SUB- AND SUPERCRITICAL FLUID EXTRACTION OF FUNCTIONAL INGREDIENTS FROM DIFFERENT NATURAL SOURCES: PLANTS, FOOD-BY-PRODUCTS, ALGAE AND MICROALGAE.

*Miguel Herrero, Alejandro Cifuentes, Elena Ibañez**

Instituto de Fermentaciones Industriales (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain

*To whom correspondence should be addressed. Phone: +34-91-5622900 (ext. 388)

Fax: +34-91-5644853

e-mail: elena@ifi.csic.es

ABSTRACT

In this review, new environmentally clean technologies for producing natural food ingredients are discussed. With the increasing interest in functional foods there has been a raise in the demand of functional ingredients obtained using "natural" processes. This work intends to provide an updated overview on the principal applications of two clean processes of great importance in food technology, such as Supercritical Fluid Extraction (SFE) and Subcritical Water Extraction (SWE), in the isolation of natural products from different raw materials: plants, food by-products and algae and microalgae. Special attention is paid to the extraction of antioxidant compounds due to the important role they can play in both, food preservation and health promotion.

KEYWORDS

SFE, SWE, plants, food by-products, algae, microalgae, antioxidant, functional food.

1. INTRODUCTION.

In recent years, there has been a growing interest in the so-called functional foods because they can provide additional physiological benefits other than nutritional and energetic (Goldberg, 1996). A functional food can be defined as the food that produces a beneficial effect in one or more physiological functions, increases the welfare and/or decrease the risk of suffer a particular disease. The beneficial effect from any functional food should be effective at the usual eating habits. Furthermore, a new type of products, derived from food, called nutraceuticals are recently being developed. These products, usually employed as food supplements, are marketed in tablets and pills and can provide important health benefits too.

Frequently, functional foods are obtained from traditional foods enriched with an ingredient able to provide or promote a beneficial action for human health. These are the so-called functional ingredients.

These ingredients are preferred by consumers to have a natural origin (i.e., non synthetic origin) being commonly extracted from natural sources such as plants, food by-products or even algae and microalgae. These types of marine sources are receiving much attention mainly for their content in interesting ingredients as polyunsaturated fatty acids (Mahajan & Kamat, 1995; Cohen & Vonshak, 1991), β-carotene and other pigments (antioxidants) (Madhava, Bhat, Kiranmai, Reddy, Reddanna & Madyastha, 2000; Bhat & Madyastha, 2000), sulphated polysaccharides (anti-viral), sterols (antimicrobials), etc. (Borowitzka & Borowiztka, 1988; Ötles & Pire, 2001; Xue et at, 2002).

Among the different compounds with functional properties, antioxidants are being the most studied (Piñero-Estrada, Bermejo-Bascós & Villar del Fresno, 2001; Bhat et al., 2000). These compounds can play an important role in Food Technology because of their usefulness as a preservation method against lipid peroxidation. Usually, food production process and storage can generate important losses of endogenous antioxidants that limits their own protection against lipid oxidation. At the same time, the important role of antioxidants in human health has been recently demonstrated thus increasing the interest in such products and their demand by the consumers (Borowitzka et al., 1988).

The traditional extraction methods used to obtain these type of products have several drawbacks; thus, they are time consuming, laborious, have low selectivity and/or low extraction yields. Moreover, these techniques employ large amounts of toxic solvents. At present, new extraction methods able to overcome the above mentioned drawbacks are being studied, among them, Supercritical Fluid Extraction (SFE) and Subcritical Water

Extraction (SWE) are among the more promising processes (King, 2000). These extraction techniques provide higher selectivities, shorter extraction times and do not use toxic organic solvents.

The goal of this review is, therefore, to provide an update overview on the principal applications of two environmentally safe technologies, SFE and SWE, for obtaining functional ingredients from natural sources as plants, by-products from the food industry, algae and microalgae.

2. SUPERCRITICAL FLUID EXTRACTION (SFE).

2.1. Principles and instrumentation.

When a certain fluid is forced to a pressure and temperature higher than its critical point (see Figure 1), it becomes a supercritical fluid. Under these conditions, the different properties of the fluid are placed between those of a gas and those of a liquid. Although a supercritical fluid density is similar to a liquid and its viscosity is similar to a gas, its diffusivity is intermediate, as can be seen in Table 1. Thus, the supercritical state of a fluid has been defined as a state in which liquid and gas are indistinguishable to each other, or as a state in which the fluid is compressible (i.e. similar behaviour to a gas) even though posses a density similar of a liquid and, therefore, has its solvating power.

Because of its different physico-chemical properties, SFE provides several operational advantages over traditional extraction methods (Anklam, Berg, Mathiasson, Sharman & Ulberth, 1998). Due to their low viscosity and relatively high diffusivity, supercritical fluids have better transport properties than liquids, can diffuse easily through solid materials and therefore allow to obtain higher extraction yields. One of the main characteristics of a supercritical fluid is the possibility to modify the density of the fluid by changing the pressure and/or the temperature. Since density is directly related to solubility (del Valle & Aguilera, 1999; Raventós, Duarte & Alarcón, 2002), by tuning the extraction pressure, the solvent strength of the fluid can be modified changing in this way the selectivity of the system. Other advantages, compared to other extraction techniques, are the use of solvents generally recognized as safe (GRAS), the efficiency of the extraction process (when working with dynamic extraction in which fresh supercritical fluid is forced to pass through the sample), and the possibility of direct coupling with analytical chromatographic techniques such as Gas Chromatography (GC) or Supercritical Fluid Chromatography (SFC). As for the solvents, there is a wide range of compounds that can be

used as supercritical fluids (see Table 2), being carbon dioxide the most commonly used because of its moderate critical temperature (31.3 ºC) and pressure (72.9 atm). Besides, supercritical CO_2 gives additional advantages to those inherent to the technique (Lang & wai, 2001). Carbon dioxide is a gas at room temperature, so once the extraction is completed, and the system decompressed, a complete elimination of CO_2 is achieved without residues and the extract obtained remains free of solvent. At industrial scale, when carbon dioxide consumption is high, the operation can be controlled to recycle it. However, supercritical CO_2 because its low polarity, can be less effective to extract the most polar compounds in natural matrices. To overcome this shortcoming, modifiers (also called co-solvents) are commonly used. Modifiers are highly polar compounds that added in small amounts can produce substantial changes on the solvent properties of supercritical CO_2 (Valcárcel & Tena, 1997).

According to the specific requirements, the design of a supercritical fluid extraction system can be relatively simple or highly complex. Basically, it is possible to differentiate between analytical instruments and preparative systems (pilot or industrial scale). The analytical systems are utilized as sample preparation prior to, for example, a chromatographic analysis to obtain usually from milligrams to grams of extracts. There are several configurations depending on their capabilities to process one or several samples at the same time, or according to their automation degree. The preparative systems are used to extract grams of compounds when working at pilot scale or kilograms at industrial scale. In these preparative systems, two different configurations can be found: for solid or liquid samples extraction.

Basically, a preparative system at pilot scale plant is formed by (see Figure 2) a solvent pump, that delivers the fluid throughout the system, a modifier pump if necessary, an extraction cell or extraction column according to the system configuration (that is, for solids or liquids, respectively), and one or more separators (also called fractionation cells) in which the extract is collected and the solvent depressurized. Likewise, the extraction cell or column and the separators are commonly equipped with an independent control of temperature and pressure, in such a way that fractionation of the extracted compounds can be carried out by a step depressurization. Therefore, different compounds can be obtained on each separator, depending on their different solubility in the supercritical fluid. Additionally, it is possible to install a refrigerated system especially designed to trap the most volatile compounds, as well as a recycling system to recycle the fluid employed.

As it has been mentioned, the most important difference between a pilot plant to process solid or liquid samples is either the extraction cell or the extraction column. This

fact conditions the type of process applied being always a batch process for solid samples and a continuous procedure for liquid samples (using, in the latter case, packed columns operating under countercurrent conditions). In liquid-sample extractions, the supercritical fluid (usually CO_2) is moving upwards while the sample feed is introduced to the system from the top of the column and moves downwards by gravity. Nowadays, carbon dioxide is the most used supercritical fluid and it has been employed in a broad range of applications. Figure 3A shows the distribution, according to Rozzi et al. (Rozzi & Singh, 2002), of the work done with supercritical fluids in the different fields between 1999-2000 according to the *Current Contents database*. More specifically, Figure 3B shows the number of papers published in the "Food Science and Technology" field between 2001 and 2003 after searching in *Food Science and Technology Abstracts (FSTA) database*, using "supercritical and fluid" as search parameters.

2.2. Plants as natural sources of functional ingredients.

Numerous vegetable matrices have been extracted using compressed fluids. Legumes, spices, aromatic plants, and even fruit beverages such as natural orange juice (Señoráns, Ruiz-Rodriguez, Cavero, Cifuentes, Ibañez & Reglero, 2001) have been processed to obtain natural antioxidant compounds.

