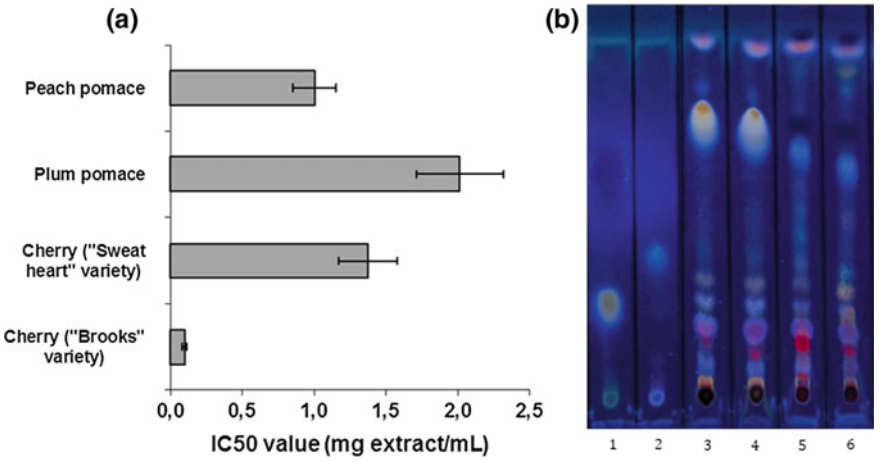

Fig. 1.15 Characterization of cherry, peach and plum extracts obtained using high-pressure technology (P = 250 bar, T = 323 K, CO_2 + EtOH 10 %). **a** Antiproliferative effect on HT29 cell line after 24 h of treatment- IC50 values; **b** Terpene profile detected by TLC (Legend: *1* perillyl alcohol standard; *2* Linalool standard; *3* Brucks cherry; *4* Sweet Hearth cherry; *5* plum; *6* peach) [adapted from Silva (2013)]

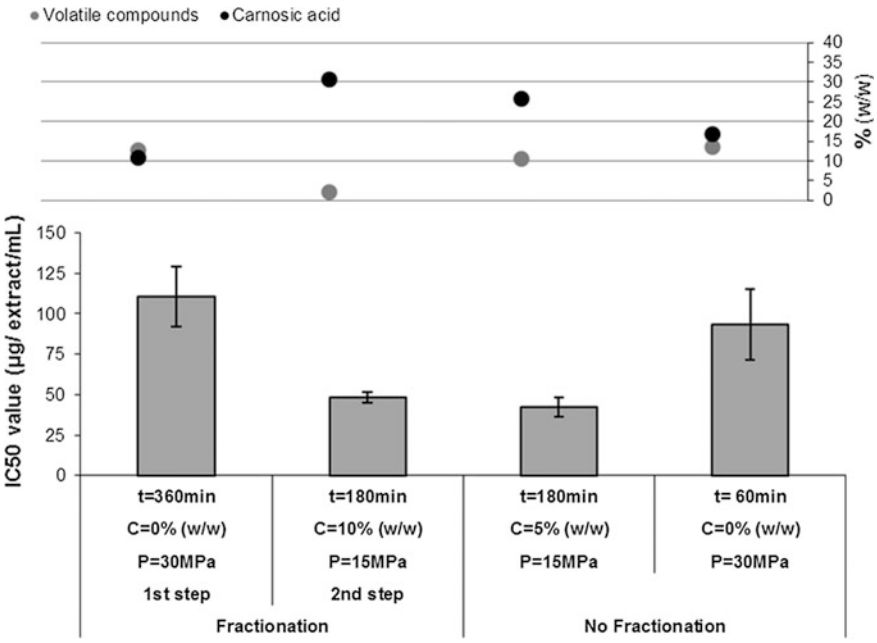

Fig. 1.16 Comparison of the antiproliferative effect in HepG2 cells of rosemary extracts obtained by supercritical fluid technology with composition in terms of carnosic acid and volatile compounds. Rosemary extracts were obtained using supercritical fluid technology under different pressure conditions and percentages of co-solvent (ethanol); (IC50 values were obtained after 48 h of treatment) (Vicente et al. 2013)

effect on growth inhibition conferred by rosemary extract. Moreover, other substances comprising the volatile oil fraction may synergize with the rosemary compound in its anti-tumoural action.

Together, these studies demonstrate the importance not only of developing methods for isolating individual compounds, but also of optimizing processes for separating fractions rich in synergistic mixtures of bioactivity.

1.6 Summary

Lipophilic compounds of various structures have shown promising bioactivity. In this chapter, conventional and new alternative techniques for the recovery and isolation of high-value lipophilic bioactives have been reviewed, with particular emphasis on supercritical fluid extraction.

References

Abad MJ, Bedoya LM, Apaza L, Bermejo P (2012) The *Artemisia L.* Genus: a review of bioactive essential oils. Molecules 17:2542–2566

Abboud J, Notario R (1999) Critical compilation of scale solvent parameters. Part I. Pure, non-hydrogen bond donor solvents. Pure Appl Chem 71:645–718

Adam F, Abert-Vian M, Peltier G, Chemat F (2012) "Solvent-free" ultrasound-assisted extraction of lipids from fresh microalgae cells: a green, clean and scalable process. Bioresour Technol 114:457–465

Adil IH, Cetin HI, Yener ME, Bayindirli A (2007) Subcritical (carbon dioxide plus ethanol) extraction of polyphenols from apple and peach pomaces, and determination of the antioxidant activities of the extracts. J Supercrit Fluids 43:55–63

Adil İH, Yener ME, Bayındırlı A (2008) Extraction of total phenolics of sour cherry pomace by high pressure solvent and subcritical fluid and determination of the antioxidant activities of the extracts. Sep Sci Technol 43:1091–1110

Ajila C, Brar S, Verma M, Tyagi R, Goudbout S, Valero J (2010) Extraction and analysis of polyphenols: recent trends. Crit Rev Biotechnol. Informa Healthcare

Al-Darmaki N, Lu T, Al-Duri B, Harris JB, Favre TLF, Bhaggan K, Santos RCD (2012) Isothermal and temperature gradient supercritical fluid extraction and fractionation of squalene from palm fatty acid distillate using compressed carbon dioxide. J Supercrit Fluids 61:108–114

Amiguet V, Kramp KL, Mao J, McRae C, Goulah A, Kimpe LE, Blais JM, Arnason JT (2012) Supercritical carbon dioxide extraction of polyunsaturated fatty acids from northern shrimp (Pandalus borealis Kreyer) processing by-products. Food Chem 130:853–858

