**COMPRESSED FLUIDS (SFE, PLE and SWE) FOR THE EXTRACTION OF
BIOACTIVE COMPOUNDS**

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ABSTRACT.

The improvement of sample preparation and extraction techniques for natural bioactive compounds determinations is of great importance nowadays. New concepts are being introduced in this field, not only related to enhancement of extraction efficiencies but also related to environmental impact. This evolution towards Green Chemistry is pushing to new extraction and sample preparation processes that should be faster, more reproducible and more environmentally friendly. In this context, compressed fluids-based sample preparation techniques (such as supercritical fluid extraction and pressurized liquid extraction) are demonstrating good capabilities. In this review, an updated knowledge on the techniques will be presented together with the main technical developments and the most notable recent applications for the extraction of bioactive compounds.

Keywords: bioactive compounds, natural samples, plants, agricultural by-products, pressurized liquid extraction, supercritical fluid extraction, subcritical water extraction

Abbreviations: ASE, accelerated solvent extraction; DHA, docosaheptaenoic acid; EAE, enzyme-assisted extraction; EPA, eicosapentaenoic acid; IL, ionic liquids; MIP, molecular imprinted polymers; PFE, pressurized fluid extraction; PHSE, pressurized hot solvent extraction; PHWE, pressurized hot water extraction; PLE, pressurized liquid extraction; PLPW, pressurized low polarity water extraction; n-3 PUFA, n-3 polyunsaturated fatty acid; RAM, restricted access media; scCO₂, supercritical CO₂; SFE, supercritical fluid extraction; SHWE, superheated water extraction; SPE, solid

phase extraction; SWE, subcritical water extraction; TG, triglycerides; UAE, ultrasound
assisted extraction; WEPO, water extraction and particle formation on-line.

1. INTRODUCTION

The interest in the search of bioactive compounds from natural origin has risen in the last two decades driven by the increasing number of scientific papers demonstrating the efficacy of such compounds against several diseases. Applications in Food Science and Technology have undergone also this evolution mainly due to the increasing evidences correlating diet and some chronic diseases. Different sources of bioactive compounds have been studied along the years, being plants, agricultural by-products and marine products among the most promising. In fact, some plant antioxidants, derived from fruits and vegetables, have already been associated to lower risks of coronary heart diseases and cancer [1, 2]. As for marine sources, they have a great potential mainly considering their huge diversity, their sometimes unique chemical structures and their ability to work as natural bioreactors, favoring the synthesis of valuable compounds depending on the cultivation conditions or through biotechnology approaches [3].

Extraction techniques are studied worldwide not only from the production point of view, but also as sample preparation techniques able to contribute meeting the requirements for the development of faster, more efficient, cheaper, high throughput and “greener” analytical methodologies. Sample preparation is typically considered one of the “bottlenecks” of any analytical procedure, not only in throughput but also in terms of greening the analysis. Sample preparation has been the most evaluated step in the analytical procedure since it is considered the most difficult to implement.

A closer look to the goals pursued by the “Green Analytical Chemistry” principles referred to sample preparation tools [4], namely: 1) reduction on the amount of sample to treat and reduction/elimination of pollutant solvents/acids (miniaturization); 2) simultaneous extraction of multiple compounds; and 3) increasing automation and

throughput determination, leads to the understanding of the important role that the techniques based on the use of compressed fluids can play.

Basic principles and main advances on these techniques, such as supercritical fluid extraction (SFE), pressurized liquid extraction (PLE) (also called accelerated solvent extraction (ASE), pressurized hot solvent extraction (PHSE) or pressurized fluid extraction (PFE)) and subcritical water extraction (SWE) (also called pressurized hot water extraction (PHWE), pressurized low polarity water extraction (PLPW) or superheated water extraction (SHWE)) [5,6] will be reviewed in the present article covering the literature published during the period 2008-2012. Principles and instrumentation of compressed fluids extraction will be briefly presented, together with some technical developments and key applications in the field of bioactives extraction.

As can be seen in Figure 1, depending on the polarity of the compressed fluid selected, different “green” pressurized sample preparation techniques can be used. All of them rely on the use of minimum amount of food-grade solvents for a selective extraction of bioactives while preserving their bioactivity and chemical structure. They all show a great versatility and efficiency since the physicochemical properties of solvents (density, diffusivity, viscosity, dielectric constant) can be modified by changing the pressure and/or temperature of the extracting fluid, that also modifies their selectivity and solvating power.

2. COMPRESSED FLUIDS. PRINCIPLES AND INSTRUMENTATION.

Despite there are several differences in the basic principles of SFE, PLE and SWE, they all have in common that must operate under medium-high pressures. In this section, their main characteristics will be addressed.

SFE is based on the use of solvents at temperatures and pressures above their critical points, while PLE and SWE operate using liquids at temperatures above their normal boiling points and pressures enough to keep the extracting fluid in the liquid state. Figure 2 shows a basic scheme of the equipment used to perform compressed fluid extractions. It consists of a container of the extraction solvent (A) (usually CO₂ for SFE, water for SWE, or other organic solvents for PLE), a pump to pressurize the fluid (B), an oven containing the extraction vessel (C), a restrictor to maintain the high pressure inside the system (D), a trapping vessel (E) and optionally a modifier pump or N₂ container (F). Nevertheless, from this starting point, the instrumentation employed might be more or less sophisticated. For instance, a dynamic extraction might require more accurate high pressure pumps in order to maintain a precise flow rate during the whole extraction procedure. Besides, in this case, a heating coil should be included inside the oven to assure that the solvent reaches the extraction cell at the set temperature. Although the extraction cell is a simple device, it should be capable of withstanding high pressures and temperatures (up to 200-250 °C, when working with pressurized liquids like water or ethanol). For operations with SWE and PLE, a nitrogen circuit can be included to purge all the system after the extraction, as well as to assure that all the extracting solvent has reached the collecting vial once the extraction is finished.

The general extraction procedure might be divided into three phases, comprising desorption of analytes from the matrix, their diffusion into the solvent that has penetrated the matrix itself, and their transfer to the rest of extracting solvent [7].

Despite the high number of advantages found in these techniques, the use of experimental designs to optimize the extraction conditions is recommended, since

undesirable effects might also take place at high temperatures, such as the degradation of bioactive compounds.

