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CHAPTER 7. SUPERCRITICAL FLUID EXTRACTION

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

Instituto de Investigación en Ciencias de la Alimentación/Institute of Food Science Research CIAL (CSIC-UAM)

C/ Nicolás Cabrera, 9 (Campus de Cantoblanco)

28049 Madrid, SPAIN

Corresponding author: elena@ifi.csic.es

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7.1. INTRODUCTION

Today, there is a wide range of classical extraction techniques that have been traditionally used to extract bioactive compounds from natural matrices. Although these techniques are routinely used, they have several recognized drawbacks; they are tedious, difficult to automation and therefore are more prone to present low reproducibility, they also have low selectivity and/or provide low extraction yields. These shortcomings can be partially or completely overcome by using the newly developed advanced extraction techniques. This new kind of extraction techniques are characterized by being faster, more selective towards the compounds to be extracted and, also very important nowadays, these techniques are more environmentally friendly. In fact, by using the considered advanced extraction techniques, the use of toxic solvents is highly limited.

Supercritical fluid extraction (SFE) is based on the use of solvents at temperatures and pressures above their critical points. SFE can be a fast, efficient, and clean method for the extraction of natural products from several matrices. The ease of tuning the operating conditions in order to increase the solvation power makes this technology a good option for the recovery of several types of substances[1, 2].

7.2. FUNDAMENTALS OF SUPERCRITICAL FLUID EXTRACTION

Supercritical fluid extraction was first introduced in 1879 by Hannay and Hogarth. Despite the advantages associated to the use of supercritical fluids as extracting agents, it was not until around 1960 that this extraction method started to be thoroughly investigated as an alternative to conventional extraction techniques such as SLE (solid–liquid extraction) and LLE (liquid–liquid extraction), both requiring large amounts of hazardous chemicals such as chlorinated solvents.

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The discovery of the critical phenomena is attributed to Charles Cagniard de la Tour in 1822.[3] Experiments on steam engines in the late 17th and early 18th centuries motivated interest in the behavior of fluids at high temperatures and pressures. The discovery of what we now call "the critical point" came about with Cagniard de la Tour's experiments in acoustics; he placed a ball in a digester barrel partially filled with liquid. Upon rolling the device, a splashing sound was generated as the solid ball penetrated the liquid-vapour interface. But heating the system far beyond the boiling point the splashing sound ceased above a certain temperature. This marks the discovery of the supercritical fluid phase. He measured the critical temperature at which the interface tension vanished, as determined by the disappearance of the meniscus, for different substances such as water, alcohol, ether and carbon bisulphide. In 1869, the term "critical point" was coined by Thomas Andrews, who further elucidated the meaning of Cagniard de la Tour's *état particulier*[3]. The important concept of universality of critical phenomena was introduced by Pierre Curie, who discovered that ferromagnetic materials become demagnetized above the critical temperature[4]. The field of critical phenomena has blossomed and now forms a keystone of modern science, both experimental and theoretical and its development exemplifies how a topic of purely fundamental research, can diversify into initially unforeseeable directions.

7.2.1. Physical properties of supercritical fluids

As the substance approaches its critical temperature, the properties of its gas and liquid phases converge, resulting in only one phase at the critical point: a homogeneous supercritical fluid. The heat of vaporization is zero at and beyond this critical point, and so no distinction exists between the two phases. On the Pressure-Temperature diagram (**Figure 7.1.A**), the point at which critical temperature and critical pressure meet is

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called the critical point of the substance. Above the critical temperature, a liquid cannot be obtained by increasing the pressure, even though a solid may be formed under sufficient pressure. The critical pressure is the vapor pressure at the critical temperature. In the vicinity of the critical point, a small increase in pressure causes large increases in the density of the supercritical phase (**Figure 7.1.B**).

----------INSERT FIGURE 7.1 HERE----------

Physical properties of supercritical fluids are between those of a gas and those of a liquid, as can be observed in **Table 7.1**, in which some data taken from Pereda, Bottini and Brignole[5] has been included. For instance, the density of a supercritical fluid is similar to a liquid while its viscosity is similar to a gas and its diffusivity is placed between gas and liquid. Thermal conductivities are relatively high in supercritical fluids and have large values near the critical point. Surface tension is close to zero in the critical point, being similar to gases and much smaller than for liquids. Many other physical properties such as relative permittivity, solvent strength, etc., highly related with density, show large gradients with pressure above the critical point. Changes in those properties are crucial when dealing with extraction since they are related to changes in solubility and mass transfer ratios.