Several studies have compared the antioxidant activity of plant extracts obtained using supercritical fluid extraction with those using traditional extraction methods. For example, eucalyptus (Fadel, Marx, El-Sawy & El-Gorab, 1999) and *Lippia alba* (Stashenko, Jaramillo & Martínez, 2004) have been used to compare different extraction methods in terms of composition and activity. The research performed with eucalyptus demonstrated the differences that exist between the composition and functional properties of extracts obtained with SFE (using supercritical carbon dioxide at 200 bar and 50 ºC) and hydrodistillation. Antioxidant activity was found to be higher for supercritical fluid extracts than for hydrodistillation extracts. In spite of the fact that the main compounds were the same in extracts, their quantitative composition changed. For example, supercritical fluid extracts had a higher content of sesquiterpenes and oxygenated compounds. As for the antioxidant activity, it seems to be related to the concentration of both, *p*-cymen-7-ol and thymol. The concentration of both compounds, mainly the amount of *p*-cymen-7-ol, on SFE extracts was higher than that found on hydrodistillation extracts. Those values were in agreement with the higher antioxidant activity found in supercritical extracts compared to extracts from hydrodistillation. A more exhaustive comparison was carried out by

Stashenko et al. (2004) with extracts obtained from *Lippia alba*. In that work, 40 compounds were identified and quantified from extracts obtained using hydrodistillation, microwave-assisted hydrodistillation and supercritical fluid (carbon dioxide) extraction. Results showed both quantitative and qualitative differences among extracts. The most complex extracts, in terms of amount of isolated compounds, were those obtained with SFE. Likewise, a significantly higher amount of sesquiterpenes were quantified in the supercritical fluids (SF) extracts as compared to those obtained with the other extraction techniques tested. However, this relation was overturned concerning to the amount of monoterpenoids detected.

Among the studies performed with leguminosae as natural sources of antioxidant compounds, those using Tamarind (*Tamarindus indica* L.) are of great interest. Tsuda et al. (Tsuda, Mizuno, Ohsima, Kawakishi & Osawa, 1995) studied the antioxidant activity of extracts obtained from tamarind seed coat using SFE (with CO_2) at different conditions. Results showed that the antioxidant activity of the extracts increased when the extraction pressure and temperature were raised. Besides, the addition of a co-solvent suitable for the food industry (ethanol) on the extraction process was studied in order to show the influence of polar compounds on the final antioxidant activity of the extracts. In any case, the addition of 10% modifier (V/V) increased the antioxidant activity of the extracts. On a later work, Luengthanaphol et al. (Luengthanaphol, Mongkholkhajornsilp, Douglas, Douglas, Pengsopa & Pongamphai, 2004) established a comparison between SF extracts and extracts obtained using solvent extraction (ethyl acetate and ethanol). The results of this study showed that the antioxidant activity of ethanol extracts was better than that of SF extracts.

However, for other matrices, supercritical fluid extraction is considered the most suitable method to produce fractions with high antioxidant activity. One example is the coriander extraction (*Coriander sativum*). Yépez et al. (Yépez, Espinosa, López & Bolaños, 2002) demonstrated the possibility to obtain odourless and flavourless extracts from coriander, with high antioxidant activity, using supercritical fluid extraction with CO_2. Extraction at moderate conditions (45 ºC and 177 bar), that means CO_2 densities close to 0.74 g/ml, provides extracts with high antioxidant activity and high yields. Moreover, Esquível et al. (Esquível, Ribeiro & Bernardo-Gil, 1999) showed that low temperatures and pressures were efficient to concentrate antioxidant compounds from savory oil (*Satureja hortensis*). In this study, a constant temperature extraction (40 ºC) was kept while changing the pressure values. The most suitable pressure was set at 120 bar considering that higher pressures did not report any improvement. Depressurization was carried out on three

collectors. Likewise, Esquível et al. (1999) suggested that it would be possible to isolate carvacol (i.e., the main component of savory oil) by optimizing the separation parameters.

Aromatic plants are among the most studied plants with antioxidant activity. Ribeiro et al. (Ribeiro, Bernardo-Gil & Esquível, 2001) studied the antioxidant activity of supercritical extracts from lemon balm (*Melissa officinalis L.*). The plant material was subjected to pressures from 100 to 180 bar and temperatures from 35 to 40 ºC. However, in this case, the solid residues were analyzed to determine their antioxidant activity instead of the supercritical carbon dioxide extracts. The results obtained showed that the best antioxidant activity was achieved setting the extraction conditions at 100 bar of pressure and 35 ºC of temperature for four hours. Therefore, it was demonstrated, according to the authors, the possibility to employ supercritical CO_2 extraction to isolate compounds with antioxidant activity from solid samples.

Ginger (*Zingiber officinale* Roscoe) is another aromatic plant which has been widely studied due to its multiple functional activity. Zancan et al. (Zancan, Marques, Petenate & Meireles, 2002) carried out a study to prove the effect of temperature and pressure as well as the addition of a co-solvent on the kinetics of ginger extraction and on the extracts antioxidant activity. By means of a factorial design it was possible to conclude that the addition of a co-solvent was not necessary to increase the mass transfer rate nor the extraction yield. The factors selected to carry out the factorial design were: extraction temperature (25 to 35 ºC), extraction pressure (200 to 250 bar) and solvent (i.e. CO_2, CO_2 + ethanol, CO_2 + isopropyl alcohol). The best results in terms of antioxidant activity were obtained when the extraction was carried out with a modifier at low temperatures and pressures and relatively long extraction times, apparently, due to the extraction of gingerols and shogaols at these conditions. These compounds are related to the ginger antioxidant activity. A recent study compares this aromatic plant with other similar plants, as rosemary (*Rosmarinus officinialis* L.) and turmeric (*Curcuma longa* L.) (Leal, Braga, Sato, Carvalho, Marques & Meireles, 2003). Extracts of these three plants were obtained using supercritical carbon dioxide with/without modifier (ethanol and/or isopropyl alcohol). Experiments were carried out at pressures between 100 and 300 bar and temperatures between 30 and 40 ºC. Once the antioxidant activity assays were performed, it was possible to conclude that the extracts which lower antioxidant activity were those from turmeric and ginger, while rosemary extracts provided higher values of antioxidant activity.

In fact, rosemary is one of the plants with higher antioxidant activity and therefore one of the most studied (Mendes et al., 1995; Quirin, 2003; López-Sebastian et al., 1998). The antioxidant activity of supercritical extracts of rosemary is extremely high even at low

concentrations, the extracts are heat-resistant, and do not change the colour, the taste, or the flavour of the food in which are used. Besides, in that food, the extracts are dissolved easily (Gerard, Quirin & shawarz, 1995). Tena et al. (Tena & Varcárcel, 1997) developed a method to obtain this type of extracts using carbon dioxide. This method was previously compared with other traditional methods formerly developed using liquid solvent sonication. SF extracts showed higher carnosic acid concentration, one of the main compounds in rosemary responsible for its antioxidant activity (Hidalgo, Ubera, Tena & Varcárcel, 1998). Therefore, the highest antioxidant activity was found in these extracts. As an additional advantage, it was found that these extracts did not show any colour. In that work Hidalgo et al., 1998), the optimum extraction conditions were: 383 bar, 120 ºC for 20 minutes. Bauman et al. (Barman, Hadolin & Rizner-Hras, 1999) studied the extraction of antioxidant components from rosemary using supercritical carbon dioxide at 100 ºC and 475 bar. The extraction yield obtained ranged from 5 to 6 %. The antioxidant activity of these extracts was compared with the activity of known synthetic antioxidants, such as BHA and BHT, providing the former much higher activity than those found in the synthetic antioxidants. In a work from our group (Ibáñez, Oca, de Murga, López-Sebastián, Tabera & Reglero, 2000), a two step extraction method was suggested, with sequential recovery of two fractions with different antioxidant activity and different chemical composition. The SFE experiments were carried out at analytical scale and the conditions selected were: 100 bar and 40 ºC for the first fraction and 400 bar and 60 ºC for the second fraction. The antioxidant compounds were preferentially extracted in the second fraction. Both fractions were collected on a device specially designed to improve the performance of the sample collection. This device consisted on a reservoir, that, by means of an extra cooling system, was capable to avoid extract losses after CO_2 decompression.

