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CHAPTER. SUPERCRITICAL FLUID EXTRACTION

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Abstract

Supercritical fluid extraction (SFE) has become one of the most popular green extraction techniques nowadays since it has demonstrated many advantages compared to traditional or classical extraction processes. Aspects such as improved selectivity, higher extraction yields, better fractionation capabilities and lower environmental impacts have been crucial to the important growth of SFE. In this chapter, fundamentals of SFE are presented together with the most important variables that can affect the extraction process and how to tune them. Moreover, interesting and new applications in different areas such as food science, pharmaceutical and others like, for instance, heavy metals recovery are presented.

1. Introduction

At present there is an increasing interest in developing processes and methodologies able to comply with the Green Chemistry Principles. Among them, extraction techniques have received a great deal of attention since new approaches are needed to solve some important drawbacks associated to the use of conventional techniques involving the extensive use of toxic organic solvents and high energy usage while providing low selectivity and low extraction yields. These shortcomings can be partially or completely overcome by using newly developed advanced extraction techniques which are faster, more selective towards the compounds to be extracted and, on the top of it, more environmentally friendly. In fact, by using the advanced extraction techniques, the use of toxic solvents is highly limited or greatly reduced.

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This is especially true for Supercritical Fluid Extraction (SFE), a technique based on the use of solvents at temperatures and pressures above their critical points. SFE can be a fast, efficient, and clean method for the extraction of compounds of interest from different matrices while being also an appropriate reaction media, among other important applications, as it will be demonstrated throughout this chapter.

2. Fundamentals of Supercritical Fluid Extraction

Supercritical fluid extraction is based on the use of a fluid at pressures and temperatures beyond its critical point, in order to achieve significant physical changes that will modify its capabilities as solvent. Although the first experimental works with supercritical phenomena as well with supercritical extraction started back in the 19th century, the increase on the interest of this technique as a potential alternative to conventional solvent-based extraction techniques is quite recent. Charles Cagniard de la Tour observed, in 1822[1,2] for the first time, changes in solvents at certain values of pressure and temperature. More than 40 years passed until Thomas Andrews presented the first definition of the term "critical point" in 1869[3]. Some years later, the first application of this knowledge to extraction was introduced by Hannay and Hogarth[4] who reported how solids could get dissolved in solvents at pressures above their critical point. These early works started to show the important implications occurred in a substance that is submitted to pressure and temperature conditions beyond its critical points, mainly derived from important physical changes that are directly responsible for their possible applications in supercritical fluid extraction. In the following section, these physical properties are described in more detail.

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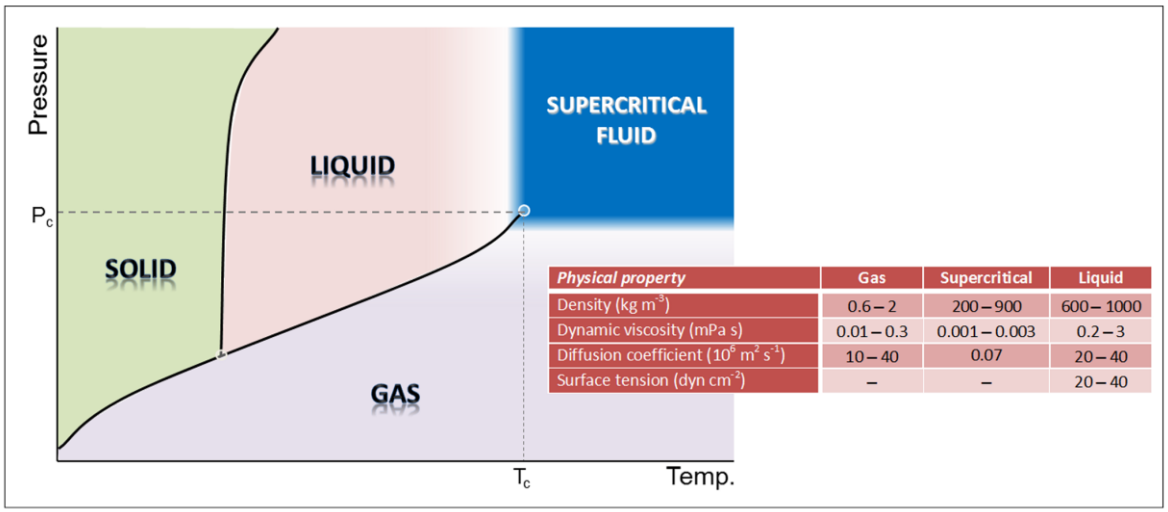
2.1. The critical point, physical peculiarities.

The critical point (determined by the critical pressure and temperature) is a particular property of a substance; when these values are reached, some changes are induced that effectively modify its physical properties. As can be seen in Figure 1 (pressure-temperature phase diagram), when the temperature of a solvent is increased at the same time that its pressure and the critical point is reached, a homogeneous supercritical fluid is obtained in which no distinction can be found between phases.



Physical property	Gas	Supercritical	Liquid
Density (kg m^{-3})	0.6 – 2	200 – 900	600 – 1000
Dynamic viscosity (mPa s)	0.01 – 0.3	0.001 – 0.003	0.2 – 3
Diffusion coefficient (10^6 m^2 s^{-1})	10 – 40	0.07	20 – 40
Surface tension (dyn cm^{-2})	–	–	20 – 40

Figure 1. Typical pressure–temperature phase diagram for a given fluid and main physical properties of fluids in the gas, liquid (at room conditions), and supercritical phase. Pc, critical pressure; Tc, critical temperature.

As can be observed in Figure 1, supercritical fluids have mixed properties between those of liquids and those of gases; for instance, the viscosity is similar to a gas whereas its density is close to values found for liquids. On the other hand, its diffusivity is intermediate between that of liquids and of gases. Other important properties are also modified in a supercritical fluid (surface tension, solvent strength, etc.), and will be responsible of the

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properties as a solvent that the fluid will present. Besides, changes in temperature and pressure beyond their critical points will also modify mainly density, effectively changing the solvent capabilities and permitting the achievement of a high degree of selectivity, as it will be described later. For a more in depth description of all the physical modifications produced in a supercritical fluid, readers are referred to other book chapters and review articles[5,6]. In practice, a wide group of compounds might be used as supercritical fluids provided they are submitted to the appropriate temperature and pressure conditions, from water to organic solvents, among others. In Table 1, some of the most-used supercritical fluids as well as their corresponding critical values are shown. As it can be observed, the critical values greatly change from a substance to another. It is clear that attaining the correct conditions may be very expensive hindering the practical applicability of some of them at pilot and industrial scales. Besides, it is also important to note that some of these substances are not safe. Considering the always increasing awareness for the development of environmentally respectful processes, the use of solvents that demand extremely high amounts of energy to be placed into a supercritical state as well as those that may not be perfectly safe or that are toxic, cannot be justified at all. For these reasons, most of supercritical fluid extraction applications developed nowadays seek to gain advantage of the mild critical temperature and pressure values of carbon dioxide (Table 1). Moreover, CO_2 is a green solvent, that is considered a GRAS (generally recognized as safe) solvent for the food industry, is cheap and easily available. Besides, the use of this fluid is not against the limitations established at present for processes generating CO_2, as the carbon dioxide employed is not produced ad hoc, but just recycled or collected from other industrial processes. Thus, the use of CO_2 in SFE processes is a way to reuse this important industrial

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by-product. Another important advantage that increases even more the interest on the use of this compound is that CO_2 is a gas at room conditions. That means in practice that after the extraction process, when the pressure is relieved, the CO_2 is automatically evaporated leaving a perfectly solvent-free extract. On the other hand, the main shortcoming related to the use of supercritical CO_2 is its very low polarity. Consequently, its ability to extract highly or medium polarity compounds is rather limited. To overcome this issue, another solvent may be employed together with CO_2 at very low proportions, in order to increase the polarity of the supercritical fluid. This added solvent is commonly termed modifier or entrainer. Ethanol or methanol mixed below a 10% of total CO_2 employed are frequently used as modifiers. In the following section, the most-influencing parameters during a supercritical fluid extraction process, including the use of modifiers, are detailed.