----------INSERT TABLE 7.1 HERE----------

The solvent strength of a supercritical fluid can be characterized, among others, by the Hildebrand solubility parameter, δ, that relates to the density of the solvent, as follows:

$$\delta = 1.25\ P_c^{1/2}\ [\rho/\rho_{liq}]$$

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where Pc is the critical pressure, ρ is the gas density and ρ_{liq} is the liquid density. At low pressures, the density of a gas is small, so, the solvating power is rather low; at near critical conditions, the density increases rapidly approaching that of a liquid and thus, the solubility parameter increases as the critical pressure is approached. This effect can be seen graphically in **Figure 7.2** in which the Hildebrand solubility parameter for CO_2 is represented as a function of the pressure for different temperatures[6]. This is one of the key features of SFE since the solvating power of the fluid can be strongly influenced by small changes in pressure and temperature either favoring the extraction of the target compounds or the precipitation of the solutes dissolved in the supercritical fluid.

----------INSERT FIGURE 7.2 HERE----------

7.2.2. Supercritical solvents

Although there is a wide range of compounds that can be used as supercritical fluids (see **Table 7.2** in which the critical properties of several solvents used in SFE are given; reproduced with permission), it is true that after the Montreal Protocol, introduced in 1987 to restrict or eliminate the manufacture and use of particularly damaging ozone depleting solvents (at present signed by 170 nations), there is a pressure worldwide for the industry to adopt new sustainable processes that do not require the use of environmentally damaging organic solvents[7]. In this sense, SFE using green solvents has been suggested as a clean alternative to hazardous processes and thus, SFE has found its growing niche.

----------INSERT TABLE 7.2 HERE----------

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Among the green solvents used in SFE, carbon dioxide (critical conditions = 30.9 °C and 73.8 bar) is undoubtedly the most commonly employed. CO_2 is cheap, environmental friendly and generally recognized as safe (GRAS). Supercritical CO_2 (SC-CO_2) is also attractive because of its high diffusivity combined with its easily tunable solvent strength. Another advantage is that CO_2 is gaseous at room temperature and pressure, which makes extract recovery very simple and provides solvent-free extracts. Also important for food and natural products is the ability of SFE using CO_2 to be operated at low temperatures using a non-oxidant medium, which allows the extraction of thermally labile or easily oxidized compounds[8]. As can be seen also in **Table 7.2**, supercritical CO_2 has a low polarity (with a low solubility parameter, around 7.5 $cal^{-1/2}$ $cm^{-3/2}$), and therefore, its efficiency to extract polar compounds from natural matrices is quite limited. To overcome this problem, polar co-solvents (methanol, ethanol) are commonly used in small amounts to increase the solubility of polar compounds in the supercritical mixture.

The widest application of supercritical fluids is extraction, especially with carbon dioxide. The first patent dealing with supercritical fluid extraction was given by Messmore in 1943,[9] although the first industrial application was developed by Zosel in 1978.[10] Since then, supercritical fluids have been used to isolate natural products, but for a long time applications relayed only on few of them. The development of processes and equipment is beginning to pay off and industries are getting more and more interested in supercritical techniques. This interest is also observed in the high amount of scientific papers dealing with supercritical fluid extraction (SFE) published in recent years. Moreover, industrial applications of SFE have experienced a strong development since the 90s in terms of patents[8, 11].

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SFE has been used in different fields such as the food, pharmaceutical, chemical, and fuel industries. Due to the absence of toxic residue in the final product among other advantages, they are especially useful for extraction in two situations: (a) extracting valuable bioactive compounds such as flavors, colorants, and other biomolecules or (b) removing undesirable compounds such as organic pollutants, toxins, and pesticides[2]. In this chapter we will focus on the use of supercritical fluids to extract valuable compounds from vegetal and marine sources and by-products from the food industry.

7.3. INSTRUMENTATION.

Supercritical fluid extraction is commonly carried out considering two basic steps: 1) extraction of soluble substances from the matrix by the supercritical fluid and 2) separation or fractionation of the extracted compounds from the supercritical solvent after the expansion.