Later on, we studied the scaling up of the process using a pilot scale plant (Señoráns, Ibáñez, Cavero, Tabera & Reglero, 2000; Ibáñez, Cifuentes, Crego, Señoráns, Cavero & Reglero, 2000). In this work, instead of a sequential extraction process in two steps, two separation cells were used to carry out the fractionation. This system allowed obtaining two different fractions in terms of analytical and functional composition. Different extraction and fractionation conditions were tested, using carbon dioxide as supercritical fluid and ethanol as modifier. The extraction conditions ranged from 300 to 350 bar and from 40 to 60 ºC. For all the experiments, the first separator was maintained at the given extraction temperature (from 40 to 60 ºC) while the fractionation pressure was set on a range from 150 to 200 bar. The temperature in the second separator was kept equal to 25 ºC in all experiments and the pressure varied between 20 and 55 bar.

Another study performed in our research group to test the effect of the CO_2 quality on the antioxidant activity of rosemary extracts (Ibáñez et al., 2001) demonstrated that the CO_2 quality significantly affected the antioxidant activity of the extracts collected. Thus, the better the quality of the CO_2 employed the higher the antioxidant activity of the extracts.

Extraction of vitamin E from natural sources has received an increasing interest due to the high antioxidant activity associated to this family of compounds. Besides its well known antioxidant activity, recent studies have demonstrated synthetic vitamin E to be less effective than natural vitamin E (Hadolin, Skerget, Knez & Bauman, 2001). Several natural sources have been used to isolate vitamin E using supercritical carbon dioxide extraction.

Hadolin et al. (2001) studied the extraction of vitamin E-rich oil from a plant (*Silybum marianum*) that naturally grows in Mediterranean area. It was pointed out that extractions at 60 ºC and 200 bar produced the most concentrated extracts in terms of α-tocopherol (0.08 %), while the extraction yield was relatively high (19 %).

Other important source of vitamin E is the wheat germ. Ge et al. (Ge, Yang, Hui, Ni, Wang & Cai, 2002; Ge, Ni, Chen & Cai, 2002) extracted vitamin E from this material at the following extracting conditions: 275 bar, 40 ºC and a CO_2 flow rate of 2 ml/min for 90 minutes. The amount of total vitamin E extracted at these conditions was higher than those obtained using traditional extraction methods (with n-hexane or chloroform/methanol mixtures). Likewise, the quantities of α, γ and δ-tocopherol were much higher using SFE. However, the n-hexane extracts, and mainly, the chloroform/methanol extracts, showed higher selectivity to β-tocopherol.

2.3. Functional ingredients from food industry by-products.

Frequently, the processes that take place in the food industry generate products (the so-called by-products) that are discarded, with the subsequent environmental problems associated to the removal of these wastes. In recent years, companies, administrations and research groups have devoted a lot of efforts trying to find some usefulness of these food by-products while reducing their environmental impact.

Supercritical fluid extraction has been a useful tool in many of these studies. Thus, several works have been developed to extract β-carotene and lycopene from by-products of tomato industry (Baysal, ersus & Starmans, 2000; Rozzi, Singh, Vierling & Watkins, 2002). These compounds are natural pigments belonging to carotenoids group and their antioxidant properties are well known, which increase even more their high added value. Baysal et al. (2000) studied the optimisation of β-carotene and lycopene extraction from

tomato paste waste employing a factorial design. By previously determining the total amount of these compounds in the tomato paste, it was possible to establish an optimum recovery of 54 % of total lycopene using as extraction conditions CO_2 at 300 bar and 55 ºC for two hours. The CO_2 flow rate was kept constant at 4 Kg/h and ethanol was used as modifier (5 %). The optimum value for β-carotene recovery was around 50 %. Extraction conditions were similar as the previous selected for lycopene extraction, except for an increase in the extraction temperature up to 65 ºC. Likewise, these authors suggested the use of higher extraction temperatures and pressures to increase extraction yields, although a 100% recovery would never be expected due to the degradation that these compounds can suffer during the extraction process. Rozzi et al. (2002) studied the extraction of lycopene of tomato seeds and skins with supercritical CO_2. In this work more extreme extraction conditions were tested with extraction temperatures ranging from 32 and 86 ºC and extraction pressures from 138 to 483 bar. The authors showed that the amount of lycopene extracted increased at higher pressures and temperatures until a maximum recovery of 61 % at 86 ºC and 345 bar. The CO_2 flow rate was 2.5 ml/min and 3 g of sample where used in an analytical system. The results presented in that study (Rozzi et al., 2002) demonstrated the possibility of lycopene extraction from tomato by-products using supercritical CO_2 without the addition of co-solvents although it is important to point out the high solvent/feed ratio (S/F = 166) used in the process.

Other interesting by-products are those from the wine industry. Their interest is related to the type and amount of phenolic compounds that are found in grape seeds and skins. Isolation of phenolic compounds from grape seeds has been attempted using supercritical carbon dioxide (Palma & Taylor, 1999; Murga, Ruíz, Beltrán & Cabezas, 2000). Palma et al. (1999) observed that the recovery of catechin and other phenolic compounds from grape seeds was higher using supercritical CO_2 with methanol as modifier than when using traditional solid-liquid extraction. Besides, supercritical fluid extraction is faster and allows fractionation of the phenolic compounds of the grape seeds (Murga et al., 2000) by changing the pressure and adding co-solvents at different percentages. In a recent work, Louli et al. (Louli, Ragoussis & Magoulas, 2004) employed supercritical fluid extraction to increase the added-value of extracts from by-products of the wine industry obtained through extraction with ethyl acetate. These extracts had antioxidant activities similar to the synthetic antioxidant BHT. The properties of the starting product were significantly improved thanks to the subsequent supercritical CO_2 extraction that cause an increase on the antioxidant activity and allows the obtention of an extract without odour and with clearer colour. These characteristics make the extracts more appropriate to use as natural

antioxidants for the food industry. The selected parameters to perform the extraction were a pressure of 150 bar and a temperature of 45 ºC. The addition of 0.5 % of co-solvent did not improve significantly the results.

The use of by-products from the olive oil industry to extract tocopherols was suggested by Ibañez et al. (Ibáñez, Palacios, Señoráns, Santa-María, Tabera & Reglero, 2000). These compounds are highly valued not only because tocopherols are well known as components of vitamin E but also because they show an important antioxidant activity and therefore can be used as natural antioxidants. In that work (Ibáñez et al., 2000), the separation of tocopherols from the olive pomace (that is, the solid residue obtained using two-phase olive oil production systems) was achieved by means of a supercritical carbon dioxide extraction with two step fractionation. On the second fractionation step, where complete depressurization took place, the CO_2 density was very low and enrichment of the extract with tocopherols was observed. The extraction was carried out at a pressure of 350 bar and at a temperature of 50 ºC, while the fractionation conditions that gave the best results were: 100 bar and 60 ºC on the first separator and 10 bar and 25 ºC on the second separator. Although the extract obtained using SFE was rich in tocopherols, these authors suggested a selective fractionation system of the different tocopherol isomers using supercritical fluid chromatography (SFC) with packed columns.

Other work that suggested the utilization of food by-products to produce vitamin E using SFE, was performed by Mendes et al. (Mendes, Pessoa & Uller, 2002). It is based on the soybean oil production and the by-product studied is obtained as a waste on the deodorization step during the industrial production of soybean oil. Considering that the soybean oil is the most widely consumed oil in the world, the use of this by-product (also called soybean sludge) has a great importance. Taking into account the interest of obtaining a tocopherol enriched residues, different extraction temperatures (from 40 to 80 ºC) and pressures (from 90 to 170 bar) were studied. The highest extraction efficiency was obtained at mild extraction conditions, being extracted the fatty acids by the supercritical CO_2. This fact allowed the tocopherol enrichment inside the reactor cell.

The extraction of natural pigments from food by-products has a major importance not only because of the increasing demand on natural ingredients to be used in the food industry but also because some of these pigments can also have associated some antioxidant activity that can increase, even more, their added-value. The possibility of extracting natural pigments with antioxidant properties using SFE has been already mentioned above. However, sometimes pigments themselves are the target compounds, for example, when extracting carotenoids from carrots and tomatoes industry, i.e., the sources

most frequently used to obtain natural carotenoids. Thus, Cadoni et al. (Cadoni, De Giorgi, Medda & Poma, 2000) described the tomato extraction (both from skin and pulp) to achieve the isolation of lycopene and β-carotene. Among the different conditions studied in this work, the best results were obtained using an extraction pressure equal to 275 bar and an extraction temperature of 80 ºC. The product obtained at these conditions had a composition of 65 % lycopene and 35 % β-carotene. However, taking into account that both compounds showed different solubility parameters on supercritical CO_2, it was possible to select a two-steps extraction to preferentially extract lycopene or ß-carotene. For instance, if extraction is performed in a first step at 275 bar and 40 ºC and in a second step at 275 bar and 80 ºC, it was possible to obtain an end-product with 87 % lycopene and 13 % β-carotene, because of β-carotene is preferably extracted at the first extraction conditions. The isolation of lycopene was also studied by Ollanketo et al. (Ollanketo, Hartonen, Riekkola, Holm & Hiltunen, 2001) with tomato skin. After considering different extraction conditions (that is, different extraction temperatures, with/without co-solvents), they achieved a 100 % lycopene recovery when performing extractions with supercritical carbon dioxide at 1.5 ml/min flow rate, 110 ºC of temperature and 400 bar as extraction pressure for 50 minutes. Results were similar with and without using acetone as modifier; leading to a lycopene recovery of 94 % in just 15 minutes of extraction.