The main valuable characteristic of **SFE** is the highly reduced (often to zero) employment of toxic organic solvents. Carbon dioxide is the solvent most commonly used to extract bioactive compounds from natural sources using SFE. Despite some other solvents have been proposed for SFE (namely: propane, butane, dimethyl ether...), none of them fulfill as well as CO₂ the principles of Green Chemistry. In fact, CO₂ has a series of interesting properties for bioactives extraction: its critical conditions are easily attainable (31.1 °C and 7.39 MPa) and is non-toxic and non-flammable solvent that, besides, is considered GRAS (Generally Recognized as Safe) for its use in the food industry. At supercritical conditions, solvents present high diffusivity whereas their solvent strength and density can be easily modified by tuning the temperature and pressure applied. Another important characteristic of this technique, when using supercritical CO₂ (scCO₂), is the possibility of attaining solvent-free extracts. Once the extraction procedure is finished, the depressurization of the system turns CO₂ gas, while the compounds extracted from the matrix precipitate. On the other hand, an important drawback of CO₂ is its low polarity that can be overcome employing polar modifiers at low amounts (1–10%), to change the polarity of the supercritical fluid and to increase its solvating power towards the analyte of interest.

The basic principle of **PLE** relies on the use of solvents to carry out extractions at high pressures and temperatures, always below their critical points, so that the liquid state of the solvent is maintained during the whole extraction process [5]. From the application of those conditions, faster extraction processes result, in which, typically, higher extraction yields are obtained with low volumes of organic solvents (e.g., 20 min using 10–50 ml of solvent in PLE can be compared with a traditional extraction step in which

10–48 h and up to 200 ml are required), decreasing in this way the dilution of the sample. These characteristics are mainly due to the improvement on mass transfer kinetics obtained under high temperature and pressure [8,9]. The use of high temperatures increases the solubility of the analytes in the solvent and decreases solvent viscosity and surface tension, thus allowing a better penetration of the solvent into the matrix. Although a wide number of commercial PLE instruments are available in the market, different applications have been also reported with home-made instruments [10–13]. In both cases, it has to be considered that given the operating pressures and temperatures usually employed, corrosive-resistant materials have to be used.

Subcritical water extraction (**SWE**) is a particular use of PLE based on the employment of hot water as pressurized liquid. Usually, temperatures higher than the water boiling point (100 °C) and lower than its critical temperature (374 °C) are used, while pressures from 3.5 to 20 MPa are employed to keep the water in liquid state during the whole extraction process. Under these conditions of pressure and temperature, physical and chemical properties of water change dramatically. Among the main parameters that can influence SWE (extraction temperature, time, pressure, the addition of an organic solvent or surfactant, and water flow rate), temperature is the main factor that affects the extraction efficiency and selectivity. An increase in temperature facilitates analyte diffusion (diffusivity of water at 25 °C is about 10 times lower than water at 200 °C), favors the mass transfer kinetics by the disruption of intermolecular forces (i.e., van der Waals forces, hydrogen bonds and dipole attractions), decreases the viscosity of water (enabling better penetration of matrix particles), and decreases the surface tension (allowing the water to better wet the sample matrix) [8]. In spite of the improvement of all these properties, the most important effect of the increase of liquid water temperature is undoubtedly the weakening of hydrogen bonds, resulting in a lower dielectric

constant (ϵ). The dielectric constant (measure of polarity) of water, at enough pressure to be maintained in its liquid phase, varies from ca. 80 at 25 °C (being extremely polar) to 25-27 when temperatures of ca. 250 °C are used [14], which falls between those of methanol ($\epsilon = 33$) and ethanol ($\epsilon = 24$) at 25 °C [14,15] (see Figure 3). As it can be observed, water dielectric constant's values resemble to those presented by others less polar solvents at room temperature, so, under these conditions, water could be used as an alternative to dissolve medium polar and even nonpolar organic compounds. Basically, the experimental set-up for SWE is similar to that one described above (see Figure 2). The advantages of home-made set up compared to commercial systems are the range of working temperature, the possibility to carry out both dynamic and static extractions and different processes (extraction, reaction, drying) just modifying the basic set-up. More information about how to build a SWE system can be obtained from Turner and Ibáñez's book chapter [8].

3. SUPERCRITICAL FLUID EXTRACTION (SFE)

3.1. Methodological and technological advances

Method optimization plays an important role in SFE; several parameters must be considered such as extraction temperature and pressure, addition, amount and type of modifier, amount of sample (as well as particle size) and use of dispersing agents. Pressure and temperature have a strong influence on the solvent properties, such as density, and therefore, are strongly related to the solubility of the target compounds in the supercritical fluid. Proportion and type of modifier are also key factors responsible for solubility of the target compounds in the supercritical fluid; in this sense, the most commonly employed organic modifier to extract bioactives is ethanol in a range of 5-10 % of CO₂ flow; other modifiers such as methanol, acetone or even small amounts of

water have been also used to isolate polar bioactive compounds. On the other hand, to extract high molecular weight non-polar compounds, vegetable oils (such as olive oil) have demonstrated its ability as co-solvents in a proportion of 10% [16]. Recently, the use of higher amount of modifier (up to 50%) has been tested to obtain fractions enriched in γ -linolenic acid from *Arthrospira platensis* (*Spirulina platensis*); using CO₂-expanded ethanol at 30 MPa, 40 °C and a ratio CO₂:ethanol 1:1 in the optimum, a recovery up to 35.3% was achieved [17], GXLs, which was used in this work for the first time in food products, have demonstrated its performance as intermediate between PLE and supercritical fluids for the extraction of medium-polar compounds. Thus, in this case gas expanded liquids (GXL) extraction is taking place, which can offer several important advantages related to an improved extraction yield with lower consumption of organic solvents compared to classic solid-liquid extractions. GXL are liquids whose volume has been increased when pressurized with a condensable gas such as CO₂. Under these conditions, at least two fluid phases or a single phase above the bubble point curve but below the critical composition exist. The properties of the liquid phase are substantially different from those at atmospheric pressure. GXLs have been shown to have improved mass transfer through reduced viscosity, increased solute diffusivity and decreased interfacial tension [18].

As for the particle size and use of dispersing agents, these parameters are more related to the efficiency of the extraction procedure and can be modified to avoid clogging and to increase extraction rate. The particle size (or crushing degree) is a very significant factor in the mass transfer rate and, therefore, will have a maximum influence in the extraction yield.

Table 1 shows a selection of reviews published since 2002 and are strongly recommended for readers to gain a deeper understanding on the factors involved in SFE extractions.

