5 Ultrasound-Assisted Extraction in Food Analysis

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5.1 INTRODUCTION

Food products are complex mixtures of vitamins, sugars, proteins and lipids, fibers, aromas, pigments, antioxidants, and other organic and mineral compounds. Before such substances can be analyzed, they have to be extracted from the food matrix. Direct analyses are generally not possible to achieve due to the complexity of food samples and necessitate the introduction of samples under a liquid form to the analysis detector. Different methods can be used for this purpose, e.g., Soxhlet extraction, maceration, elution, steam distillation, cold pressing, and simultaneous distillation-extraction. Nevertheless, many food ingredients are well known to be thermally sensitive and vulnerable to chemical changes. Losses of some compounds, low extraction efficiency, time- and energy-consuming procedures (prolonged heating and stirring in boiling solvent, use of large volumes of solvents, etc.) may be encountered using these extraction methods. These shortcomings have led to the use of new sustainable ''green'' techniques in extraction, which typically involve less solvent and energy, such as ultrasound-assisted extraction (UAE) [1], supercritical fluid extraction [2], headspace method [3], microwave extraction [4], controlled pressure drop process [5], accelerated solvent extraction [6],

and subcritical water extraction [7]. Extraction under extreme or nonclassical conditions is currently a dynamically developing area in applied research and industry. Alternatives to conventional extraction procedures may increase production efficiency and contribute to environmental preservation by reducing the use of solvents, fossil energy, and generation of hazardous substances.

Ultrasound is a key technology in achieving the objective of sustainable green chemistry. Ultrasound is well known to have a significant effect on the rate of various processes in the chemical and food industry. Much attention has been given to the application of ultrasound for the extraction of natural products that typically needed hours or days to reach completion with conventional methods. Using ultrasound, full extractions can now be completed in minutes with high reproducibility, reducing the consumption of solvent, simplifying manipulation and workup, giving higher purity of the final product, eliminating post-treatment of wastewater, and consuming only a fraction of the fossil energy normally needed for a conventional extraction method such as Soxhlet extraction, maceration, or steam distillation. Several classes of food components such as aromas, pigments, antioxidants, and other organic and mineral compounds have been extracted and analyzed efficiently from a variety of matrices (mainly animal tissues, food, and plant materials).

UAE is a research area that has an impact in several fields of modern chemistry. The main benefits are decrease of extraction time, energy, and solvent used. The advantages of using ultrasound energy for extraction also include more effective mixing and micromixing, faster energy and mass transfer, reduced thermal and concentration gradients and extraction temperature, selective extraction, reduced equipment size, faster response to process extraction control, faster start-up, increased production, and elimination of process steps. Extraction processes performed under the action of ultrasound are believed to be affected in part by cavitation phenomena and mass transfer enhancement.

This chapter presents a complete picture of current knowledge on UAE in food analysis. It provides the necessary theoretical background and some details about extraction by ultrasound, the technique, the mechanism, some applications, and environmental impacts.

5.2 BASIC PRINCIPLES

5.2.1 Importance of the Extraction Step

In general, any analytical procedure for food components from vegetables, fruits, spices, or other complex food matrices comprises two steps: extraction (e.g., single-step solvent extraction, Soxhlet extraction, steam distillation, and simultaneous distillation–extraction) and analysis (e.g., gas chromatography, gravimetry, etc.). While the analysis step is complete after only 15 to 30 min, extraction takes at least several hours. It is frequently carried out by prolonged heating and stirring in boiling solvent. Thus, the principal limiting step of a food analysis operation is the extraction of the analyte from the matrix, which consists of transferring the desired compounds into solvent. The conventional solvent extraction procedure represents 70% of the total processing time (Figure 5.1). It is thus important to shorten this limiting step. The choice of the technique is the result of a compromise between efficiency and reproducibility of extraction, ease of procedure, together with considerations of cost, time, degree of automation, and safety.

5.2.2 Ultrasound Cavitation

The ultrasound frequency range can be divided basically into diagnostic and power ultrasound. Diagnostic ultrasound plays a very important role in modern measuring techniques. It involves high-frequency ultrasound in the range 2–10 MHz. A typical application is to measure the velocity and absorption coefficient of the acoustic wave in a medium. It is an easy, fast, noninvasive, and nondestructive way of gaining structural and chemical information. Low-power ultrasound can be used to characterize acoustic properties of foodstuffs like, for instance, velocity of sound, attenuation, reflection, and scattering. In pure food compounds (oil, water, sugar, etc.), the attenuation and

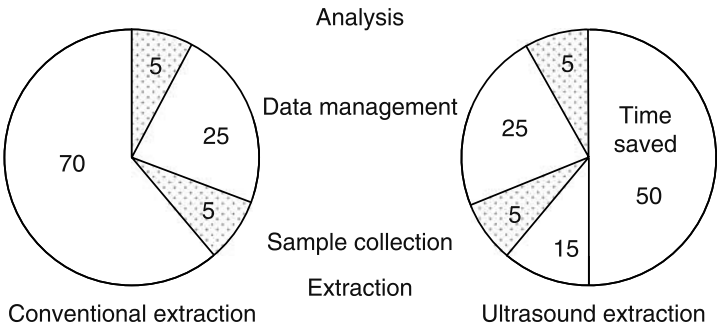

FIGURE 5.1 Relative consuming time of different steps for a food analytical procedure.

velocity of sound can be measured relatively easily and the adiabatic compressibility can be calculated. With multiphase products (most food products) it is not as straightforward and a lot of computational data treatment is required for useful results. Ultrasonic spectroscopy is a technique where a very short ultrasonic pulse (broadband) is transmitted into a product. The ultrasonic spectra of the original pulse and its echoes are recorded and the change in frequency is a result of various physical properties like particle size, concentration, temperature, etc. Ultrasonic imaging is being used to scan fruits for bruises and diseases. For instance, certain diseases cause the inside of pears to turn brown and hollow. A laboratory scale apparatus has been developed with which fruits are knocked on with small hammer-like devices. The reverberation is recorded with polymeric transducers and analyzed. The system was capable of measuring the ripeness of mangos in a nondestructive way [8–11].