Using carrots as natural source of carotenoids, Barth et al. (Barth, Zhou, Kute & Rosenthal, 1995) carried out the optimization of the supercritical carbon dioxide extraction using a factorial design. The influence of different extraction conditions towards carotenoid isolation was studied. Considering extraction temperatures of 30, 40 and 50 ºC, extraction pressures of 300, 400 and 500 bar and the addition of co-solvent (5 and 10 % ethanol), the authors concluded that the optimum extraction conditions were achieved working at 50 ºC, 300 bar and 10 % ethanol as modifier. Moreover, the quantitative data obtained from SFE extracts were compared with other extracts obtained employing traditional solvent extraction methods. In this study, it was confirmed that the amount of carotenoids extracted (including those with provitamin A activity) was higher in SFE extracts than in traditional solvent extracts. Besides, the traditional solvent extraction was completed after 6 hours while the supercritical extraction was finished after 1 hour.

2.4. Extraction of functional ingredients and other compounds of interest from algae and microalgae.

In the search of feasible new sources of natural antioxidants that can be used in the food industry, algae and microalgae have been suggested as possible raw material. Both organisms are widely known and consumed in certain regions, and numerous health benefits have been associated to their use. Therefore, algae and microalgae are potentially a great source of natural compounds that could be used as ingredients for preparing functional foods. Different compounds with antibacterial, antiviral and antifungical activity can be found in this type of organisms (Borowitzka et al., 1988; Ötles et al., 2001; Xue et al., 2002), along with compounds with antioxidant activity that, as has been already mentioned, is nowadays one of the most important field of activity for the food industry.

Subra et al. (Subra & Boissinot, 1991) proved that starting from a complex matrix as an algae (*Dilophus ligulatus*) it was possible to obtain extracts with different compositions and yields by changing the extraction pressure. Therefore, depending on the type of compounds of interest, the optimum extraction conditions could be tailored.

Many algae and microalgae are rich in polyunsaturated fatty acids. An increase in the consumption of these type of compounds has been associated to a decrease in the incidence of cardiovascular diseases (Cohen at al., 1991). Cheung (1999) studied the effect of the extraction conditions to obtain fatty acids from *Hypnea charoides* algae, using supercritical CO_2, and suggested the usefulness of this extraction technique to convert this specie of algae in a non-conventional source of ω-3 fatty acids. Temperature ranges from 40 to 50 ºC and pressure ranges from 241 and 379 bar were studied. Although, in general, the lipid recovery increased according to a pressure and temperature increase, also the ratio of unsaturated fatty acids was increased. Concerning to the extraction of ω-3 fatty acids, their solubility was shown to depend on their chain length.

Several microalgae species have been used to obtain natural compounds of interest for the food industry using supercritical fluid extraction. Mendes et al (Mendes et al, 1995a; Mendes et al., 1995b; Mendes, Nobre, Cardoso, Pereira & Palavra, 2003) applied this technique to several microalgae species to extract, for example, diolefines from *Botrycoccus braunii* cells. This organism can store high amount of long chain hydrocarbons (i.e. 25-31 carbon atoms), that can be utilized as substitutes of paraffinic and natural waxes. The authors proved that the solubility of these type of compounds in CO_2 increased with the pressure and found that extraction at 300 bar provided the best conditions also in terms of extraction speed.

Likewise, it was proved the feasibility of this technique to obtain carotenoids from microalgae *Chlorella vulgaris*. High pressure values allowed a high carotenoids extraction yield. When microalgae cells were crashed, a small increase in temperature values

improved slightly the carotenoid extraction if the pressure was maintained at high values. However, similar temperature increase at low pressures produced an opposite effect. The optimum conditions were fixed at 55 ºC for extraction temperature and 350 bar for extraction pressure (Mendes et al., 1995b).

Dunaliella salina is a microalgae specie able to produce 14 % of β-carotene relative to its dry weight. Therefore, it can be an important source of this interesting compound because of its important activities against lung and prostate cancer. The optimum extraction yield for β-carotene extraction with supercritical CO_2 was obtained by working at 300 bar and 40 ºC (Mendes et al., 2003).

Other microalgae specie studied by Mendes et al. has been *Arthospira* (*Spirulina*) *maxima* (Mendes et al., 2003). This microalgae is capable to produce high amounts of γ-linolenic acid (GLA). The recovery of GLA with pure CO_2, and with CO_2 plus ethanol as co-solvent, was compared with the results obtained using traditional organic solvents extraction. It was observed that, although both CO_2 and n-hexane provided similar extraction yields, the CO_2 allowed a higher recovery of GLA. The maximum extraction yield was obtained using CO_2 with 10 % of ethanol as modifier and performing the extraction at 350 bar and 60 ºC.

Other specie belonging to Cyanobacterium, *Spirulina platensis*, was studied by Qiuhui (1999) to determinate the amount of lipids and GLA present in the microalgae. It was estimated that the maximum extraction yield was obtained at 350 bar of pressure, in good agreement with the works by Mendes et al. (2003). The temperature was set at 40 ºC and the CO_2 flow rate was fixed at 24 Kg/h for 4 hours.

3. SUBCRITICAL WATER EXTRACTION (SWE).

3.1. Principles and instrumentation.

Subcritical water extraction (SWE), i.e., extraction using hot water under pressure, has recently emerged as a useful tool to replace the traditional extraction methods. SWE is an environmentally-clean technique that, in addition, provides higher extraction yields to extract solid samples (Luque de Castro, Jimenez-Carmona & Fernández-Pérez, 1999). SWE is carried out using hot water (from 100 ºC until 374 ºC, the latter being the water critical temperature) under high pressure (usually up to 10 bar) enough to maintain water in the liquid state. The most important factor to take into account in this type of extraction procedures is the dielectric constant (ε). This parameter can be modulated easily, within a

wide range of values, by only tuning the extraction temperature. Water at room temperature is a very polar solvent, with a dielectric constant close to 80. However, this level can be significantly decreased to values close to 27 when water is heated up to 250 ºC (see Figure 4) while maintaining its liquid state applying pressure. This dielectric constant value is similar to that of ethanol being therefore appropriate to solubilize less-polar compounds (Miller & Hawthorne, 2000).

The experimental set-up needed to use this technique is quite simple (see Figure 5). Basically, the instrumentation consists on a water reservoir coupled to a high pressure pump to introduce the solvent into the system, an oven, where the extraction cell is placed and where the extraction takes place, and a restrictor to maintain the pressure along the extraction line. Extracts are collected in a collector vial placed at the end of the extraction system. Additionally, the system can be equipped with a coolant device for a fine control of the temperature. Also the instrumentation can include a nitrogen circuit to purge the system once the extraction is completed.

Although this technique has been mainly used in batch processes, there is a work published about the on-line coupling of a SWE system to a HPLC equipment via a solid phase trapping (Li, Yang, Gan, Eaton, He & Jones, 2000).

Different applications of SWE to extract compounds from plants, algae and microalgae are next discussed. No applications of SWE to food industry by-products have been found so far in literature.

3.2. Extraction from plants using SWE.

Subcritical water extraction has been widely applied to extract different compounds from several vegetable matrices. One of the most deeply studied materials has been rosemary (*Rosmarinus officinalis* L.). Ibañez et al. (Ibáñez, Kuvatová, Señoráns, Cavero, Reglero & Hawthorne, 2003) studied the extraction of antioxidant compounds of rosemary by SWE testing a wide range of temperatures. Also in this work, an exhaustive characterization of the extracts obtained was carried out. Several temperatures, from 25 to 200 ºC were tested to study the extraction selectivity towards antioxidant compounds. There was a clear effect of water temperature on the extraction yield, increasing at higher extraction temperatures. The authors verified that the most polar compound (i.e., rosmanol) was the main compound extracted at low temperatures (25 ºC). When the extraction was performed at 200 ºC, a decrease on the capability of water to dissolve the most polar compounds was observed, while a high concentration of other kind of compounds, as

carnosic acid of medium-lower polarity, was obtained. Besides, the possibility to obtain antioxidant extracts comparable to those achieved using supercritical carbon dioxide extraction was demonstrated.

In addition to antioxidants from rosemary, the SW extraction of aroma compounds has been also studied for this plant (Basile, Jiménez-Carmona & Clifford, 1998) and other plants as savory (*Satureja hortensis*) and peppermint (*Mentha piperita*) (Kuvatová, Lagadec, Miller & Hawthorne, 2001).