Andıran F, Ayhan A, Tanyel FC, Abbasoğlu O, Sayek İ (2000) Regenerative capacities of normal and cirrhotic livers following 70 % hepatectomy in rats and the effect of α-Tocopherol on cirrhotic regeneration. J Surg Res 89:184–188

Andrass CD, Simanndi B, Orsi F, Lambrou C, Missopolinou-Tatala D, Panayiotou C, Domokos J, Doleschall F (2005) Supercritical carbon dioxide extraction of okra (*Hibiscus esculentus L*) seeds. J Sci Food Agric 85:1415–1419

Arnaiz E, Bernal J, Martin MT, Garcia-Viguera C, Bernal JL, Toribio L (2011) Supercritical fluid extraction of lipids from broccoli leaves. Eur J Lipid Sci Technol 113:479–486

Astorg P (1997) Food carotenoids and cancer prevention: an overview of current research. Trends Food Sci Technol 8:406–413

Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, Jahurul MHA, Ghafoor K, Norulaini NAN, Omar AKM (2013) Techniques for extraction of bioactive compounds from plant materials: a review. J Food Eng SI Extr Encapsul

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils—a review. Food Chem Toxicol 46:446–475

Batista A, Vetter W, Luckas B (2001) Use of focused open vessel microwave-assisted extraction as prelude for the determination of the fatty acid profile of fish—a comparison with results obtained after liquid-liquid extraction according to Bligh and Dyer. Eur Food Res Technol 212:377–384

Baysal T, Ersus S, Starmans DAJ (2000) Supercritical CO2 extraction of beta-carotene and lycopene from tomato paste waste. J Agric Food Chem. American Chemical Society

Bendich A (1994) Recent advances in clinical research involving carotenoids. Pure Appl Chem 66:1017–1024

Briganti S, Picardo M (2003) Antioxidant activity, lipid peroxidation and skin diseases. What's new. J Eur Acad Dermatol Venereol 17:663–669

Brunner G (2005a) Supercritical fluids: technology and application to food processing. J Food Eng IV Iberoamerican Congr Food Eng (CIBIA IV)

Brunner G (2005b) Supercritical fluids: technology and application to food processing. J Food Eng 67:21–33

Burt S (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 94:223–253

Burton-Freeman B, Reimers K (2011) Tomato consumption and health: emerging benefits. Am J Lifestyle Med 5:182–191

Cadoni E, de Giorgi MR, Medda E, Poma G (1999) Supercritical CO2 extraction of lycopene and beta-carotene from ripe tomatoes. Dyes Pigm 44:27–32

Capuzzo A, Maffei ME, Occhipinti A (2013) Supercritical fluid extraction of plant flavors and fragrances. Molecules 18:7194–7238

Carvalho RHR, Galvao EL, Barros JAC, Conceicao MM, Sousa EMBD (2012) Extraction, fatty acid profile and antioxidant activity of sesame extract (*Sesamum Indicum L.*). Braz J Chem Eng 29:409–420

Catchpole OJ, Von Kamp JC, Grey JB (1997) Extraction of squalene from shark liver oil in a packed column using supercritical carbon dioxide. Ind Eng Chem Res. American Chemical Society

Catchpole OJ, Grey JB, Noermark KA (2000a) Fractionation of fish oils using supercritical CO_2 and CO_2+ethanol mixtures. J Supercrit Fluids 19:25–37

Catchpole OJ, Simoes P, Grey JB, Nogueiro EMM, Carmelo PJ, Nunes da Ponte M (2000b) Fractionation of lipids in a static mixer and packed column using supercritical carbon dioxide. Ind Eng Chem Res. American Chemical Society

Catchpole OJ, Tallon SJ, Eltringham WE, Grey JB, Fenton KA, Vagi EM, Vyssotski MV, Mackenzie AN, Ryan J, Zhu Y (2009) The extraction and fractionation of specialty lipids using near critical fluids. J Supercrit Fluids 20th Year Anniversary Issue J Supercrit Fluids

Cavalcanti RN, Forster-Carneiro T, Gomes MTMS, Rostagno MA, Prado JM, Meireles MAA (2013) Chapter 1 uses and applications of extracts from natural sources. Nat Prod Extr Principles Appl. The Royal Society of Chemistry

Cavero S, Garcia-Risco MR, Marin FR, Jaime L, Santoyo S, Senorans FJ, Reglero G, Ibanez E (2006) Supercritical fluid extraction of antioxidant compounds from oregano: chemical and functional characterization via LC-MS and in vitro assays. J Supercrit Fluids 38:62–69

Cheah ELC, Chan LW, Heng PWS (2006) Supercritical carbon dioxide and its application in the extraction of active principles from plant materials. Asian J Pharm Sci 1:59–71

Chemat F, Tomao V, Virot M (2008) Ultrassound-assisted extraction in food analysis. In: OTLES S (ed) Handbook of food analysis instruments. CRC Press

Chen S, Spiro M (1994) Study of microwave extraction of essential oil constituents from plant materials. J Microw Power Electromagn Energy 29:231–241

Cheung PCK, Leung AYH, Ang PO (1998a) Comparison of supercritical carbon dioxide and soxhlet extraction of lipids from a brown seaweed, *Sargassum hemiphyllum* (Turn) C. Ag. J Agric Food Chem. American Chemical Society

Cheung PCK, Leung AYH, Ang PO (1998b) Comparison of supercritical carbon dioxide and soxhlet extraction of lipids from a brown seaweed, *Sargassum hemiphyllum* (Turn) C. Ag.†. J Agric Food Chem 46:4228–4232

Chew BP (1995) Antioxidant vitamins affect food animal immunity and health. J Nutr 125:1804S–1808S

Ciurlia L, Bleve M, Rescio L (2009) Supercritical carbon dioxide co-extraction of tomatoes (*Lycopersicum esculentum L.*) and hazelnuts (*Corylus avellana L.*): a new procedure in obtaining a source of natural lycopene. J Supercrit Fluids 49:338–344

Cunnane SC (2003) Problems with essential fatty acids: time for a new paradigm? Prog Lipid Res 42:544–568

da Porto C, Decorti D, Tubaro F (2012) Fatty acid composition and oxidation stability of hemp (*Cannabis sativa L.*) seed oil extracted by supercritical carbon dioxide. Ind Crops Prod 36:401–404

