Chapter

Application of Green Extraction Techniques for Natural Additives Production

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Abstract

During the last decades, consumers have increased the demand for healthier natural foods with lower presence of chemical additives. One reason of this choice is the controversy about chemical additives possible adverse effects. To fulfill market needs, different techniques have been developed to extract compounds from various raw materials to produce natural additives with different properties (preservatives, emulsifiers, or colorants) and bioactivities. In addition, the growing concern about the effects of climate change has led the development of more sustainable techniques to carry out the extraction. The use of new alternative nonconventional, emerging, or green extraction methodologies has gained considerable attention during the last decade. These novel techniques have been applied to minimize any negative changes in the nutritional, physicochemical or sensory properties of the natural source, while at the same time reducing the environmental impact of the process and gaining competitiveness of the world market. For this purpose, new green extraction methods have been proposed and optimized for the reduction of the consumption of raw materials, solvents, and energy. In this chapter, a revision of different types of green extraction techniques is compiled together with the main factor that can affect extraction-process feasibility and the main challenges and future trends for their development.

Keywords: Natural additives, green extraction techniques, conventional extraction, solvent, energy

1. Introduction

The use of additives in the food industry has become a routine process due to the ability of this type of compound to improve the organoleptic properties (flavor, aroma, color) of foodstuff or to extend its shelf-life due to its bioactivities (mainly, antioxidant and antimicrobial) [1]. Food additives are the most useful tool to improve foodstuff quality. According to the European Food Safety Authority (EFSA), food additives are defined as "substances that are not normally consumed

as food nor used as intrinsic ingredients of food, which has a technological purpose" [2]. In this context, the nature of some additives, known to be potentially hazardous if consumed in excess, has derived to an increasing consumer trend to avoid these types of products. One possible effect of these molecules is the potential allergic reactions or health risks associated with a frequent consumption. Compounds that have been subjected to this controversy are sulfites, nitrosamines or palm oil, whose presence in food have been sometimes forbidden (*e.g.*, sulfites in wines sold in the USA) [3, 4], leading to an ideological current against synthetic additives [5]. This phenomenon collides with a growing interest for developing a green and sustainable economy and reducing generated wastes. For this reason, and the food industry has focused its efforts research on new effective and feasible extraction methods of natural compounds from different raw materials (including by-products) that could be used as additives [6]. These compounds belong to different classes, being the most studied phenolic compounds (*i.e.* phenolic acids, flavonoids, tannins), pigments (*i.e.* chlorophylls, carotenoids, anthocyanins), vitamins, polysaccharides, proteins and unsaturated fatty acids [7–11]. They have different bioactivities, depending on the chemical structure of the molecule (**Figure 1**). For example, phenolic compounds, pigments or vitamins are recognized as potent antioxidants and antimicrobials, whereas pigments like β-carotene will additionally provide color to the product. In addition, there are other aromatic compounds such as terpenoid carvacrol, obtained from thyme and oregano, *d*-limonene from citrus tree or curcumin from turmeric with beneficial properties which can be also used as food additives [12].

Studies pointed out that natural additives may have higher bioactivity than artificial ones. For example, it has been observed that phenolic compounds (phenolic acids and flavanols) have comparable or even more potent antioxidant activity than currently used artificial additives like propyl gallate or butylhydroxytoluene in meat, fish and bakery products [13–16]. Hence, there is scientific evidence to propose their use as alternative condiments. On this matter, many of these compounds and/or extracts, like essential oils, are already approved and generally recognized as safe (GRAS) flavoring food additives by the *Food* and Drug Administration (FDA) and EFSA [2, 17]. Some examples of additives directly extracted from natural sources approved by EFSA include plant and algal pigments, tocopherols, or antioxidant-rich extracts like those from rosemary. On the other hand, some natural additives, like tannic acid, are approved for their use in animal feed but are not yet approved for human consumption [18].

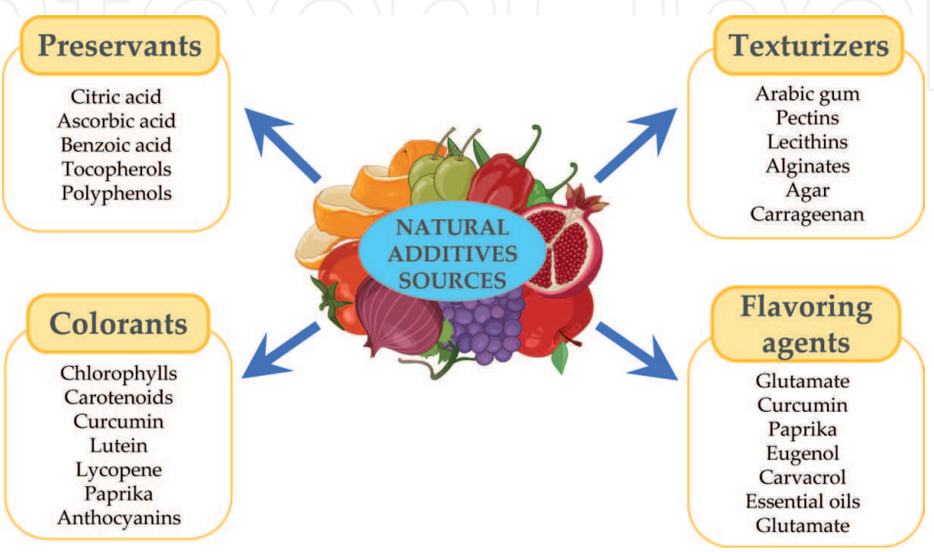

Figure 1.
Properties and main applications of major additives of natural origin in foods.