Table 1. Critical properties of some of the most-employed fluids used in supercritical fluid extraction.

Fluid	*Critical value*			
	Solubility parameter δ_{SFC} (MPa $^{1/2}$)	**Density (kg m^{-3})**	**Temperature (ºC)**	**Pressure (MPa)**
Carbon dioxide	15.34	470	31.2	7.38
Water	27.61	322	101.1	22.05
Methanol	18.20	272	-34.4	8.09
Ethylene	11.86	200	10.1	5.11
Ethane	11.86	200	32.4	4.88
n-butene	10.64	221	-139.9	3.65
n-pentane	10.43	237	-76.5	3.37

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3. Parameters affecting the extraction process

Although the selection of the supercritical solvent to be employed may be envisioned as the most-influencing parameter in the extraction, there are a number of important parameters that will significantly affect the solvent strength and the mass transfer processes generated during the extraction and, thus, the outcome of an extraction process. In this section, the most important parameters are briefly described and commented.

3.1. Raw material

The raw material is herein defined as the sample to be extracted. For SFE applications either solid or liquid samples might be employed, although in each case the considerations given as well as the instrumentation needed is slightly modified. Considering solid samples, the physical state of the sample may have a strong influence. Particle size and porosity will have a great impact on mass transfer by increasing the surface contact although the humidity of the sample may also hamper the extraction process. In general, the use of dried samples allows attaining better results, although exceptions exist. The correct parameters have to be experimentally set. If the sample size is too small, the formation of preferential channels inside the extraction cell is possible. To avoid this problem, dispersion agents may be used to produce homogeneous extractions.

In the case of liquid samples, counter-current extractions are commonly employed to increase contact between the sample and the supercritical fluid. In these applications, the liquid sample is introduced in the upper part of a packed extraction column whereas the

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supercritical CO_2 is introduced from the bottom. By correctly selecting the introducing point (height) as well as the inert column packing material that increases the surface contact, the mass transfer may be optimized.

3.2. Solubility (Pressure and Temperature)

Extraction pressure and temperature are probably the most influencing parameters in terms of solubility of a substance in the supercritical fluid. In general, it can be said that higher density of the supercritical fluid will be obtained through an increase in pressure and will lead to an enhanced solubility of sample components. On the other side, an increase on temperature will decrease the density (for a given pressure) although will also promote the transfer of solutes from the sample to the supercritical fluid due to the increment on their vapor pressure. Thus, the selection of the temperature and pressure values to be employed in a process should be carefully made according to the aim of the process as well as the targeted compounds. For natural complex samples, the use of experimental designs that allow the statistical observation of the influence of the different parameters involved as a function of one or more response variables is frequent. Response surface methodology (RSM) or simplex centroid designs (SCD) are often selected.

3.3. Polarity/Use of Modifiers

As it has been already mentioned, CO_2 is the most-widely employed supercritical fluid nowadays, although its low polarity limits somehow its application to the extraction of low polar/lipophilic compounds. In order to increase the range of potential applications, a modifier might be employed together with the supercritical CO_2. Typically, organic

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solvents such as methanol or ethanol are employed as modifiers, at concentration below 10 % related to the amount of CO_2 used for the extraction, although water has been also employed in some applications. This way, it is possible to increase the solubility of sample components with higher polarity. Under these conditions, the physical state of the solvent mixture is more complex, above all because the modifiers might not be in their supercritical state and, thus, different phases may be coexisting during the extraction procedures. Other modifiers have been also used to help in the extraction of very low polarity components, such as oils mixed with CO_2 at very low proportions. Lastly, it has to be noted that when using modifiers the possibility of attaining solvent-free extracts is lost because these solvents are not gases at room conditions.

3.4. Solvent:feed ratio

The solvent-to-feed ratio to be employed has a critical importance on the supercritical process. Once the pressure and temperature conditions have been defined, it is important to study the effect of the solvent-to-feed ratio or the influence of the CO_2 flow rate. This flow rate should be high enough to maximize the extraction yield but also low enough to allow good contact with the sample in order to minimize the amount of CO_2 employed, and thus, the operational costs. As it can be deduced, this parameter is particularly important when extracting liquids under counter-current conditions, as in those cases, the ratio will define the contact time allowed between the sample and the supercritical CO_2.

4. Instrumentation for Supercritical Fluid Extraction

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Nowadays, there exists a wide range of commercial instruments from bench-top to industrial scales to carry out supercritical fluid extractions. However, it is common to find applications based on in-house made equipment. The basic instrumentation needed to build a SFE instrument will slightly vary depending on the application, solids or liquids extraction. In Figure 2, the basic components of a SFE extractor are depicted.

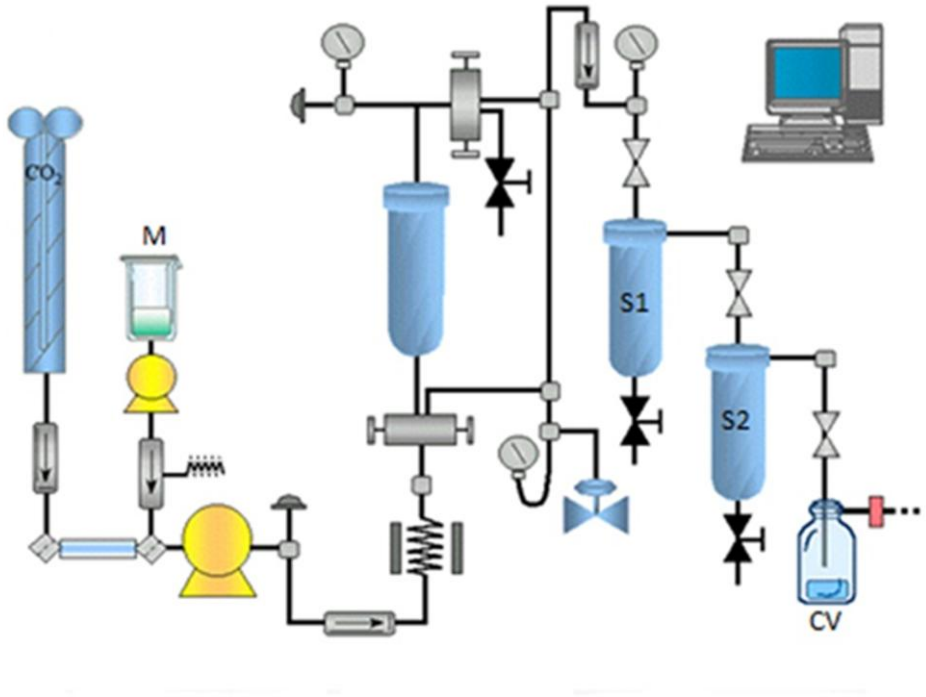

Figure 2. Basic instrumentation needed for a supercritical fluid extraction equipment. M, modifier; S1, separator 1; S2, separator 2; CV, collection vessel.