Power ultrasounds, having frequencies between 20 kHz and 100 MHz, are now well-known to have significant effects on the rate of various physical and chemical processes (Figure 5.2). Cleaning and solubilization are the more developed applications and a large variety of ultrasound baths exist for chemical laboratory use. The effect of ultrasonic waves on solid samples is widely used for the extraction of aromas from plant materials or metal impurities from soils. Degassing and stripping are widely used for flavor analysis and in environmental and polymer research. Other interesting ultrasound applications involve homogenization, emulsification, sieving, filtration, and crystallization. The most interesting effect of ultrasound-based operational units is the reduction of processing time and increase of product quality. All these effects are attributed to acoustic cavitation: When a liquid is irradiated by ultrasound, micro-bubbles form, grow, and oscillate extremely fast, and eventually collapse powerfully if the acoustic pressure is high enough. These collapses, occurring near a solid surface, generate micro-jets and shock waves that result in cleaning, erosion, and fragmentation of the surface. Power ultrasound involves the mechanical and chemical effects of cavitation. The mechanism can be explained by two competing theories. The hot spot theory

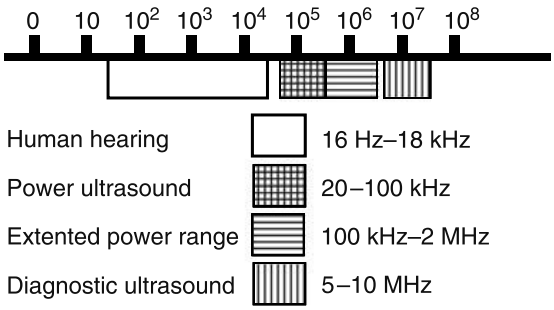

FIGURE 5.2 Frequency ranges of sound.

assumes that high pressures and temperatures generated in the bubbles during the last nearly adiabatic compression, just before collapse, are responsible for the breakage of molecular bonds and formation of radicals. On the other hand, the electrical theory involves micro-discharges due to high electrical fields generated by deformation and fragmentation of the bubbles [12–14].

5.2.3 Instrumentation

The two most common ultrasound equipments that are used for extraction are the ultrasonic cleaning bath and the more powerful probe system. For small extraction volumes, an ultrasound horn with the tip submerged in the fluid can be sufficient. Large volumes of fluids have to be sonicated in an ultrasound bath or in continuous or recycled-flow sonoreactors (Figure 5.3).

Recently, the new methodology of continuous-flow systems has been used in analytical chemistry. Most UAE applications have been developed in discrete systems using a bath or an ultrasonic probe, particularly in extraction of food samples. Less frequent has been the design of online UAE systems in the same field [15]. However, it is noted that the last approach is considerably faster. It consists in an open system, in which fresh solvent flows continuously through the sample. This induces the displacement of mass transfer equilibria toward the solubilization of analytes into the liquid phase. The coupling of the extraction step to the analytical steps, which would overcome the dilution effect, has not been performed yet despite its ease of implementation. The extract is then driven to the continuous manifold for online achievement of the analytical process, which involves preconcentration, derivatization, filtration, and detection (using flame atomic-absorption spectrometer [FAAS], gas chromatography-mass spectrometry (GC-MS), or other techniques). The main advantages of using online UAE are the reduction of sample contamination as well as analyte losses (because less sample manipulation is needed), a reduction of reagent consumption and concentration, compared with off-line (batch or discontinuous reactor) UAE. In addition, the online method removes the centrifugation or filtration step required to separate the liquid phase from the sample particles and thus significantly reduces the duration of sample preparation.

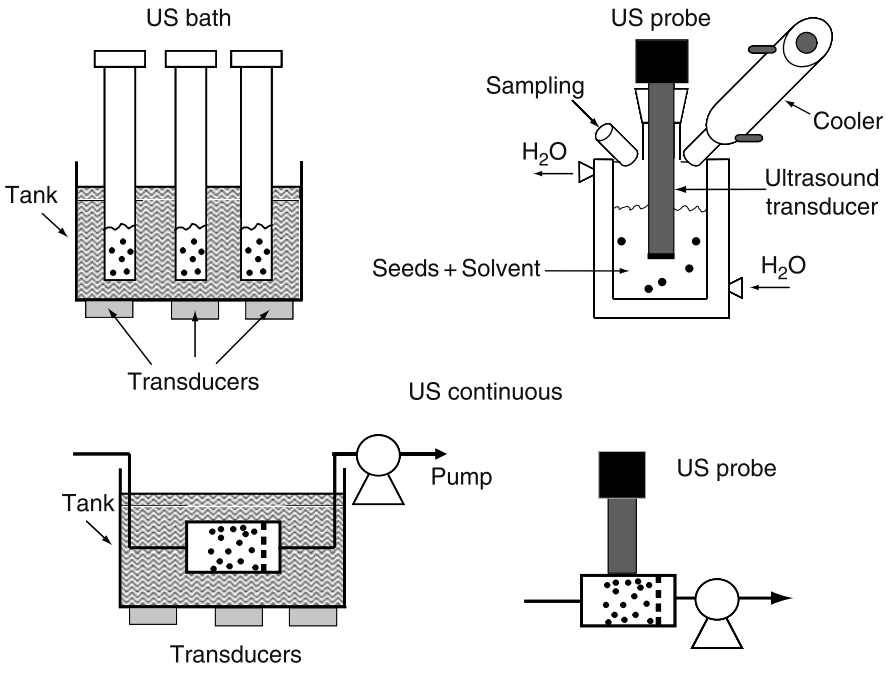

FIGURE 5.3 Some current concepts of UAE.

While most of the research effort in UAE has concentrated on ultrasound itself, some studies have also examined the coupling between ultrasound and other techniques. For instance, UAE is being employed in combination with microwave energy [16], supercritical fluid extraction [17], or simply with conventional methods such as Soxhlet extraction [18]. When combined with supercritical fluid extraction, UAE enhances the mass transfer of the species of interest from the solid phase to the solvent used for extraction. Soxhlet extraction can also be improved by ultrasound when applied at the cartridge zone before siphoning, thus permitting the removal of lipid fractions from very compact matrices. The efficiency of combining microwave and ultrasound has been clearly shown in applications such as extraction of copper and the Kjeldahl method for determination of total nitrogen in food [19].

