“Extraction techniques for the determination of phenolic compounds in food”

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ABSTRACT

Considering the importance of phenolic compounds as potential antioxidants and their complex chemical structure and distribution, it is of outmost importance to be able to correctly assess their content in food commodities and therefore to understand their possible biological effects. In this chapter an updated overview of the extraction methods used to determine phenolic compounds in foods is presented, ranging from more traditional to advanced extraction processes. The main extraction protocols employed to extract the different type of food phenolics (including phenolic acids and flavonoids) are discussed considering different applications. New developments in the field of extraction techniques applied to phenolics' extraction are reviewed and compared to the traditional processes. Therefore, the goal of this contribution is to provide the reader with a broad view on the different extraction protocols and techniques that are used to extract phenolic compounds from different kind of foods.

5.08.1. INTRODUCTION

In the last 15 years, researchers and food producers have been increasingly interested in phenolic compounds, mainly because of their potential effects as antioxidants. Phenolic compounds have a great advantage compared to other antioxidants, since they are widespread in plant-based foods and therefore are consumed in important amounts on a daily basis (the estimated rate of consumption of phenolic acids is 25 mg to 1 g per day¹ while flavonoid intake can range from 50 to 800 mg a day, both depending on the diet, meaning consumption of vegetables, fruit, grains, tea, coffee, red wine, etc.), and they have been suggested as playing an important role in the prevention of several diseases associated to oxidative stress, such as cancer, cardiovascular and neurodegenerative diseases.²⁻⁴

Food industry has released several functional food products based on their phenolics content, usually higher than traditional foods but, at present, a strong focus should be paid to the establishment of the evidence of the action of phenolic compounds consumption on health and to identify the compounds responsible of such activity. By covering first the nature and distribution of phenolics in foods and diet, researchers should be able to understand the relationship between the intake of such compounds and the risk of development of certain diseases. Important aspects to consider are the different bioavailability of phenolics,⁵ their transformation due to processing and/or storage,⁶ their possible interaction with gut microbiota,⁷ etc.

Since all these properties are strongly dependent on the phenolics chemical structure, a considerable effort should be put in the correct extraction, separation and identification of such compounds from foods. Undoubtedly, considering the importance of phenolics correct characterization, extraction techniques involving an isolation of intact structures

should be always preferred, together with other general aspects required nowadays such as the lower use of organic solvents, the miniaturization, the possible automation, the effectiveness, selectivity, etc.

In this book chapter an updated overview of the extraction methods used to determine phenolic compounds in foods is presented, ranging from more traditional to advanced extraction processes. Conventional extraction techniques are often characterized by using large amounts of organic solvents and are based on manual protocols which are labor-intensive and highly dependent on the operator, therefore, not perfectly reproducible. Techniques such as liquid-liquid extraction (LLE), solid-liquid extraction (SLE) or Soxhlet extraction are among the most used techniques. In order to overcome some of the above mentioned drawbacks, new extraction processes have been developed with the aim to provide characteristics, such as enhanced selectivity, automation, lower consumption of organic solvents, higher extraction efficiency, etc. Techniques such as supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), subcritical water extraction (SWE), ultrasounds assisted extraction (UAE), microwave assisted extraction (MAE), solid phase extraction (SPE), solid-supported liquid-liquid extraction (SSLLE), high hydrostatic pressure extraction (HHPE), matrix solid-phase dispersion (MSPD) and counter-current chromatography (CCC) are some of the extraction techniques belonging to the group of advanced extraction techniques that have been applied to phenolic compounds extraction, isolation and fractionation. Therefore, the goal of this contribution is to provide the reader with a broad view on the different extraction protocols and techniques that are used to extract phenolic compounds from different kind of foods.

5.08.2. TYPES AND DISTRIBUTION OF PHENOLIC COMPOUNDS IN FOODS

Plants are able to synthesize a multitude of organic molecules known as secondary metabolites;⁸⁻⁹ they are synthesized both under normal development and in response to conditions such as plant infection, wounding, UV-radiation, extreme temperatures, etc.¹⁰⁻¹² These compounds can be classified into different groups as a function of the number of phenol rings they contain and of the structural elements that bind these rings to one another. Figure 1 shows the chemical structures of different phenolic compounds and classified them into phenolic acids, flavonoids, stilbenes and lignans.⁵

< Insert Figure 1 near here >

Phenolic acids

Two types of phenolic acids can be distinguished as plant metabolites: hydroxybenzoic acids and hydroxycinnamic acids. Although the basic skeleton is the same, the numbers and positions of the hydroxyl groups on the aromatic rings establish the variety of phenolic acids that can be found in different foods.