Daintree LS, Kordikowski A, York P (2008) Separation processes for organic molecules using SCF technologies. Adv Drug Delivery Rev Drug Delivery Appl Supercrit Fluid Technol

Danh L, Han L, Triet N, Zhao J, Mammucari R, Foster N (2013) Comparison of chemical composition, antioxidant and antimicrobial activity of lavender (*Lavandula angustifolia L.*)

essential oils extracted by supercritical CO_2, Hexane and hydrodistillation. Food Bioprocess Technol 6:3481–3489
de Paz E, Martin A, Duarte CMM, Cocero MJ (2012) Formulation of beta-carotene with poly-(e-caprolactones) by PGSS process. Powder Technol 217:77–83
Delgado-Zamarreno MM, Bustamante-Rangel M, Martinez-Pelarda D, Carabias-Martinez R (2009a) Analysis of beta-sitosterol in seeds and nuts using pressurized liquid extraction and liquid chromatography. Anal Sci 25:765–768
Delgado-Zamarreno MM, Bustamante-Rangel M, Sierra-Manzano S, Verdugo-Jara M, Carabias-Martinez R (2009b) Simultaneous extraction of tocotrienols and tocopherols from cereals using pressurized liquid extraction prior to LC determination. J Sep Sci 32:1430–1436
Denery JR, Dragull K, Tang CS, Li QX (2004) Pressurized fluid extraction of carotenoids from *Haematococcus pluvialis* and *Dunaliella salina* and kavalactones from *Piper methysticum*. Anal Chim Acta 501:175–181
Destandau E, Michel T, Elfakir C (2013) Chapter 4 microwave-assisted extraction. Nat Prod Extr Principles Appl. The Royal Society of Chemistry
Dey S, Rathod VK (2013) Ultrasound assisted extraction of beta-carotene from *Spirulina platensis*. Ultrason Sonochem 20:271–276
Díaz-Reinoso B, Moure A, Domínguez H, Parajó JC (2006) Supercritical CO_2 extraction and purification of compounds with antioxidant activity. J Agric Food Chem 54:2441–2469
Dunford NT, Irmak S, Jonnala R (2010) Pressurised solvent extraction of policosanol from wheat straw, germ and bran. Food Chem 119:1246–1249
Edris AE (2007) Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytotherapy Res 21:308–323
Egydio JA, Moraes AM, Rosa PTV (2010) Supercritical fluid extraction of lycopene from tomato juice and characterization of its antioxidation activity. J Supercrit Fluids 54:159–164
Eh AL, Teoh SG (2012) Novel modified ultrasonication technique for the extraction of lycopene from tomatoes. Ultrason Sonochem 19:151–159
Esclapez MD, Garcia-Perez JV, Mulet A, Carcel JA (2011) Ultrasound-assisted extraction of natural products. Food Eng Rev Food Eng Rev. Springer-Verlag
Fernandes P, Cabral JM (2007) Phytosterols: applications and recovery methods. Bioresour Technol 98:2335–2350
Fernandez-Ronco MP, De Lucas A, Rodriguez JF, Garcia MT, Gracia I (2013) New considerations in the economic evaluation of supercritical processes: Separation of bioactive compounds from multicomponent mixtures. The journal of supercritical fluids special issue—10th international symposium on supercritical fluids special issue—10th international symposium on supercritical fluids
Fiori L, Solana M, Tosi P, Manfrini M, Strim C, Guella G (2012) Lipid profiles of oil from trout (*Oncorhynchus mykiss*) heads, spines and viscera: trout by-products as a possible source of omega-3 lipids? Food Chem 134:1088–1095
Folmer BM, Barron D, Hughes E, Miguet L, Sanchez B, Heudi O, Rouvet M, Sagalowicz L, Callier P, Michel M, Williamson G (2009) Monocomponent hexa- and dodecaethylene glycol succinyl-tocopherol esters: self-assembly structures, cellular uptake and sensitivity to enzyme hydrolysis. Biochem Pharmacol 78:1464–1474
Fornari T, Vicente G, Vázquez E, García-Risco MR, Reglero G (2012) Isolation of essential oil from different plants and herbs by supercritical fluid extraction. J Chromatogr A 1250:34–48
Friedman M, Henika PR, Mandrell RE (2002) Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes*, and *Salmonella enterica*. J Food Prot® 65:1545–1560
Friedrich W (1987) Handbuch der vitamine. Urban & Schwarzenberg, Munchen
Fritsche K (2006) Fatty acids as modulators of the immune response. Annu Rev Nutr 26:45–73
Fujii K (2012) Process integration of supercritical carbon dioxide extraction and acid treatment for astaxanthin extraction from a vegetative microalga. Food Bioprod Process 90:762–766

Garcia C, Talarico L, Almeida N, Colombres S, Duschatzky C, Damonte E (2003) Virucidal activity of essential oils from aromatic plants of San Luis, Argentina. Phytotherapy Res 17:1073–1075

Ge Y, Yan H, Hui B, Ni Y, Wang S, Cai T (2002a) Extraction of natural vitamin E from wheat germ by supercritical carbon dioxide. J Agric Food Chem. American Chemical Society

Ge Y, Yan H, Hui B, Ni Y, Wang S, Cai T (2002b) Extraction of natural vitamin E from wheat germ by supercritical carbon dioxide. J Agric Food Chem 50:685–689

Gerster H (1993) Anticarcinogenic effect of common carotenoids. Internat J Vit Nutr Res J Vit Nutr Res

Geusens P, Wouters C, Nijs J, Jiang Y, Dequeker J (1994) Long-term effect of omega-3 fatty acid supplementation in active rheumatoid arthritis. Arthritis Rheum 37:824–829

Golmakani MT, Mendiola JA, Rezaei K, Ibanez E (2012) Expanded ethanol with CO_2 and pressurized ethyl lactate to obtain fractions enriched in γ-Linolenic Acid from Arthrospira platensis (Spirulina). J Supercrit Fluids 62:109–115

Haeger K (1974) Long-time treatment of intermittent claudication with vitamin E. Am J Clin Nutr 27:1179–1181

He HP, Cai Y, Sun M, Corke H (2001) Extraction and purification of squalene from amaranthus grain. J Agric Food Chem. American Chemical Society

Herrera MC, de Castro MD (2005) Ultrasound-assisted extraction of phenolic compounds from strawberries prior to liquid chromatographic separation and photodiode array ultraviolet detection. J Chromatogr A 1100:1–7