5.3 ULTRASOUND-ASSISTED EXTRACTION: IMPORTANT PARAMETERS AND MECHANISM

5.3.1 Influence of Operating Conditions

Proper selection of the solvent is the key to successful UAE. Solvent choice is dictated by the solubility of the analytes of interest, the interactions between the solvent and matrix, and the intensity of ultrasound cavitation phenomena in the solvent. Important physical parameters related to UAE are presented in this section.

Ultrasound power, temperature, and extraction time affect not only the extraction yield but also the composition of the extract. According to Palma and Barroso [20], a higher temperature for UAE means a higher efficiency in the extraction process due to the increase of the number of cavitation bubbles and a larger solid-solvent contact area. However, this effect is decreased when the temperature is near the solvent's boiling point. It is also important to prevent the degradation of thermolabile compounds. For instance, polyphenols and isocyanates are conventionally extracted at 4°C and increasing the extraction temperature will automatically decrease the quantity and quality of the extract. Wu et al. [21] suggest that the optimal duration for the UAE of ginseng saponins from ginseng root is about 2 h. Short ultrasound treatment (less then 30 min) was found to improve the extraction process [22]. The ultrasound power is one of the parameters to optimize to reach a compromise between extraction time and solvent volume. Li et al. [23] pointed that the relative yield of soybean oil at a power density of 47 W/cm^2 was approximately five times higher than at 16 W/cm^2.

Generally, the highest efficiency of UAE, in terms of yield and composition of the extracts, can be achieved by increasing the ultrasound power, reducing the moisture of food matrices to enhance solvent-solid contact, and optimizing the temperature to allow a shorter extraction time.

5.3.2 Influence of the Food Matrix

Food tissues consist of cells surrounded by walls. Some cells exist in the form of glands (external or internal) that are filled with the target products (generally secondary metabolites). A characteristic of such glands (when external) is that their skin is very thin and can be very easily destroyed. For internal glands, it is the degree of milling of the plant material that plays an important role.

Conventional solvent extraction may be thought of as a transfer of solutes from one phase (e.g., a solid phase) into another (the solvent). The food matrix can be compared to a grain constituted of an impermeable core covered by a solvent boundary layer (Figure 5.4). Secondary metabolites are extracted in three steps: desorption from the matrix surface or release from internal glands, diffusion through the boundary layer to the boiling solvent, and solubilization in the solvent. The extraction recovery can be limited by one or several steps.

The phenomena at play in UAE could be visualized by referring to our original investigations where we designed a series of solid–liquid extraction steps using various extraction procedures on a single source carvi, a food spice material [24]. The effects of such extraction processes on the

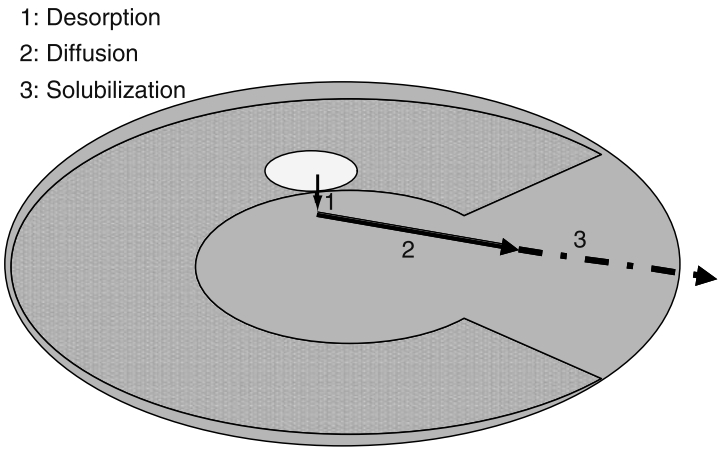

FIGURE 5.4 Schematic representation of the individual steps in the extraction process.

physical microstructure of the material being extracted were closely monitored using scanning electron microscopy. The various extraction methods (Soxhlet, maceration, and ultrasound) produced distinguishable physical changes on the extracted matrix (caraway seeds). Figure 5.5a is a micrograph of the untreated seeds, broken cryogenically, which can be compared with structures of the treated seeds in Figure 5.5b and c. After a few hours of conventional extraction or maceration in hexane (69°C), the cell walls seemed thicker but intact and most of the cells were totally free from any component released out of the cell. After 30 min of ultrasound extraction (20°C), cells and cell walls were affected to different degrees. We observed a huge perforation of the particles' external surface and some waste material is dispersed, showing that all the cell walls were finally broken and converted into undefined cell shapes. There was clear evidence of explosions occurring at the cell level as a consequence of the sudden enhancement in micromixing, generated in that case by localized mass transfer caused by ultrasound power.

Ultrasound has focused its power, at the beginning of extraction, on cuticular layer destruction and oil exudation. Then, it deflected this power against cell walls perforation mainly due to the high resistance of the particles in the medium toward ultrasound energy.

When the glands were subjected to more severe thermal stresses and localized high pressures induced by cavitation, as in the case of UAE, the pressure buildup within the glands could have exceeded their capacity for expansion, and caused their rupture more rapidly than in control experiment. In general, the SEM observations pointed two distinct extraction mechanisms for conventional and ultrasound procedures, respectively. The first involves diffusion of the plant extract components across the unbroken gland wall due to the temperature increase in the medium, and the other one, exudation of oil from damaged cell walls and even cells, due to a strong ultrasonic mechanical effect, which generally triggers an instantaneous release of the plant extract components into the surrounding solvent.

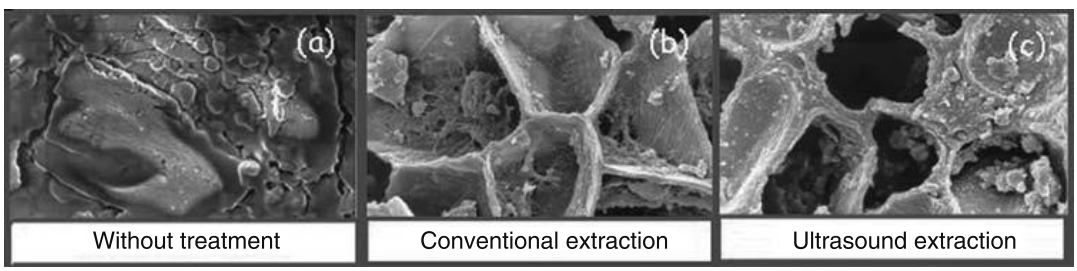

FIGURE 5.5 Electron micrograph of carvi seeds (untreated, conventional extraction, and UAE).