The basic instrumentation to carry out supercritical fluid extractions should be composed of materials that are capable to withstand high pressures, typically as high as 500 bar (although systems requiring extractions pressures as high as 700 bar have been also used). The equipment needed is different depending if the application deals with solid or liquid samples. **Figure 7.3** shows the two schemes corresponding to a SFE extractor for solid and liquid samples. As is can be observed, the main differences are related to the extraction cell itself. Whereas the solid samples extractor has an extraction vessel of a given internal volume (see, **Figure 7.3.A**), the liquid samples extraction plant uses an extraction column in which the extraction is performed in countercurrent mode (**Figure 7.3.B**). Countercurrent extraction is performed introducing the sample in the system from the top of the column and the pressurized solvent from the bottom side;

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in this process, the components distribute between the solvent and the liquid sample which flows countercurrent through the separation column. Depending on the separation factor between components to be extracted, the desired contact time between the solvent and the sample can be varied by adjusting the height of the sample introduction into the extraction column or modifying the performance of the separation column, in terms of height and diameter, or of the packing material (structured/random, packing dimensions, surface area, etc.). Different methods have been published in the literature concerning the modeling of a countercurrent supercritical fluid extraction system. For an in-depth understanding of CC-SFE, readers are referred to previous papers published by Brunner[12, 13] and Reverchon[14]. Other factors such as solvent-to-feed ratio are of crucial importance in this type of extractions, as will be discussed in the following section.

----------INSERT FIGURE 7.3 HERE----------

As can be seen in **Figure 7.3**, both systems are composed by a tank for the extracting solvent, usually CO_2, a pump to pressurize the gas to the desired extraction pressure, a restrictor or valve to maintain the high pressure inside the system, and a trapping vessel (or separation cells, also called fractionation cells) for the recovery of the extracts. Different factors should be optimized in order to avoid losses of extracted compounds, one of them is the trapping method, which selection should be done considering analyte volatility and polarity, volatility of the extracting agent, volatility of modifier (if used) and solvent flow rate, among other parameters; different trapping methods are available, such as solid trapping, liquid trapping, cool trapping, etc. In pilot or industrial systems, collection of the extracted analytes is done by rapidly reducing the pressure, increasing the temperature or both. In this case, depressurization after the extraction could be

performed in cascade considering that each separation vessel could have a particular temperature and pressure in order to have some of the extracted compounds precipitated and separated.

Additionally, the system may include another pump to introduce an organic modifier (co-solvent) that are sometimes needed to extend the solvent capabilities of, for instance, supercritical CO_2, allowing the extraction and recovery of more polar compounds.

Regarding the extraction mode, at small scale, solid samples can be extracted in dynamic or static modes or even in a combination of both. Under static conditions, the supercritical fluid is introduced in the extraction vessel and is kept in contact with the sample for a given extraction time. Once the desired time is achieved, the extract is released through the pressure restrictor to the trapping vessel. On the other hand, in a dynamic process, the supercritical fluid is continuously entering the extraction vessel and flowing through the sample to the separators for a cascade fractionation. In the combined mode, a static extraction is performed for a period of time, and subsequently a dynamic extraction is carried out. Medium and large scale SFE are generally carried out in dynamic conditions: the supercritical solvent flows through the solid material extracting the target compounds until the substrate is depleted. On the other hand, liquid samples, according to the design of the extractors, are commonly extracted in a continuous mode.

7.4. PARAMETERS AFFECTING THE EXTRACTION PROCESS

The extraction of the soluble substances from the matrix can be described considering several steps, each one influenced by several factors that should be optimized. When

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dealing with solid samples, there is, at the beginning of the extraction process, a diffusion of the solvent into the matrix leading to an absorption of the supercritical solvent and therefore to a decrease of the mass transfer resistance; after this step, soluble compounds are dissolved into the supercritical fluid and are further transferred by diffusion first into the surface of the solid and later to the bulk of the fluid phase. The extraction process ends with the transport of the solute and the bulk fluid phase and their removal from the extractor. The kinetics of the extraction process can be followed by determining the amount of extract against extraction time, providing an extraction curve as the one shown in **Figure 7.4**. A typical extraction curve can be divided in two parts, the first one (I) may be a straight line whose slope is given by the equilibrium solubility (considering a constant mass transfer) while the second part (II) is non-linear and may approach a limiting value, corresponding to the maximum amount of extractible compounds.

----------INSERT FIGURE 7.4 HERE----------

For liquid samples, steps are similar although further complexity is introduced by including the dimensions of the column and the size and structure of the packing material in the countercurrent column. Moreover, theoretical calculations of the efficiency of the separation, based on experimental measurements, are sometimes necessary to adjust the experimental conditions for challenging separations.

Following, an explanation of the main factors influencing the SF extraction process is presented.