Herrero M, Cifuentes A, Ibanez E (2006a) Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae—a review. Food Chem 98:136–148

Herrero M, Cifuentes A, Ibanez E (2006b) Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae: a review. Food Chem 98:136–148

Herrero M, Mendiola JA, Cifuentes A, Ibanez E (2010) Supercritical fluid extraction: recent advances and applications. J Chromatogr A Extr Tech

Herzi N, Bouajila J, Camy S, Cazaux S, Romdhane M, Condoret JS (2013) Comparison between supercritical CO_2 extraction and hydrodistillation for two species of eucalyptus: yield, chemical composition, and antioxidant activity. J Food Sci 78:C667–C672

Hodge L, Salome C, Hughes J, Liu-Brennan D, Rimmer J, Allman M, Pang D, Armour C, Woolcock A (1998) Effect of dietary intake of omega-3 and omega-6 fatty acids on severity of asthma in children. Eur Respir J 11:361–365

Hooper L, Thompson RL, Harrison RA, Summerbell CD, Ness AR, Moore HJ, Worthington HV, Durrington PN, Higgins J, Capps NE (2006) Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. BMJ Brit Med J (Int Edn) 332

Huang L, Zhong T, Chen T, Ye Z, Chen G (2007) Identification of beta-sitosterol, stigmasterol and ergosterin in A. roxburghii using supercritical fluid extraction followed by liquid chromatography/atmospheric pressure chemical ionization ion trap mass spectrometry. Rapid Commun Mass Spectrom 21:3024–3032

Huang W, Li Z, Niu H, Li D, Zhang J (2008) Optimization of operating parameters for supercritical carbon dioxide extraction of lycopene by response surface methodology. J Food Eng 89:298–302

Ibáñez E, Palacios J, Señoráns FJ, Santa-María G, Tabera J, Reglero G (2000) Isolation and separation of tocopherols from olive by-products with supercritical fluids. J Am Oil Chem Soc 77:187–190

Jacques PF, Chylack LT (1991) Epidemiologic evidence of a role for the antioxidant vitamins and carotenoids in cataract prevention. Am J Clin Nutr 53:352S–355S

Jahaniaval F, Kakuda Y, Marcone MF (2000) Fatty acid and triacylglycerol compositions of seed oils of five Amaranthus accessions and their comparison to other oils. J Am Oil Chem Soc J Am Oil Chem Soc. Springer-Verlag

Jaime L, Rodriguez-Meizoso I, Cifuentes A, Santoyo S, Suarez S, Ibanez E, Senorans FJ (2010) Pressurized liquids as an alternative process to antioxidant carotenoids' extraction from *Haematococcus pluvialis* microalgae. Lwt-Food Sci Technol 43:105–112

Jones PJ, Macdougall DE, Ntanios F, Vanstone CA (1997) Dietary phytosterols as cholesterol-lowering agents in humans. Can J Physiol Pharmacol 75:217–227

Kagliwal LD, Patil SC, Pol AS, Singhal RS, Patravale VB (2011) Separation of bioactives from seabuckthorn seeds by supercritical carbon dioxide extraction methodology through solubility parameter approach. Sep Purif Technol 80:533–540

Kassama LS, Shi J, Mittal GS (2008) Optimization of supercritical fluid extraction of lycopene from tomato skin with central composite rotatable design model. Sep Purif Technol 60:278–284

Kawahito Y, Kondo M, Machmudah S, Sibano K, Sasaki M, Goto M (2008) Supercritical CO_2 extraction of biological active compounds from loquat seed. Sep Purif Technol 61:130–135

Kelly GS (1999) Squalene and its potential clinical uses. Altern Med Rev J Clin Ther 4:29–36

Kerrola K (1995) Literature review: isolation of essential oils and flavor compounds by dense carbon dioxide. Food Rev Int 11:547–573

Khor HT, Chieng DY (1997) Lipidaemic effects of tocotrienols, tocopherols and squalene: studies in the hamster. Asia Pac J Clin Nutr 6:36–40

Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA, Wang J, Yu B, Zhang J, Bryant SH (2015) PubChem substance and compound databases. Nucleic Acids Res

King JW (2000) Sub- and Supercritical fluid processing of agrimaterials: extraction, fractionation and reaction modes. Supercrit Fluids Fundam Appl

King JW, Dunford NT (2002a) Phytosterol-enriched triglyceride fractions from vegetable oil deodorizer distillates utilizing supercritical fluid fractionation technology. Sep Sci Technol 37:451–462

King JW, Dunford NT (2002b) Phytosterol-enriched triglyceride fractions from vegetable oil deodorizer distillates utilizing supercritical fluid fractionation technology. Sep Sci Technol. Taylor & Francis

King JW, Srinivas K (2009) Multiple unit processing using sub- and supercritical fluids. J Supercrit Fluids 20th Year Anniversary Issue J Supercrit Fluids

Kohlmeier L, Hastings SB (1995) Epidemiologic evidence of a role of carotenoids in cardiovascular disease prevention. Am J Clin Nutr 62:1370S–1376S

Kokolakis AK, Golfinopoulos SK (2013) Microwave-assisted techniques (MATs); a quick way to extract a fragrance: a review. Nat Prod Commun 8:1493–1504

Konwarh R, Pramanik S, Kalita D, Mahanta CL, Karak N (2012) Ultrasonication—a complementary "green chemistry" tool to biocatalysis: a laboratory-scale study of lycopene extraction. Ultrason Sonochem 19:292–299

Koo S, Cha K, Song DG, Chung D, Pan CH (2012) Optimization of pressurized liquid extraction of zeaxanthin from *Chlorella ellipsoidea*. J Appl Phycol J Appl Phycol. Springer Netherlands

Kotnik P, Škerget M, Knez Ž (2007) Supercritical fluid extraction of chamomile flower heads: comparison with conventional extraction, kinetics and scale-up. J Supercrit Fluids 43:192–198

Krichnavaruk S, Shotipruk A, Goto M, Pavasant P (2008) Supercritical carbon dioxide extraction of astaxanthin from *Haematococcus pluvialis* with vegetable oils as co-solvent. Bioresour Technol 99:5556–5560

Kunisaki M, Bursell SE, Umeda F, Nawata H, King GL (1998) Prevention of diabetes-induced abnormal retinal blood flow by treatment with d-α-tocopherol. BioFactors 7:55–67