The first part of any extractor is devoted to the extraction process itself, composed by a temperature-controlled extraction cell or column able to withstand the high pressures needed to perform the extractions, a CO_2 pump and a modifier pump. In the case of liquid samples, the extraction column is frequently equipped with different ports for the introduction of the sample at variable heights. In this latter case, another additional pump is

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needed to introduce the sample into the system. The CO_2 pump is the one setting the pressure inside the extraction chamber, so that the supercritical CO_2 is always under the desired conditions, which is maintained using a restrictor or a back pressure regulator. The second part of the extractor is focused on extract recovery. It may be composed by a collection cell or by several fractionation vessels in order to perform cascade depressurization.

This basic equipment may be further developed into more complicated systems, for instance installing a system for CO_2 recycling or by a variety of devices depending on the scale of the extractor. More details can be found elsewhere[6, 7].

5. Applications

5.1. Food Science

SFE has been widely used in Food Science; in fact, the first industrial application was the extraction of caffeine from coffee beans by Zosel[8, 9]. Since then a high variety of samples, type of materials, target compounds and procedures have been published.

Two clear trends co-exist in the applications of supercritical fluids to food science: removal of unwanted compounds and extraction of valuable compounds. Both operating trends will be discussed in the following sections.

5.1.1. Removal of unwanted compounds

When dealing with the removal of unwanted compounds, SFE can be used with different approaches: to remove external toxic compounds from different raw materials and to

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eliminate or reduce unwanted compounds naturally present in the sample. In some cases, both the extraction residue and the extract can be used in different applications. Some examples of each approach are discussed:

Removal of external contaminants:

This is probably the main use of SFE as sample preparation. One of the main areas of application of SFE in the last few years has been in food pollutants analysis, mainly pesticide residues and environmental pollutants[10]. A common characteristic of these works is the extremely high selectivity of SFE in the isolation of the low polarity pesticides; this fact makes SFE probably the technique of choice to isolate pesticides from low fat food[11]. In fact, in the last years, SFE is being used as sample preparation method for multiresidue analysis, for example Valverde *et al.*[12] developed a method to analyze 22 pesticides by GC-ECD/NPD from rice, wild rice and wheat; in their work, CO_2 at 20 MPa and 50 ºC was used in combination with methanol as modifier and results were compared with classical extraction using ethyl acetate as extracting solvent, providing the use of SFE with better results than the conventional approach.

Beside pesticides, some other examples of pollutants that can be extracted in foods and other matrices by SFE are PAHs (Polycyclic Aromatic Hydrocarbons)[13], halogenated dioxins and biphenyls (PCBs)[14, 15] veterinary drugs[16, 17], etc. An interesting application by Choi *et al.*[17] has been the extraction of polar and nonpolar fluoroquinolone antibiotics (enrofloxacin, danofloxacin, and ciprofloxacin) in pork by using Na_4EDTA and sea sand in combination with CO_2 at 80 °C, 30 MPa and 30% methanol. The interest in controlling the presence of drug residues in livestock products has raised important public health concerns

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(related to toxic effects, development of resistant strains of bacteria, allergic hypersensitivity reactions, etc.) as well as environmental and industrial (cheese or yoghurt production, etc.) problems.

Removal of naturally occurring toxins: several kinds of toxins can be present in food depending on their origin, namely, mycotoxins, algal toxins or plant toxins. In many cases, these toxins are large polar compounds that cannot be extracted by supercritical fluids, but not always. Some examples are the isolation of toxins from *Acorus calamus*[18] or from *Podophyllum hexandrum* rizhomes[19], where SFE provided much higher recoveries of some toxins, using neat CO_2, than conventional Soxhlet.

Removal of unwanted compounds from food matrices: sometimes compounds found naturally in foods are not toxic but they decrease the overall quality of the food; this is the case of the presence of free fatty acids in several oils such as olive oil[20], soybean oil[21], yuzu oil[22] which are related to the quality of the fruits prior to oil extraction. Deacidification processes can be conducted by countercurrent SFE with advantages compared to conventional chemical processes providing two fractions, the deacidified oil in the raffinate fraction, and free fatty acids and volatile compounds in the separators.

Removal and use of both fractions: the very first example of this process is the removal of caffeine from coffee[9], in this example both fractions are used: decaffeinated coffee and caffeine. Nowadays not only coffee can be used as source of caffeine, but also tea[23] and other herbs like mate herb[24]. In both cases, mild pressures combined with temperatures close to 60 ºC must be used to increase extraction ratio. Another example is the removal of odorant volatile compounds from winemaking inactive dry yeast preparation[25]. Inactive dry yeasts are used as supplement to enhance wine fermentation, but during the inactivation of

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yeast several odorant compounds are synthesized; the use of 20 MPa, 60 ºC and ethanol as co-solvent provided an inactive dry yeast preparation free of odorant compounds and an extract rich in "toasted" flavor that could be used in bakery products.

Another example using liquid matrices together with countercurrent extraction is the fractionation of wine to obtain three valuable fractions: dealcoholized wine, ethanol and wine. First, the recovery of aroma from wine was attained in a countercurrent packed column (white and red wines were investigated) using very low CO_2/wine ratios. Then, the aroma-free wine recovered from the bottom of the extraction column was dealcoholized by applying different extraction conditions. The results obtained from these studies permit the design of a two-step countercurrent CO_2 extraction process at 9.5 MPa and 40°C, in which the different CO_2/wine ratios employed in each step lead to the recovery of aroma or the removal of ethanol. One example of countercurrent extraction apparatus can be seen in Figure 3.

A similar approach has been also used for the fractionation of essential oils[26], recovery of used oils[27], extraction of tocopherols from oil production byproducts[28] or recovery of alkoxyglycerols from shark liver oil[29]

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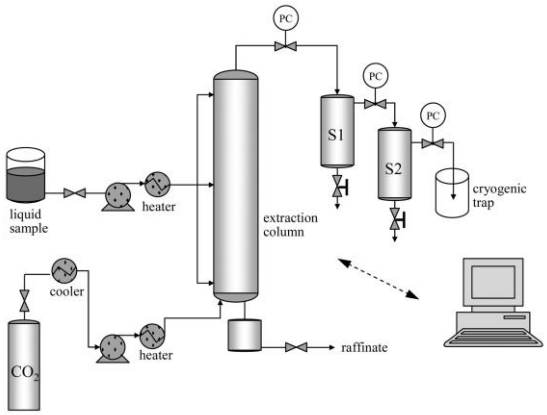

Figure 3. Experimental CC-SFE device. Reprinted with permission from Vázquez, L.; Fornari, T.; Señoráns, F. J.; Reglero, G.; Torres, C. F. Supercritical Carbon Dioxide Fractionation of Nonesterified Alkoxyglycerols Obtained from Shark Liver Oil. *J. Agric. Food Chem.* **2008**, 56 (3), 1078–1083. Copyrights (2008) American Chemical Society.