In the last years several sample preparation methods combining ionic liquids (IL) and supercritical fluids are emerging. Room-temperature ionic liquids have been revealed as a new type of green solvent, generally comprising quaternary nitrogen cations. These solvents have interesting properties (e.g., negligible vapor pressure, thermal stability, tunable viscosity, and miscibility with water and organic solvents). They have been proposed as environmentally friendly solvents for “green chemistry” because they are good substitutes for traditional volatile and flammable organic solvents. However, there is some controversy about the greenness of the ILs due to their incomplete physical, chemical and toxicological data. Ionic liquids have become the partner of supercritical CO₂ in many applications that have been reviewed recently by Keskin et al. [30]. Although more research is needed for a routine use of ILs in SFE, the developments of new applications using ILs is increasing and the number of publications have risen exponentially since the mid 2000’s. The main field for the combination of ionic liquids and SFE is their use in reactions. Their variable water and organic solvent miscibility allows the development of convenient extraction methods. Their characteristics can be easily modified (even changing pressure and temperature) and tuned to a targeted process by adjusting the cation/anion couple. Basically, the most studied methods consist on enzymatic reactions in ionic liquid media followed by extraction of the desired compounds using scCO₂; this scheme has been proposed for a wide range of biocatalyzed reactions, mainly using lipases for different esterification purposes [31]. Enzymes in supercritical media are not only used in combination with ionic liquids. Nowadays, the main trend in this field is the so-called “Enzyme-assisted extraction”

(EAE) that is used with liquids and supercritical phases. Even if its main application has been in plant matrices to break cell walls at larger scale, the possibilities offered by this new approach for sample preparation are huge, mainly related to the increase in the extraction efficiency and/or selectivity of the extraction process. For a more in depth knowledge in this field, readers are referred to recent reviews of Sowbhagya and Chitra [32] and Subramaniam [33].

On the other hand, in the last few years, other technological developments have been studied in order to improve SFE extraction efficiency for sample preparation; among them, the combination SFE+UAE (ultrasound assisted extraction) has gained interest due to the higher extraction yields that can be obtained in shorter times. Its mechanism of action has been recently deciphered by Van Iersel et al [34]. The authors describe that ultrasound irradiation of high-pressure fluids leads to an improvement of mass and heat transfer in high-pressure fluids due to the creation of an extremely fast and local phase separation, which propagates through the mixture with a velocity equal to the speed of sound, in the vicinity of the critical point. The combination of UAE+SFE has been used, among others, for carotenoids extraction, showing an extraction yield up to 2.5 times higher than using only SFE [35]. Pilot scale devices have been also designed for a larger scale extraction of bioactive compounds [36].

In a recent paper by Klejdus et al. [37], a new methodology was proposed to isolate and identify natural bioactive substances in biological matrices based on the use of a new SPE/SFE hybrid extraction. A new extractor device was designed allowing the insertion of the SPE cartridge into the cell; after a careful optimization of the extraction and elution conditions, it was possible to recover 13 phenolic compounds from different cyanobacteria and microalgae.

Sample collection has been also improved through the optimization of the factors involved in the effectiveness of the trapping method. Traditionally the collection of the extracts has been carried out using collection vessels where depressurization takes place, causing CO₂ gasification and extract precipitation. Since CO₂ gas is vented through an exhaust pipe, it is common to have losses of compounds (mainly volatiles) together with the CO₂ gas. Thus, trapping can result in significant loss of material or can increase the possibility of adding noise to the global analytical processes. Although different trapping methods have been developed, such as solid trapping, liquid trapping or a combination of both, for a correct selection of the most appropriate approach it is crucial to consider different factors such as analyte volatility and polarity, volatility of the extracting agent, volatility of modifier (if used), solvent flow rate, etc. Novel sample collectors use centrifugal forces to retain extracts while allowing their drying; one example of this use is the equipment developed in 2008 by KD Scientific® that can be coupled to SFC and SFE systems [38]. The system consists on a flexible eluent tube that directs the flow from the SFC system into the fraction collection tube while the rotor is spinning. Extract (liquids or solids, even volatiles) are trapped in the bottom of the collection containers due to the centrifugal force created by the rotor at 1500 rpm.

3.2. Applications

Since the end of the 1970s, supercritical fluids have been used for sample preparation and to isolate compounds from natural products, but for a long time applications rely only on few products. In the last years, many applications have been developed to extract a wide variety of bioactive compounds from very different sources [5]; Table 2 shows some of the most relevant applications of SFE published in the period 2008-2012. SFE has been mainly used to isolate bioactive non-polar compounds, such as

lipids and carotenoids. Lipids have been isolated from many natural sources such as dairy products, oils, algae or microalgae. Normally, pressures about 10-30 MPa and temperatures around 40-50 °C [5] have been used. Another important application of SFE has been the extraction of essential oils from plants. Essential oils have a complex composition, containing from a few dozen to several hundred constituents, especially hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, aldehydes, ketones, acids, phenols, oxides, lactones, acetals, ethers and esters). Besides their fragrance, the mixture of compounds confers several bioactivities such as antimicrobials, antioxidant, etc. Among the most well-known advantages of SFE towards the extraction of essential oils is the use of low temperatures that allows preserving the integrity of the sample. Pourmortazavi & Hajimirsadeghi reviewed in 2007 all the factors affecting the essential oil extraction from several plants by means of SFE [22].

But not only non-polar bioactive compounds are extracted using supercritical fluids; as mentioned, the addition of small amounts of modifiers allows obtaining fractions enriched in polar compound, i.e. phenolic compounds, metal-ligand complexes, etc. The normally working pressures and temperatures are similar to those used to extract non-polar compounds, but the presence of modifiers (ethanol, methanol, water, acetone or mixtures of them) favors the extraction of flavonoids, lignan, simple phenolics, among others [5,21]. Even if it is true that SFE may provide lower yields than other conventional extraction methods such as Soxhlet, extractions are faster, as reviewed by Stalikas [69].

New applications have been recently developed dealing with the addition of derivatizing agents to promote the extraction of, for instance, bioactive, pharmaceutical compounds and metals. In these applications, the addition of the derivatizing agent helps improving

the later detection of the target analytes while increasing their extraction rate by increasing the solubility of the analyte-ligand complex. Urea complexation is a useful technique to enrich unsaturated fatty acids from mixtures. Upon crystallization, urea forms inclusion complexes with some long-chain aliphatic compounds. Saturated fatty acids form complexes readily, their formation being less efficient with increasing number of double bonds or in the presence of branched chains. Lin et al [70] used urea complexation to enrich n-3 PUFA content of triglycerides (TG) in Menhaden oil under supercritical carbon dioxide. Through the saponification of Menhaden oil followed by urea inclusion, 80.1 wt% of n-3 PUFA could be concentrated, containing 29.4 wt% EPA and 41.8 wt% DHA, under the following conditions: pressure up to 10 MPa, temperature equal to 50°C, 10% of ethanol as co-solvent and 5 h conversion time; to carry out the conversion of n-3 PUFA onto TG an immobilized 1,3-regiospecific lipase was selected. Authors conclude that under supercritical conditions the conversion is 40% higher than that under ambient conditions after 5 h.