Some studies have been conducted to compare SWE to traditional extraction methods (such as Soxhlet extraction). Thus, clove (*Syzygium aromaticum*) extractions were performed by Clifford et al. (Clifford, Basile & Al-Saidi, 1999) who demonstrated that the amount of eugenol and eugenyl acetate recovered using subcritical water at 150 ºC was similar to those achieved using Soxhlet extraction and hydrodistillation. These compounds are well known to possess antioxidant properties similar to other natural compounds such as α-tocopherol (Lee & Shibamoto, 2001).

Interestingly, in general the use of subcritical water extraction provides a number of advantages over traditional extraction techniques (i.e. hydrodistillation, organic solvents solid-liquid extraction). These are, mainly, low extraction times, higher quality of the extracts (mostly for essential oils), lower costs of extractant agent, environmentally cleaner technique, and better and adjustable selectivity that can be easily changed by tuning the extraction temperature. These advantages have been verified on several plants as laurel (Fernández-Pérez, Jiménez-Carmona & Luque de Castro, 2000), fennel (Gámiz-García & Luque de Castro, 2000), oregano (Soto Ayala & Luque de Castro, 2001) and kava (Kuvatová, Miller & Hawthorne, 2001), among others.

Ozel et al. (Ozel, Gogus & Lewis, 2003) studied the extraction of essential oil from *Thymbra spicata* considering the influence of several factors such as temperature (100, 125, 150 and 175 ºC), pressure (20, 60 and 90 bar) and flow rate (1, 2 and 3 ml/min) for dynamic extractions. In this work, it was proved that the best extraction yields (3.7 %) were obtained at 150 ºC and 60 bar using a flow rate of 2 ml/min for 30 minutes. Later, an analysis of the extracts, by means of a two-dimensional gas chromatography-TOF-Mass spectrometry demonstrates the type of compounds selectively extracted under the mentioned conditions. Moreover, the essential oils of *Timbra spicata* were found to inhibit mycelial growth of the several fungi species (Ozel et al., 2003).

3.3. Extraction from microalgae using SWE.

As it has been mentioned, microalgae are nowadays considered as a new source of functional ingredients that can be used by the food industry to produce new foods able to provide additional benefits on consumer's health.

SWE has been already applied to extract antioxidant compounds from microalgae *Spirulina platensis* (Herrero, Ibáñez, Señoráns & Cifuentes, 2003). Likewise, another version of this technique has been described (Basile et al., 1998; Denery, Gradull, Tang & Li, 2004) by replacing the water by another environmentally clean solvent such as ethanol, capable to be used in the food processing industry. Denery et al. (2004) studied the carotenoids extraction from microalgae *Haematococcus pluvialis* and *Dunaliella salina* using ethanol. The extraction yield was shown to be similar to that obtained using traditional extraction techniques.

4. CONCLUSIONS.

Nowadays, new natural sources for functional ingredients are being searched by the food industry. The final goal is to develop new products that can provide, besides the energetic and nutritional basic requirements, an additional benefit to human health. Among the more interesting compounds that can be extracted from natural sources, antioxidants are the most intensely studied because they can have a double functionality, that is, they can be useful as a food preservation method while providing important health benefits for humans. With the objective of isolating antioxidant compounds from natural sources, several plant varieties have been studied along with different natural sources as algae, microalgae and food-by-products.

At the same time there is a clear need in developing new extraction processes, environmentally clean, safe and selectively enough to extract natural food ingredients with a high yield. Thus, supercritical fluid technology has risen on importance, mainly when using carbon dioxide as supercritical fluid. SFE allows obtaining extracts free of toxic residues that can be directly used without any further treatment, and with a composition tuneable by changing the extraction conditions (that, of course, affects the extraction selectivity). A new technology that is currently growing on interest and applications, and that can be considered as complementary to supercritical fluid extraction, is subcritical water extraction. Thanks to this technique, intermediate polar compounds can be isolated with a high selectivity, what can be achieved modifying the extraction temperature. At the same time, the extraction procedures are faster than traditional extraction methods without the use of any organic solvent.

ACKNOWLEDGEMENTS

M.H. thanks MCYT for a FPI grant. Authors thank MCYT (project AGL2002-04621-C02-02) for financial support.

5. LITERATURE CITED.

Anklam, E., Berg, H., Mathiasson, L., Sharman, M. &. Ulberth, F. (1998). Supercritical fluid extraction (SFE) in food analysis: a review. *Food Additives & Contaminants*. 15, 729-750.

Barth, M.M., Zhou, C., Kute, K.M. & Rosenthal, G.A. (1995). Determination of optimum conditions for supercritical fluid extraction of carotenoids from carrot (*Daucus carota* L.) tissue. *Journal of Agricultural and Food Chemistry*. 43, 2876-2878.

Basile, A., Jiménez-Carmona, M.M. & Clifford, A.A. (1998). Extraction of rosemary by superheated water. *Journal of Agricultural and Food Chemistry*. 46, 5205-5209.

Bauman, D., Hadolin, M., Rizner-Hras, A. & Cnes, Z. (1999). Supercritical fluid extraction of rosemary and sage antioxidants. *Acta Alimentaria*. 28, 15-28.

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

Bhat, V.B. & Madyastha, K.M. (2000). C-Phycocyanin: A potent peroxyl radical scavenger *in vivo* and *in vitro*. *Biochemical & Biophysical Research Communications*. 275, 20-25.

Borowitzka, M.A. & Borowitzka, L.J. (1988) Micro-algal biotechnology, Cambridge University Press. Cambridge (UK). pp. 96-100.

Cadoni, E., De Giorgi, M.R., Medda, E. & Poma, G. (2000). Supercritical CO_2 extraction of lycopene and β-carotene from ripe tomatoes. *Dyes and pigments*. 44, 27-32.

Cheung, P.C.K. (1999). Temperature and pressure effects on supercritical carbon dioxide extraction of n-3 fatty acids from red seaweed. *Food Chemistry*. 65, 399-403.

Clifford, A.A., Basile, A. & Al-Saidi, S.H. (1999). A comparison of the extraction of clove buds with supercritical carbon dioxide and superheated water. *Fresenius Journal of Analytical Chemistry*. 364, 635-637.

Cohen, Z. & Vonshak, A. (1991). Fatty acid composition of *Spirulina* and *Spirulina*-like cyanobacteria in relation to their chemotaxonomy. *Phytochemistry*. 30, 205-206.

Del Valle, J.M. & Aguilera, J.M. (1999) Review: high pressure CO_2 extraction. Fundamentals and applications in the food industry. *Food Science & Technology International.* 5, 1-24.

Denery, J.R., Dragull, K., Tang, C.S. & Li, Q.X. (2004). Pressurized fluid extraction of carotenoids from *Haematococcus pluvialis* and *Dunaliella salina* and kavalactones from *Piper methysticum. Analytica Chimica Acta*. 501, 175-181.

Esquível, M.M., Ribeiro, M.A. & Bernardo-Gil, M.G. (1999). Supercritical extraction of savory oil: study of antioxidant activity and extract characterization. *Journal of Supercritical Fluids*. 14, 129-138.

Fadel, H., Marx, F., El-Sawy, A. & El-Gorab, A. (1999). Effect of extraction techniques on the chemical composition and antioxidant activity of *Eucalyptus camaldulensis* var. Brevirostris leaf oils, *Z. Lebensm. Unters. Forsch. A*. 208, 212-216.

Fernández-Pérez, V., Jiménez-Carmona, M.M & Luque de Castro, M.D. (2000). An approach to the static-dynamic subcritical water extraction of laurel essential oil: comparison with conventional techniques. *Analyst*. 125, 481-485.

Gámiz-García, L. & Luque de Castro, M.D. (2000). Continuous subcritical water extraction of medicinal plant essential oil: comparison with conventional techniques. *Talanta*. 51 1179-1185.

Ge, Y., Ni, Y., Chen, Y. & Cai, T. (2002). Optimization of the supercritical fluid extraction of natural vitamin E from wheat germ using response surface methodology. *Journal and Food Science*. 67, 239-243.

Ge, Y., Yang, H., Hui, B., Ni, Y., Wang, S. & Cai, T. (2002). Extraction of natural vitamin E from wheat germ by supercritical carbon dioxide, *Journal of Agricultural and Food Chemistry*. 50, 685-689.

Gerard, D., Quirin, K.W. & Schwarz, E. (1995). CO_2 extracts from rosemary and sage, *Food Mark. and Technology*. 9, 46-55.

Goldberg, I., (1996). Functional foods. Designer foods, pharmafood, nutraceuticals. Chapman and Hall. Londres, Gran Bretaña. p. 3

Hadolin, M., Skerget, M., Knez, Z. & Bauman, D. (2001). High pressure extraction of vitamin E-rich oil from *Silybum marianum*. *Food Chemistry*. 74, 355-364.