Compound	Applications	Current & Potential Natural Sources	E-Number	Ref.
Benzoic acid	Antioxidant & antimicrobial preservatives	Cranberries, blueberries (*Vaccinium* sp.)	E-210	[21]
Ascorbic acid	Antioxidant	Peppers, kiwifruit, citrus, rosehip.	E-300	[22]
Citric acid	Antioxidant, acidifier	Citrus	E-330	[23]
Tartaric acid	Acidifier	Grapes	E-334	[24]
Chlorophylls	Colorant	Green leaves from alfalfa, nettles, spinach, green microalgae	E-140, E-141	[25]
Carotenoids (β-carotene)	Colorant, antioxidant	Pigmented vegetables (carrots, palm fruit), red microalgae	E160a	[26]
Curcumin	Colorant, flavoring, antioxidant	Turmeric (*Curcuma longa*)	E-100	[27]
Lutein	Colorant, antioxidant	Kale (*Brassica oleracea*), spinach, *Calendula officinalis*, *Tagetes erecta*	E-160b	[28]
Paprika extract (capsaicin, captaxanthin, capsorubin)	Colorant, flavoring	Red peppers (*Capsicum* sp.)	E-160c	[29]
Lycopene	Colorant	Tomato peels	E-160d	[30]
Anthocyanins	Colorant	Red grape skin, pomegranate, black currant	E-163	[31]
Glutamate	Flavoring	Wheat gluten, de-oiled soybeans	E-620	[32]
Limonene	Odorant, antimicrobial	Citrus peels	—	[33]
Eugenol	Flavoring, antimicrobial preservative	Clove	—	[34, 35]
Carvacrol	Flavoring, antimicrobial preservative	Oregano, thyme, rosemary	—	[35, 36]
Rosemary extract (carnosic acid, carnosol)	Preserver (antioxidant, antimicrobial), flavoring	Rosemary	E-392	[37]
Lecithins	Emulsifiers, lubricants	Soybean, sunflower kernels, rapeseed	E-322	[38]
Alginates	Texturizer, binder, thickener	Brown seaweeds	E-401, E-402	[39]
Agar	Texturizer, binder, softener	Red seaweeds	E-406	[40]
Carrageenan	Texturizer, binder, softener	Red seaweeds	E-407	[41]
Pectins	Thickener, texturizer	Apple pomace, citrus peels	E-440	[42]

Compound	Applications	Current & Potential Natural Sources	E-Number	Ref.
Arabic gum	Thickener, texturizer	*Acacia nilotica*	E-414	[43]
Tannic acid	Antioxidant, plasticizer, flavoring	Grape seeds, skins	—	[44, 45]
Phytosterols	Functional, health	Soybean	E-499	[46]
Tocopherols	Health, antioxidant	Vegetal oils, cereal germs, rapeseed, soybean	E-306, E-307, E-308, E-309	[47]

[*]Described natural sources are indicated and evidenced in the used references, but chemical or microbiological synthesis as well as enzymatic transformations from natural sources may be the most extended production method.

Table 1.
Summary of main natural additives from vegetable sources[*] and EU E-number reference, if they are at present approved for use in the EU.

In parallel, the effectiveness and new potential applications of additives like food active packaging and their addition into novel food matrices are the focus of study by many research groups. To release new functional food to market, health claims and properties must be supported by scientific evidences to be legally labeled as functional food [19]. Thereupon, a great number of compounds or extracts may be obtained from natural sources, such as vegetables, fruits, algae or even by-products for their application as food and feed additives but also as cosmetic ingredients [20]. **Table 1** collects a list of selected natural additives that may be directly extracted from natural sources. Although these compounds are of natural origin and may be extracted from natural sources, efficient yields, costs and steady production has led the chemical and biotechnology industry to manufacture them by biological or chemical synthesis. Representative examples are ascorbic acid and citric acid. Ascorbic acid is a potent antioxidant and an essential vitamin with many uses in agriculture and food industry. While it is present in a wide number of fruits and vegetables (*i.e.* citrus, peppers, kiwifruit), the current production approach comprises microbiological synthesis with either bacteria or fungi, chemical synthesis from *d*-sorbitol, or a combination of both [48]. Natural colors, pigments such as chlorophylls, carotenoids and anthocyanins, are more feasible to obtain from their natural sources [49]. It is of great importance to carry out optimization process according to raw material and compounds of interest. This fact leads the increase of the operation performance and the reduction of costs. Therefore, developing optimum and environmental-friendly extraction procedures and methods is of great importance to value natural sources in a cost-effective way.

2. Conventional extraction technologies: advantages and drawbacks

Extracting compounds from complex vegetable matrixes requires the application of several techniques to extract and isolate the selected molecules. Thus, it is important to choose the best fitting extraction technique to the specific additive in need of isolation. Conventional extraction techniques, which have been applied for many years, include maceration, Soxhlet extraction, distillations, infusions, and cold-pressed extractions [50]. They are usually not eco-friendly due to the large amounts of solvents and energy required for their application [51], which also provides a safety concern for the workers and consumers involved, as well as the lack of sustainability, and green extraction protocols [52]. Besides, some

of these techniques are also very time-consuming which aggravates the energy spending problem as the equipment must remain working for long periods of time, being one of the biggest expenses heating or cooling. Lastly, the yields obtained using these methodologies are usually not as rentable as the ones produced using more innovative extraction techniques, that are faster and more efficient than the previously available protocols. For this reason, conventional methodologies are started to be conjugated with new technologies resulting in Soxhlet or distillations assisted by ultrasound or microwave technologies to respond to the current needs of the industry.

Nevertheless, these techniques are still used nowadays because they allow the extraction of compounds in a cost-effectively manner with simpler equipment [52], like the distillation of essential oils [53], or they achieved a better stability of the extracted compound to be used as additive, as is the case cold pressing for oils extraction [54].

2.1 Maceration

Maceration is one of the most known and used conventional extraction techniques [55]. It is a solid–liquid extraction achieved by applying heat and agitation to a previously selected solvent, with a convenient polarity, that is in contact with the sample of interest [52, 56].

Maceration has few advantages. It can be performed using low-cost and simple equipment compared with other conventional and innovative techniques. Besides, a large range of molecules can be extracted by changing the protocols and adapting the variables like solvent [57], temperature, agitation, and time in order to optimize the extraction of the desired compounds [58]. Furthermore, this extraction technique is still used due to its easy scale-up to several applications in the industry.

In comparison, maceration also has some major drawbacks. It often requires long extraction times, large volumes of solvent (mostly organic solvents), high temperatures which translate in a big amount of energy spent, and it has to be coupled with several filtration or centrifugation steps in order to separate the extract from the biomass [52, 59–62].

2.2 Soxhlet extraction

Soxhlet is a reference extraction method to evaluate the performance of other liquid–solid extraction methodologies [56]. This technique, developed in 1879, uses a particular type of condenser known as the Soxhlet apparatus [50]. The traditional Soxhlet extractor is composed by a thimble-holder where the sample is placed inside the thimble, and a distillation flask where fresh solvent is added. When the solvent reaches the boiling point, it vaporizes and enters the matrix, solubilizing compatible compounds. After that, the solvent hits the cooling tubes of the condenser and condense back into the initial flask with the extracted compounds. This operation repeats until the full extraction is completed [50, 52].