7.4.1. Raw material (particle size, porosity, location of the solute, moisture content)

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Despite the raw material is normally imposed by the process, there are several factors to take into account. It is well known the influence of the physical state of the sample (solid, liquid) on the outcome of the extraction. When dealing with solid samples, other factors such as particle size, shape, and porosity of the solid material are of crucial importance since they have direct effects on the mass transfer rate of the process. In order to increase the extraction rate, the solid matrix must be comminuted to increase the mass transfer area. On the other hand, very small particles must be avoided. Their use can compact the bed, increasing the internal mass transfer resistance and causing channeling inside the extraction bed. As a result, the extraction rate decreases due to inhomogeneous extraction[2].

The crushing degree was a very significant factor in the extraction of carotenoids from *Haematococcus pluvialis* microalga[15]. It was demonstrated how an increase in the crushing procedure produced an enhancement in the carotenoid extraction yield. This effect could be due to an increase of the mass transfer rates as a consequence of the lower particle size as well as to the increase of carotenoids in the medium as a result of the disruption of cells in the heavier crushing procedure[15]. Although supercritical solvents have a diffusivity in the matrix higher than liquids, a decrease in the sample particle size generally produces an increase in the extraction yield obtained, mainly due to the increase in the contact surface between sample and solvent, thus increasing the mass transfer. Nevertheless, in some applications, that is, when dealing with samples of high water content, the use of dispersing agents (e.g., diatomaceous earth) to avoid sample clogging together with hydromatrix to absorb the liquid portion from the sample can be useful. In general, drying the raw material is recommended; however, in some cases the presence of water is necessary to favor the interaction of the solvent with the

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solute, as in the extraction of caffeine from green coffee beans, or due to its role in the swelling of the cell, which facilitates the flow of the solvent into the cell[2].

In the case of liquid samples two main strategies are used: a) to trap the liquid on a solid support (e.g. sepiolite) and treat them like a solid or b) to perform column countercurrent extractions (see **Figure 7.3.B**). The first strategy is mainly used at small scale since the employment of solid supports can increase the extraction costs. As mentioned, during countercurrent extraction, the liquid sample is continuously added on a column by the top or the middle point, while supercritical phase is supplied by the bottom point. This strategy has been very useful for oil refining; for example, Hurtado-Benavides et al. studied the effect of different factors, such as the type, size and structure of the column packing on the efficiency and performance of the countercurrent system for the SFE of olive oil[16]. Results demonstrated the influence of these factors on the mass transfer ratio. For instance, authors showed that the use of a column packing with high surface area provide similar results than decreasing the mean particle size of a solid raw materials.

7.4.2. Solubility (Pressure and Temperature)

As previously mentioned, there are several physical parameters of the supercritical fluid that are highly dependent on the pair Pressure–Temperature. The design of processes using supercritical solvents is strongly dependent on the phase equilibrium scenario, which is highly sensitive to changes in operating conditions. Therefore, phase equilibrium engineering, that is, the systematic application of phase equilibrium knowledge to process development, plays a key role in the synthesis and design of these processes[5].

In general, both the yield of a solute and the separation selectivity highly depend on solubility properties which, as it has been previously shown, are determined by the operating pressure and temperature. At SFE conditions, the solvent capacity increases with pressure at constant temperature, therefore, the content of the target compound in the raw material will decrease with pressure after a certain extraction time. In general terms, increasing the pressure leads to an exponential increase of the solubility close to the critical point (higher densities).

As a general rule, a component with high vapor pressure has higher solubility in a supercritical medium or its solubility is better if the bulk density of the SCF is increased, that is, by increasing the extraction pressure. In SFE processes using CO_2, the component solubility is lowered as the polarity and/or the molecular weights of the solutes are increased.

Increasing the temperature, at constant pressure, have two opposite effects: it reduces the solvent power of CO_2 by a decrease of the density, and, on the other hand, it increases the vapor pressure of solutes which can be easily transferred to the supercritical phase. The increase or decrease on the solubility of the solute in the supercritical solvent will depend on the operating pressure. In fact, near the critical pressure, the effect of fluid density is predominant, thus, a moderate increase in temperature leads to a large decrease in the fluid density, and therefore, to a decrease in solute solubility. However, at high pressures, the increase in the vapor pressure prevails, thus and the solubility increases with the temperature. This is called a retrograde behavior of the solid solubility, as can be seen in **Figure 7.5**.

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In many cases, instead of setting up the conditions a priori by using thermodynamic models, experimental designs are employed as a strategy to set up robust extraction processes[8]. For instance, the use of response surface methodology (RSM) allowed the simultaneous graphical optimization of the extraction temperature, pressure and time of different natural product such as passiflora seed oil[17], algal fatty acids[18]. Although the extraction yield can be selected as response variable, the particular composition of the extracts can be also optimized. In the extraction of passiflora seed oil[17], fourteen experiments plus six replicates in the centerpoint were carried out to test 3 variables at 5 levels.