5.1.2. Extraction of functional food ingredients

5.1.2.1. From plants

One of the most widely studied applications of the use of supercritical fluids is obtaining functional food ingredient from plants. Notably, there is an important increase in the number of published works in the last decade about the use of supercritical fluids for the recovery of bioactive compounds, mainly with antioxidant activity. Aromatic plants, fruits, legumes and seeds have been used as source of natural antioxidant compounds. Table 2 summarizes the more remarkable studies published in the last five years (2009-13) for the SFE of bioactive compounds from plants.

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320 An important application is the SFE of essential oil from medicinal herbs. Essential oils have been traditionally employed in the manufacture of foodstuffs, cosmetics, cleaning products, herbicides, fragrances, and insecticides. Depending on the location and the community knowledge, several of these plants have been used in traditional medicine as diuretics, expectorants, digestives, among others uses[30,31].

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Table 2. Remarkable recent published works (2009–2013) dealing with the use of SFE for the extraction of bioactive components from plants.

Source	Bioactive compound of interest	Related functional bioactivities	Extraction conditions			Reference
			Solvent	P (MPa)/T (°C)	Extraction time (min) / Mode	
Amaranth seeds	Squalene, tocopherols	Antioxidant activity	CO_2 + ethanol	65/40	180/dynamic	(32)
Braccharis dracunculifolia leaves	Artepillin C	Antioxidant activity	CO_2	40/60	20 + 260/static + dynamic	(33)
Camellia sinensis	Fatty acids and antioxidants	Antioxidant activity	CO_2	32/45	90/static	(34)
Ginger (*Zingiber officinale R.*)	Phenolic compounds	Antioxidant activity	CO_2 Propane	CO_2: 25/60 Propane: 10/60	180/dynamic	(35)
Green Tea Leaves	Caffeine	Stimulant	CO_2 + ethanol	23/65	120/dynamic	(36)
Hemerocallis disticha	Lutein, zeaxanthin	Antioxidant activity	CO_2	60/80	30 + 30 / static + dynamic	(37)
Magnolia officinalis	Honokiol and Magnolo	antioxidant, anti-inflammatory activities	CO_2	40/80	60 + 40/ static + dynamic /	(38)
Mangifera indica leaves	Phenolic compound	Antioxidant activity	CO_2 + ethanol	40/55	180/dynamic	(39)
Mitragyna speciosa	Alkaloids	NI	CO_2 + ethanol	30/65	45/dynamic	(40)
Olive leaves	Phenolic compounds	Cytotoxic activity	CO_2 + ethanol	15/40	120/dynamic	(41)
Oregano Leaves (*Origanum vulgare*)	Essential oil	Anti-inflammatory activity	CO_2	30/40.	NI/dynamic	(42)
Persea indica	Diterpene ryanodanes	Insecticidal antifeedant activity	CO_2	20/50	660/dynamic	(43)
Pumpkin (*Cucurbita maxima*)	Carotenoids	Antioxidant activity	CO_2 + ethanol	25/80	60/dynamic	(44)
Rosemary (*Rosmarinus officinalis*)	Carnosic acid, Carnosol, Rosmarinic acid	Antiproliferative colon cancer cells activity	CO_2 + ethanol	150/40	300/dynamic	(45)
Rosemary	Phenolic	Antioxidant activity	CO_2	30/40	300/dynamic	(46)

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(*Rosmarinus officinalis*)	compounds					
Rosemary (*Rosmarinus officinalis*)	Volatile compounds, carnosic and carnosol	Antioxidant activity for use in edible oils	CO_2 + ethanol	15/40	180/dynamic	(47)
Rosemary + spinach leaves *(50%)*	Phenolic diterpenes and carotenoids	Antioxidant activity	CO_2	30/40	300/dynamic	(48)
Satureja hortensis L	Phenolic compounds	Antioxidant activity	CO_2 + ethanol	45/ 40	60/dynamic	(49)
Spearmint (*Mentha spicata L*.)	Essential oil	Antioxidant activity	CO_2	9/35	30/static	(50)
Strawberry (*Arbutus unedo*)	Total phenolics	Antioxidant activity	CO_2 + ethanol	60/48	60/dynamic	(51)
Thyme *(Thymus vulgaris, vulgaris, Thymus hyemalis, Thymus zygis)*	Thymol, carvacrol, borneol, linalool	Antiviral activity	CO_2	30/40	480 min/dynamic	(52)
Usnea arbata L.	Usnic acid	Antibacterial activity	CO_2	30/40	NI/dynamic	(53)

NI: Not indicated

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Essential oils have a complex composition containing a few dozen to several hundreds of components, especially hydrocarbons (terpenes and sesquiterpenes), and oxygenates (alcohols, aldehydes, ketones, acids, phenols, oxides, lactones, acetals, ethers and esters). Besides their fragrance, the mixture of compounds confers several bioactivities (e.g., antimicrobial and antioxidant). Among the most well-known advantages of SFE towards the extraction of essential oils is the use of low temperatures that preserve the integrity of the sample. Recently, Fornari *et al.*[31] reviewed the advances in SFE of essential oils and accomplished an analysis of the effect of matrix and process conditions.

As can be observed from the information presented in Table 2, the bioactives extracted belong to a wide range of compound classes, from polar phenolic compounds to carotenoids, alkaloids, and other pigments and essential oils. As mentioned, in order to extend the polarity range of compounds extracted, ethanol and methanol have been used as modifiers. Usually, quantities of up to 20%[54-49] have been employed, although percentages as low as 2 - 5% have shown to be useful to extract, for instance, polyphenols and terpenoids[55, 32.]

Other less polar bioactive compounds can be potentially recovered by using small amounts of modifiers or even using pure CO_2 at higher pressures. Compounds such as carotenoids, with low polarity, generally need to be extracted using high pressures due to their low solubility in CO_2. These components are basically interesting by their antioxidant activities and coloring properties at the same time. Results of the study of SFE of carotenoids from Pumkim (*Cucurbita maxima*)[56] showed that the total amount of carotenoids extracted increased by increasing pressure from 25 to 35 MPa and temperature from 40 to 70 °C. The highest pressure tested (35 MPa) presented the highest yield (109.6 mg/g), with a 73.7%

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recovery. In fact, 60 MPa of pressure was employed for the extraction of lutein and zeaxanthin from *Hemerocallis disticha*. Also, the addition of a co-solvent to SC-CO_2 was proven to improve the extraction efficiency[37]. Although so far the antioxidant activity is the most studied feature of the extracts obtained by supercritical fluids, other biological activities such as anti-inflammatory, antiviral, antibacterial, cytotoxicity and anti-proliferative activity against cancer cells are started to be explored[38-53]. Santoyo *et al.*[52] evaluated the antiviral properties of supercritical CO_2 extracts obtained from thyme species (*Thymus vulgaris, Thymus hyemalis and Thymus zygis*) against the herpes simplex virus type 1 (HSV-1) at different stages during virus infection. Results indicated that when cells were pre-treated with the thyme extracts, an important reduction of virus infectivity was achieved; being *T. zygis* extract more effective than the other thyme species. Meanwhile, Valdes *et al.*[45], studied the effect of rosemary extracts rich on polyphenols (rosmarinic acid, carnosol, carnosic acid) obtained using SFE (15 MPa, 40°C, 7% ethanol as modifier) on the gene expression of human SW480 and HT29 colon cancer cells. This study showed that rosemary extracts, more specifically, carnosol/carnosic acid-enriched extracts, showed the strongest effect on the proliferation of both cell lines.