5.4 ULTRASOUND-ASSISTED EXTRACTION: MAIN APPLICATIONS IN FOOD ANALYSIS

Among newer techniques used in extraction technology, UAE of food components has been employed as a new tool to improve the yield and quality of extraction products and to reduce the duration of analytical procedures. The first applications were related to the determination of metals in foods. Since then, numerous other compounds have been efficiently extracted such as aromas, antioxidants, oils, pigments, etc.

5.4.1 Flavors and Fragrances

Natural flavors and fragrances have been used probably since the discovery of fire. Egyptians, Phoenicians, Jews, Arabs, Indians, Chinese, Greeks, Romans, and even Mayas and Aztecs all possessed a fragrance culture of great refinement. Fragrances are complex mixtures of volatile substances generally present in low concentrations. Flavors or aromas are obtained from a variety of aromatic plant materials including flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots. Their yield and quality depend mainly on the cultivar (chemotype and genetic variability), environment (fertilization, climatic conditions, and crop protection), and physiological stage (plant development stage). These aromatic compounds are produced by plants as by-products or indeed as final metabolites and stored in certain organs of the plant:

- Thyme, sage, and rosemary (Lamiaceae family): in glandular cells, hairs, and scales.
- Cinnamon, laurel, and cassia (Lauraceae family): in essential oil and resin cells.
- Caraway, anis, and coriander (Umbellifers family): in essential oil channels occurring in the intercellular space of plant tissue.
- Lemon, orange, and bergamot (Rutaceae family): in lysigenous secretory reservoirs formed inside the plant.

Conventional flavor and fragrance extraction techniques have important drawbacks, such as low yields and formation of by-products because of the low stability of the target compounds. For steam distillation and hydrodistillation methods, the steam is percolated through the flask containing the aromatic plants and the aromas evaporate. The elevated temperatures and prolonged extraction time can cause chemical modifications of the aromatic components and often a loss of the most volatile molecules. To obtain high-quality extracts from aromatic plant materials, several innovative methods are available and a large number of studies have dealt with the benefits of ultrasonic power. Typical analytes, extraction conditions, and detection devices are summarized in Table 5.1. Prior to extraction, there are many parameters to optimize: plant moisture, particle size, ultrasonic design, temperature, and solvent [25].

Vanillin has been extracted and quantified by sonoelectroanalysis with ethylacetoacetate in two different samples [26]. Vanillin concentrations were in close agreement with HPLC-UV quantification. Vanillin was also extracted from *Vanilla planifolia* in dry ethanol in 0.99% yield in comparison with a maximal yield of vanilla "crude" extract (14.3%) obtained in $EtOH/H_2O$ (40/60). UAE is a useful tool for the rapid quantification of several aromatic compounds in wine [25,27,28], honey, citrus flowers [29,30], and carvone [24]. In addition, US assistance enabled the precise quantification of 37 volatile compounds in brandies and 16 in alcoholic oak extracts [31]. These good results can be explained by the mild conditions and specific mechanical effects of sound waves, according to Chemat et al. [24] and Shotipruk et al. [32].

5.4.2 Metals

Metals occurring in food are important in the fields of nutrition, toxicity, and control of the manufacturing process. Several metal ions have a nutritional value since they are involved in

TABLE 5.1
Flavor and Fragrance Extracts

Matrix	Analyte	Extraction Conditions and Remarks	Detection (Connection)	References
Aged brandies and red wine	Aroma compounds	USB, 20°C, 3-step extraction in CH_2Cl_2. ST: 3×10 min. Mean values (μg/L) for monoterpenoids: 291 (linalool), 248 (α-terpineol), 397 (citronellol)	GC-FID (off-line) GC-MS (off-line)	[31,33]
Caraway seeds	Carvone and limonene	USH, 20 kHz, 150 W, 20°C, *n*-hexane. ST: 60 min. Yd (mg/g): 17 (carvone) 16 (limonene). USAE gave a better quality of extracts with an increased yield for carvone	GC-FID (off-line) GC-MS (off-line) SEM (off-line)	[24]
Citrus flowers	Volatile compounds	USB, 25°C, *n*-pentane: Et_2O. ST: 10 min. Among extracted compounds, linalool was the major (% of the total peak area): 51.6 (orange), 11.3 (lemon), 75.2 (tangerine), 80.6 (sour orange)	GC-MS (off-line)	[30]
Vanilla	Vanillin	USH, 20 kHz, 750 W, 25°C, EtOH or EtOH/H_2O. ST: 1–2 min	HPLC-DAD (off-line) Sonoelectro-analysis (online)	[26,34]
Greek saffron	Safranal	USB, 35 kHz, 25°C, H_2O: Et_2O. ST: 5×10 min. Safranal ranged between 40.7 and 647.7 mg/100 g saffron	GC-FID (off-line) GC-MS (off-line)	[35]
Honey	Aroma compounds	USB, 25°C, H_2O, *n*-pentane: Et_2O, ST: 2×10 min. UAE allows isolation of several compounds and is the best extraction technique	GC-MS (off-line)	[29,30]
Must and wine	Aroma compounds	USB, 48 kHz, 20°C, 3-step extraction with CH_2Cl_2. ST: 3×10 min. Results were higher than those obtained by traditional method and several compounds were extracted	GC-FID (off-line)	[27]
Peppermint leaves	Menthol	USB, 40 kHz, 22°C, H_2O, ST: 60 min, Yd: 17.8 μg/g (2% of total product). The amount of menthol released can be enhanced (12%) with temperature increasement (39°C)	GC-FID (off-line) SEM (off-line)	[32]
White wine	Aroma compounds	USB, *n*-pentane: Et_2O, $MgSO_4$, ST: 30 min. The method described enables the rapid quantification of 24 wine compounds	GC-FID (off-line)	[28]
Wine	Volatile compounds	USB, 40 kHz, 25°C, CH_2Cl_2, ST: 15 min. 12 compound concentrations are done and extend from 0.422 to 168 mg/L. Linalool and α-terpineol were not detected	GC-FID (off-line) GC-MS (off-line)	[25]

USB, ultrasonic bath; USH, ultrasonic horn; ST, sonication time; Yd, yield.

various biological mechanisms such as enzyme functioning. However, in elevated concentrations, metal ions may have adverse and toxic effects. The qualitative and quantitative knowledge of metals such as Sn or Cu is of great importance because they are possibly involved in cancer and cardiovascular diseases. An actual trend of great health interest is Al speciation because this element

might favor the development of Alzheimer's disease. Another important point is the determination of metal traces in seafood samples which can be used as biomarkers to monitor the environmental pollution.