Herrero, M., Ibáñez, E., Señoráns, F.J. & Cifuentes, A. (2003) Accelerated solvent extracts from *Spirulina platensis* microalga: determination of their antioxidant activity and analysis by Micellar Electrokinetic Chromatography. *Journal of Chromatography A* (submitted for publication)

Hidalgo, P.J., Ubera, J.L., Tena, M.T. & Valcárcel, M. (1998). Determination of carnosic acid content in wild and cultivated *Rosmarinus officinalis. Journal of Agricultural and Food Chemistry*. 46, 2624-2627.

Ibañez, E., Cifuentes, A., Crego, A.L., Señoráns, F.J., Cavero, S. & Reglero, G. (2000). Combined use of supercritical fluid extraction, micellar electrokinetic chromatogrphy, and reverse phase high performance liquid chromatography for the analysis of antioxidants from rosemary (*Rosmarinus offininalis* L). *Journal of Agricultural and Food Chemistry*. 48, 4060-4065.

Ibáñez, E., Kuvatová, A., Señoráns, F.J., Cavero, S., Reglero, G. & Hawthorne, S.B. (2003). Subcritical water extraction of antioxidant compounds from rosemary plants. *Journal of Agricultural and Food Chemistry*. 51, 375-382.

Ibáñez, E., López-Sebastián, S., Fernández, J., Tabera, J., Bueno, J.M., Ballester, L. & Reglero, G. (2001). Influence of the CO_2 quality in the antioxidant activity of rosemary extracts dearomatized by supercritical fluid extraction. *Food Science and Technology International*. 7, 177-182.

Ibáñez, E., Oca, A.,de Murga, G., López-Sebastián, A., Tabera, J. & Reglero, G. (1999). Supercritical fluid extraction and fractionation of different preprocessed rosemary plants. *Journal of Agricultural and Food Chemistry*. 47, 1400-1404.

Ibáñez, E., Palacios, J., Señorans, F.J., Santa-María, G., Tabera, J. & Reglero, G. (2000). Isolation and separation of tocopherols from olive by-products with supercritical fluids *Journal of the American Oil Chemist Society*. 77, 187-190.

King, J.W. (2000). Advances in critical fluid technology for food processing, *Food Science & Technology Today*. 14, 186-191.

Kuvátová, A., Lagadec, A.J.M., Miller, D.J. & Hawthorne, S.B. (2001). Selective extraction of oxygenates from savory and peppermint using subcritical water. *Flavour and Fragrance Journal.* 16, 64-73.

Kuvátová, A., Miller, D.J. & Hawthorne, S.B. (2001). Comparison of subcritical water and organic solvents for extracting kava lactones from kava root. *Journal of. Chromatography A*. 923, 187-194.

Lang, Q. & Wai, C.M. (2001). Supercritical fluid extraction in herbal and natural product studies-a practical review, *Talanta.* 53, 771-782.

Leal, P.F., Braga, M.E.M., Sato, D.N., Carvalho, J.E., Marques, M.O.M. & Meireles, M.A.A. (2003). Functional properties of spice extracts obtained via supercritical fluid extraction. *Journal of Agricultural and Food Chemistry*. 51, 2520-2525.

Lee, K.G. & Shibamoto, T. (2001). Antioxidant property of aroma extract isolated from clove buds (*Syzygium aromaticum* L. Merr et Perry). *Food Chemistry*. 74, 443-448.

Li, B., Yang, Y., Gan, Y., Eaton, C.D., He, P. & Jones, A.D. (2000). On-line coupling of subcritical water extraction with high-performance liquid chromatography via solid-phase trapping. *Journal of Chromatography A*. 873, 175-184.

Lopez-Sebastián, S., Ramos, E., Ibáñez, E., Bueno, J.M., Ballester, L., Tabera, J. & Reglero, G. (1998). Dearomatization of antioxidant rosemary extracts by treatment with supercritical carbon dioxide. *Journal of Agricultural and Food Chemistry*. 46, 13-19.

Louli, V., Ragoussis, N. & Magoulas, K. (2004). Recovery of phenolic antioxidats from wine industry by-products. *Bioresource Technology*. 92, 201-208.

Luegthanaphol, S., Mongkholkhajornsilp, D.,Douglas, S., Douglas, P.L., Pengsopa, L. & Pongamphai, S. (2004). Extraction of antioxidants from sweet Thai tamarind seed coat-preliminary experiments. *Journal of Food Engineering*. 63, 247-252

Luque de Castro, M.D., Jiménez-Carmona, M.M. & Fernández-Pérez, V. (1999). Towards more rational techniques for the isolation of valuable essential oils from plants. *TRAC, Trends in Analytical Chemistry*. 19, 708-716.

Madhava, C., Bhat, V.B., Kiranmai, G., Reddy, M.N., Reddanna, P. & Madyastha, K.M. (2000). Selective Inhibition of Cyclooxygenase-2 by C-Phycocyanin, a Biliprotein from *Spirulina platensis***,** *Biochemical & Biophysical Research Communications*. 277, 599-603.

Mahajan, G. & Kamaţ, M. (1995). γ-Linoleic acid production from *Spirulina platensis*. *Applied Microbiology and Biotechnology*, 43, 466-469.

Mendes, M.F., Pessoa, F.L.P.& Uller, A.M.C. (2002). An economic evaluation based on an experimental study of the vitamin E concentration present in deodorizer distillate of soybean oil using supercritical CO_2. *Journal of Supercritical Fluids*. 23, 257-265.

Mendes, R.L., Coelho, J.P., Fernandes, H.L., Marrucho, I.J., Cabral, J.M.S., Novais, J.M. & Palavra, A.F. (1995). Applications of supercritical CO_2 Extraction to microalgae and plants. *Journal of Chemistry Technology & Biotechnology*. 62, 53-59.

Mendes, R.L., Coelho, J.P., Fernandes, H.L., Marrucho, I.J., Cabral, J.M.S., Novais, J.M. & Palavra, A.F. (1995)a. Applications of supercritical CO_2 Extraction to microalgae and plants. Journal of Chemistry Technology and Biotechnology. 62, 53-59.

Mendes, R.L., Fernandes, H.L., Coelho, J.P., Reis, E.C., Cabral, J.M.S., Novais, J.M. & Palavra, A.F. (1995)b. Supercritical CO_2 extraction of carotenoids and other lipids from *Chlorella vulgaris*. *Food Chemistry*. 53, 99-103.

Mendes, R.L., Nobre, B.P., Cardoso, M.T., Pereira, A.P. & Palabra, A.F. (2003). Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. *Inorganica Chimica Acta*. 356, 328-334.

Miller, D.J. & Hawthorne, S.B. (2000). Solubility of liquid organic flavor and fragance compounds in subcritical (hot/liquid) water from 298 K to 473K. *Journal of Chemical Engineering Data*. 45, 315-318.

Murga, R., Ruíz, R., Beltrán, S. &. Cabezas, J.L. (2000). Extraction of natural complex phenols and tannins from grape seeds by using supercritical mixtures of carbon dioxide and alcohol. *Journal of Agricultural and Food Chemistry*. 48, 3408-3412.

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

Ötles, S. & Pire, R. (2001). Fatty acid composition of *Chlorella* and *Spirulina* microalgae species, *J. AOAC.* 84, 1708-1714.

Ozel, M.Z., Gogus, F. & Lewis, A.C. (2003). Subcritical water extraction of essential oil from *Thymbra spicata*. *Food Chemistry*. 82, 381-386.

Palma, M.& Taylor, L.T. (1999). Extraction of polyphenolic compounds from grape seeds with near critical carbon dioxide. *Journal of Chromatography A*, 89, 117-124.

Piñero-Estrada, J.E., Bermejo Bascós, P. & Villar del Fresno, A.M. (2001). Antioxidant activity of different fractions of *Spirulina platensis* protean extract, *Il Farmaco.* 56, 497-500.

Qiuhui, H. (1999). Supercritical carbon dioxide extraction of *Spirulina platensis* component and removing the stench. Journal of Agricultural and Food Chemistry. 47, 2705-2706.

Quirin, K.W. (2003). SFE of natural antioxidants from rosemary and sage, *Innovations in Food Technology*. 18, 186-191.

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

Ribeiro, M.A., Bernardo-Gil, M.G. & Esquível, M.M. (2001) *Melissa officinalis* L.: study of antioxidant activity in supercritical residues. *Journal of Supercritical Fluids*. 21, 51-60.

Rozzi, N.L. & Singh, R.K. (2002). Supercritical fluids and the food industry, *Comprehensive reviews in food science and food safety*. 1, 33-44.