Considering the great variations among bioactive compounds and the huge number of plant species, recently Azmir *et al.*[30] adapted from Farnsworth *et al.*[57], a strategy to build up a standard and integrated approach to screen out these compounds with potential benefits for human health. Selection of plant species, evaluation of toxicity, preparation of sample (extraction) and elemental analysis, biological testing, isolation of active compounds and *in-vivo* analysis are among the steps proposed before marketing the bioactive compounds. Extraction step is critical and a large number of factors have to be properly adjusted in

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order to optimize the process; as mentioned above, the use of experimental designs is of great help in order to minimize the number of experiments needed to determine the optimum extraction conditions. Taguchi, Box-Behnken or central composite experimental designs have been used, among others, for the optimization of response variables involved in the SFE extraction of bioactives from plants[58]. Ramandi *et al*.[59] applied a full factorial design for screening the extraction of fatty acids from *Borago officinalis L.* flowers before optimization using a central composite design. Temperature, pressure, volume of modifier and static extraction time were selected as factors to study their influence on the yield of the extracted oil. Caldera *et al*.[60] optimized the SFE of antioxidants (carnosol and carnosic acid) from rosemary (*Rosmarinus officinalis L.*). 2^3 full factorial design was used to select important variables before optimization of the selected factors by Box–Behnken design. Three factors (temperature, pressure and static extraction time) were studied in this experiment. Extraction pressure, dynamic extraction time as well as modifier volume were the factors studied to maximize the recovery of essential oils from *Myrtus comunis* leaves[61] whereas extraction pressure, temperature, and time were the parameters selected in the extraction of *Garcinia mangostana*[62]. In this latter case, total extraction yield and radical scavenging activity of the extracts were chosen as response variables and the composition and amount of co-solvent used as modifier were kept constant.

5.1.2.2. From marine products

The high biodiversity of the marine environments makes the ocean an extraordinary source of high-value compounds that can be obtained from algae, microalgae, and other marine-related organisms such as crustaceans, fish, and their by-products[63, 64]. Table 3 summarizes

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the most relevant literature recently published (from 2009 to 2013) dealing with the recovery of valuable compounds from marine sources using SFE.

Marine sources, especially fish oil and fish by-products, provide the major natural dietary source of ω-3 PUFAs (polyunsaturated fatty acids), mainly EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), which have been associated to a lower incidence of cardiovascular diseases due to their potential biological properties, such as anti-inflammatory, antithrombotic and antiarrhythm[65, 66.] Recently, using a fish oil (*Pseudoplatystoma corruscans*) with low ω-3 fatty acids content (10%), Lopes *et al.*[67] studied the possibility, under different temperatures and pressures, of fractionating the TAGs with respect to EPA and DHA and demonstrate that the fractionation is improved by using fish oil with lower ω-3 fatty acids content as the basis.

The applicability of SFE technology to add value to fish industry waste products has been also demonstrated by using different fish by-products and some marine invertebrate as raw materials to obtain ω-3 PUFAs. Yamaguchi *et al.*[68] reported for the first time the application of SFE to crustacean waste. These authors extracted mainly triglycerides from the Antarctic Krill and analyzed the effects of temperature (40-80°C) and pressure (25.5 MPa) on oil extraction with SC-CO_2. Later, Hardardottir and Kinsella[69] studied the extraction of lipids from rainbow trout in a range of pressures and temperatures of 13.8 - 34.5 MPa and 40-50°C, respectively. Also, the addition of 10% ethanol as co-solvent was evaluated, showing a significant increase in the solubility of the lipids in SC-$CO_{2.}$ Tanaka and Ohkubo[70] reported data from SC-CO_2 extraction of carotenoids and lipids from salmon roe. These researchers used pressures and temperatures ranging from 9.8-31.4 MPa and 40-80°C, respectively.

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425 **Table 3.** Remarkable recent published works (2009–2013) dealing with the use of SFE for the extraction of bioactive components from marine products and by-products.

Marine Source	Bioactive compound of interest	Related functional bioactivities	Extraction conditions			Reference
			Solvent	P (MPa)/T (°C)	Extraction time (min) / Mode	
Arthrospira platensis (*Spirulina platensis*)	Fatty acids, γ-linolenic	Anti-inflammatory, reduce risk of certain cardiovascular diseases	CO_2:ethanol (1:1)	30/40	90/ dynamic	(71)
Brazilian red-spotted shrimp waste (shell and tail)	ω-3 PUFAs, Astaxanthin	Antioxidant activity, Anti-inflammatory, reduce risk of certain cardiovascular diseases	CO_2 + ethanol	30/50	20 + 100/ static + dynamic	(72)
Brazilian red-spotted shrimp waste (heads, shell and tail)	ω-3 PUFAs, Astaxanthin	Antioxidant activity, Anti-inflammatory, reduce risk of certain cardiovascular diseases	CO_2	40/60	20+200/static + dynamic	(73)
Chlorella vulgaris	Lutein	Antioxidant activity	CO_2 + ethanol	40/40	45/dynamic	(74)
Chlorella vulgaris C-C	Polyphenols and Flavonoids	Antioxidant and anti-cancer activity	CO_2 + ethanol	31/50	20/static	(75)
Fish by-products (off cuts from hake, orange roughy and salmon, and livers from jumbo squid)	ω-3 PUFAs,	anti-inflammatory, reduce risk of certain cardiovascular diseases	CO_2	25/40	90/dynamic	(63)
Fish by-product (*Indian mackerel skin*)	ω-3 PUFAs	Anti-inflammatory, reduce risk of certain cardiovascular diseases	CO_2	35/75	180/ 10 static cycles of 18 min	(76)

Fish oil (*Pseudoplatystoma corruscans)*	ω-3 PUFAs	Anti-inflammatory, reduce risk of certain cardiovascular diseases	CO_2	20/40	30/ static + dynamic	(67)
Haematococcus pluvialis	Astaxanthin	Antioxidant activity for use in edible oils	CO_2 + ethanol	50/75	60 + 150/static + dynamic	(77)
Monoraphidium sp. GK12	Astaxanthin	Antioxidant activity	CO_2 + ethanol	20/60	60/static	(78)
Nannochloropsis oculata	Lipids, zeaxanthin	Anti-inflammatory, reduce risk of certain cardiovascular diseases, Antioxidant activity	CO_2 + ethanol	35/50	NI/dynamic	(79)
Northern shrimp by-products (heads, shell and tail)	ω-3 PUFAs	Anti-inflammatory, reduce risk of certain cardiovascular diseases	CO_2	35/40	90/dynamic	(80)
Saragssum Muticum	Phorotannins	Antioxidant activity	CO_2 + ethanol	15.2/60	90/dynamic	(81)
Scenedesmus almeriensis	Lutein and β-carotene	Antioxidant activity	CO_2	40/60	300/dynamic	(82)
Schizochytrium limacinum	Fatty acids DHA	Anti-inflammatory, reduce risk of certain cardiovascular diseases	CO_2 + ethanol	35/40	30/ Urea complexation + static	(83)
Striped weakfish (*Cynoscion striatus*) wastes	Polyunsaturated fatty acids (PUFA)	Anti-inflammatory, reduce risk of certain cardiovascular diseases	CO_2	30/60	150/dynamic	(84)

NI: Not indicated

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Authors observed that at constant temperature, the oil extraction yield increased with the pressure; the highest oil recovery (about 60%) was achieved under the maximum conditions tested. In general, they observed that the low molecular weight triglycerides were easily extracted easily at low pressures and triglycerides of high molecular weight were readily extracted at high pressures. Another interesting work recently developed by Sánchez-Camargo *et al*.[72] studied the effect of the addition of ethanol on the extraction yields of lipids and astaxanthin from redspotted shrimp waste (*farfantepenaeus paulensis*). Results showed that the extraction yields increase considerably with the increase in the amount of ethanol in the solvent mixture, reaching maximum recoveries of 93.8% and 65.2% for lipids and astaxanthin, respectively, when employing 15% ethanol. Besides, increasing the amount of ethanol resulted in increase in the concentration of the ω-3 fatty acids in the lipids of the extract.