Extracting metals from food samples is complicated by the strong interactions typically occurring between the food matrix and analytes. Several methods are available to transfer the desired analytes into the liquid phase, the most common being leaching. Other methods such as digestion, calcination, ashing, etc. can also be used when leaching is not sufficient. These methods infer some drawbacks and drastic conditions like high temperatures or pressures, use of concentrated acids, sample losses by manipulation, or volatilization. In addition, these methods are generally hazardous and time consuming.

Table 5.2 shows a sample of US-assisted metal extractions carried out on several matrices. The acid-leaching procedure assisted by ultrasound appears rapid, accurate, and effective [36–38]. It offers rapid sample preparation and mild conditions compared with the tedious and time-consuming acid digestion. Filgueiras et al. [39] described a fast UAE (only 7 min) for Mg, Mn, and Zn. According to Krishna and Arunachalam [40], the procedure required only 15 min to estimate trace elements in mussel and lichen and allows the preparation of about 35 samples by working day. Another alternative is to combine the benefits of UAE with a flow injection (FI) manifold coupled with an FAAS. FI permits a preconcentration step for a higher sensitivity. Thus, the handling and analytical steps are shortened. The risk of contamination is reduced and the centrifugation step required in the off-line technique is totally removed.

For faster procedures, the sampling frequency is another key parameter to optimize. According to Del Carmen Yebra et al. [41], continuous UAE coupled with an FI-FAAS allows a total sample frequency of 46 and 18 samples per hour for copper and iron determination in seafood samples, respectively. Calcium determination in seafood samples can also be carried out at a frequency of 40 samples per hour with the same technique [42].

Another focus in the current works is the method described by Cava-Montesinos et al. [43], where an online FI method coupled with Cold vapour atomic absorption spectrometry (CV-AFS) was used in order to determine mercury contents in fish samples. The method presented by Šuchman and Bednář [44] allows direct chloride analysis in meat products on the average extraction time of 7 min.

The US leaching technique avoids the use of strong acids, which is a great advantage for US probes and analytical instruments. It also presents the advantage of requiring only little solvent and sample (as low as a few micrograms) while offering a rapid, precise, and reproducible metal extraction procedure in different food samples.

5.4.3 Antioxidants

Plant and food antioxidants are able to rapidly scavenge free radicals, thereby inhibiting deleterious oxidative processes such as lipid peroxidation that are responsible for food deterioration, accumulation of toxic products, and off-flavor compounds. Thus, the knowledge of their properties and concentrations in food is most desirable. Traditional extraction methods such as maceration, mix-stirring, or refluxing require large volumes of solvent and are often time consuming. In addition, they often require drastic conditions (high temperature or pressure) that are not fully compatible with the general chemical instability of potent antioxidants. Hence, special care (in terms of light exposure, temperature, pH, etc.) is needed during handling in order to prevent antioxidant-rich extracts from oxidation. For instance, some authors have performed direct HPLC analysis of wine or cider for a simple and rapid determination of polyphenols [57,58]. However, direct analysis is not always possible and a preliminary preparation step is often necessary. The feasibility of UAE in the analysis of polyphenols and other antioxidants has been investigated on many matrices (Table 5.3). In most cases, the yields are increased with US assistance. According to Tsanova-Savova et al. [59], 5 min of sonication is equivalent to 1 h of mechanical stirring for