Simplex centroid design is another popular possibility, and it has been used to determine the optimum temperature, pressure, dynamic extraction time and modifier volume that maximize the yield of the essential oil of valerian (*Valeriana officinalis* L.) attained by SFE using supercritical carbon dioxide[19]. With this strategy four independent variables were tested at five levels by using only 18 experiments.

7.4.3. Use of Modifiers

As previously mentioned, CO_2 is largely the most used solvent to perform SFE. Its main drawback is its low polarity. As many other substances its dielectric constant may change with density, but even at high densities, CO_2 has a limited ability to dissolve high-polarity compounds. To solve this problem, small amounts of co-solvents (modifiers) are added to CO_2 current. The addition of modifiers to CO_2 can improve the extraction proficiency by raising the solubility of the solutes. Two mechanisms have been proposed by Pereira and Meireles[2] to explain the effects:

- solute–co-solvent interactions, caused by increase in solvent polarity.
- matrix swelling that facilitates the contact of the solute by the solvent.

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The effect is not only dependent on the nature of modifier used, but also on the type of matrix, and the target solutes.

As a general rule, the amount of modifier used is lower than 10-15 %. The most used modifiers are methanol and ethanol. It must be taken into account that modifiers are not gases at room conditions and therefore, liquid residues are obtained in extracts and remaining matrix after SFE. This is the main reason for not recommending the use of methanol in the extraction of natural products since the presence of this toxic solvent can preclude the further use of the extracts, for instance, in food applications. Ethanol is a GRAS solvent widely employed as a co-solvent for natural products extraction, although its final use will be determined by its affinity towards the target compounds. Considering only toxicity and polarity, water can be suggested as an interesting modifier, but its presents several drawbacks such as the increase in the formation of ice blockages due to the Joule–Thompson effect in the separator vessel; the possible ionization and hydrolysis of compounds; and the foam formation, attributed to the coextraction of saponins[2].

Sometimes modifiers are not only used to increase the polarity of the extractant phase but also to improve the extraction rate of non-polar solvents. For example, Sun and Temelli demonstrated the ability of vegetable oils to enhance the yield of carotenoids (non-polar and low volatile) from carrot; without a co-solvent, the extraction yield had a very small variation with changes in pressure and temperature, but when canola oil was employed, extraction yields increase by 3-4 times[20].

7.4.4. Solvent flow rate (solvent-to-feed ratio)

Solvent ratio is the most important parameter for supercritical fluid extraction, once the extraction pressure and temperature have been selected. Solvent flow rate must be high

enough to provide a good extraction yield in short time, but it should also grant enough contact time among solvent and solutes. Moreover, it must be considered that higher solvent flow rate promotes an elevation of the operational and capital costs, which should be carefully studied for industrial applications[2].

Solvent to feed ratios are also highly important when dealing with countercurrent column extractions. Generally, the efficiency of the column decreased as the CO_2 flow rate increased, since the HTU (height of a transfer unit) increased with increasing CO_2 loading, as demonstrated by Hurtado-Benavides et al.[16] and Brunner et al.[21]

7.5. APPLICATIONS

7.5.1. Plants

SFE has been widely employed to extract interesting compounds from natural matrices, such as plants. In fact, there exist a high number of published works in which the use of this extraction technique is described for the attainment of bioactive compounds from those materials. As examples, in **Table 7.3**, the most remarkable and recent works published dealing with the use of SFE to extract bioactive components from plants are summarized. Besides, the reader is referred to other review papers that can be found in the literature in order to gain a deeper insight on the less recent applications[2, 8, 22-25].

As it can be observed from the information presented on the Table, as expected, all the applications dealt with extraction of solid materials. Most of the applications are directed to the extraction of compounds possessing a particular bioactivity. In this regard, antioxidant compounds have been the most-studied. The bioactives extracted belong to a wide range of compounds, from more polar phenolic compounds to alkaloids, carotenoids and other pigments and essential oils. Considering that CO_2 is the

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supercritical fluid frequently selected, and bearing in mind that bioactive compounds present on natural samples often possess a relatively high polarity, the use of organic modifiers to extract these components is very common. Ethanol and methanol are the solvents most-frequently used, although the use of others such as diethylamine and water has been also explored. Normally, proportions of up to 20% have been employed for the modifiers, although proportions as low as 5% have been shown to be useful to extract for instance polyphenols[26]. Different is the case of the extraction of essential oils. In those studies, neat CO_2 is employed as the polarity of supercritical carbon dioxide is low enough to extract the less polar compounds that are part of the essential oils. Other less polar bioactives could be potentially recovered by using small proportions of modifiers or even using only CO_2. In this regard, carotenoids are natural pigments which polarity is very low. These components are basically interesting by their antioxidant activities. In general, high pressures are employed to extract these compounds when using neat CO_2. In fact, 600 bar of pressure were employed for the extraction of lutein and zeaxanthin from *Hemerocallis disticha*[27].