Kwon KT, Uddin MS, Jung GW, Sim JE, Chun BS (2010) Supercritical carbon dioxide extraction of phenolics and tocopherols enriched oil from wheat bran. Int J Biol Life Sci 6:117–122

Lee J, Ye L, Landen J, Eitenmiller RR (2000) Optimization of an extraction procedure for the quantification of vitamin E in tomato and broccoli using response surface methodology. J Food Compos Anal 13:45–57

Leighton TG (2007) What is ultrasound? Prog Biophys Mol Biol Eff Ultrasound Infrasound Relevant Hum Health

Leitner W, Poliakoff M (2008) Supercritical fluids in green chemistry. Green Chem 10:730–735
Li BB, Smith B, Hossain M (2006) Extraction of phenolics from citrus peels: I. Solvent extraction method. Sep Purif Technol Sep Purif Food Ind
Li TSC, Beveridge THJ, Drover JCG (2007) Phytosterol content of sea buckthorn (*Hippophae rhamnoides L.*) seed oil: Extraction and identification. Food Chem 101:1633–1639
Li Y, Radoiu M, Fabiano-Tixier AS, Chemat F (2013) From laboratory to industry: scale-up, quality, and safety consideration for microwave-assisted extraction. Microwave-Assist Extr Bioact Compd. Springer
Lianfu Z, Zelong L (2008) Optimization and comparison of ultrasound/microwave assisted extraction (UMAE) and ultrasonic assisted extraction (UAE) of lycopene from tomatoes. Ultrason Sonochem 15:731–737
Lim G-B, Lee S-Y, Lee E-K, Haam S-J, Kim W-S (2002) Separation of astaxanthin from red yeast *Phaffia rhodozyma* by supercritical carbon dioxide extraction. Biochem Eng J 11:181–187
Liu J (2005) A combination of mitochondrial nutrients for relieving stress, preventing and improving stress-related disorders. Google Pat
Liu GM, Xu X, Gong Y, He L, Gao YX (2012) Effects of supercritical CO_2 extraction parameters on chemical composition and free radical-scavenging activity of pomegranate (*Punica granatum L.*) seed oil. Food Bioprod Process 90:573–578
London RS, Sundaram G, Murphy L, Goldstein P (1983) The effect of alpha-tocopherol on premenstrual symptomatology: a double-blind study. J Am Coll Nutr 2:115–122
Longo C, Leo L, Leone A (2012) Carotenoids, fatty acid composition and heat stability of supercritical carbon dioxide-extracted-oleoresins. Int J Mol Sci 13:4233–4254
Lopes BLF, Sanchez-Camargo AP, Ferreira ALK, Grimaldi R, Paviani LC, Cabral FA (2012) Selectivity of supercritical carbon dioxide in the fractionation of fish oil with a lower content of EPA plus DHA. J Supercrit Fluids 61:78–85
López M, Arce L, Garrido J, Rios A, Valcarcel M (2004) Selective extraction of astaxanthin from crustaceans by use of supercritical carbon dioxide. Talanta 64:726–731
Lu B, Zhang Y, Wu X, Shi J (2007) Separation and determination of diversiform phytosterols in food materials using supercritical carbon dioxide extraction and ultraperformance liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry. Anal Chim Acta 588:50–63
Lucchesi ME, Chemat F, Smadja J (2004) Solvent-free microwave extraction of essential oil from aromatic herbs: comparison with conventional hydro-distillation. J Chromatogr A 1043:323–327
Lungershausen YK, Abbey M, Nestel PJ, Howe PR (1994) Reduction of blood pressure and plasma triglycerides by omega-3 fatty acids in treated hypertensives. J Hypertens 12:1041–1046
Luque De Castro MD, Priego-Capote F (2010) Soxhlet extraction: past and present panacea. J Chromatogr Extr Tech
Machmudah S, Winardi S, Sasaki M, Goto M, Kusumoto N, Hayakawa K (2012) Lycopene extraction from tomato peel by-product containing tomato seed using supercritical carbon dioxide. J Food Eng 108:290–296
Macias-Sanchez MD, Fernandez-Sevilla JM, Fernandez FGA, Garcia MCC, Grima EM (2010) Supercritical fluid extraction of carotenoids from Scenedesmus almeriensis. Food Chem 123:928–935
Marubayashi S, Dohi K, Ochi K, Kawasaki T (1986) Role of free radicals in ischemic rat liver cell injury: prevention of damage by alpha-tocopherol administration. Surgery 99:184–192
Mason TJ, CHEMAT F, VINATORU M (2011) The extraction of natural products using ultrasound or microwaves. Curr Org Chem 15:237–247
Mayne ST (1996) Beta-carotene, carotenoids, and disease prevention in humans. FASEB J 10:690–701
McClements DJ, Decker EA, Weiss J (2007) Emulsion-based delivery systems for lipophilic bioactive components. J Food Sci 72:R109–R124

Mendes RL, Nobre BP, Cardoso MT, Pereira AP, Palavra ANF (2003) Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. Inorg Chim Acta Protagonists Chem. Frausto Da Silva

Mendiola JA, Herrero M, Castro-Puyana M, Ibáñez E (2013) Supercritical fluid extraction. Nat Prod Extr Principles Appl 21:196

Meydani SN, Wu D, Santos MS, Hayek MG (1995) Antioxidants and immune response in aged persons: overview of present evidence. Am J Clin Nutr 62:1462S–1476S

Mezzomo N, Mileo BR, Friedrich MT, Martinez J, Ferreira SR (2010) Supercritical fluid extraction of peach (*Prunus persica*) almond oil: process yield and extract composition. Bioresour Technol 101:5622–5632

Moghadasian MH, Frohlich JJ (1999) Effects of dietary phytosterols on cholesterol metabolism and atherosclerosis: clinical and experimental evidence. Am J Med 107:588–594

Molero G, MARTINEZ DE LA OSSA (2000) Quality of wheat germ oil extracted by liquid and supercritical carbon dioxide. J Am Oil Chem Soc 77:969–974

Moura PM, Prado GHC, Meireles MAA, Pereira CG (2012) Supercritical fluid extraction from guava (*Psidium guajava*) leaves: Global yield, composition and kinetic data. J Supercrit Fluids 62:116–122

Moyler DA (1993) Extraction of essential oils with carbon dioxide. Flavour Fragrance J 8:235–247

Mustafa A, Turner C (2011) Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review. Anal Chim Acta 703:8–18