Rozzi, N.L., Singh, R.K., Vierling, R.A. & Watkins, B.A. (2002). Supercritical fluid extraction of lycopene from tomato processing by-products, *Journal of Agricultural and Food Chemistry*. 50, 2638-2643.

Señoráns, F.J., Ibáñez, E., Cavero, S., Tabera, J. & Reglero, G. (2000). Liquid chromatographic-mass spectrometric analysis of supercritical fluid extracts of rosemary plants. Journal of Chromatography A. 870, 491-499.

Señorans, F.J., Ruiz-Rodríguez, A., Cavero, S., Cifuentes, A., Ibáñez, E. & Reglero, G. (2001). Isolation of antioxidant compounds from orange juice by using countcurrent supercritical fluid extraction (CC-SFE), *Journal of Agriculture and Food Chemistry*. 49, 6039-6044.

Soto Ayala, R. & Luque de Castro, M.D. (2001). Continuous subcritical water extraction as a useful tool for isolation of edible essential oils. *Food chemistry*. 75, 109-113.

Stashenko, E.E., Jaramillo, B.E. & Martínez, J.R. (2004). Comparison of different extraction methods for the analysis of volatile secondary metabolites of *Lippia alba* (Mill.) N.E. Brown, grown in Colombia, and evaluation of its in vitro antioxidant activity, *Journal of Chromatography A*. 1025, 93-103.

Subra, P. & Boissinot, P. (1991). Supercritical fluid extraction from a brown alga by stagewise pressure increase. *Journal of Chromatography*. 543, 413-424.

Tena, M.T. & Varcárcel, M. (1997). Supercritical fluid extraction of natural antioxidants from rosemary: comparison with liquid solvent sonication. *Analytical Chemistry*. 69, 521-526.

Tsuda, T., Mizuno, K., Ohshima, K., Kawakishi, S. & Osawa, T. (1995). Supercritical Carbon Dioxide extraction of antioxidative component from Tamarind (*Tamarindus indica* L.) seed coat. *Journal of Agriculture and Food Chemistry*. 43, 2803-2806.

Valcárcel, M. & Tena, M.T. (1997). Applications of supercritical fluid extraction in food analysis. *Fresenius Journal of Analytical Chemistry*. 358, 561-573.

Xue, C., Hu, Y., Saito, H., Zhang, Z., Li, Z., Cai, Y., Ou, C., Lin, H. &. Imbs, A.B. (2002). Molecular species composition of glycolipids from *Spirulina platensis*. *Food Chemistry*. 77, 9-13.

Yépez, B., Espinosa, M., López, S. & Bolaños, G. (2002). Producing antioxidant fractions from herbaceaus matrices by supercritical fluid extraction. *Fluid phase equilibria*. 197, 879-884.

Zancan, K.C., Marques, M.O.M., Petenate, A.J. & Meireles, M.A.A. (2002). Extraction of ginger (*Zingiber officinale* roscoe) oleoresin with CO_2 and co-solvents: a study of the antioxidant activity of the extracts. *Journal of Supercritical Fluids*. 24, 57-76.

FIGURES.

Figure 1. Typical phase diagram for a pure compound.

Figure 2. Diagram of a supercritical fluid extraction pilot plant equipped with two fractionation cells. (1) CO_2 Pump; (2) Modifier pump; (3) Solid samples extraction cell; (4) Fractionation cell 1; (5) Fractionation cell 2; (6) Valve.

Figure 3. A) Graphical representation of the results obtained of a literature search in *Current Contents* database, using "supercritical fluid" and "carbon dioxide" as search parameters, between 1999-2000 (Rozzi et al.). B) Graphical representation of SFE applications in Food Science and Technology based on a literature search in *Food Science and Technology Abstracts (FSTA)* database, using "supercritical and fluid" as search parameters between 2001 and 2003.

Figure 4. Graphical representation of dielectric constant of water vs temperature.

Figure 5. Diagram of a subcritical water extraction system. SR: Solvent Reservoir; PV: Purge Valve; RV: Pressure Relief Valve; EC: Extraction Cell; SV: Static Valve; CV: Collector Vial; WV: Waste Vial.

Table 1. Range values of several physicochemical properties of gases, liquids and supercritical fluids

State of fluid	Density (ρ, g/cm^3)	Diffusivity (D_{AB}, cm^2/s)	Viscosity (μ, g.s/cm)
Gas p = 1 atm; T 21 ºC	10^{-3}	10^{-1}	10^{-4}
Liquid p = 1 atm; T = 15-30 ºC	1	$<10^{-5}$	10^{-2}
Supercritical p = p_c; T = T_c	0.3 – 0.8	10^{-3} – 10^{-4}	10^{-4} - 10^{-3}

Table 2. Critical properties of several solvents used in SFE.

Solvent	Critical Property			
	Temperature (ºC)	Pressure (atm)	Density (g/ml)	Solubility parameter δ_{SFC} ($cal^{-1/2}cm^{-3/2}$)
Ethene	10.1	50.5	0.200	5.8
Water	101.1	217.6	0.322	13.5
Methanol	-34.4	79.9	0.272	8.9
Carbon dioxide	31.2	72.9	0.470	7.5
Ethane	32.4	48.2	0.200	5.8
Nitrous oxide	36.7	71.7	0.460	7.2
Sulfur Hexafluoride	45.8	37.7	0.730	5.5
n-Butene	-139.9	36.0	0.221	5.2
n-Pentane	-76.5	33.3	0.237	5.1

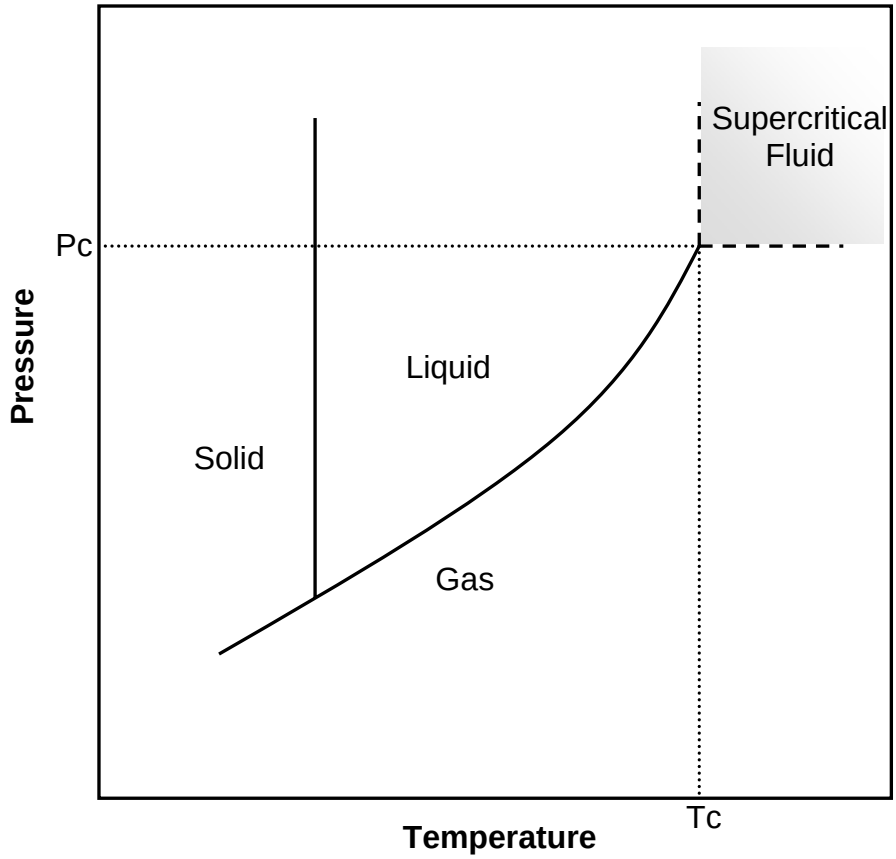

Supercritical
Fluid
Pc
Pressure
Liquid
Solid
Gas
Tc
Temperature

Figure 1.