TABLE 5.2
Metal Extraction

Matrix	Analyte	Extraction Conditions and Remarks	Detection (Connection)	References
Fish and mussel	Cd, Cu, Zn	USB, 56°C, HNO_3: HCl: H_2O_2. ST: 30 min. Yd (mg/kg), for fish and mussel, respectively: 0.06, 0.55 (Cd), 1.16, 4.24 (Cu) and 15.45, 52.30 (Zn)	GFAAS (off-line) FAAS (off-line)	[36]
Fish samples	Hg	USB, 50 Hz, 50 W, 50°C, HCl: H_2SO_4: HNO_3: H_2O_2. Total Hg concentrations ranged between 0.74 (anchovy) and 6.1 mg/kg (mussel)	CV-AFS (online)	[43]
Fruits and vegetables	Cd	USB, 20°C, HNO_3, ST: 1–2.5 min, flow rate: 3.5 mL/min. Concentrations of Cd found in fruits and vegetables samples ranged between 0.118 (banana) and 0.640 μg/g (lettuce)	FI-FAAS (online)	[45,46]
Juices and soft drinks	Al	USB, 35 kHz, 80°C, HNO_3: H_2SO_4: H_2O_2, ST: 20 min, Yd (μg/mL) extended from 2.15 to 12.0 for 18 different juice and soft drink samples	ETAAS (off-line)	[47]
Lettuce and cabbage	Ca, Mg, Mn, Zn	USB, 47 kHz, 25°C, H_2O, HNO_3, detergent, ST: 10 min, Yd for lettuce and cabbage, respectively: 1.68, 0.89% (Ca), 0.280, 0.180% (Mg), 165.69, 32.35 μg/g (Mn), 112.26, 26.36 μg/g (Zn)	FAAS (off-line)	[48]
Meat	Fe, Zn	USB, 40 kHz, 20°C, HNO_3 and/or HCl, ST: 0.5–5 min, flow rate: 3.5 mL/min. Contents (μg/g) ranged from 58.8 (pig muscle) to 277.8 (rabbit liver) for Fe, and from 58.8 (chicken muscle) to 195.7 (lamb muscle) for Zn	FI-FAAS (online)	[49,50]
Meat products	Chlorides	USB, 500 W, 60°C, H_2O, ST: 5 min. Yd (g NaCl/kg) extend from 16.5 (salami) to 41.2 (herkules salami) for different thermal meat products	Potentiometry (online)	[44]
Mussel	Cd, Pb	USH, HNO_3. ST: 15 s at 10% amplitude for Cd and 180 s at 60% for Pb. Contents ranged from 0.60 and 0.79 μg/g for Cd, and from 2.03 to 2.81 for Pb for five samples	ETAAS (off-line)	[38]
Mussel	Cd, Pb, Cu, Fe	USB, 40 kHz, 20°C, HNO_3 for Cd and Pb, or HNO_3/HCl for Cu and Fe. ST: 2–5 min, flow rate: 3.5 mL/min. Yd (μg/g): 0.383–0.559 (Cd), 0.49–1.0 (Pb), 1.2–3.6 (Cu), 212.5–257.1 (Fe)	FI-FAAS (online)	[51–53]
Mussel tissue samples	Hg	USH, HCl. ST: 3–5 min, amplitude: 20%–70%. Content of methylmercury and inorganic mercury ranged from 0.053 to 0.243 μg/g for four samples	FI-CV-AAS (off-line)	[37]
Plant samples	Mg, Mn, Zn	USH, 20 kHz, 100 W, <50°C–60°C, HCl, 30% amplitude, ST: 3 min. Concentration (μg/g) ranges found in various samples: 20.1–46.5 for Zn, 28.4–731 for Mn and 1439–2989 for Mg	FAAS (off-line)	[39]
Raw pork meat	Pb, Ca, Cu, Cd, Cr, Fe, Zn, Mg	USH, 20 kHz, 400 W, ambient temperature, HNO_3, duty cycle 0.1 s, amplitude 70%, ST: 10 min, Yd (mg/kg) for 1 sample: 0.17 (Pb), 0.022 (Cd), 1.8 (Cu), 9.1 (Cr), 166 (Ca), 219 (Mg), 16.6 (Fe), 19.4 (Zn)	GFAAS (off-line) FAAS (off-line)	[54]
Rice	As	USH, 25°C, H_2O + enzymes (10 mg of α-amylase than 30 mg of protease). ST: 1–2 min. Yield of total as extracted: 149 ng/g for Spanish white rice and 56 ng/g for Indian basmati rice	ICP-MS (off-line) HPLC-ICP-MS (off-line)	[55]
Seafood	Ca, Cu, Fe	USB, 40 kHz, 20°C, HNO_3, ST: 0.5–3 min, flow rate: 6 mL/min. Values (μg/g) ranged from 5.9 (hake) to 18.8 (mussel) for Cu, 37.2 (crab) to 256.2 (mussel) for Fe, 1576.73 (tuna) to 6849.35 mg/kg (prawn) for Ca	FI-FAAS (online)	[41,42]
Seafood	Se	USH, HNO_3, ST: 3 min at 50% amplitude. Contents ranged from 0.95 (meagrin) to 2.61 μg/g (edible crab)	ETAAS (off-line)	[56]

TABLE 5.3
Antioxidant Extracts

Matrix	Analyte	Extraction Conditions and Remarks	Detection (Connection)	References
Amaranthus caudatus seed	Tocopherols, vitamin E isomers	USB, 25°C, 2-step extraction: ST: 30 or 60 min in MeOH than 30 min in hexane. Total tocopherols content varies from 51.81 to 63.7 mg/kg	HPLC-UV (off-line)	[61,62]
Bulgarian fruits	(+)-Catechin, (−)-epicatechin	USB, MeOH/H_2O, ST: 5 min, 15 fruits were studied and total catechins ranged between 4.3 and 195.3 mg/kg. No catechins were detected in melon	HPLC-FLD (off-line)	[59]
Eucommia ulmodies Oliv. *(E. ulmodies)*	Chlorogenic acid	USB, 50 kHz, 160 W, MeOH. ST: 3×30 min. Concentration ranged from 0.07% (fresh bark) to 0.71% (fresh leaves) with a good recovery	HPLC-UV (off-line)	[63]
Olive leaves	Oleuropein	USH, 20 kHz, 450 W, 40°C, EtOH/H_2O, duty cycle: 70%, amplitude: 30%, flow rate: 5 mL/min, ST: 25 min. The main found compounds ranged from 488 (verbacoside) to 22610 mg/kg (oleuropein)	GC-MS (off-line) HPLC-DAD (online)	[64]
Extra virgin olive oil	Polyphenols	USH, 20 kHz, 400 W, *n*-hexane, amplitude: 10%, duty cycle: 30%, flow rate: 2.4 mL/min. Total Yd of polyphenols in various oil samples ranged between 124 and 1267 μg CAE/mL	DAD-UV-vis spectrometer (online)	[65]
Fruit juices	Ascorbic acid (vitamin C)	USH, 20 kHz, 25 W/cm, 25°C, Phosphate buffer solution, ST: <2 min, Yield: 31 mg/100 mL	UV-vis spectrometer (off-line)	[66]
Tea leaves, grape seeds, and soybeans	Catechins, epicatechins, and isoflavones	USH, 200 W, 24 kHz, 60°C, MeOH or EtOH, ST: 10–20 min. Content ranges: 0.22–0.46 mg/g (catechin), 0.07–3.36 mg/g (epicatechin) (Ref. [15]) and 72.58–550.45 μg/g (isoflavones) (Ref. [13])	HPLC-DAD-FLD, HPLC-DAD or -MS (off-line)	[67,68]
Pistachio hulls, coconut shells	Phenolic compounds	USB, H_2O, ST: 45 min, Yd: 34.2 mg TAE/gdw for pistachio hulls USB, 150 W, 25 kHz, 30°C, EtOH/H_2O. ST: 60 min, Yd: 406.93 mg TAE/L for coconut shells	UV-vis spectrometer (off-line)	[69,70]
Rosemary leaves	Carnosic acid	USB, Acetone. ST: 3×5 min, Yd: 26.2 mg/g USB, 40 kHz, 47°C–53°C, EtOH. ST: 45 min, Yd is about 18 mg/g	MS (off-line) HPLC-UV or −DAD (off-line)	[60,71]
Strawberries	Phenolic compounds	USH, 20 kHz, 100 W, HCl, ST: 2 min, duty cycle and radiation amplitude: 20%. Contents ranged from 0.12 (syringic acid) to 566 mg/kg (gallic acid) for strawberries	HPLC-DAD (off-line)	[72, 73]
Yellow sweet clover	Coumarins	USB, EtOH. ST: 60 min, Yd (mg/g): 3.569 (coumarin), 1.269 (*o*-coumaric acid), 8.092 (melilotic acid)	HPLC-DAD (off-line)	[74]
Pastinaca sativa fruits	Furano-coumarins	USB, 60°C, ST: 3×30 min in petroleum ether than 3×30 min in MeOH. Total content ranged from 0.264 (phellopterin) to 14.444 mg/g (imperatorin)	HPLC-UV (off-line)	[75]