This type of extraction presents several advantages. Firstly, the constant renovation of the solvent in contact with the matrix, allows for a disequilibrium between the compounds in the sample and the lack of them in the solvent, favoring the extraction of these compounds. Secondly, the temperature of the system is maintained throughout the process. Soxhlet extraction also does not require filtration or centrifugation of the final extracts, being perfectly separated from the original biomass. And lastly, it allows for the treatment of several samples in parallel at a relatively low cost and easy operational processes, considering that the basic equipment is quite affordable and simple [52, 63].

However, Soxhlet extraction also presents some disadvantages, as a large amount of organic solvents required, long periods of extraction until the final number of cycles is completed [52], the high temperatures employed to boil the solvents that can degrade the compounds [50], and this technique cannot be accelerated by adding agitation [55, 63].

Nevertheless, the Soxhlet extraction has continued to evolve to try to compensate some of these disadvantages, by automating the process, aiming to shorten the extraction times, and even recently Soxhlet extraction has been coupled to innovative technologies like high-pressure Soxhlet extraction, supercritical fluid-Soxhlet extraction, and automated Soxhlet extraction or by applying auxiliary energies such as ultrasounds or microwaves, that results in higher efficiency than the conventional Soxhlet extraction [52, 63].

2.3 Distillation

Distillation is one of the oldest extraction techniques that has been used for over 5000 years and is still currently used. It main application is to separate liquid mixtures through the boiling point of each component of the mixture after which condensation steps take place [64]. Another ancient application is to create "distilled waters" from plant materials [65]. Distillation is still used in chemical refineries to separate and purify compounds, due to its simplicity and easy scale up [66]. Even though distillation processes are still quite common, they have many drawbacks. For example, the necessity for the consumption of large amounts of energy for long periods of time, as well as the high temperatures used which can degrade the additive of interest. In addition, the large amounts of solvent required [52] and the long extraction times [66].

Regarding the extraction of compounds from natural products, distillation is mostly used to isolate volatile molecules from mixtures of compounds of even several biological matrices, but it is only efficient in the case of thermostable molecules due to the high temperatures employed. Even so, steam distillation is still currently the most used technique in the food industry for extracting volatile compounds and essential oils to be used as food additives. Distillation can be divided into three types: water, water-steam, and steam distillations, and the extraction of essential oils is based on the latest, which the steam goes into a recipient containing the plant matrix, releasing the essential oils from the samples, then the essential oil is cooled and condensed, generating two different phases that can be separated [53].

2.4 Infusions

Infusions are very short macerations, where the plant is put in contact with boiling or sometimes cold water, for short periods of time. Therefore, infusions contain the readily soluble active chemical compounds that were present in the crude plant, in a diluted concentration. This methodology is used to obtain fresh infusions with the phytochemicals from aromatic or medicinal plants which can be further used as food additives [55]. Many infusions nowadays are prepared starting from a very concentrated infusion and diluting one volume of it to 9 volumes of water. To prepare the concentrated infusions the most common strategy is a percolation or modified maceration. In a modified maceration it is added 25% ethanol to the extraction solvent during or after the maceration process. The final solution is then diluted with water to resemble the scent and the potency of a normal fresh infusion.

One of the greatest susceptibilities of this method is that infusions are very prone to fungus and bacterial growth, due to the large amount of water they contain, so they have a very short shelf life and need to be used right away. For that reason, infusions are rarely used in industrial fields [67].

2.5 Cold pressing technique

This technique relies on pure pressure applied to the ground plant material to squeeze out what they contain. When the plant is well dried and grounded, this extraction technique is a viable, simple and eco-friendly option to consider for the extraction of some oils. During the process, cold pressers apply a large amount of pressure which translates into a mechanical rupture of the oil glands in the plant. The oil is extracted independently of its polarity and is rich in several lipophilic compounds. This process is still used in some industries because of its simplicity and easy scalability and for the fact that the obtained oils are more stable and resistant to oxidative stress than oils that have been refined or processed in any other way [54]. However, this technique can only be used to produce some specific vegetable and seed oil and it is not suitable to produce essential oils. Furthermore, the final product can many times contain other chemicals or contaminants [50].

3. Green extraction techniques

The application of green extraction techniques to obtain food natural additives is gaining great interest in recent years due to the growing demand of healthier and more sustainable products. **Figure 2** collects the main advantages of the green extraction techniques applied to natural sources.

3.1 Ultrasound assisted extraction (UAE)

Ultrasound assisted extraction (UAE) is used in different fields such as biomedicine or food technology, in which UAE is applied to obtain compounds of interest or as a pre-step in numerous technological procedures. There are several parameters to consider optimizing the method including ultrasound power intensities, frequency, wavelength and time. High frequency and low power ultrasounds are used in medical science. However, low frequency and high power ultrasounds are used in food industry [68].

Low frequency and high-power conditions produce cell disruption and the subsequent release of compounds present in the matrix [69–71]. This liberation of the

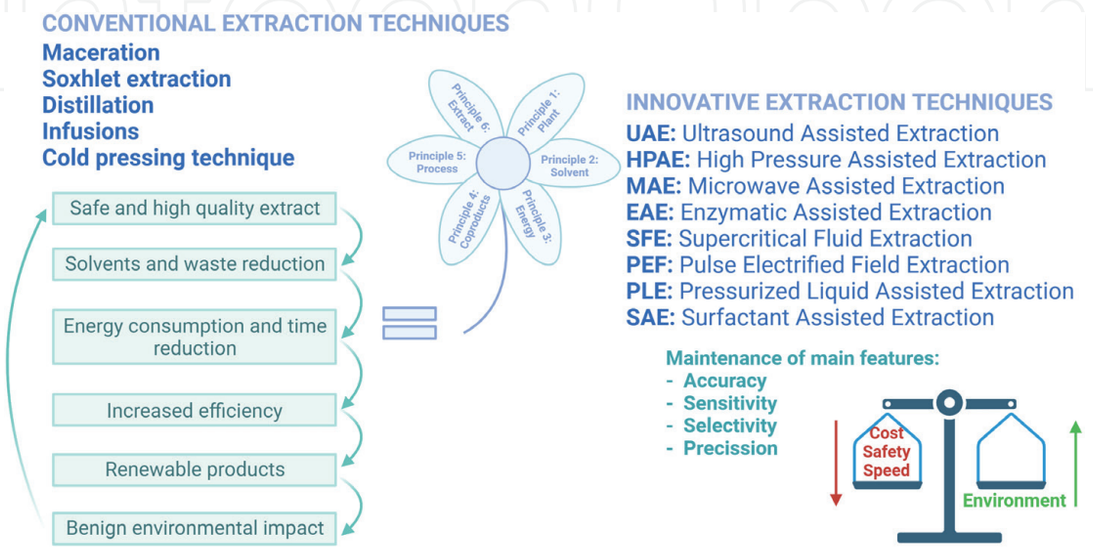

Figure 2.
Conventional and green extraction techniques development.

compounds is based on the principle of cavitation. This physical–chemical process comprises generating bubbles that grow in such a way that they explode causing the rupture of the cell wall of plants, and the consequent release of the substances in their interior [72]. Another important characteristic of ultrasonic-assisted extraction is that the hot spots created by cavitation bubbles during the extraction process hardly generate heating and have a great capacity to cool down while the process is taking place, in this way UAE is cataloged as an extraction method suitable for extracting thermolabile compounds. However, when the extraction is longer than five minutes, at high powers it is necessary to use some refrigeration to keep the temperature constant [73].