Regarding the extraction times needed to obtain interesting compounds from plants, this parameter might vary a lot among applications. A combination of a relatively short static extraction period followed by a dynamic extraction step is frequently employed in order to increase the yield of extraction of the aimed compounds.

In any case, what it is interesting during process optimization is the employment of chemometric tools in order to determine the optimum extraction conditions for the different parameters involved. In this regard, the application of an experimental design is of great help in order to have enough experimental data to subsequently determine the optimum conditions for each studied parameter according to the response variables selected. Taguchi[28], Box-Behnken[54] or central composite experimental designs[55] have

been used, among others, for the optimization of variables involved in the SFE extraction of bioactives from plants. Extraction pressure and dynamic extraction time as well as modifier volume were the factors studied to maximize the recovery of essential oils from *Myrtus comunis*[55], whereas extraction pressure, temperature and time were the parameters selected in the extraction of *Garcinia mangostana*[54]. In this latter case, total extraction yield and radical scavenging activity of the extracts were chosen as response variables and the composition and proportion of cosolvent was kept constant.

Response surface methodology has been also employed. This method allows not only the visualization of the best conditions obtained for the studied factors, but also the graphical observation of the influence of the different factors studied on the response variables observed[55].

----------INSERT TABLE 7.3 HERE----------

7.5.2 Marine products

The discovery and development of marine bioactives is a relatively new area compared to those derived from terrestrial sources. Although some plants have demonstrated to be interesting sources of bioactive compounds (see previous section), the potential of other sources from marine nature have been also pointed out since the high diversity observed in the marine environments from a chemical and biological point of view makes the ocean an extraordinary source of high value compounds. In this regard, SFE has been widely employed for extracting bioactive compounds from algae, microalgae and other marine-related organism such as crustaceans, fish or their by-products[2, 8, 25, 56-60]. Thus, **Table 7.4** summarizes the most relevant literature recently published (from 2010 to 2012) dealing with the recovery of valuable compounds from marine sources using

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SFE. As can be observed in this table, the main application of SFE developed in the last two years dealt with the extraction of ω-3 PUFAs and carotenoids.

The possibility of obtaining ω-3 PUFAs from marine sources has been highly studied in the last years considering the important properties, such as anti-inflammatory, antithrombotic, antiarrhythmic, etc., attributed to some of them[78-81]. Marine sources, especially fish oil and fish by-product provide the major natural dietary source of ω-3 PUFAs, mainly eicosapentaenoic (EPA) and docosahexaenoic acids (DHA). SFE using non-polar CO_2 is especially well suited to extract this kind of compounds as can be observed in Table 4. Regarding fish oil, Lopes et al. (2012) studied the possibility, under different temperatures and pressures, of fractionating the TAGs with respect to EPA and DHA from a fish oil with a low ω-3 fatty acids content (10 %) in order to demonstrate that the probability of fractionating the oil with respect to these fatty acids improves by using a fish oil with a lower ω-3 fatty acids content as the basis[61]. The applicability of SFE technology for valorizing waste products of the fish industry is also demonstrated by the use of different fish by-products and some marine invertebrate (sea urchin) as raw material to obtain ω-3 PUFAs (see Table 7.4). For instance, an interesting work developed by Sánchez-Carmargo et al.[63] demonstrate that the addition of ethanol improve significantly the extraction yields of lipids and astaxanthin from redspotted shrimp waste compared to the extraction without ethanol as co-solvent[64]; data obtained in this study showed that the extraction yields increases considerably with increasing the % of ethanol in the ethanol/scCO_2 mixture reaching maximum recoveries of 93.8 and 65.2 % for lipids and astaxanthin respectively, when employing 15 % ethanol. Besides, increasing the % ethanol resulted in an increase in the concentration of the ω-3 fatty acids in the lipids of the extract[63].