Moreover, complexation in supercritical media has demonstrated to be a very useful tool for pharmaceuticals; in this sense, Shinde et al reviewed the use of cyclodextrins to prepare inclusion complexes of certain drugs [56] and showed how solubility can be enhanced about 70 times by complexation with methyl- β -cyclodextrin. Special attention must be paid to the drug since extraction conditions are highly dependent on drug's structure (for example, 15 °C and 45 MPa were the conditions for piroxicam and 100 °C and 10 MPa for borneol).

Complexation and extraction with supercritical fluids has been also used, for example, to isolate radioactive metals; in this case, SFE with organic ligands has been employed to recover actinides and other radionuclides from diverse matrices, including oxides of these elements or by-products [71]. This application not only requires important

optimization of complexation and extraction conditions, but also safe facilities, such as those designed by Kumar et al [52].

A novel interesting trend in the application of SFE is the development of *integrated processes*. These processes can be defined as those that combine more than one specific unit process into a single piece of equipment. For example, extraction can be followed by conversion, such as feeding SC-CO₂ and oil from the extractor to an enzymatic reactor for hydrolysis and/or esterification. The main challenge in this approach is the compatibility of the optimal operating conditions for the extraction and reaction steps [16,43]. Different solvents other than CO₂ are under study in integrated processes of biocatalysis+extraction; in this sense, dimethyl ether seems to be a promising solvent mainly for hydrophobic compounds. Some of its uses have been recently reviewed by Catchpole et al [72].

4. PRESSURIZED LIQUID EXTRACTION (PLE)

4.1. Methodological and technological advances

As already mentioned for SFE, method development in PLE also involves the optimization of different factors influencing the outcome of the extraction process for each particular sample. As it has been already stated, temperature has a critical importance on the extraction procedure. Theoretically, the highest temperature could provide the best results in terms of extraction yield. Nevertheless, when dealing with bioactive compounds, this parameter has to be closely examined and optimized since it is widely known that high temperatures might have negative effects on the bioactivity of some thermo-labile compounds. Even if it has been repeatedly observed that bioactive extracts might be obtained at high temperatures (maintaining the solvent's liquid state), this parameter should be studied and selected for each type of matrix/bioactive being

extracted. On the other hand, possible formation of new components in the extracts, as a result of the occurrence of different reaction during the extraction process, has to be considered [73,74]. This point, that is clear shortcoming when the natural composition of a sample is sought, has been considered by some authors as an additional advantage in terms of bioactivity of the achieved extracts [75]. In fact, it has been observed how during PLE extraction at high temperatures (135-160 °C) of microalga *Chlorella vulgaris*, an increased pyropheophytin formation was obtained. This chlorophyll derivative, not naturally present on the sample, might have potent antimutagenic activity, and consequently, according to authors, PLE extracts' properties might be enhanced by the formation of this kind of components [75].

Parameters such as pressure and time have been repeatedly reported to possess a less critical effect. Different works have pointed out the null influence of the extraction pressure beyond the point in which the solvent is maintained liquid [7, 76]. Thus, in this sense, most applications devoted to the use of PLE to obtain bioactive natural products set an extraction pressure high enough to maintain the solvents in the liquid state, and its influence is not further studied [26]. The extraction time influence will directly depend on the PLE mode applied, static or dynamic. Most commercial instruments only allow performing static extractions in which a certain volume of solvent, under the desired conditions of pressure and temperature, is maintained in contact with the sample for a given time. Thus, under static conditions equilibria between those sample components still bound to the matrix and those already solubilized in the solvent might be reached. If this is the case, the efficiency of the extraction procedure will not be increased beyond this point. A solution that has been widely employed to partially solve this shortcoming and to increase the total yield is the use of sequential extractions of the same sample, using several consecutive static extraction cycles. For instance, by using

four consecutive extraction cycles, it was possible to increase the extraction yield of phenolic compounds from parsley, as compared to an equivalent increase of the static extraction time using only one cycle [77]. However, this approach does not always provide with the same results, as in some applications one extraction cycle is enough to extract the target compounds [78].

Under the dynamic mode, fresh solvent is continuously introduced in the extraction cell. Consequently, the equilibria might be displaced and the efficiency of the extraction procedure might be increased. This approach was shown to provide higher extraction yields than static extractions for the isolation of diterpenes taxanes from *Taxus Canadensis* [79], and for the extraction of phenolic compounds from different *Pinus* species [80], among others.

As for the solvent selection, although this is obviously a quite straight-forward approach, care must be taken in the possible change in behavior under PLE conditions, compared to room temperature; this is especially important in the case of water, as previously mentioned.

As for the most commonly used solvents in PLE, those regarded as GRAS such as ethanol, water or its mixtures are preferred, although others more toxic and harmful such as petroleum ether or dichloromethane have been also used [81] for the extraction of very non-polar target compounds. This is, for instance, the case of the extraction of volatiles and essential oils that are more efficiently extracted with solvents such as n-hexane [82-84]. However, as it has been already mentioned, one of the most important trends in this field is based on the search and application of new environmentally green and food-grade solvents. In this regard, the possibility of using bioethanol (obtained as the fermentation product of glucose from renewable biomass such as edible starch and non-edible cellulose) to obtain bioactive gingerols from ginger (*Zingiber officinalis*) has

been successfully explored [85]. These compounds could be efficiently recovered using 70% bioethanol at 100 °C using a static extraction time of 5 min. More recently, the employment of ethyl lactate as a low polarity solvent for the extraction of α -linolenic acid from a microalga has been also explored [17]. The ability of this solvent combined with ethanol was assessed and the capabilities of this alternative food-grade solvent for some applications were demonstrated.

Another important niche of research on this topic in the near future is the application of ILs as pressurized solvents. Already applied in other kinds of extractions, such as liquid phase microextraction [86], solid phase microextraction [87], liquid-liquid extraction [88], microwave assisted extraction [89] ultrasound-assisted extraction [90] or, as already mentioned, supercritical fluid extraction [30], ILs present some characteristics that make them potentially applicable in PLE, such as high solvent power, high chemical and thermal stability, non-flammability and non-volatility [91]. In this sense, Wu et al. [92] already demonstrated the usefulness of pressurized ILs to extract flavonoids from *F. sophorae Inmaturus* herb. Main parameters influencing the outcome of the extraction were the IL selection and its concentration; in fact, optimum conditions for the recovery of rutin and quercetin from this plant included the use of a 1 mol/L concentration of [C4mim][Cl] at 120 °C using a single static extraction cycle of 5 min [92]. However, much work remains to be done in this field, as nowadays there is a huge variability of ionic liquids and the information available from an extraction point of view is still scarce, as it has been already pointed out. Besides, a potential problem that should be solved is the low volatility of these components, which would make difficult to separate the ionic liquid from the interesting compounds after the extraction procedure.