Among the advantages of this technology are reduction of solvents consumption, temperature and time, low equipment investment and easy implementation, so it can be basically industrially employed in local companies [74]. One of the main disadvantages of UAE is that heating can degrade the additives present in the sample. Other common applications of UAE in food industry are cooking of meat and vegetable, drying of dehydrated products, degassing of juices, sterilization and in the formation of emulsions among others [72]. This is due to UAE can reduce the activity of enzymes and microorganisms without modifying the organoleptic characteristics and the presence of bioactive compounds in food. **Table 2** collects some studies supporting the application of UAE to obtain bioactive compounds to be used as food additives from natural sources.

Several are the parameters that have influence on the UAE extraction. They include the type of the reactor, the ultrasonic intensity and frequency, the extraction time and temperature, the solvent proportion and nature [90]. In this sense, the intensity or power is proportional to the ultrasonic amplitude, however, a greater ultrasonic amplitude is not directly related to a better efficiency of the process. On the contrary, it could be related to certain problems such as those that cause the erosion of the probe and reduce the formation of cavitation which can even promote the degradation of the extracted compounds [91]. Regarding the frequencies used, they must be selected together with the ultrasonic intensity to obtain the desired cavitation. Higher extraction yields are reported in the low frequency range (20–40 kHz). Regarding temperature, high temperatures help to interrupt the interaction of the solvent and the matrix and improve the diffusion rates of the solvent, while low temperatures improve cavitation. Extraction time is another variable to consider. Long extraction time improves extraction yields; however, it can cause changes in the extracted compound. In addition, the nature of the solvent has influence in the UAE, so that a viscous solvent reduces cavitation and a volatile solvent can be evaporated if the extraction is carried out at a higher temperature for a long period. Finally, the size of the matrix, its interaction with the solvent and the ratio of solvent to matrix are also parameters to consider when UAE is used [91].

3.2 High pressure assisted extraction (HPAE)

High pressure assisted extraction (HPAE) is a novel technique used for extraction of active ingredients from plant materials. Its mechanism of action is based on two principles: the isostatic principle and the Le Chatelier's principle. Isostatic principle establishes that the pressure is exerted uniformly on the matrix regardless of its shape or constitution. In turn, according to Le Chatelier's principle, by applying a force (pressure) that alters the equilibrium, the system acts trying to minimize said disturbance [92]. To carry out this type of extraction, it is necessary to apply pressures ranging from 100 to 800 MPa, even in some cases reaching values of 1000 MPa, which has been proved to be more effective. However, HPAE cannot be applied for the extraction of all compounds since this technique can cause some

Compound	Raw material	Conditions					Yield	Ref.
		Freq.	Intensity	t	T	Solvent		
		kHz	W/cm^3	min	°C			
Phenolics	*Nephelium lappaceum* L. fruit peel	—	20	20	50	H_2O	5.53 mg GAE/g	[75]
Phenolics	*Plinia cauliflora* (jabuticaba) peel	25	150	10	30	EtOH	92.8 mg GAE/g	[76]
Phenolics	Microalgae	40	700	60	75	EtOH	9.8 mg GAE/g	[77]
Phenolics	*Nannochloropsis* spp.	24	400	5	21	EtOH	50%	[78]
Phenolics	*Ascophyllum nodosum*	20	750	25	21	0.06 M HCl	143.12 mg GAE/g	[79]
Phenolics	*Malva sylvestris* leaves	20	110	49	48	EtOH	279.9 mg GAE/g	[80]
Phenolics	*Elaeocarpus serratus* L. leaves	40	300	120	21	EtOH	92.4 mg GAE/g	[81]
Capsaicinoids	Peppers	20	360	10	50	MeOH	448 μmol/kg	[82]
Vitamin C	*Citrus sinensis* (orange) peels	20	400	30	21	EtOH	53.78 mg AA/100 g	[83]
Carotenoids	*Punica granatum* (pomegranate) wastes	20	130	30	51.5	Sunflower oil and soy oil	93.8%	[84]
Carotenoids	*Daucus carota* (carrots)	20	22.5	20	40	Sunflower oil	334.75 mg/L	[85]
β-Carotene	*Daucus carota* (carrot) wastes	20	100	50	50	—	83.32%	[86]
Sulfated polysaccharide	*Nizamuddinia zanardinii*	20	196	58	70	EtOH	3.51%	[87]
Fucoidan	*Fucus evanescens*	35	150	15	23	H_2O	3.63%	[88]
Polysaccharides	*Silvetia compressa*	—	3.8	—	50	EtOH	23%	[89]

Abbreviations: EtOH: ethanol; MeOH: methanol; T: temperature; t: time; Freq: frequency.

Table 2.
Different experimental conditions carried out with ultrasound assisted extraction (UAE).

structural changes in foods, such as cellular deformation, cellular membrane damage or protein denaturation. The parameters that must be optimized to increase the extraction yield are type and amount of solvent, temperature, pressure, extraction time and number of cycles [93].

Among the advantages of HPAE are the improvement of the mass transfer rate, the enhancement of solvent permeability in cells as well as secondary metabolite diffusion. Other advantages include shorter extractions times, the process may be performed at room temperature (avoiding thermal degradation of heat labile components) and higher extraction yields. In addition, the use of solvents with different polarity allows to extract a great variety of compounds [94].

Some studies can already be observed that support the application of HPAE to obtain bioactive compounds derived from both plants and food (**Table 3**). However, research in the field of HPAE is in its initial stages, and more in-depth studies are still needed to determine the full potential of HPAE.