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Although SFE has been also applied to carry out the lipid extraction from microalgae, such as *Nannochloropsis oculata*[71] *a*nd *Schizochytrium limacinum*,[75] the main application of this technology using algae and microalgae as natural source has been the extraction of antioxidant compound, namely carotenoids, isoflavones, polyphenols and flavonoids as can be observed in **Table 7.4**.

Traditionally, carotenoids have been extracted using organic solvent, however, different studies have discussed the use of SFE for their recovery. Different works have demonstrated the extraction of carotenoides, such as lutein and b-carotene[73] or canthaxanthin and astaxanthin[74] using neat SC-CO_2, however most of the applications presented in Table 4 employed certain amount of a co-solvent (ethanol or methanol) to modify the polarity of the SC-CO_2. Using the mixture co-solvent/SC-CO_2 the extraction efficiency of carotenoids was improved. Besides the extraction of carotenoids, other works described in the literature deal with their purification by using supercritical anti-solvent precipitation (SAS). For instance, this methodology has been employed to the purification of zeaxanthin from the ultrasonic[82] or soxhlet[83] extract of the microalga *Nannochloropsis oculata.* In addition, Liau et al. developed an interesting process considering SFE of lipids and carotenoids from *Nannochloropsis oculata* and SAS of carotenoid-rich solution[71]. Although in this approach both processes were considered independently, the combination of both may favor the simultaneous extraction and purification of carotenoids.

As mentioned above, other antioxidant compounds different from carotenoids, such as isoflavones, polyphenols and flavonoids have been also extracted by SFE using methanol or ethanol as co-solvent from algae, microalgae and cyanobacteria[76, 77].

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----------INSERT TABLE 7.4 HERE----------

7.5.3 Agricultural and food by-products

Industrial activities generate a large variety of by-products that normally do not have any commercial value. The conversion of these by-products into valuable material through, for instance, the extraction of high value compounds can provide enormous benefits from an environmental and economic point of view. In this sense, SFE has been widely used to value agricultural and food by-products[1, 8, 25, 84, 85]. A high variety of agricultural and food by-products have been employed as source of bioactive compounds as can be observed in **Table 7.5**. Several of the studies shown in this table use sophisticated chemometrics tools in order to select the most appropriate extraction conditions as well as to study the influence of each experimental parameter (temperature, pressure or percentage of co-solvent) in the extraction procedure. Among them, factorial experimental design[86], response surface methodology[69, 87-89], central composite non-factorial design[90] and mathematical modelling[91] haven been employed.

As it can be observed in the table, the main bioactive compounds extracted by SFE from agricultural and food by-products are polyphenols and carotenoids with antioxidant properties, but also fatty acids, essential oils, and tocopherols. This fact demonstrates, as mentioned before, the versatility of SFE towards the extraction of lipohilic and hydrophilic compounds when a co-solvent is added to CO_2. For instance, for extracting polyphenols the addition of a moderately polar modifier is critical, so that ethanol is usually added at relative low levels (10-20 %) although extraction using up to 60 % has been also reported[88]. Ethanol in the most used co-solvent, but other compounds can be also employed; for instance, Castro-Vargas et al.[53] compared the extraction yield of phenolic compounds from guava seeds by SFE with CO_2 and with ethyl acetate and

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ethanol as co-solvent. The phenolic fraction yield increased directly with solvent polarity (CO_2, CO_2/ethyl acetate and CO_2/ethanol).

Most of the works presented in Table 5 dealing with the SFE extraction of polyphenols measure the extraction efficiency by total phenolic content, however, some other studies measure the levels of specific compounds such as resveratrol[104] or kaempferol glycosides[88].

Regarding carotenoids, different SFE methodologies have been developed to extract lycopene which has the highest antioxidant activity among all dietary antioxidants and play an important role in the prevention of oxidative and age-related diseases[108, 109]. It represents the most abundant carotenoid in tomatoes, accounting for more than 80 % of the pigment present in fully red-ripe fruits. The SFE extraction of lycopene has been mainly carry out from tomato by-products[86, 106, 107] (see Table 5) however, it has been also extracted from pink guava, a tropical fruit rich in lycopene[105]. Usually, lycopene recovery does not exceed 20 % of the total amount of carotenoids in the absence of a co-solvent. This percentage is considerably increased when a vegetable oil is added as co-solvent. As examples, Lenucci et al.[107] demonstrated that the addition of an oleaginous co-matrix consisting of roughly crushed hazelnuts to the lyophilised tomato matrix made possible to increase the lycopene recovery from 35 to approximately 80 % in the oleoresin, whereas Machmudah et al.[106] shown how the presence of tomato seed oil helped to improve the recovery of lycopene by SFE from dried tomato peel by-products from 18 to 56 %.