Macroalgae, microalgae and cyanobacteria have been also used as natural sources for the extraction of lipids and antioxidants, namely carotenoids, isoflavones, polyphenols, and flavonoids [85]. Due to their polarity, these compounds have been traditionally extracted using organic solvents. However, most of the applications presented in Table 3 employed certain amount of a co-solvent (ethanol or methanol) to modify the polarity of the SC-CO_2.

For instance, Wang *et al*.[77] extracted carotenoids (astaxanthin) from *Haematococcus pluvialis* and studied its antioxidant potential in sunflower oil. An increasing co-solvent amount resulted in an improved astaxanthin yield at 40 MPa and 65 °C. Since carotenoids volatility is very low, the use of modifiers is gene rally recommended instead of increasing the pressure above 50 MPa. The addition of the extract to sunflower oil showed a significant increase in the oxidation stability of the sample at low temperatures, resulting in

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a higher inhibitory effect on the peroxide formation. On the other hand, the use of high amounts of modifier (up to 50%) was tested to obtain fractions enriched in γ-linolenic acid from the cyanobacteria *Arthrospira platensis* (*Spirulina platensis*); using CO_2-expanded ethanol at 30 MPa, 40°C and a ratio CO_2: ethanol 1:1 in the optimum, a recovery up to 35.3% was achieved[71].

One recent interesting area of research is the supercritical fluid extraction of phenolic compounds (phenols, flavonoids) from marine sources. For instance, Wang *et al.*[75] used SFE to extract the active components (flavonoids as antioxidants) from a novel microalga, *Chlorella vulgaris* C-C. Authors compared SC–CO_2 at 31 MPa, 50°C, using 50% aqueous ethanol mix as modifier, and ultrasound assisted extraction (UAE) with 50% aqueous ethanol, and reported that polyphenol and flavonoid content obtained under SFE conditions were 29.1 and 3.7-fold higher than those obtained using UAE, respectively. This resulted in a higher antioxidant activity and better inhibition of lung cancer metastasis.

5.1.2.3. From food by-products

Food industrial processing generates a large quantity and variety of by-products and wastes ranging from manure to packing residuals; this fact has raised important environmental concerns mainly related to their disposal and/or elimination. A strong research has emerged towards the development of suitable alternatives for these by-products, aimed to create high-value products. Their conversion into valuable materials by, for instance, the extraction of high-value compounds can provide enormous benefits from an environmental and economic point of view. SFE has been widely used, among other applications, to add

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value to agricultural and food by-products[86-87] that have been employed as source of bioactive compounds (Table 4).

The main bioactive compounds extracted by SFE from agricultural and food by-products have been polyphenols and carotenoids with antioxidant properties, but also fatty acids, phytosterols and essential oils. Polyphenols extraction is generally carried out by using ethanol as co-solvent in amounts ranging between 10-20%, although extraction using up to 60% has been reported[88] Most of the published works about polyphenols extraction measured the efficiency of the extraction of these bioactive compounds using Folin-Ciocalteu methodology and thus expressing their outcomes as Total Phenolic Content (TPC); however, some studies measure the levels of specific compounds such as resveratrol[89], kaempferol glycosides[88] and chlorogenic acid[90]. Recently, olives processing by-products[41, 91], vineyard[89, 92] and winemaking residues[93] have been recognized as a potential sources of polyphenols with high antioxidant activity. Peralbo-Molina & Luque de Castro[94] reviewed the potential of these residues from the Mediterranean agriculture and agrifood industry.

Regarding carotenoids, lycopene is the most studied pigment and antioxidant extracted from food by-products, it is the most abundant carotenoid in tomatoes, accounting for more than 80% of the pigments present in fully red ripe fruits[95 - 96]. SFE extraction of carotenoids has been mainly carried out from tomato by-products (skins, seeds and tomato paste waste), although it has been also extracted from water melon, pink guava, apricot by-products and carrot press cakes[95, 97]. Extraction temperature is a critical variable affecting extraction efficiency of SC-CO_2 extractions.

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Table 4. Remarkable recent published works (2009–2013) dealing with the use of SFE for the extraction of bioactive components from food by-products

Food by-product	Compounds of interest	Related functional bioactivities	Extraction conditions			Reference
			Solvent	P (MPa)/T (°C)	Extraction time (min) / Mode	
Banana peel	Carotenoids, fatty acids, phytosterols, triterpenes	NI	CO_2	30/50	220/dynamic	(98)
Grape bagasse	Polyphenols	Antioxidant activity	CO_2 + ethanol	35/40	10 + 340/ static + dynamic	(93)
Grape by-products (seed, stem, skin and pomace)	Resveratrol	Antioxidat activity	CO_2 + ethanol	40/35	180/dynamic	(89)
Grape seed	Proanthocyanidins	Antioxidant activity	CO2 + ethanol	30/50	60 min/dynamic	(92)
Guava seeds *(Psidium guajava)*	Phenolic compounds	Antioxidant activity	CO_2 + ethanol	30/50	30/static x 4 cycles	(99)
Jabuticaba (*Myrciaria cauliflora*)	Polyphenols and antocyanins	Antioxidant activity	CO2 + ethanol	30/60	NI/ dynamic	(100)
Melon seeds	Phytosterol-enriched oil	NI	CO_2	40/80	30 + 180/ static + dynamic	(101)
Olive oil mill waste	Phenolic compounds	Antioxidant activity	CO_2	35/40	60/dynamic	(91)
Orange (*Citrus sinensis L. Osbeck*) pomace	Flavonoids, phenolic acids and terpenes	Antioxidant activity, Antimicrobial activity	CO_2 + ethanol	30/50	300/dynamic	(102)
Palm kernel cake	Palm oil	NI	CO_2	41.36/ 70	60/dynamic	(103)
Peach (*Prunus persica*) almond	Oleic and Linoleic acid	LDL cholesterol redactor	CO_2 + ethanol	30/50	150/dynamic	(104)
Red pepper (*Capsicum annum L.*) by-products	Vitamin E and provitamin A	Different protective effects	CO_2	24/60	120/ dynamic	(105)

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Spent coffee grounds and coffee husks	Caffeine and chlorogenic acid	Antioxidant activity	CO_2 + ethanol	30/60	Spent coffee grounds: 270/dynamic Coffee husks: 150/dynamic	(90)
Tea seed cake	Kaempferol glycoside	Antioxidant activity	CO_2 + ethanol	45/80	150/dynamic	(88)
Tea stalk and fiber wastes	Caffeine	Stimulant	CO_2 + ethanol	25/65	180/dynamic	(106)
Sugarcane residue	Octacosanol, phytosterols	Hypocholesterolemic effect	CO_2	35/60	360/dynamic	(107)
Tomato juice	Lycopene	Antioxidant activity	CO_2	35/40	5 + 180 or 360/ static + dynamic	(96)
Tomato peel and seeds	Lycopene	Antioxidant activity	CO_2	40/90	180 /dynamic	(108)
Tomato Skin	Lycopene	Antioxidant activity	CO_2+ ethanol+ olive oil+ water	35/75	NI/dynamic	(109)
Wheat bran	Alkylresorcinols	Antioxidant activity	CO_2	40/80	215/dynamic	(110)