Nobre BP, Palavra AF, Pessoa FLP, Mendes RL (2009) Supercritical CO_2 extraction of trans-lycopene from Portuguese tomato industrial waste. Food Chem 116:680–685

Nyam KL, Tan CP, Lai OM, Long K, Man YBC (2010) Optimization of supercritical fluid extraction of phytosterol from roselle seeds with a central composite design model. Food Bioprod Process 88:239–246

Nyam K, Tan C, Lai O, Long K, Che Man Y (2011) Optimization of supercritical CO_2 extraction of phytosterol-enriched oil from Kalahari melon seeds. Food Bioprocess Technol Food Bioprocess Technol. Springer-Verlag

Oddy W, de Klerk N, Kendall G, Mihrshahi S, Peat J (2004) Ratio of omega-6 to omega-3 fatty acids and childhood asthma. J Asthma 41:319–326

Okoh OO, Sadimenko AP, Afolayan AJ (2010) Comparative evaluation of the antibacterial activities of the essential oils of *Rosmarinus officinalis L.* obtained by hydrodistillation and solvent free microwave extraction methods. Food Chem 120:308–312

Ollanketo M, Hartonen K, Riekkola ML, Holm Y, Hiltunen R (2001) Supercritical carbon dioxide extraction of lycopene in tomato skins. Eur Food Res Technol 212:561–565

Olmedilla B, Granado F, Blanco I, Vaquero M (2003) Lutein, but not α-tocopherol, supplementation improves visual function in patients with age-related cataracts: a 2-y double-blind, placebo-controlled pilot study. Nutrition 19:21–24

Ostlund RE (2002) Phytosterols in human nutrition. Annu Rev Nutr. Annual Reviews

Oufnac DS, Xu Z, Sun T, Sabliov C, Prinyawiwatkul W, Godber JS (2007) Extraction of antioxidants from wheat bran using conventional solvent and microwave-assisted methods. Cereal Chem J 84:125–129

Palavra AMF, Coelho JP, Barroso JG, Rauter AP, Fareleira JMNA, Mainar A, Urieta JS, Nobre BP, Gouveia L, Mendes RL, Cabral JMS, Novais JM (2011) Supercritical carbon dioxide extraction of bioactive compounds from microalgae and volatile oils from aromatic plants. In: The journal of supercritical fluids selected papers from the 9th conference on supercritical fluids and their applications, 05–08 Sept 2010, Sorrento (Italy)

Palma M, Barbero GF, Pineiro Z, Liazid A, Barroso CG, Rostagno MA, Prado JM, Meireles MAA (2013) Chapter 2 extraction of natural products: principles and fundamental aspects. Nat Prod Extr Principles Appl. The Royal Society of Chemistry

Pasquet V, Cherouvrier JR, Farhat F, Thiery V, Piot JM, Berard JB, Kaas R, Serive B, Patrice T, Cadoret JP, Picot L (2011) Study on the microalgal pigments extraction process: performance of microwave assisted extraction. Process Biochem 46:59–67

Pereira C, Meireles A (2010) Supercritical fluid extraction of bioactive compounds: fundamentals, applications and economic perspectives. Food Bioprocess Technol Food Bioprocess Technol. Springer-Verlag

Perera CO, Yen GM (2007) Functional properties of carotenoids in human health. Int J Food Prop. Taylor & Francis

Peres VF, Saffi J, Melecchi MI, Abad FC, De Assis Jacques R, Martinez MM, Oliveira EC, Caramao EB (2006) Comparison of soxhlet, ultrasound-assisted and pressurized liquid extraction of terpenes, fatty acids and vitamin E from piper gaudichaudianum Kunth

Perretti G, Troilo A, Bravi E, Marconi O, Galgano F, Fantozzi P (2013) Production of a lycopene-enriched fraction from tomato pomace using supercritical carbon dioxide. J Supercrit Fluids 82:177–182

Pingret D, Fabiano-Tixier AS, Chemat F (2013) Chapter 3 ultrasound-assisted extraction. Nat Prod Extr Principles Appl. The Royal Society of Chemistry

Plaza M, Amigo-Benavent M, del Castillo MD, Ibanez E, Herrero M (2010a) Neoformation of antioxidants in glycation model systems treated under subcritical water extraction conditions. Food Res Int 43:1123–1129

Plaza M, Santoyo S, Jaime L, Garcia-Blairsy Reina G, Herrero M, Senorans FJ, Ibanez E (2010b). Screening for bioactive compounds from algae. J Pharm Biomed Anal Nat Bioact Compd Nutrigenomics

Priego-Capote F, Delgado De La Torre MDP (2013) Chapter 5 accelerated liquid extraction. Nat Prod Extr Principles Appl. The Royal Society of Chemistry

Pronyk C, Mazza G (2009) Design and scale-up of pressurized fluid extractors for food and bioproducts. J Food Eng 95:215–226

Ranasinghe L, Jayawardena B, Abeywickrama K (2002) Fungicidal activity of essential oils of *Cinnamomum zeylanicum* (L.) and *Syzygium aromaticum* (L.) Merr et LM Perry against crown rot and anthracnose pathogens isolated from banana. Lett Appl Microbiol 35:208–211

Rao CV, Newmark HL, Reddy BS (1998) Chemopreventive effect of squalene on colon cancer. Carcinogenesis 19:287–290

Rao AV, Rao LG (2007) Carotenoids and human health. Pharmacol Res Nutr Pharmacol

Reverchon E (1997) Supercritical fluid extraction and fractionation of essential oils and related products. J Supercrit Fluids 10:1–37

Reverchon E, de Marco I (2006) Supercritical fluid extraction and fractionation of natural matter. J Supercrit Fluids 38:146–166

Reverchon E, Senatore F (1992) Isolation of rosemary oil: comparison between hydrodistillation and supercritical CO_2 extraction. Flavour Fragrance J 7:227–230

Riccioni G (2009) Carotenoids and cardiovascular disease. Curr Atherosclerosis Rep 11:434–439

Rodriguez-Meizoso I, Jaime L, Santoyo S, Senorans FJ, Cifuentes A, Ibanez E (2010) Subcritical water extraction and characterization of bioactive compounds from *Haematococcus pluvialis* microalga. J Pharm Biomed Anal Nat Bioact Compd Nutrigenomics

Routray W, Orsat V (2012) Microwave-assisted extraction of flavonoids: a review. Food Bioprocess Technol Food Bioprocess Technol. Springer-Verlag