Compound	Raw material	Conditions				Yield	Ref.
		P	t	T	Solvent		
		MPa	min	°C			
Flavonoids	*Ficus carica* L. (fig)	600	18–29	—	40% EtOH	35%	[94]
Phenolics	*Olea europaea* (olive)	10.3	110	60	EtOH	386.42 mg GAE g/ extract	[95]
Phenolics	*Chenopodium formosanum*	600	5	25	70% EtOH	567–642 mg GAE/g	[96]
Melanoidins	*Allium sativum* (garlic)	300	5	25	DW	(595.14 ± 12.14 μg/ mg melanoidins	[97]

Abbreviations: EtOH: ethanol; P: pressure; T: temperature; t: time; DW: distilled water; DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical method; GAE: gallic acid equivalent.

Table 3.
Different experimental conditions carried out with high pressure assisted extraction (HPAE).

3.3 Microwave assisted extraction (MAE)

Microwave assisted extraction (MAE) is a technique which combines conventional solvent extraction with microwave heating. Microwaves are composed of electric and magnetic fields with spectral frequency ranging from 300 to 300,000 MHz [98]. Many reports have been published on the extraction of secondary metabolite from plants using MAE. Some of them are collected in **Table 4**. The advantages of this technique include high efficiency, rapid temperature rise, short extraction time, better process monitoring, and low energy consumption and cost [104]. One of the disadvantages of MAE is the degradation of some compounds due to the heat produced by irradiation. The efficiency of the MAE depends on factors such as the extractant nature, the power of microwave irradiation, the temperature, and the extraction time as well as the characteristics of the matrices and the solvent-food relationship. The extraction efficiency usually behaves directly proportional to the microwave power due to the local heating that contributes to the rupture of the matrix. However, there is a limit in the microwave power that can cause a decrease in the extraction efficiency. In this sense, Alara and co-workers point out that a power between 400 and 500 W in the microwave, the extraction amount of phenolic compounds is greater than that achieved using a power upper 500 W [111]. This is because overexposure to radiation from the microwaves produce overheating and degradation of compounds [112]. On the other hand, the choice of solvent influences the extraction of compounds. It was reported that a mixture of organic solvents and water has a desirable impact on the extraction efficiency. On the contrary, it was observed that the presence of water in organic solvents leads to greater penetration of the extractant in the matrix molecules promoting microwave heating and causing a positive impact on the general efficiency and extraction time compared to MAE that uses only organic solvents [113].

Also, the toxicity of the solvent is another important factor that must be evaluated with respect to the selection of a suitable extractant for MAE [114] since some theories highlight the efficiency and selectivity of MAE depends on the dielectric constant of the solvent mixture [115]. The polarity of organic solvents increases with the addition of water, the temperature within the sample increases due to better absorption of microwave energy, and extraction increases. The extraction time is another parameter that influences the extraction by MAE. Long times of extraction decreases the yield due to the alteration of the structural integrity of the

Compound	Raw material	Conditions of extraction				Yield	Ref.
		T	t	Power	Solvent ratios		
		°C	min	W			
Polysaccharides	*Potentilla anserina*	63.3	76.8	369	Water:raw material 14.5:1	13.33%	[98]
Polysaccharides	*Actinidia chinensis* (kiwi)	80	120	480	EtOH (80%) 1:10	2.92%	[99]
Polysaccharides	*Ascophyllum nodosum*	120	15	—	EtOH (80%) 1:10	16.08%	[100]
Polysaccharides	*Palmaria palmata*	70	10	500	Water 1:70	17.01%	[101]
Polysaccharides	*Ribes nigrum*	30	41	414	Water 1:30	10.59%	[102]
Essential oil	*O. vulgare* (oregano)	60	50	600	Water 20:1	7.1%	[103]
Crocetin	*Crocus sativus* L. (saffron)	96	30	2.45 GHz	59.59% EtOH	228 mg/g	[104]
Polyphenols	*Malus domestica* (apple) skin	150	90	60	Water 1:10	50.4 mg GAE/g	[105]
Phenolics	*Hibiscus sabdariffa (hibiscus)*	164	12.5	850	EtOH (45%)	42.4%	[106]
Phenolics	*Aristotelia chilensis*	100	2	800	MeOH (60%)	54.3 mg/g	[107]
Usnic acid	*Cladonia foliacea*	80	5	—	ACE 1:10	4.2 mg/g	[108]
Flavonolignan	*Silybum marianum*	30	12	600	EtOH (80%) 1:25	79%	[109]
Vicine	*Vicia faba* (beans)	30	0.5	1140	MeOH (50%)	—	[110]

Abbreviations: EtOH: ethanol; MeOH: methanol; ACE: acetone; T: temperature; t: time; GAE: gallic acid equivalent.

Table 4.
Different experimental conditions carried out with microwave-assisted extraction (MAE).

chemically active principles. In this way, the extraction time in most of the MAE process is ranged from a few minutes to 30 min. Nevertheless, when MAE is used without solvent, longer extraction times can be employed. In this case, extraction cycles can be used to reduce the degradation of the compounds [116]. Additionally, the agitator effect influences the extraction process, reducing the negative effects of the S/F ratio on extraction recovery [116].

3.4 Enzyme assisted extraction (EAE)

Enzymes are protein molecules whose function is to catalyze chemical reactions. Due to this ability to accelerate reactions, enzymes have always been important to food technology and are widely used to transform raw materials into improved food

products such as starch processing, meat processing, dairy industry, wine industry and manufacturing of predigested foods [117]. However, with the advancement of technology, new applications have been developed as well as new sources for obtaining enzymes, being microbial enzymes the preferred source due to the advantages they present, among which it is worth highlighting an easy, profitable and constant production [118]. Among the novel applications of enzymes is the extraction of compounds of interest from different raw materials to be used as additives in the food industry. However, they are typically used for a feedstock pretreatment that makes conventional solvent extraction or distillation more efficient. Enzymes promote access to the substances of interest, so its applications include, among others, the extraction of flavor and color from plant materials as a pre-treatment of the raw material before subjecting the plant material to hydro-distillation/solvent extraction [119].

This entire process must be optimized to obtain the extract with the highest yield, that is, in a more purified and profitable way. To carry out this optimization, it is necessary to know the mechanism of action of the enzymes and, therefore, their optimal conditions of activity, being pH and temperature of vital importance. Another important factor to take into account is the majority composition of the raw material, which will determine the type of enzyme to be used (lipases, celluloses, proteases) [120]. This system has been investigated for extracting lipids, proteins, polysaccharides, phenols and oils among others (**Table 5**).

This technique has the advantages over other traditional methods of having a high selectivity and efficiency, being an environmentally friendly process with a minimum consumption of energy and chemical products, having good performance and the possibility of recycling the process. However, it also has some drawbacks such as the cost of the enzymes, the need for storage tanks that may require long-term incubation, the lack of knowledge about optimal or compatible enzyme formulations for cell disruption and the inability to fully hydrolyze the bonds in the plant cell wall [136].