500 NI: Not indicated

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While high temperatures can improve the extraction of some carotenoids, it can also induce thermal degradation or isomerization of the compounds during extraction[109]. As for the pressure, values in the range of 20 to 40 MPa provided the best recoveries of carotenoids such as lycopene and β-carotene. The interaction between temperature and pressure is certainly important in order to maximize carotenoids' extraction yield when SC-CO_2 is used as solvent; however, some studies affirm that the effects of temperature are more significant compared to pressure, for example, in maximizing lycopene recovery[95, 108]. Due to the low solubility of certain carotenoids in CO_2, the type of modifier and its percentage in the mix with CO_2 is a crucial parameter affecting carotenoids' extraction yield. Ethanol and some edible oils like almond, peanut, hazelnut, olive, and sunflower seed oil have been used as co-solvents[95]. The effect of the addition of ethanol, water and olive oil as different co-solvents on the lycopene extraction yield from tomato skin from a tomato processing plant was investigated by Shi *et al.*[109]; the recovery of lycopene increased when the co-solvent was increased from 5% (w/w) to 15% (w/w), in the following order: olive oil (58.2%) > ethanol (51.7%) > water (48.8%).

5.2. Pharmaceutical

Pharmaceutical industries are facing important challenges nowadays, mainly related to the development of production processes with very low environmental impact; in particular, they are urged to reduce the use of volatile organic compounds in drugs synthesis/manufacturing as well as to avoid residues in the finished product. In general terms, the main use of supercritical fluids in pharmaceutics deals with the extraction of bioactive compounds from a mixture (purification from reactions, quantification of active

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enantiomer, extraction from natural matrices, etc.) or with the extraction of the matrix itself. In this case, crystallization and particle formation have undergone an enormous development in recent years[111]. Other benefits of supercritical fluid technologies, strictly related to the above-mentioned new paradigm in pharmaceutics, are linked to the reduced complexity of the process which stems from a reduction of the number of steps as well as to the improved process understanding and control[112]. Despite all the advantages that supercritical fluids can provide to the pharmaceutical industry, extraction is only a minor field in this area; other uses of supercritical fluids are described for their interest although they are not specifically related to SFE:

- Particle generation and co-precipitation: In the pharmaceutical industry, fine particles (μm or nm) with uniform narrow size range are of particular interest. Various supercritical (SCF) processes for particle formation include:

  i) Rapid expansion of supercritical solutions (RESS): involves a fast depressurization of saturated supercritical fluid-drug solution through a heated nozzle into a low pressure vessel that produces a rapid nucleation of the substrate in form of very small particles[113].

  ii) Supercritical antisolvent (SAS) precipitation: a solution composed of a solute and a solvent is injected into the antisolvent (supercritical fluid). While the solvent and the antisolvent are miscible, the solute is quasi non-soluble in the mixture and consequently the mixture is supersaturated and solute particles precipitate[114].

  iii) Particles from Gas Saturated Solutions (PGSS): is a process similar to RESS but in this case the substances are not soluble in the supercritical fluid but they are melted forming a dispersion; then, the Joule-Thomsom effect associated to depressurization cools the dispersion and small particles are obtained[115].

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iv) Aerosol solvent extraction system (ASES): drug and polymer are dissolved or dispersed in an organic solvent which is sprayed into a supercritical phase; the organic solvent, soluble in the supercritical gas phase, is extracted resulting in the formation of solid microparticles of drug+polymer[116].

v) Solution enhanced dispersion by supercritical fluids (SEDS): it allows simultaneous dispersion, solvent extraction and particle formation. The drug solution meets the supercritical carbon dioxide in a coaxial nozzle of the SEDS apparatus, producing a supersaturated solute. The turbulent, high-velocity flow speeds both mixing and particle formation. The supercritical carbon dioxide disperses and mixes the drug solution, acting as an anti-solvent at the same time[117].

- Co-formulation of drug and excipient is one of the emerging concepts in the pharmaceutical industry, in this case some of the above mentioned techniques are used to prepare formulations with drug and polymer[118] or drugs into liposomes[119]. Attending to the extraction capabilities of supercritical fluids and its use in the pharmaceutical industry, one of the main areas of interest is in solvent removal. Residual solvent removal by superctitical fluids exploits the great diffusivity of the compressed gas as well as the easy evaporation of organic solvent into the supercritical phase. The efficiency of the process is a function of the solid/solvent and the solvent/supercritical fluid affinity[112]. For example, Kluge *et al.*[120] proved that crystallization from oil in water emulsions may be used as a purification step; they used SFE to remove the solvent and control crystallization rate of phenanthrene. In this process solvent is extracted before the onset of crystallization, therefore different methods of solvent extraction, such as dilution with water or SFE, affect the process primarily by providing different initial

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conditions for the crystallization step. SFE processed emulsions showed a low residual solvent content, especially in comparison to simple dilution of the system. This causes a higher supersaturation of the oil phase, thus accelerating the self-nucleation of droplets. Both effects are in good agreement with the observation that smaller particles have been obtained at the higher suspension density (see Figure 4). This process has been named as supercritical fluid extraction of emulsions (SFEE).

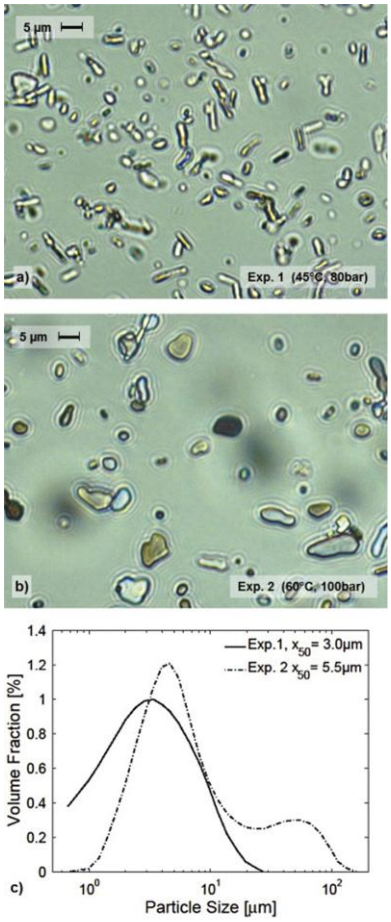

Figure 4. Crystallization upon supercritical fluid extraction of emulsions (SFEE): (a) and (b) Phenanthrene crystals obtained at different operating conditions, (c) corresponding particle size diagram. Reproduced with permission from Kluge *et al.* [109].

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SFE can also be used in a combined process of solvent removal and sterilization of drugs. The process described by Howell *et al.*[121] demonstrated that it is possible to inactivate difficult to kill spores while removing, in the same process, organic solvent. The process was carried out directly from dispensing vials containing drug, a biological indicator and one sterilant (peracetic acid) and using SC-CO_2 as extracting agent. Recovery of drug and analysis of two drugs treated by the process (acetaminophen and paclitaxel) showed no increase in degradation products. After processing, no residual peracetic acid was detected. The process operates at a temperature of about 37 °C (±2 °C) and pressure of about 8 MPa and has a full cycle time of less than 90 min. While much remains to be done before this process could be commercially applicable, the procedure is promising, especially for the preparation of drugs that are easily susceptible to hydrolysis in the presence of water.