In an effort to limit the use of organic solvents, Chang et al. [93] developed an extraction protocol based on the use of surfactants as extraction fluids under PLE conditions. This method was applied to the extraction of different flavonoids from *Costus speciosus* flowers before micellar electrokinetic chromatography (MEKC). This surfactant-assisted pressurized extraction was carried out with SDS and Triton X-100 during 30 min under pressures between 2-3 MPa. Although authors did not study the influence of the extraction temperature, this approach could be further developed in the future combining different surfactants and solvents in order to increase the efficiency of bioactives extraction.

On the other hand, analyte recovery in PLE is not as critical as in SFE since most of the automatic systems available in the market recover the solutes in solution in a closed vial, thus minimizing solute losses.

4.2. Applications

Although the majority of PLE applications developed so far are aimed to the extraction of contaminants from different natural, food and environmental samples, this technique has also demonstrated its usefulness for the extraction of bioactive compounds from natural matrices. Table 3 shows some of the most relevant PLE applications (published during the period 2008-2012) devoted to the extraction of bioactives. As it can be observed, plants are, by far, the samples more frequently studied, although different papers have been published dealing with the extraction of bioactive compounds from algae and microalgae, as well as from other natural matrices. PLE has been mostly used to obtain antioxidants such as phenolic compounds and carotenoids.

Generally, commercial instruments are mostly used working in static extraction mode and employing extraction times up to 20 min. It is also common to find applications in

which the sample is re-extracted several consecutive times, in order to fully extract the target compounds. This strategy was shown to be useful to extract anthocyanins from strawberry [95], sweet potato [102] and grape skins [112], phenolic acids from food industry byproducts [96], flavonoids from spinach [118] or alkaloids [128], among others, performing 3 consecutive static extraction cycles.

As it can be observed in Table 3, temperatures ranging from 100 to 160 °C have been widely applied to extract natural bioactives. Nevertheless, the use of higher temperatures should not be discarded. In fact, the use of PLE with ethanol at 200 °C was shown to be the most appropriate process to obtain extracts with the highest antioxidant activity from rosemary compared to other pressurized extraction methods including subcritical water extraction and supercritical fluid extraction [77].

PLE has been widely compared to other extraction techniques such as maceration, UAE [75], solvent partitioning [133], or Soxhlet extraction [85] for the extraction of bioactive compounds, providing better results than these more conventional techniques. Advantages associated are mainly based on the attainment of higher extraction efficiencies, involving significantly less volume of solvents, less total extraction time and easing the automation of the process.

In terms of automation, one important trend is the development of new systems and approaches to couple PLE to other processes on-line. For example, UAE, PLE and SPE may be combined to increase the extraction efficiency and purity of the desired compounds in the obtained extracts [134]. Although some off-line hyphenations have been presented, the in-line use remains to be fully explored. Similarly, in-cell enzyme pre-treatments prior extraction might be a good alternative to increase the extraction yield on particular compounds thanks to the release of components from the natural matrix being extracted. Although this approach might be best suited for water

extractions, the employment of PLE with hydroalcoholic solutions, for instance, cannot be discarded. This strategy has been followed in other research fields different to bioactive natural products extraction, to speed-up enzymatic hydrolysis [135]. The possibility of using in-cell SPE materials to retain either the compound of interest or the interferences can facilitate the isolation of the target compounds since it includes a new element of selectivity in the system. This approach can be even improved by using specifically designed materials such as molecularly imprinted polymers (MIPs) or restricted access materials (RAMs) than can fit only to the molecule of interest; although at present no applications can be found in the field of isolation of bioactives, it is expected to be a growing field of research in a near future. Special devices have been designed for this purpose and are described elsewhere [136]; they are mainly based on the design of modular approaches and cell assemblies for simultaneous extraction and clean-up of different types of samples, including bioactive compounds extraction. As mentioned before, different types of approaches (trap and release, class separation, dual-mode clean-up or matrix retainer and trap) can be intended bearing in mind the idea of isolation and purification of bioactive compounds.

5. SUBCRITICAL WATER EXTRACTION (SWE)

5.1. Methodological and technological advances

Although SWE might be seen just as a variation of PLE, the use of water as extraction solvent is highly relevant from an environmental point of view, since it is considered the greenest solvent that can be used in an extraction process. Water has essentially negligible environmental effect, since it is non-toxic to health and the environment and it is safe to work with. For this reason, it is common to refer to SWE independently. Generally, one of the major limitations of SWE is the low water solubility of certain

compounds and the instability of some of them and/or matrices towards elevated temperatures. Some authors have investigated the use of modifiers or additives to improve the extraction yields; for instance, Mukhopadhyay and Panja carried out the extraction of high amounts of natural sweeteners from licorice by SWE with 0.01 % w/v ammonia [137] and Arapitsas and Turner [138] developed a fast extraction method of anthocyanins from red cabbage using pressurized hot water containing 5 % of ethanol [138]. Other authors have studied how to extend the applicability of SWE to compounds exhibiting limited water solubility by pH control, thus Euterpio et al. employed SWE for extracting curcumin from turmeric rhizomes by adjusting the water pH [139].

Regarding the type of extraction, the most frequent method used in SWE is static mode in which an equilibrium between the sample components and the water phase (in which the components are solubilized) is reached. This implies that a careful optimization of the static extraction time is of outmost importance. For the extraction of bioactive compounds from natural sources short static times (from 5 to 20 min) are generally applied. When dynamic mode is used, heated and pressurized water will flow into the extraction cell continuously. This is theoretically more favorable for the complete extraction, however extracts may be diluted and more costly procedures will have to be applied to remove the water. In this sense, a new on-line process has been developed involving the subcritical water extraction plus drying of the extracts in a single step [140]. This methodology (called WEPO) combines a continuous flow of water through the sample with the continuous production of an aerosol from the extract assisted by sc-CO₂ nebulization system which is instantaneously dried by a hot N₂ current. The applicability of this process to obtain in one step dried extract has been recently demonstrated in the extraction of bioactive compounds with high antioxidant activity from rosemary leaves [12] and from fresh onion [141]. Moreover, its greenness has

been recently assessed, in comparison to SFE and PLE, by using LCA (life cycle assessment) [142]. In this work, the interest of using such tools to assess the environmental performance of an extraction method to realize which are the main bottlenecks associated to a particular process has been demonstrated.

From an environmental point of view, the combination of subcritical water extraction and enzymatic hydrolysis using a thermostable beta-glucosidase to catalyze hydrolysis of quercetin glucosides in onion waste has demonstrated to be a viable process preferred (in terms of primary energy consumption and global warming) over more conventional extraction based on methanol extraction and hydrochloric acid hydrolysis [143,144].