In most of the papers dealing with SFE of lycopene from seeds, pulp and tomato skin, the extraction if preceded by the removal of the humidity from the raw material by using some drying process to further increase the extraction yield of lycopene. However

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Egydio et al.[86] developed a SFE methodology to extract lycopene from tomato juice without the need to dry the raw material. The recovery from the pulp of centrifuged tomato juice increased significantly after substituting the water for ethanol before SFE extraction.

----------INSERT TABLE 7.5 HERE----------

7.6. FUTURE TRENDS AND CONCLUSIONS

In the present chapter we have tried to demonstrate that SFE is nowadays one of the most popular alternative methods for extracting valuable compounds from different natural raw materials such as plants, marine products and agricultural by-products. Advantages of the use of such technology have been underlined as well as the parameters that can be modified to optimize the process in terms of yields and/or purity of the target compounds. Recent applications have been summarized allowing identifying both, the target compounds and the key raw materials that have been studied lately. In this sense, it seems that compounds or extracts with associated antioxidant activity are the most popular, mainly because of their suggested relationship with the improvement of health status. Other bioactivities such as anti-inflammatory and antimicrobial have become also of interest. As for the target compounds, carotenoids, phenolic compounds, ω-3 PUFAs and essential oils are among the most widely studied.

Although SFE has been recognized as an advantageous process from an environmental point of view, sustainability and eco-friendliness of a particular process is a goal that has to be approached through the application of, among other tools, life cycle analysis (LCA). LCA should be employed to efficiently calculate the impact on the environment of the different available procedures. Future research in this interesting area is expected.

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Moreover, more focus is needed in terms of economic considerations of SFE processes at large scale. Pioneer works of Meireles have set the basis for a better understanding of process economics; interested readers are referred to an interesting review on this topic.[2] As more advantages are associated to the use of SFE as a viable process for natural products extraction, a wider range of experimental conditions are tested, including sub- and supercritical conditions, and a higher number of solvents are included, trying to cover a wide range of polarities. In this sense, new developments using solvents other than carbon dioxide are every time more common, including, for example, the employment of supercritical ethane to extract all-trans-lycopene from tomato industrial wastes[110], or the extraction of lipids from fermentation biomass using near-critical dimethylether (DME)[111]. DME has shown, for instance, important advantages associated to the extraction of wet biomass because of its high solubility in water. This mutual solubility of water and DME enables the co-extraction of water and lipids that can be easily separated afterwards but that allows the processing of the material without a previous drying step.

Other solvents with great possibilities to be used in SFE are the so-called Gas-expanded liquids (GXLs), understanding a GXL like a mixed solvent composed of a compressible gas (such as CO_2 or ethane) dissolved in an organic solvent. CO2-expanded liquids (CXLs) are the most commonly used class of GXLs. By just modifying the CO_2 composition, a continuum of liquid media ranging from the neat organic solvent to SC-CO_2 is generated, the properties of which can be adjusted by tuning the operating pressure. Moreover, CXLs can be created at relatively mild pressures with a substantial replacement of the organic solvent with CO_2. Therefore, GXLs combine the beneficial properties of compressed gases (such as the improved mass transport) and of traditional solvents (large solvating power), leading to a new class of tunable solvents that are

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often the ideal type of solvents for a given application. Although these novel solvents have been applied to some processing applications, including gas antisolvent (GAS) processes, particle deposition, etc., just few examples demonstrated the ability of such solvents in extracting valuable compounds from natural matrices[112]. Other solvents such as ionic liquids (ILs) have started to be explored combined with supercritical fluids. The most obvious benefit of coupling ILs and SC fluids is in the integration of reaction and extraction processes into the same system; that is, linking the possibility of carrying out a reaction in the most favorable phase (the ionic liquid) while the reaction products are extracted into the supercritical phase for easy recovery[113].

In this sense, it is foreseen an important development of green processing platforms able to perform, using green solvents such as supercritical carbon dioxide and water, multi-unit operations consisting on raw material pre-treatment, reactions (biocatalysis, transesterification), extraction and biofuel conversion, etc. New technologies involving the combined use of enzymes, disruption methods such as ultrasounds[114], or membrane separation[115] with supercritical fluids can undoubtedly revolutionize the concept of process sustainability, approaching it to a more promising green biorefinery platform able to give new answers to the demands posted nowadays.

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