5.3. Other applications

5.3.1 Heavy metals recovery

SFE is a promising technique for metal recovery. Chelation combined with solvent extraction is one of the most widely used techniques for separation of metal ions from solid and liquid samples, however these solvent extraction procedures are usually time and labor intensive. In addition, solvent extraction techniques require large amount of organic solvents and often creates environmental problems. In recent years, there has been an increasing interest in extracting metal ions by using SFE. When CO_2 is used to extract chelated complexes, CO_2 and the chelating agent can be easily separated by simply lowering the pressure of the system[122]. Nejad *et al.*[122] optimized the extraction of some lanthanides by SFE using bis(2,4,4-trimethylpentyl)dithiophosphinic acid (Cyanex 301) as

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a chelating agent and tributylphosphate (TBP) as co-extractant. They used a fractional factorial design, 2^{5-1} for process optimization considering five experimental factors: amount of Cyanex 301, flow rate, temperature, pressure and amount of TBP, being pressure the most significant factor. Their results showed that La^{3+}, Ce^{3+} and Sm^{3+} ions could be quantitatively extracted from the solid matrix by using the following conditions: amount of Cyanex 301, 0.14 g, flow rate, 4 ml min−1, temperature, 40ºC, pressure, 25 MPa, and amount of TBP, 30 μl.

The possible combination of (food residues + heavy metal) extraction has been demonstrated by Albarelli *et al.*[123]. These authors analyzed the effects of SC-CO_2 on waste banana peels for copper adsorption. SC-CO_2 was used for antioxidants recovery and in an emerging biomass treatment to increase the efficiency of the subsequent heavy metal-removal step. Adsorption studies showed similar behaviors for fresh and extracted samples, demonstrating that banana peels can successfully be used for the adsorption of copper ions after being subjected to supercritical fluid extraction (SFE) for antioxidant recovery, enabling a promising alternative process chain focused on the integral use of waste banana peels.

5.3.2 Biopesticides production

The interest for biopesticides has been growing rapidly since the awareness for sustainability, climate change and organic farming has risen dramatically. Biopesticides, according to the United States Environmental Protection Agency (USEPA) include naturally occurring substances and microorganisms that control pests and pesticidal substances produced by plants containing added genetic material. The production of

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biopesticides is included in the philosophy of Green Chemistry, a current within the Chemistry which seeks safer products with cleaner processes, in this sense supercritical fluids can provide important advantages[124].

Supercritical fluids are used at different stages and in different approaches in the production of biopesticides. In this review the focus will be on the application of SFE to biopesticides but readers can refer to Martín *et al.*[124] for other uses of supercritical fluids for biopesticides. There are two main families of biopesticides that are commonly extracted by supercritical fluids, pyrethrins and azadirachtins:

- Pyrethrins are the most widely used natural domestic insecticides, extracted from pyrethrum flowers (genre Chrysanthemum) and are comprised mainly by pyrethrin, jasmolin and cinerin. The first application of SFE to obtain pyrethrins was patented in 1981[125]; in general better results are obtained at low temperatures and mild pressures. In a recent study, Cai *et al.*[126] compared the results obtained by using hexane and supercritical CO_2, their results showed that the main chemical compounds in pyrethrum flower extracts were β-farnesene, β-cubebene, ethyl palmitate and ethyl linoleate, besides six pesticidal active compounds of pyrethrins (cinerin I, jasmin I, pyrethrin I, cinerin II, jasmin II and pyrethrin I). The supercritical extract was very similar to the one obtained with n-hexane, still containing waxes and oil, which could be eliminated by cascade depressurization.
- Azadirachtins are tetranortriterpenoids obtained from the tree *Azadirachta indica* (neem), formed by a group of closely related compounds including azadirachtin, salannin, gemudin and nimbin. They are very active as insecticides but have very low toxicity for vertebrates. In fact, Chen *et al.* [127] found that the synergism of azadirachtin,

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oil and other active components in neem SFE extracts could increase the bioactivity against insects. The extraction of one of those azadirachtins, nimbin, was optimized by Zahedi *et. al* [128] who found that optimal conditions to extract nimbin from neem seeds were 40 ºC and 20 MPa, with methanol as co-solvent (10%).

Beside these well-known pesticides, there are several essential oils extracted by supercritical fluids which are being assayed as pesticides. Extracts of thyme (*thymus vulgaris*) and savory (*Satureja hortensis*) obtained at 12 MPa and 50°C have proven insecticidal activity comparable to traditional pesticides[129, 130]. But not only insecticide activity of essential oils obtained by SFE has been assayed, Liang *et al.*[131] compared the acaricidal effect of traditional extracts (hydrodistillation and organic solvent extraction) and SFE (18.0 MPa at 40 °C using ethanol as cosolvent) of *Artemisia absinthium*. The supercritical extracts exhibited stronger antifeedant effects than the traditional ones (up to 8 times more active) with moderate selective phytotoxic effects[132].

6. Future trends

In the present chapter we have tried to present the most recent applications of SFE in different fields, including not only the extraction of valuable compounds from different natural raw materials such as plants, marine products, and agricultural by-products but also new and recent advances in different areas such as food science, pharmaceutical and environmental science. The information is provided as a tool for readers to develop new processes at lab and pilot scale, to discover new ways for sample preparation, to learn how to deal with SFE optimization and how to tune the different parameters involved in the

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process and to be able, at the end, to contribute to the development of future emerging technologies able to fulfil the requirements of green chemistry processes. Bearing this in mind, new emerging technologies, for instance the use of supercritical fluids in particle formation, sterilization, heavy metals removal or biopesticides production have been included.

Even if SFE is now a real option for product development, mainly those related to new foods, food ingredients or pharmaceutical products, there is still a long way to go to be able to implement and demonstrate the sustainability and eco-friendliness of a particular SFE process; to do so, different tools to evaluate the environmental impact of the different procedures are needed, like those based on life-cycle analysis (LCA). Moreover, more focus is needed in terms of economic considerations of SFE processes at large scale.

Even though in the present chapter applications based on the use of supercritical CO_2 (plus some modifiers) are mainly presented for their interest and applicability, the future trends in the SFE field point out to the use of a wider range of experimental conditions (including sub- and supercritical conditions), and a higher number of solvents such as supercritical ethane, near-critical dimethyl ether (DME), gas expanded liquids (GXLs) or combinations of ionic liquids (ILs) and supercritical fluids. Readers are referred to [6, 133, 134] for more information on new solvents and approaches.

Finally, it is expected an important development of green processing platforms based on the use of green solvents such as supercritical CO_2 and water, multi-unit operations consisting of raw material pre-treatment, reactions, extraction, and biofuel conversion, etc. For a really interesting revision of this important field of research, readers are referred to review of

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Catchpole *et al.*[135] where recent developments in integrated processing using supercritical fluids for bioseparations are presented.

Together, all the ideas presented in this chapter and in many other interesting reviews and papers suggested throughout it, can be used towards the real development of process sustainability, providing with new answers to the most challenging demands posted today.

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