5.2. Applications

The potential of SWE to extract bioactive compounds from natural matrices, such as plants or algae, has been already demonstrated. Table 4 shows a selection of some of the most remarkable SWE applications to obtain bioactive compounds from natural sources published during the period 2008-2012. As can be seen, a quite large number of recent works described in the literature have been applied to the extraction of antioxidants from rosemary [12], grape pomace [145], grape skin [146], seabuckthorn leaves [147], pomegranate seed [148], rice bran [149], olive leaves [108], Citrus unshiu peel [150], bitter melon [151] or onion skin [152], among others. Not only antioxidant compounds have been extracted by SWE but also other bioactive compounds possessing different activities. For instance, SWE has enabled to obtain extract with anti-inflammatory properties from hop (*Humulus lupulus*) [153] or antimicrobial [119,154] or antiviral activity [126,155] from algae. In addition, SWE has also been employed as a final step to separate dietary fibers from the residue obtained by supercritical CO₂ of citrus,

demonstrating the importance of sequential methodologies for the recovery of valuable compounds from citrus fruit waste [157].

Recently, the possibility to obtain antioxidant compounds in SWE extracts not naturally present in the original matrix as consequence of using high temperature has been demonstrated [73, 156] both, in glycation model systems and in real samples. In both cases, neoformed antioxidants derived from Maillard or caramelization reactions were produced during SWE, which could increase the interest on the bioactive extract obtained, although undoubtedly modifies the natural profile of bioactive compounds in the sample.

5. CONCLUSIONS

At present, with the important introduction of mass spectrometry in all laboratories it is true that the requirements for sample preparation have changed considerably. Since modern MS, combined with LC and/or GC, is able to provide with higher sensitivities and selectivities, sample preparation techniques have become simpler; for instance, there is no big need for enrichment or exhaustive clean-up since both, sensitivity and selectivity of the new systems have increased enormously.

On the other hand, when dealing with research in bioactive compounds linked to natural products, biomedicine, food science, and -omics technologies, several clues have to be considered to optimize the sample preparation step: 1) we are dealing with natural matrixes, all of them very complex samples; 2) most of the analytes are non-volatile or semi-volatile and the matrixes include solid and liquid samples; 3) most of the bioactive compounds are present in low concentrations in the samples; 4) in most cases information is needed for a further processing of the natural matrix in order to extract the compound/s of interest. With all this information it is possible to understand the

(still) important role of sample preparation in the extraction of bioactives. We have a long way to go to be able to achieve the degree of simplicity that is undoubtedly observed in other areas of analytical chemistry in terms of sample preparation.

Therefore, for bioactive compounds extraction the first step is, usually, a solvent extraction to enrich the target bioactive compounds from the complex matrix (plant, by-product, marine source, etc.). In the present manuscript, we have been discussing the advantages of some environmentally friendly techniques such as SFE, PLE, SWE as alternatives to more conventional extraction techniques such as Soxhlet. All of these techniques have in common the drastic reduction in the amount of solvents used since other physical processes, such as pressure and temperature, are applied to improve the efficiency and to speed up the extraction process. These techniques allow the reduction of energy consumption and of the amount of wastes generated and they can be easily miniaturized and automated, thus increasing the throughput. They can also constitute the first step towards a larger scale production process, often so necessary in this type of research. All of these techniques can be seen as “green pressurized sample preparation” techniques working with green (or food-grade) solvents.

Technological and methodological advances presented, even separated by techniques, can be understood from the need of developing more selective sample preparation procedures that achieve better clean-up (from very complex samples) and improved analysis at lower concentrations (typical of bioactive components). In the manuscript we presented several general approaches that can be applied to all techniques using compressed fluids: different designs (in-cell) for improving clean-up or selectivity of the extraction process; more selective solvents (ionic liquids); cleaner and greener solvents (ethyl lactate); integrated processes (use of enzymatic assisted extraction plus pressurized fluids for a more selective extraction of the compounds of interest and

susceptible to being enlarged to a pilot scale process), in-situ derivatization plus extraction for higher requirements in terms of sensitivity (or lower detection limits), etc. Therefore, we think there is a huge area of research in front of us that can contribute to the development of new methodologies for higher throughput, while complying with the rules of Green Chemistry, and also provide with new tools for the future development of more sustainable and clean industrial processes towards extraction of bioactive compounds from natural sources.

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FIGURE CAPTIONS.

Figure 1. “Green” techniques based on the use of compressed fluids for extraction of bioactive compounds. ϵ , dielectric constant.

Figure 2. Scheme of the typical compressed fluid extractor. The different parts of the system are named as: solvent reservoir (A), pump (B), heated extraction cell (C), static/dynamic valve (D), collection vessel (E) and modifier/N₂ container (F)

Figure 3. Dielectric constant of water as a function of temperature at 20 MPa. Solid circles superimposed with the plot are the values corresponding to different organic solvent at 25 °C and 0.1 MPa (values given in parentheses). Reproduced with permission from [132] (American Geophysical Union).

Table 1. Summary of some of the most relevant reviews published in SFE, SWE and PLE in the last 10 years.

Technique	Title	Year	Reference
SFE	Steps of supercritical fluid extraction of natural products and their characteristic times	2012	[19]
SFE	Extraction of volatile oils by supercritical fluid extraction: Patent survey	2011	[20]
SFE	Supercritical fluid extraction: Recent advances and applications	2010	[21]
SFE, PLE, SWE	Use of compressed fluids for sample preparation: Food applications	2007	[5]
SFE	Supercritical fluid extraction in plant essential and volatile oil analysis	2007	[22]
SFE	Supercritical CO ₂ extraction and purification of compounds with antioxidant activity	2006	[23]
SFE, PLE	Extraction methods and chemical standardization of botanicals and herbal preparations	2004	[24]
PLE	Natural dyes extraction from cochineal (<i>Dactylopius coccus</i>). New extraction methods	2012	[10]
PLE	Application of accelerated solvent extraction in the analysis of organic contaminants, bioactive and nutritional compounds in food and feed	2012	[25]
PLE	Techniques to extract bioactive compounds from food by-products of plant origin	2012	[26]
PLE	Pressurized liquid extraction as a green approach in food and herbal plants extraction: A review	2011	[7]
SWE	Pressurized hot water extraction (PHWE)	2010	[15]
SWE	Pressurised hot water as a novel extractant of natural products: A review	2010	[27]
SWE	Extraction of functional substances from agricultural products or by-products by subcritical water treatment.	2008	[28]
SWE	Pressurized hot water extraction of bioactive or marker compounds in botanicals and medicinal plant materials	2006	[14]
SWE, SFE	Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review	2006	[6]
SWE	Extractions with superheated water	2002	[29]

Table 2. Most remarkable advancements in SFE published during the time period 2008-2012.