3.5 Supercritical fluid extraction (SFE)

Extraction by supercritical fluids is characterized by bringing the fluid to conditions of temperature and pressure above the critical point, at which time the extracting agent behaves as a liquid and a gas simultaneously. Among the advantages of this method are the absence of toxic residues in the final product, high selectivity, short times, low consumption of solvents, high stability of the product obtained and that the remaining biomass can be treated with other techniques to continue the extraction. This technique can also be used to remove undesirable compounds such as pollutants, toxins, and pesticides [136].

The extraction process takes place in several stages. At the beginning, the plant matrix absorbs the supercritical solvent, producing a swelling of the cell structure with the consequent dilation of the intercellular channels. This results in a decrease in resistance to mass transfer. In addition, there is a simultaneous transfer of matter from the internal matrix to the surface. After that, these compounds are transported from the surface to the supercritical solvent and finally removed from the solvent [137].

To increase extraction performance, several parameters need to be optimized. These are fundamentally temperature, pressure and co-solvent type and all depend on the compound to be extracted. The most widely used solvent is CO_2 for its thermodynamic and heat transfer properties. Furthermore, it has a low critical point (31°C, 73 bar). In addition, the polarity of CO_2 can be modified by using co-solvents such as ethanol, so that the polar components are also extracted [138].

Several reviews have been published on SFE fundamentals, experimental design and specific applications on food processing, surface coating analysis, vegetable matrices, extraction of metals as complexes, functional ingredients from natural

Compound	Raw material	Enzyme	Conditions			Yield	Ref.
			T	pH	t		
			°C	—	h		
Proteins	*Glycine max* (soy)	Protease	50	9.0	1	97%	[121]
Proteins	*Brassica napus* (rapeseed)	Pectinase, cellulase, β-glucanase	48	10.0	4	83.0%	[122]
Proteins	*Arachis hypogaea* (peanut)	Alcalase	60	9.5	1.5	88.2%	[123]
Lipids	*Rubus idaeus* (raspberry)	Proteases	60	9.0	2	38%	[124]
Polysaccharides	*Allium sativum* (garlic)	Cellulase	45	5.0	1.3	35.3%	[125]
Polysaccharides	*Medicago sativa* (alfalfa)	Cellulase, pectin, pectase	52.7	3.9	2.73	5.1%	[126]
Phenols	*Vitis vinifera* (grape) marc seeds	Pectinase	48	3.5	2.7	18–20 mg/g	[127]
Phenols	*Brassica oleracea* (cauliflower)	Viscozyme	35	4.0	0.5	0.6 mg/g	[128]
Phenols	*Ulmus pumila* (elm tree)	Cellulase, pectinase, β-glucosidase	52.6	4.6	1	16.04 mg/g	[129]
Oils	*Citrullus lanatus* (watermelon) seeds	Protex	47.1	7.9	7.8	97.9%	[130]
Oils	Yellow mustard flour	Protex	60	4.5	3	91.0%	[131]
Vitamin C	*Malpighia emarginata* (acerola)	Celluclast	50	4.5	2	23.4 g/L	[132]
Carotenoids	*Capsicum annum* (pepper)	Viscozyme L	60	4.5	1	87.0%	[133]
Carotenoids	*Capsicum annum* (pepper)	Viscozyme L	50	4.5	5	78.0%	[134]
Lycopene	*Solanum lycopersicum* (tomato)	Pectinase	60	5.0	0.4	1.1 mg/g	[135]

Abbreviations: T: temperature; t: time.

Table 5.
Different experimental conditions carried out to obtain additives by enzyme-assisted extraction.

sources, constituents of fish oil and decontamination of hazardous substances [139]. **Table 6** shows some examples. Despite the large number of studies using this extraction technique, the comparatively high cost of investment has kept this expertise from being broadly considered as an alternative. Nevertheless, new studies have proved that SFE is an economically viable choice [152]. Moreover, the interest in SFE is not only at the laboratory level as an analytical tool but also in industrial processing, mainly decaffeination of coffee or tea, extraction of essential oils, extraction of high added value compounds and fatty acids [153].

Compound	Raw material	Conditions					Yield	Ref.
		Pressure	T	Flow	t	Mass		
		bar	°C		h	g	mg/g	
Lipids	*Hypnea charoides*	241–379	40–50	1 mL/min	2	2	58 mg/g	[140]
Vitamin E	*Spirulina platensis*	361	83	50 mL/min	1.5	75	29.4 mg/g	[141]
Protein	*Spirulina platensis*	350	40	400 g/min	4	—	—	[142]
Astaxanthin	*Haematococcus pluvialis*	435	65	167 mL/min	3.5	240	87.4%	[143]
Lycopene	*Solanum lycopersicum* (tomato)	300	60	1.44 cm/min	6	1	213 mg/g	[144]
Capsaicinoids	*Capsicum chinensis* (chili pepper)	150	60	2 mL/min	1.4	2.5	0.5%	[145]
Flavonoids	*Mentha spicata* (mint)	200	60	15 g/min	1	30	60.57 mg/g	[146]
Phenols	*Vitis lambrusca*	160	45	0.2 mL/min	0.3	3	12.3%	[147]
Decaffeination	Green tea	300	80	1500 mL/min	2	10	70.2%	[148]
Decaffeination	*Ilex paraguariensis* (yerba mate)	300	60	15.83 g/min	4.25	0.65	99.97%	[149]
Aroma	Vinegar	350	50	0.42 g/min	2	200	96.6%	[150]
Sulforaphane	*Brassica oleracea* (cauliflower)	250	60	2 g/min	3	25	0.47%	[151]

Abbreviations: T: temperature; t: time.

Table 6.
Different experimental conditions carried out with supercritical-fluid extraction (SFE).

The extraction through supercritical fluids presents greater efficiency in terms of increased yields and shorter extraction times compared to conventional methods. There are several factors that could limit its effectiveness, including solvent type, temperature, pressure, extraction time and particle size of the matrix to be studied [153]. Among them, it seems that the effectiveness of an SFE depends mainly on temperature and pressure. When temperature and pressure variations are considered, these have important repercussions on fluid hydrodynamics, solubility, and mass transfer [146]. In this sense, the low temperature is crucial for the conservation of bioactive compounds in the extracts and to achieve higher global/specific yields or greater bioactive capacities in the extracts. Numerous studies apply an extraction temperature range of 40–50°C [154–157]. Regarding pressure, there is evidence that the optimum range is between 200 and 400 bars [154, 158–161]. Additionally, an increase in pressure can modify the solubility of the solute, therefore, it is interesting to control the composition of the extract by pressure [162]. In addition, the optimal conditions of pressure and temperature can be influenced by the origin of the biomass and their morphology. The type of modifier and its proportion are also of extreme importance within the extraction, since they determine the solubility of the analytes, ethanol being the most applied modifier [155]. The size of the biomass particle, shape and porosity are also important factors in terms of the mass transfer rate [152]. The use of small particles would have the advantage of increasing the accessible solute, but could lead to clogging of the extraction, making the use of dispersing agents useful in such cases.