Rozzi NL, Singh RK, Vierling RA, Watkins BA (2002) Supercritical fluid extraction of lycopene from tomato processing by-products. J Agric Food Chem. American Chemical Society

Rubio-Rodriguez N, Beltran S, Jaime I, de Diego SM, Sanz MT, Carballido JR (2010) Production of omega-3 polyunsaturated fatty acid concentrates: a review. Innovative Food Sci Emerg Technol 11:1–12

Rubio-Rodríguez N, de Diego SM, Beltrán S, Jaime I, Sanz MT, Rovira J (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. J Supercrit Fluids 47:215–226

Ruen-Ngam D, Shotipruk A, Pavasant P, Machmudah S, Goto M (2012) Selective extraction of lutein from alcohol treated *Chlorella vulgaris* by supercritical CO_2. Chem Eng Technol 35:255–260

Sabio E, Lozano M, Montero De Espinosa V, Mendes RL, Pereira AP, Palavra AF, Coelho JA (2003) Lycopene and b-carotene extraction from tomato processing waste using supercritical CO_2. Ind Eng Chem Res. American Chemical Society

Sadraei H, Asghari G, Hajhashemi V, Kolagar A, Ebrahimi M (2001) Spasmolytic activity of essential oil and various extracts of *Ferula gummosa* Boiss. on ileum contractions. Phytomedicine 8:370–376

Sahena F, Zaidul ISM, Jinap S, Karim AA, Abbas KA, Norulaini NAN, Omar AKM (2009a) Application of supercritical CO_2 in lipid extraction—a review. J Food Eng 95:240–253

Sahena F, Zaidul ISM, Jinap S, Karim AA, Abbas KA, Norulaini NAN, Omar AKM (2009b) Application of supercritical CO_2 in lipid extraction: a review. J Food Eng 95:240–253

Sajfrtova M, Lickova I, Wimmerova M, Sovova H, Wimmer Z (2010) Beta-sitosterol: supercritical carbon dioxide extraction from sea buckthorn *(Hippophae rhamnoides L.)* seeds. Int J Mol Sci 11:1842–1850

Sajilata MG, Singhal RS, Kamat MY (2008) The carotenoid pigment zeaxanthin—a review. Compr Rev Food Sci Food Saf 7:29–49

Sanchez-Camargo AP, Meireles MAA, Lopes BLF, Cabral FA (2011) Proximate composition and extraction of carotenoids and lipids from Brazilian redspotted shrimp waste (*Farfantepenaeus paulensis*). J Food Eng 102:87–93

Sanchez-Camargo AP, Meireles MAA, Ferreira ALK, Saito E, Cabral FA (2012) Extraction of omega-3 fatty acids and astaxanthin from Brazilian redspotted shrimp waste using supercritical CO_2+ethanol mixtures. J Supercrit Fluids 61:71–77

Sánchez-González L, Vargas M, González-Martínez C, Chiralt A, Cháfer M (2011) Use of essential oils in bioactive edible coatings: a review. Food Eng Rev 3:1–16

Santoyo S, Plaza M, Jaime L, Ibanez E, Reglero G, Senorans J (2011) Pressurized liquids as an alternative green process to extract antiviral agents from the edible seaweed *Himanthalia elongata*. J Appl Phycol J Appl Phycol. Springer Netherlands

Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, Burton TC, Farber MD, Gragoudas ES, Haller J, Miller DT (1994) Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. JAMA 272:1413–1420

Serra AT, Seabra IJ, Braga MEM, Bronze MR, de Sousa HC, Duarte CMM (2010) Processing cherries (*Prunus avium*) using supercritical fluid technology. Part 1: recovery of extract fractions rich in bioactive compounds. J Supercrit Fluids 55:184–191

Sesso HD, Buring JE, Norkus EP, Gaziano JM (2005) Plasma lycopene, other carotenoids, and retinol and the risk of cardiovascular disease in men. Am J Clin Nutr 81:990–997

Shang YF, Kim SM, Lee WJ, Um BH (2011) Pressurized liquid method for fucoxanthin extraction from *Eisenia bicyclis* (Kjellman) Setchell. J Biosci Bioeng 111:237–241

Shi J, Yi C, Xue SJ, Jiang Y, Ma Y, Li D (2009) Effects of modifiers on the profile of lycopene extracted from tomato skins by supercritical CO_2. J Food Eng 93:431–436

Shi J, Xue SJ, Ma Y, Jiang Y, Ye X, Yu D (2012) Green separation technologies in food processing: supercritical-CO_2 fluid and subcritical water extraction green technologies in food production and processing. In: Boye JI, Arcand Y (eds). Springer US

Sihvonen M, Jarvenpaa E, Hietaniemi V, Huopalahti R (1999) Advances in supercritical carbon dioxide technologies. Trends Food Sci Technol 10:217–222

Silva I (2013) Evaluation of chemotherapeutic potential of natural extracts using 3D models of colon cancer

Silva J, Abebe W, Sousa S, Duarte V, Machado M, Matos F (2003) Analgesic and anti-inflammatory effects of essential oils of *Eucalyptus*. J Ethnopharmacol 89:277–283

Simopoulos AP (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 56:365–379

Simopoulos AP (2008) The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med (Maywood) 233:674–688

Simsek M, Sumnu G, Sahin S (2012) Microwave assisted extraction of phenolic compounds from sour cherry pomace. Sep Sci Technol. Taylor & Francis

Smith T (2000) Squalene: potential chemopreventive agent. Expert Opin Investig Drugs. Expert Opinion

Smith TJ, Yang GY, Seril DN, Liao J, Kim S (1998) Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis by dietary olive oil and squalene. Carcinogenesis 19:703–706

Snodderly DM (1995) Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. Am J Clin Nutr 62:1448S–1461S

Soria AC, Villamiel M (2010) Effect of ultrasound on the technological properties and bioactivity of food: a review. Trends Food Sci Technol 21:323–331

Sovová H (2012) Modeling the supercritical fluid extraction of essential oils from plant materials. J Chromatogr A 1250:27–33

Stahl W, Sies H (2005) Bioactivity and protective effects of natural carotenoids. Biochim Biophy Acta (BBA)-Mol Basis Dis 1740:101–107

Starmans DAJ, Nijhuis HH (1996) Extraction of secondary metabolites from plant material: a review. Trends Food Sci Technol 7:191–197