Product	Compounds of interest	Solvent	T(°C)/P(MPa)	Mode / cycles	Sample dispersion	Extraction time (min)	Ref.
<i>Arthrospira platensis</i> (<i>Spirulina platensis</i>)	Fatty acids γ -linolenic	CO ₂ :ethanol (1:1)	40 / 300	Dynamic	Sea sand	60	[17]
<i>Boletus edulis</i>	Fatty acids	CO ₂	40 / 35	Dynamic	--	214	[39]
<i>Borago officinalis</i>	Fatty acids	CO ₂ + methanol	65 / 35	Static dynamic	Sea sand	10+10	[40]
<i>Camellia sinensis</i>	Fatty acids and antioxidants	CO ₂	45 / 32	Static	--	90	[41]
<i>Chamaecyparis obtusa</i>	Essential oil	CO ₂	50 / 12	Dynamic	Diatomaceous earth	90	[42]
Corn and Fish oils	Fatty acid ethyl esters	CO ₂	40 / 160	Dynamic	--	Continuous	[43]
<i>Evodia rutaecarpa</i>	Evodiamine, rutaecarpine	CO ₂ + methanol	62 / 28	Dynamic	Sea sand	78	[44]
<i>Hemerocallis disticha</i>	Lutein, zeaxanthin	CO ₂	80 / 60	Static + dynamic	Spe-ed Matrix	30 + 30	[45]
Kale, spinach	Polyphenols, flavonoids	CO ₂ + 5 % methanol	50 / 25.8	Static	--	30	[46]
<i>Lamiaceae</i> plants	Essential oils	CO ₂	40 / 30	Dynamic	--	90	[47]
<i>Lippia dulcis</i>	Hernandulcin and other sesquiterpenes	CO ₂	35 / 120	Static + dynamic	Sea sand	60	[48]
<i>Magnolia officinalis</i>	honokiol and magnolol	CO ₂	80 / 40	Static + dynamic	--	60 + 40	[49]
<i>Mitragyna speciosa</i>	Alkaloids	CO ₂ + ethanol	65 / 30	Dynamic	--	45	[50]
<i>Nelumbo nucifera</i>	Alkaloids	CO ₂ + diethylamine + water	70 / 30	Dynamic	--	60	[51]
Nuclear waste	Radioactive Elements	CO ₂ + modifier	45 / 260	Dynamic	Cellulose	300	[52]
Olive leaves	Phenolic compounds	CO ₂ + ethanol	40 / 15	Static	--	120	[53]
Olive leaves	Oleuropein	CO ₂ + methanol	100 / 30	Dynamic	Diatomaceous earth	90	[54]
Peach kernels	Fatty acids	CO ₂	50 / 30	Static	--	150	[55]

Pharmaceutical preparations	Piroxicam	CO ₂	15 / 450	Methyl- β -cyclodextrin + Static	--	30	[56]
<i>Psidium guajava</i>	Total phenols	CO ₂ + ethanol	50 / 30	Static / 4 cycles	Diatomaceous earth	30	[57]
Rosemary (<i>Rosmarinus officinalis</i>)	Phenolic compounds	CO ₂	40 / 30	Dynamic	--	300	[58]
Rosemary (<i>Rosmarinus officinalis</i>)	Phenolic compounds	CO ₂ + ethanol	40 / 15	Static	--	120	[59]
<i>Salvia desoleana</i>	Sclareol	CO ₂	40 / 25	Dynamic	--	240	[60]
<i>Salvia officinalis</i>	Essential oil	CO ₂	40 / 30	Dynamic	--	80	[61]
<i>Schizochytrium limacinum</i>	Fatty acids DHA	CO ₂ :ethanol	40 / 350	Urea complexation + static	--	30	[62]
Sea buckthorn (<i>Hippophae rhamnoids</i>)	tocopherols, lycopene and β -carotene	CO ₂	35 / 40	Static	--	60	[63]
Spearmint (<i>Mentha spicata</i> L.)	Essential oil	CO ₂	35 / 9	Static	--	30	[64]
Spearmint (<i>Mentha spicata</i>)	essential oil	CO ₂	50 / 30	Dynamic	--	180	[65]
Spinach	Lutein	CO ₂ + ethanol	50 / 30	Static	Cellulose	90	[66]
strawberry (<i>Arbutus unedo</i>)	Total phenolics	CO ₂ + ethanol	48 / 60	Dynamic	--	60	[67]
Thyme (<i>Thymus vulgaris</i>)	Volatiles	CO ₂	40 / 9	Dynamic	Sea sand	240	[68]

Table 3. Most remarkable PLE applications devoted to natural bioactives extraction published during the time period 2008-2012.

Product	Compounds of interest	Solvent	T(°C)/P(MPa)	Mode / Cycles	Sample dispersion	Extraction time (min)	Ref.
Phenolic compounds							
<i>Hylocereus undatus</i> flowers	Flavonoids	Methanol	120 / 10	Static / 1	Diatomaceous earth	15	[94]
Strawberry	Anthocyanins	Methanol/water/formic acid 80:19:1	40 / n.i.	Static / 3	Diatomaceous earth		[95]
Potato wastes	Phenolic acids	methanol/water 90:10	160 / 10	Static / 3	Ottawa sand	5	[96]
Oak wood	Phenolic compounds	Methanol	150 / 10	Static / 1	Diatomaceous earth	5	[97]
Olive leaves	Oleuropein	Ethanol	115 / 10	Static / 2		10	[98]
Olive leaves	Phenolics	Ethanol	150 / 10	Static / 1	Sea sand	20	[99]
Cinnamon and peppermint	Phenolic compounds	75% acetone	100 / 10	Static / 3	Diatomaceous earth	10	[100]
Wheat products	Alkylresorcinols	1-propanol/water 3:1	100 / 10	Static / 3	Ottawa sand	10	[101]
Sweet potato	Anthocyanins	75% acidified methanol	80 / 15	Static / 3	Sea sand	5	[102]
<i>Heracleum leskowi</i>	Coumarins	Methanol	110 / n.i.	Static / 1	--	10	[81]
<i>Fructus schisandrae</i>	Lignans	Ethanol	160 / 15	Static / 1	--	10	[103]
<i>Citrus reticulata</i>	Flavones	70% methanol	160 / 10	Static / 1	--	20	[104]
<i>Myrciaria cauliflora</i> skins	Anthocyanins	Ethanol	80 / 5	Static / 1	--	9	[105]
Apricots	Polyphenols	Methanol/water 70:30	60 / 10	Static / 1	--	60	[106]
Rosemary	Phenolic compounds	Ethanol	150 / 10	Static / 1	Sea sand	20	[107]
Olive leaves	Phenolic compounds	Ethanol	150 / 10	Static / 1	Sea sand	20	[108]
Oregano	Phenolic compounds	Ethanol	200 / 10	Static / 1	Sea sand	20	[109]
Honey	Phenolics	Ethanol/water/HCl 70:25:5	40 / 10	Static / 3	--	15	[110]
Rosemary, marjoram	Phenolic components	56 % methanol	129 / 10	Static / 1	Diatomaceous earth	5	[111]