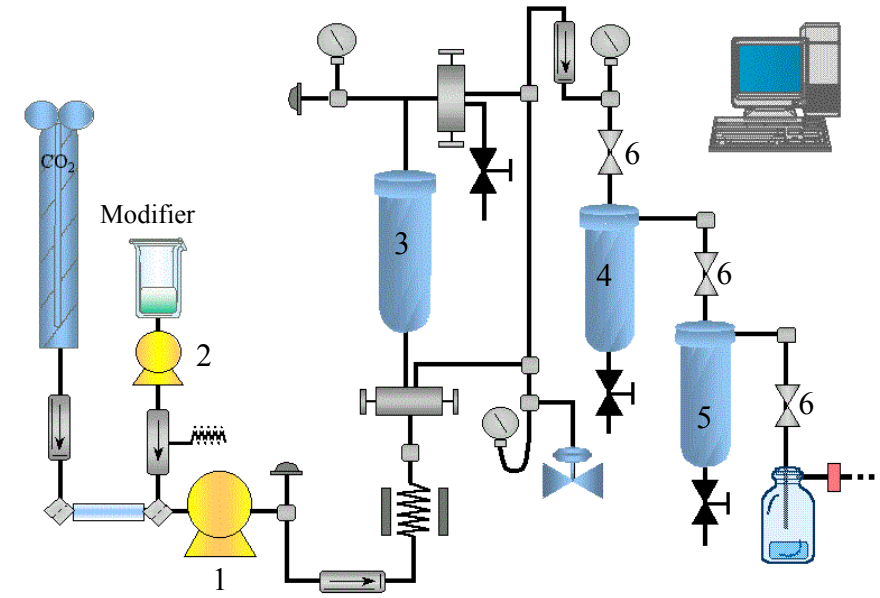
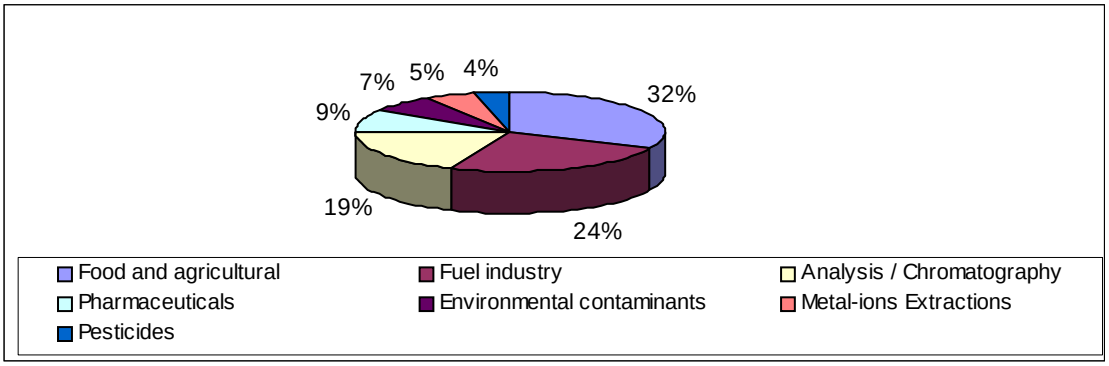


Figure 2.

A)


32%
24%
19%
9%
7%
5%
4%
Food and agricultural
Fuel industry
Analysis / Chromatography
Pharmaceuticals
Environmental contaminants
Metal-ions Extractions
Pesticides

B)

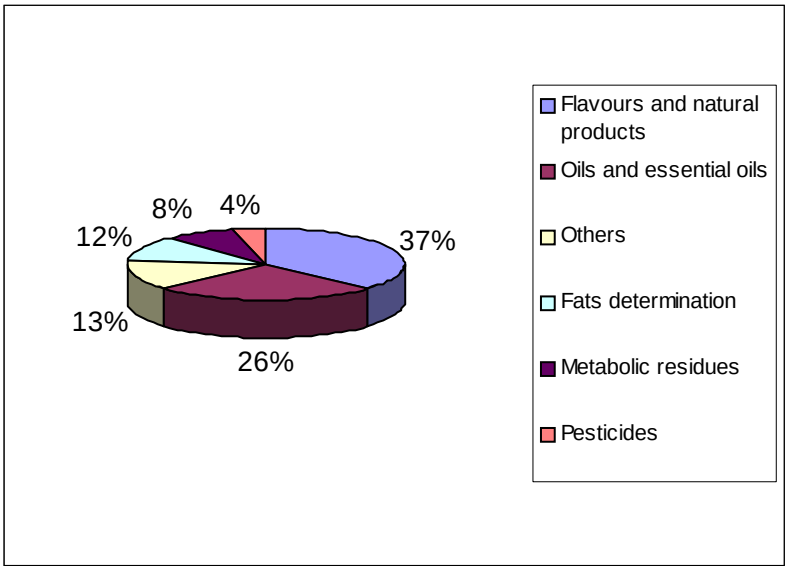

37%
26%
13%
12%
8%
4%
Flavours and natural products
Oils and essential oils
Others
Fats determination
Metabolic residues
Pesticides

Figure 3.

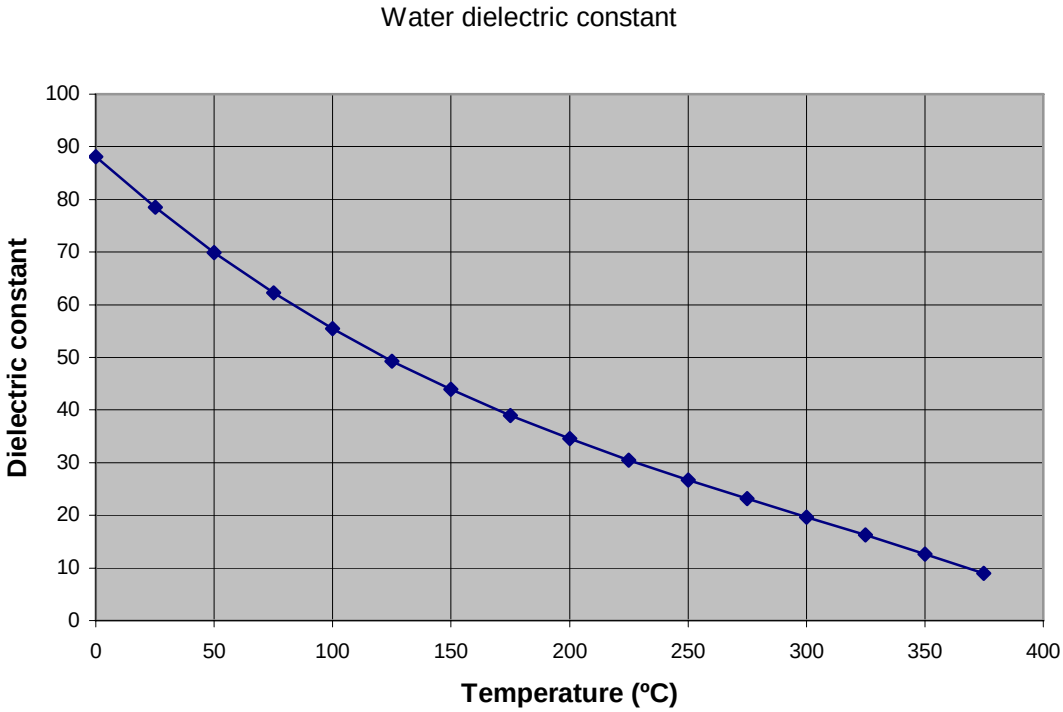

Water dielectric constant
Dielectric constant
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
350
400
Temperature (ºC)

Figure 4.

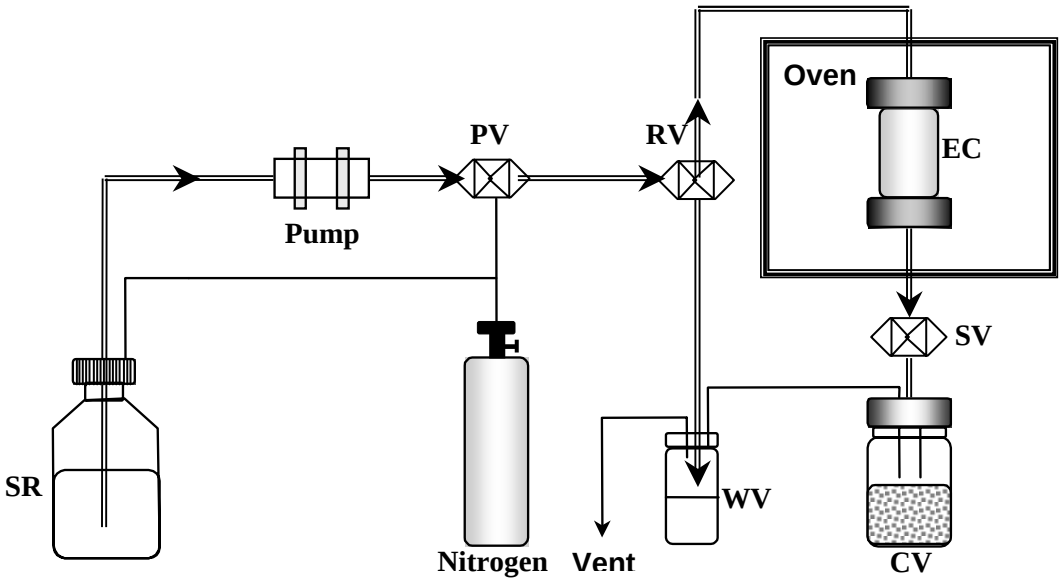


Figure 5.