Szentmihalyi K, Vinkler P, Lakatos B, Illes V, Then M (2002) Rose hip (*Rosa canina L.*) oil obtained from waste hip seeds by different extraction methods. Bioresour Technol 82:195–201

Tam CU, Yang FQ, Zhang QW, Guan J, Li SP (2007) Optimization and comparison of three methods for extraction of volatile compounds from *Cyperus rotundus* evaluated by gas chromatography–mass spectrometry. J Pharm Biomed Anal 44:444–449

Tang SK, Qin CR, Wang HG, Li SF, Tian SJ (2011) Study on supercritical extraction of lipids and enrichment of DHA from oil-rich microalgae. J Supercrit Fluids 57:44–49

Tao Y, Sun DW (2013) Enhancement of food processes by ultrasound: a review. Crit Rev Food Sci Nutr. Taylor & Francis

Topal U, Sasaki M, Goto M, Hayakawa K (2006) Extraction of lycopene from tomato skin with supercritical carbon dioxide: effect of operating conditions and solubility analysis. J Agric Food Chem. American Chemical Society

Vagi E, Simandi B, Vasarhelyine KP, Daood H, Kery A, Doleschall F, Nagy B (2007) Supercritical carbon dioxide extraction of carotenoids, tocopherols and sitosterols from industrial tomato by-products. J Supercrit Fluids 40:218–226

Vasapollo G, Longo L, Rescio L, Ciurlia L (2004) Innovative supercritical CO_2 extraction of lycopene from tomato in the presence of vegetable oil as co-solvent. J Supercrit Fluids 29:87–96

Vasquez L, Torres CF, Fornari T, Senorans F, Reglero G (2007) Recovery of squalene from vegetable oil sources using countercurrent supercritical carbon dioxide extraction. J Supercrit Fluids 40:59–66

Vicente G, Molina S, Gonzalez-Vallinas M, Garcia-Risco MR, Fornari T, Reglero G, De Molina AR (2013) Supercritical rosemary extracts, their antioxidant activity and effect on hepatic tumor progression. In: The journal of supercritical fluids special issue—10th international symposium on supercritical fluids special issue—10th international symposium on supercritical fluids

Vidovic S, Mujic I, Zekovic Z, Lepojevic Z, Milosevic S, Jokic S (2011) Extraction of fatty acids from boletus edulis by subcritical and supercritical carbon dioxide. J Am Oil Chem Soc J Am Oil Chem Soc. Springer-Verlag

von Schacky C, Angerer P, Kothny W, Theisen K, Mudra H (1999) The effect of dietary omega-3 fatty acids on coronary atherosclerosis. Ann Intern Med 130:554–562

Wang L, Weller CL (2006) Recent advances in extraction of nutraceuticals from plants. Trends Food Sci Technol 17:300–312

Wang L, Weller CL, Schlegel VL, Carr TP, Cuppett SL (2008) Supercritical CO_2 extraction of lipids from grain sorghum dried distillers grains with solubles. Bioresour Technol 99:1373–1382

Wang Y, Sun D, Chen H, Qian L, Xu P (2011) Fatty acid composition and antioxidant activity of tea (*Camellia sinensis L.*) seed oil extracted by optimized supercritical carbon dioxide. Int J Mol Sci 12:7708–7719

Weber A, Catchpole O, Eltringham W (2008) Supercritical fluid assisted, integrated process for the synthesis and separation of different lipid derivatives. J Sep Sci 31:1346–1351

Xiao W, Han L, Shi B (2008) Optimization of microwave: assisted extraction of flavonoid from radix astragali using response surface methodology. Sep Sci Technol. Taylor & Francis

Xiao X-H, Yuan Z-Q, Li G-K (2013) Preparation of phytosterols and phytol from edible marine algae by microwave-assisted extraction and high-speed counter-current chromatography. Sep Purif Technol 104:284–289

Xu LL, Zhan XR, Zeng ZW, Chen R, Li HF, Xie T, Wang SL (2011a) Recent advances on supercritical fluid extraction of essential oils. Afr J Pharm Pharmacol 5:1196–1211

Xu X, Dong J, Mu X, Sun L (2011b) Supercritical CO_2 extraction of oil, carotenoids, squalene and sterols from lotus (*Nelumbo nucifera Gaertn*) bee pollen. Food Bioprod Process 89:47–52

Yamaguchi K, Murakami M, Nakano H, Konosu S, Kokura T, Yamamoto H, Kosaka M, Hata K (1986). Supercritical carbon dioxide extraction of oils from Antarctic krill. J Agric Food Chem. American Chemical Society

Yamashita T, Oda E, Sano T, Yamashita T, Ijiru Y, Giddings J, Yamamoto J (2005) Varying the ratio of dietary n−6/n−3 polyunsaturated fatty acid alters the tendency to thrombosis and progress of atherosclerosis in apoE−/−LDLR−/−double knockout mouse. Thromb Res 116:393–401

Yi C, Shi J, Xue SJ, Jiang Y, Li D (2009) Effects of supercritical fluid extraction parameters on lycopene yield and antioxidant activity. Food Chem 113:1088–1094

Zhang HF, Yang XH, Wang Y (2011) Microwave assisted extraction of secondary metabolites from plants: current status and future directions. Trends Food Sci Technol Spec Sect Recent Dev Food Sci Technol Jpn

Zhao L, Zhao G, Chen F, Wang Z, Wu J, Hu X (2006) Different effects of microwave and ultrasound on the stability of (all-E)-astaxanthin. J Agric Food Chem. American Chemical Society

Zhou DY, Tong LEI, Zhu BW, Wu HT, Qin LEI, Tan HUI, Murata YOSH (2012) Extraction of lipid from abalone (*Haliotis discus hannai Ino*) Gonad by supercritical carbon dioxide and enzyme-assisted organic solvent methods. J Food Process Preserv 36:126–132

Zhu BW, Qin L, Zhou DY, Wu HT, Wu J, Yang JF, Li DM, Dong XP, Murata Y (2010) Extraction of lipid from sea urchin (*Strongylocentrotus nudus*) gonad by enzyme-assisted aqueous and supercritical carbon dioxide methods. Eur Food Res Technol Eur Food Res Technol. Springer-Verlag

Ziegler RG (1989) A review of epidemiologic evidence that carotenoids reduce the risk of cancer. J Nutr 119:116–122

Zigoneanu IG, Williams L, Xu Z, Sabliov CM (2008) Determination of antioxidant components in rice bran oil extracted by microwave-assisted method. Bioresour Technol 99:4910–4918