TABLE 5.4
Oil Extraction

Matrix	Analyte	Extraction Conditions and Remarks	Detection (Connection)	References
Adlay seed	Oil	USH, 20 kHz, 110 W, 40°C, sCO_2, P: 20 MPa, flow rate: 3.0 L/h. ST: 210 min. Extraction yields were 96.36% for oil	GC-MS (off-line)	[78,79]
Almond	Oil	USH, 20 kHz, 50 W, 55°C, sCO_2, P: 280 Bar, flow rate: 20 kg/h. ST: 8.5 h. Yield is enhanced by about 20% and kinetics by about 30%	Gravimetric determination (off-line)	[80]
Almond, apricot, and rice bran	Oil	USB, 42 kHz, 80 W, H_2O, ammonium sulphate, *t*-butanol. ST: 6–8 min. Recovery (%): 87–89 (almond), 72–80 (apricot), 76–88 (rice bran)	Gravimetric determination (off-line)	[81]
Bakery products	Fat	USH, 20 kHz, 400 W, *n*-hexane. ST: 6 min, duty cycle: 0.8 s, amplitude: 100%, flow rate: 2 mL/min. Found values ranged from 4.81 (Egg cakes) to 35.22% (Snack corn)	FTIR, gravimetric determination (off-line)	[82,83]
Oleaginous seeds	Fat	USH, 20 kHz, 100 W, 75°C, *n*-hexane, duty cycle: 0.5 s, amplitude: 40%. Yd: 99% of fat recoveries in 90 min on sunflower seeds	GC-FID (off-line)	[76]
Soybean	Oil	USH, 20 kHz, 20–50 W/cm, 25°C, hexane: isopropanol. ST: 3 h. Oil yield was 12.21%	GC-FID, SEM, gravimetric (off-line)	[77,84]

the extraction of catechins (flavonols) from Bulgarian fruits. By using an ultrasonic horn, 15 min are also sufficient to extract carnosic acid from rosemary leaves compared with 3 h of shaking in a water bath [60].

5.4.4 Oil and Fat

Many papers have reported on the UAE of oil and fat from various food samples (Table 5.4). According to Luque-García and Luque de Castro [76], extraction from oleaginous seeds is difficult. Indeed, only 75%–85% of the oil is solubilized in the solvent. The rest of the oil content is strongly bound to the matrix and cannot be extracted without additional treatments. For instance, Li et al. [77] described the UAE of soybean oil in optimized yield and reduced operating time in comparison with conventional maceration.

Another growing trend in the development of a new extraction process is to combine traditional and novel techniques. Luo et al. and Hu et al. [78,79] performed a US-assisted supercritical fluid extraction (SFE) device for adlay seed extraction. The procedure resulted in decreased temperature and operating time and a higher extraction yield. For instance, US-assisted SFE required a temperature of 40°C, a pressure of 20 MPa, and a flow rate of 3 L/h during 3.5 h instead of 45°C, 25 MPa, 3.5 L/h during 4 h for conventional SFE.

Finally, Luque-García et al. [76] have reported a device consisting of a U.S.-assisted Soxhlet apparatus that is an attractive alternative to traditional Soxhlet extraction. Using this device, only 90 min are needed to obtain the same yield (99% of fat recovery) as in 12 h of traditional Soxhlet extraction.

5.5 COMPARISON WITH TRADITIONAL AND RECENT EXTRACTION TECHNIQUES

Advantages and drawbacks of UAE have been compared to traditional and recent extraction techniques as conventional Soxhlet extraction, SFE, accelerated solvent extraction (ASE), and microwave-assisted extraction (MAE) (Table 5.5).

TABLE 5.5
Advantages and Drawbacks of Traditional and Recent Extraction Techniques

Name	Soxhlet	Supercritical Fluid Extraction	Accelerated Solvent Extraction	Microwave-Assisted Extraction	Ultrasound-Assisted Extraction
Brief description	Sample is contained in an extraction cartridge and percolated with recondensed vapors of the solvent	Sample is placed in a high pressure vessel and crossed continuously by the supercritical fluid	Sample is heated by a conventional oven and crossed by the extraction solvent under pressure	Sample is immersed in solvent and submitted to microwave energy	Sample is immersed in solvent and submitted to ultrasound using a US probe or US bath
Extraction time	3–48 h	10–60 min	10–20 min	3–30 min	10–60 min
Sample size	1–30 g	1–5 g	1–30 g	1–10 g	1–30 g
Solvent use	150–500 mL	2–5 mL (solid trap) 30–60 mL (liquid trap)	15–60 mL	10–40 mL	50–200 mL
Investment	Low	High	High	Moderate	Low
Advantages	Easy to handle, no filtration necessary, high matrix capacity	Fast extraction, low solvent consumption, concentration of the extract, no filtration necessary, possible high selectivity	Fast extraction, no filtration necessary, low solvent consumption	Fast extraction, easy to handle, moderate solvent consumption	Easy to use
Drawbacks	Long extraction time, large solvent volume	Many parameters to optimize	Possible degradation of thermolabile analytes	Extraction solvent must absorb microwave energy, filtration step required	Large solvent volume, filtration step required

5.5.1 Soxhlet

Extraction of solid material is traditionally performed by Soxhlet extraction. This method proceeds by iterative percolation of the sample with recondensed vapors of solvent. It has been one of the most used solid-liquid extraction techniques for a long time and is currently the principal reference method. Soxhlet extraction of solid materials has undeniable advantages such as uninterrupted extraction with repeated percolation with fresh solvent, non-necessary filtration step, and possible recycling of solvent. This technique nevertheless displays some disadvantages: poor extraction of polar lipids, long operating time, large solvent volumes, operation at the solvent's boiling point, and inadequacy for thermolabile analytes [85].