3.6 Pulse electrified field extraction (PEF)

Electric pulse is one of the newest techniques in the field of thermal food processing. Its mechanism of action is based on causing the permeability of cell membranes in a short period of time and with low energy consumption. This is achieved by applying short duration pulses (μs to ms) of moderate electric voltage (typically 0.5–20 kV/cm) to a substrate of choice placed between two electrodes, which is commonly used for preservation, enzyme and microbial inactivation purposes [163].

These characteristics have led to different studies being carried out in order to improve the extraction performance of bioactive compounds, such as polyphenols, anthocyanins and vegetable oil from plant tissues and their by-products, as well as soluble intracellular matter of microorganisms [164]. However, low to mild PEF treatment intensities are often considered an effective pretreatment method for enhancement of secondary metabolite extraction yields in cell cultures and plant systems [165].

Critical parameters of the process are electric field intensity, treatment time (number of pulses x pulse duration), pulse waveform, conductivity, pH and ionic strength of the medium [166]. Among the advantages of this method are avoidance of undesirable changes in a biological material, which are typical of other techniques such as thermal, chemical and enzymatic ones. Moreover, it is able of killing microorganism [167].

As it can be observed in **Table 7**, most PEF-assisted extraction studies from by-products have targeted the polyphenolic compounds; nonetheless, extraction of polysaccharides, proteins, phytosterols, alkaloids, seed and germ oil among others have also been investigated. In general, according to the studies available so far, it has been demonstrated that the application of moderate electric field pulse technology either as pretreatment step or as continuous extraction system improves the extraction in the area of phytochemical extraction from by-products.

3.7 Pressurized liquid assisted extraction (PLE)

This technique is also known as Accelerated Solvent Extraction (ASE), Pressurized Hot Water Extraction (HSPE), Pressurized Fluid Extraction (PFE) or Enhanced Solvent Extraction (ESE) [178]. This technique, firstly described in 1996 as a new emerging environmentally-friendly technique, presents the following advantages [179]: reduced use of solvents, lower energy consumption and short time periods (5–10 min). It consists of an extraction procedure which uses organic liquid solvents applied at high pressures (normally up to 200 bar) and temperatures (normally up to 200°C) to extract target compounds, this is, PLE is based on applying high pressures to heat the extraction solvent above its boiling point while using low volume of organic solvents [178, 180].

Extraction efficiency and selectivity is significantly affected by the following parameters, namely: i) extraction solvent; ii) temperature; iii) pressure; iv) static extraction time and number of cycles and v) sample weight [178]. Temperature and pressure have a significant effect on PLE, therefore, they are the most important for PLE optimization. Many studies support that the use of solvents at high temperatures and pressures improve the yield in comparison to other conventional techniques [178, 180, 181].

On the other hand, two types of instrumentation can be used in PLE: static and dynamic. However, generally, the equipment is very similar between them, except for some small differences such as a more sophisticated high-pressure pump and a pressure restrictor in the case of dynamic PLE. The extraction design equipment can contain the following basic parts: i) solvent supply; ii) a pump; iii) a heater; iv) a pressure vessel where occurs the extraction and v) a collected vessel for the extract

Compound	Raw material	Conditions						Yield	Ref.
		E	Power	Pulse t	No. pulses	Mass	Flow		
		kWh/kg	kV/cm	μs	—	g	mL/min	mg/g	
Proteins	*Saccharomyces cerevisiae*	—	40	8.3	500	0.5% w/w	166	1%	[168]
Polysaccharide	*Agaricus bisporus* (common mushroom)	—	38.4	2	136	9% w/w	336	7.9 mg/g	[169]
Polysacharide	*Zea mays* (corn) silk		30	6		50% w/w	25	7.3%	[170]
Pigments	*Beta vulgaris* (red beetroot)	7	1	10	270	—	48	90%	[171]
Betanine	*Beta vulgaris* (red beetroot)	2.5	7	2	5	—	—	90%	[171]
Anthocyanins	*Solanum tuberosum* (potato)	13.5	3.4	3	35	14 g	—	0.66 mg/g	[172]
Anthocyanins	*Citrus sinensis* (orange) peel	3.77	3.4	3	105		—	65.8 mg/g	[173]
Anthocyanins	*Vitis vinifera* (grape) skin	10	3.0	15	30	—	—	14.05 mg/g	[174]
Phenols	*Citrus sinensis* (orange)	—	7	3	20	100	—	3.1 mg/g	[173]
Phenols	*Agaricus bisporus* (common mushroom)	—	38.4	2	68	9% w/w	498	51%	[169]
Oil	*Zea mays* (corn) germ	0.62	0.6	—	120	2	1	88.4%	[175]
Copra	*Cocos nucifera* (coconut)	—	2.5	575	20	—	—	20%	[176]
Alkaloids	*Solanum tuberosum* (potato) peel	18.5	0.75	3	200	—	—	1.85 mg/g	[177]

Abbreviations: E: energy; T: temperature; t: time.

Table 7.
Different experimental conditions carried out with pulse electrified extraction (PEF).

[182]. Regarding static PLE, the process occurs in one or several extraction cycles with replacement of solvent in between. When the static time finishes (normally between 5 and 10 min), part of solvent in the extraction cells is replaced with fresh solvent (new extraction cycle). After the last cycle, the nitrogen gas is used for purging the sample cell and to remove the remaining solvent. As regards dynamic PLE, the extraction solvent is continuously pumped through the extraction vessel containing the sample [183]. Currently, static PLE is the most used and there are not equipment of dynamic PLE in the market [184]. This system has been employed to recover different target compounds that can be used as food additives, including proteins, phenolic compounds or fatty acids, among others (**Table 8**).