Grape skin	Anthocyanins	Ethanol	100 / 15	Static /3	Glass beads	5	[112]
Different plants		40% Ethanol	50 / 13	Static /2	Sea sand	10	[113]
Red grape pomace	Procyanidins	50% Ethanol	80-140 / 6.8	Static /1	Sea sand	10	[114]
Microalgae	Phenolic acids	80 % methanol	130 / 13	Static /2	--	10	[115]
Apple	Phenolic antioxidants	60% ethanol	102 / 10	Static /1	Diatomaceous earth	5	[116]
Onion	Flavonoids	60% methanol	40 / 10	Static /2	Ottawa sand	5	[117]
Spinach	Flavonoids	70% ethanol	150 / 13	Static /3	Ottawa sand	5	[118]
Parsley	Phenolic compounds	50% Ethanol	160 / 10	Static /4	Ottawa sand	5	[77]
Carotenoids and tocopherols							
<i>Chlorella vulgaris</i>	Carotenoids	Ethanol	150 / 15	Static /1	Sea sand	20	[119]
Carrot wastes	Carotenoids	Ethanol	60 / 5	Static /2	--	5	[120]
<i>Eisenia bicyclis</i>	Fucoxanthin	90% Ethanol	110 / 10	Static /1	Sea sand	5	[121]
<i>Chlorella vulgaris</i>	Lutein	90% Ethanol	160 / 10	Static /1	--	n.i.	[75]
<i>Haematococcus pluvialis</i>	Astaxanthin	Ethanol	100 / 10	Static /1	Sea sand	20	[122]
Shrimp waste	Astaxanthin	Acidified ethanol	87 / 4.9	Static /1	--	14	[123]
<i>Chlorella elipsoidea</i>	Zeaxanthin	Ethanol	115 / 10	Static /1	--	23.3	[124]
<i>Phormidium</i>	Carotenoids	Ethanol	150 / 10	Static /1	Sea sand	20	[125]
Sterols							
<i>Himathalia elongata</i>	Fucosterol	Ethanol	100 / 10	Static /1	Sea sand	20	[126]
Seeds and nuts	β -sitosterol	Methanol	50 /11	Static /2	Hydromatrix celite	5	[127]
Alkaloids							
<i>Macleaya microcarpa</i>	Quaternary benzo[c]phenanthridine	Ethanol	80 / 15	Static /3	Glass beads	10	[128]
<i>Bupleurum falcatum</i> roots	Saikosaponins	70% methanol	120 / 10	Static /1	Diatomaceous earth	10	[129]
Essential oils							

Turmeric	Volatiles	n-hexane	147 / 7	Static /1	Diatomaceous earth	17	[82]
Thyme	Essential oil	n-hexane	100 /6	Static /1	Neutral glass	10	[83]
Other food and natural bioactive components							
Grape seed oil	Vitamin E	Hexane	100 / 10	Static /3	--	30	[84]
Fermented red rice	Monacolin K	Ethyl lactate	120 / 10	Static /3	--	7	[130]
Radix Saposhnikoviae	Chromones	50% ethanol	140 /10	Static /1	--	8	[131]
Cereals	Tocopherols and tocotrienols	Methanol, acetonitrile	50 /11	Static /1	Hydromatrix celite	5	[132]

Table 4. Remarkable SWE applications to obtain bioactives compounds from natural sources published during the time period 2008-2012.

Product	Compounds of interest	Solvent	T(°C)/P(MPa)	Mode	Sample dispersion	Extraction time (min)	Ref.
Liquorice roots	Mono-ammonium glycyrrhizate (MAG)	Water containing 0.01 % ammonia	110 / 0.5	Static /1 cycle (stirring at 350 rpm)	n.i	90	[137]
Red cabbage	Anthocyanins	Water/ethanol/formic acid (94:5:1 v/v/v)	99 / 5	Static/1 cycle	n.i	2	[138]
Turmeric rhizomes	Curcumin	Phosphate-buffered water at ph 1.6	197 / 5	Dynamic/0.5 mL/min	Sea sand		[139]
Rosemary	Phenolic compounds	Water	200 / 10	Static /1 cycle	Sea sand	20	[12]
Onion		Water	200/n.i	Dynamic/0.2 ml/min	Sea sand	20	
Rosemary	Phenolic compounds	Water	120 / 8	Dynamic/0.3 ml/min	n.i	45	[141]
Grape pomace	Phenolic compounds/ flavonoids	Water	200/ 8	Dynamic/0.2 ml/min	Sea sand	40	[142]
Grape Skin	Phenolic compounds	Water	Static (1 cycle) + Dynamic (1-2 ml/min)	n.i	30 + 100	[145]	
Seabuckthorn leaves	Phenolic compounds/ flavonoids	Water	140/11.6	Static/3 cycles	Glass beads	5	[146]
Pomegranate seed	Phenolic compounds	Water	120/15	Static/1 cycle	n.i	15	[147]
Rice brand	Phenolic compounds	Water	150/10	Static /1 cycle (stirring at 120 rpm)	n.i	30	[148]
Olive leaves	Phenolic compounds	Water	220/6	Static /1 cycle	n.i	5	[149]
Citrus unshiu peel	Flavonoids	Water	200 / 10	Static /1	Sea sand	20	[108]
Bitter melon	Phenolic compounds	Water	160/10	Static /1	Diatomaceous earth	10	[150]
Onion skin	Flavonoids	Water	200/10	Dynamic/2 ml/min	n.i	120	[151]
Hop (Humulus lupulus)	Flavonoids	Water	165/10	Static /1	Diatomaceous earth	15	[152]
Chlorella vulgaris	Prenylflavonoids	Water	150/10	Static /6	Sea sand	5	[153]
Haematococcus pluvialis	Carotenoids/PUFAs	Water	200/10	Static/1	Sea sand	20	[119]
Himanthalia elongata	Fatty acids/vitamin E/phenols	Water	200/10	Static/1	Sea sand	20	[154]
	Polysaccharides	Water	100/10	Static/1	Sea sand	20	[126]

Haematococcus pluvialis			100/10			20	
Dunaliella salina.	Polysaccharides	Water	160/10	Static/1	Sea sand	15	[155]
Microalgae, algae, plants	Neoantioxidants	Water	100 or 20/10	Static/1	Sea sand	20	[156]
Glycation model system	Neoantioxidants	Water	200/10	Static/1		20	[73]

*n.i: no indicated; PUFAs: Polyunsaturated fatty acids