Using a UAE versus conventional Soxhlet extraction presents the advantage that the whole extraction process is accelerated. In fact, the extraction efficiency can be equivalent [86] or higher [87] than that obtained with conventional Soxhlet extraction and, in some cases, allows the extraction of thermolabile compounds that are typically degraded during the conventional procedure [85]. However, some drawbacks must be pointed out: the renewal of the solvent is not possible during the process and a filtering step is necessary.

5.5.2 Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is an important method for the extraction of solid materials using a supercritical fluid [88,89]. Carbon dioxide is the most widely used solvent in SFE because it is nontoxic, nonflammable, cheap, easily eliminated after extraction, and endowed with a high solvating capacity for nonpolar molecules. Other possible solvents are Freon, ammonia, and some organic solvents [90]. In a typical SFE procedure, the supercritical fluid continuously enters the solid matrix where it dissolves the material of interest. The extraction can be achieved with a remarkably high selectivity by adjusting the solvating capacity of the supercritical fluid by changing the pressure and temperature. Major advantages of SFE include preconcentration effects, cleanness and safety, quantitativeness, expeditiousness, and simplicity [91]. The application of SFE from plant, animal, and oil has been reviewed [92]. Compared to UAE, SFE processes are more precise in many cases [71]. The use of CO_2 as supercritical fluid extractor limits the polluting hazards. The drawback of SFE versus UAE is the need of more expensive equipment with the difficulty of extracting polar molecules without adding modifiers to CO_2. Indeed, UAE permits the extraction of a wide variety of compounds using polar or nonpolar solvents and much simpler equipment.

5.5.3 Accelerated Solvent Extraction

Accelerated solvent extraction (ASE) makes use of the same solvents as traditional extraction methods while operating at elevated temperatures and pressures. ASE, generalized to pressurized fluid extraction, is now well accepted as an alternative to Soxhlet extraction [93]. The sample is contained in an extraction cartridge heated in a conventional oven and crossed by the extraction solvent. The extraction is performed statically for a short period. When the extraction is complete, compressed gas shifts the solvent from the cartridge to the collecting vessel. ASE allows rapid extraction with small solvent volumes by using high temperatures (up to 200°C) for increased solvent diffusivity [94,95] and high pressure (up to 20 MPa) to keep the solvent in its liquid state [96]. ASE provides a wide range of applications but the high extraction temperature may lead to degradation of thermolabile compounds [94]. Fisher et al. [97] pointed out that the main drawbacks of ASE are a strong background interference and high detection limit. Moreover, the equipment is expensive. Compared to ASE, UAE allows an important reduction of extraction time, solvent volume, and sample manipulations [98].

5.5.4 Microwave-Assisted Extraction

Microwave-assisted extraction (MAE) has attracted growing interest as it allows the efficient use of microwave energy to extract valuable compounds from solid samples. There are two types of laboratory MAE systems: closed microwave extraction vessels under controlled pressure and temperature and focused microwave ovens at atmospheric pressure [95]. The fundamentals of microwave-enhanced chemistry, including the theory behind sample preparation and new instruments, has been described by Kingston and Haswell [99]. MAE consists in subjecting a solid sample to microwave irradiation in a solvent. The process is based upon the fast localized heating of the solid sample without heating the vessels. The boiling point of the solvent is then rapidly reached, thus resulting in a short extraction time. The main benefits of MAE are short extraction time, low solvent consumption, high extraction yield, and a simple process that ensures reproducibility at low cost. Pan et al. [100] have found that MAE extraction improves the efficiency of the extraction of polyphenols and caffeine from green tea leaves. However, the moisture content of samples is a defining parameter for the recovery yield. When using dried samples, the recovery yield drops dramatically. Furthermore, Molins et al. [101] have reported that the use of hexane as the sole solvent in presence of a completely dry sample was not satisfactory. Indeed, the efficiency of MAE is typically low when the solvent lacks a significant dipole moment for microwave energy

absorption. The methods are therefore limited in terms of solvents and nature of the solid material. Compared to MAE, UAE may eventually be simpler [102] and faster [103]. In addition, UAE is not restricted by the solvent and type of matrix used, or by the moisture content.

5.6 ULTRASOUND-ASSISTED EXTRACTION: ENVIRONMENTAL IMPACT

UAE is a clean method that avoids the use of large quantity of solvent and voluminous extraction vessels like Soxhlet and maceration. The reduced environmental impact of UAE is clearly advantageous in terms of energy and time. The energy required to perform the three extraction methods are, respectively, 6 kW · h for maceration at the solvent's boiling point (electrical energy for mechanical mixing and for heating), 8 kW · h for Soxhlet (electrical energy for heating), and 0.25 kW · h for UAE (electrical energy for ultrasound supply). The power consumption was determined with a Wattmeter at the ultrasound generator supply and the electrical heater power supply. Regarding environmental impact, the calculated quantity of carbon dioxide rejected in the atmosphere is higher in the case of Soxhlet (6400 g CO_2/100 g of extracted solid material) and maceration (3600 g CO_2/100 g of extracted solid material) than for UAE (200 g CO_2/100 g of extracted solid material). These calculations have been carried out based on the following assumptions: To obtain 1 kW · h from coal or fuel, 800 g of CO_2 will be rejected in the atmosphere during combustion of fossil fuel. UAE is thus proposed as an ''environmentally friendly'' extraction method suitable for extraction prior to food analysis.

5.7 FUTURE TRENDS

UAE of food components is increasingly efficient at directly transferring knowledge into technology for commercial development. UAE makes use of physical and chemical phenomena that are fundamentally different from those applied in conventional extraction techniques. This novel process can extract analytes under a concentrated form (low volumes of solvent) and free from any contaminants or artefacts. The new systems developed to date clearly indicate that UAE offers net advantages in terms of yield, selectivity, operating time, energy input, and preservation of thermolabile compounds.

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