Parameters such as the characteristics of the matrix, the solvent type, the extraction time, and temperature show a big influence on the extraction efficiency of PLE. It is pursued an appropriate combination of analyte and solvent to achieve high diffusion rates and mass transfer, the use of binary solvents such as ethanol: water or methanol: water is more efficient and respectful with the environment than pure reagents [193]. Regarding the characteristics of the matrix, they affect the recovery rate of the compounds, including the nature of the target compounds, the relative binding behavior of the analyte with the solvent, the particle size, and the moisture content [181]. Additionally, temperature and pressure significantly influence the

Compound	**Raw material**	**Conditions**					**Yield**	**Ref.**
		P	**T**	**Solvent**	**Static cycles**	**t**		
		MPa	**°C**			**min**		
Proteins	*Punica granatum* (pomegranate) peels	10.34	120	E 70% (v/v)	4, s.t. 3 min	12	9%	[185]
Phenolic compounds	*Hibiscus sabdariffa (hibiscus)*	10.34	200	E 100% (v/v) E	1	20	—	[186]
Fatty acids	*Anacardium occidentale* (cashew)	8	80	E-Pr	4, s.t. 5 min; 4, s.t. 10 min	60	19.2–33.1%	[187]
Cafestol and Kahweol diterpenes	*Coffea arabica* (coffee) beans	10.34	70	E (99.3%)	3, s.t. 8 min	24	9.78%	[188]
Anthocyanins	*Phaseolus vulgaris* L. (black beans) hulls	10	60	E-CA (30:70) (v/v)	1	26	3.96 mg C3GE/g DW	[189]
Phenolic, antioxidant and antimicrobial compounds	*Psidium guajava* L. (pineapple guava) peels	10	80	E E-W	1	60	42% 50%	[190]
Antihyaluronidase	*Padina pavonica*	15	60	W	2, s.t. 10 min	20	0.04 mg/mL (IC50)	[191]
Polysaccharides	*Campomanesia xanthocarpa* (gabiroba) pulp	15	120	UW (pH 6.9)	1	15	5.70% of pectin	[192]

Abbreviations: P: pressure; T: temperature; t: time; C3GE: cyanidin-3-glucoside equivalents, DW: dry weight; s.t.: static time. W: water, E: ethanol, CA: citric acid, Pr: propane, UW: Ultrapure water.

Table 8.
Different experimental conditions carried out with pressurized liquid extraction (PLE).

selectivity and efficiency of PLE. The use of high temperatures under reduced pressure helps to break the matrix structure by overcoming the molecular bonding forces [194] In this way the use of high temperatures reduces the activation energy and overcomes the interactive forces (cohesive and adhesive) between the matrix and the solvent molecules to cause desorption. However, the use of high pressure causes bubbling problems during extraction, causing low solubility rate [181].

3.8 Surfactant assisted extraction (SAE)

Surfactants are components able to lower the surface tension between two liquids (gas–liquid) or between liquid and solid. They are usually amphiphilic organic compounds: with a polar group in the head part (hydrophilic) and a nonpolar group in the queue part. Their distinctive characteristic is their capacity to lower surface tension of a solvent. Generally, the higher surfactant concentration in the solution, the lower surface tension. This occurs due to adsorption of the surfactant at the interface. Surfactants can be classified following two criteria: according to its structure or its hydrophilic–lipophilic balance (HLB).

Regarding its structure, some authors divide surfactants in three groups: i) ionic: within this group are anionic and cationic; ii) nonionic (without electric charge); iii) amphoteric (they contain anionic and cationic groups in the molecule) and v) no hydrocarbons. Among them, nonionic surfactants are the most used [195]. Regarding its balance, Griffin and co-workers introduced an arbitrary scale for classifying surfactants according to HLB. The HLB scale has a ranges from 0 to 20, so that a molecule is hydrophilic when HLB value is above 9, and lipophilic when HLB value is below 9 [196].

One of the main parameters used in surfactant assisted extraction (SAE) is the adsorption efficiency (pC_{20}). The higher $pC_{20,}$ the lower concentration of surfactant required to surface tension of pure solvent to decrease to 20 mM/m. Ideally, a surfactant employed to extract a compound of interest should have a high adsorption efficiency. A study verified that pC_{20} was higher when the surfactant consists of an anionic and nonionic mixture than when the surfactant was only anionic [197]. Nowadays, SAE is not used as an individual technique to extract bioactive compounds in food or nutraceutical industry. Generally, it is used as a complement in other type of extractions, mainly with MAE. A study employed surfactant-MAE method to obtain high yield bioactive compounds from fig leaves [198]; another study used a MAE - solid–liquid extraction approach using a surfactant to extract bioactive phenolic compounds from *Vitis vinifera* leaves [199]. Also, different ionic liquid based surfactants were used together with MAE to extract flavonoids from *Mangifera* sp. and *Passiflora* sp. leaves [200].

4. Conclusions

There are many natural compounds with possible potential applications as preservatives to act either antioxidants, antimicrobials or to provide specific flavor, aroma, or color to foods. A great number of compounds can be obtained from natural sources, such as vegetables, fruits, plants, macroalgae and microalgae for their application as food and feed additives but also as cosmetic ingredients.

To isolate the molecules of interest to be used as food additives, it is necessary to apply adequate extraction techniques which preserve intact the *compounds*. Among them, green extraction techniques are becoming popular due to the society growing demand in sustainable products. Green extraction methods, which comprise UAE, MAE, EAE, PEP and high-pressure methods (SFE and PLE), have some advantages over the traditional extraction methods such as lower solvent and resources consumption, as well as matching and extraction yields improvement, in some cases. Besides,

these techniques are in line with the green aspects of sample preparation, they avoid the use of harmful reagents and minimize the use of organic solvents. In this context, several studies investigating the effects of the extraction parameters of these novel techniques on yield and composition pointed the importance of optimizing the extraction time, temperature and solvent type depending on the characteristics of the matrix. Also, in the case of UAE and MAE, other parameter to considered are the frequency and the ultrasonic intensity (UAE) and power of microwave irradiation (MAE). Higher extraction yields are reported in the low frequency range (20–40 kHz). Moreover, European politics on circular economy highlight the importance of food revalorization. By-products represent a rich source of valuable additives with high antimicrobial and antioxidant activities. And in many cases, these additives are being obtained through the employment of environmentally friendly extraction techniques. Thus, these techniques are being used in various types of industries due to the benefit in the decrease of the time needed to achieve sample extraction.

Abbreviations

DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical method
GAE	Gallic Acid Equivalent
GRAS	Generally Recognized as Safe
EFSA	European Food Safety Authority
FDA	*Food* and Drug Administration
LDL	Low-density lipoprotein
UAE	Ultrasound Assisted Extraction
HPAE	High Pressure Assisted Extraction
MAE	Microwave Assisted Extraction
EAE	Enzyme Assisted Extraction
SFE	Supercritical Fluid Extraction
PEF	Pulse Electrified Field extraction

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