Hydroxybenzoic acids are present in small amounts in edible plants except in some red fruits, black radish and onions, which can have a concentration of several tens of milligrams per kilogram fresh weight.¹³ Also, tea is an important source of gallic acid, containing up to 4.5 g/kg fresh wt.¹⁴ Hydroxybenzoic acids can be also found as components of complex structures such as hydrolysable tannins (gallotannins in mangoes and ellagitannins in red fruits such as strawberries, raspberries, etc.).¹⁵

The hydroxycinnamic acids are present in nearly all plants; acids such as caffeic, *p*-coumaric, ferulic and sinapic are usually found in bound forms glycosilated or as esters of quinic acid (for example chlorogenic acid which is caffeic acid esterified with quinic

acid, that is found in many fruits and coffee), shikimic acid and tartaric acid. Table 1 shows the content of polyphenols in food.⁵ As can be seen, caffeic acid is the most abundant phenolic acid in most fruits (mainly found in outer parts of ripe fruit). Ferulic acid is the most abundant phenolic acid in cereal grains, which is in fact its main dietary source; it can be found in wheat grains,^{16,17} rice, oat, maize,¹³ etc.

< Insert Table 1 near here >

Flavonoids

Flavonoids share a common structure consisting of 2 aromatic rings (A and B) bound together by 3 carbon atoms that form an oxygenated heterocycle (ring C) (see Figure 2 for the basic structure of flavonoids). They can be divided into 6 subclasses as a function of the type of heterocycle involved: flavonols, flavones, isoflavones, flavanones, anthocyanidins and flavanols (catechins and proanthocyanidins) (Figure 3).

< Insert Figure 2 near here >

< Insert Figure 3 near here >

Flavonols

They are the most ubiquitous flavonoids in foods, the main representatives are quercetin, kaempferol and myricetin. They are present mainly in onions¹⁸ at concentrations up to 1.2g/kg fresh wt, but also in kale, leeks, broccoli, etc. (Table 1). They can also be found in red wine and tea in their glycosylated form with glucose or rhamnose. Fruit often contains between 5 and 10 different flavonol glycosides,¹⁹ that accumulate in the outer and aerial part (leaves and skin). It is described that big

differences in concentration can be found in different fruits, even coming from the same tree since their biosynthesis is favored by sunlight exposure.²⁰ Similar behaviour is found in leafy vegetables, in which the concentration is also higher in the green outer leaves, as compared to the less colored inner ones.²¹ Flavonols can also be found in beverages such as apple and cranberry juices and tea.²²

Flavones

Flavones are less common than flavonols in fruits and vegetables. They consist basically of glycosides of luteolin and apigenin. Few sources of flavones have been identified, mainly parsley, celery and sweet bell pepper (Table 1). Cereals such as millet and wheat contain glycosides of flavones.²³ On the other hand, polymethoxylated flavones such as tangeretin, nobiletin and sinensetin are found in large amounts in citrus skin. Figure 4 shows some of the structures of the polymethoxylated flavones in citrus compounds.

< Insert Figure 4 near here >

Isoflavones

Isoflavones are a subclass of flavonoids described as phytoestrogens since they exhibit the ability to bind to estrogens receptors and, although they are not steroids, they have hydroxyl groups in positions 7 and 4' in a configuration analogous to the one in the estradiol molecule. Isoflavones differ from other flavonoids in the position of the benzene ring B in C3. Isoflavones are found in leguminous plants, being soya and its processed products the main source in the human diet. Isoflavones from soybeans include 3 main molecules: genistein, daidzein and glycitein, which occur mainly as

acetyl or malonyl glycosides such as genistin, daidzin and glycitin. Thousands of isoflavonoids have been described,²⁴ being soybeans the most studied source. One important aspect to consider is the effect of processing on the isoflavonoids structure since, for, example, fermentation (to obtain miso and tempeh) and heat treatment can induce the hydrolysis of glycosides to aglycones, which are highly heat resistant. See Table 1 for average contents.

Flavanones

Flavanones are found at high concentrations in citrus fruits, being the main aglycones naringenin in grapefruit, hesperitin in oranges and eriodictyol in lemons (see Figure 4 for chemical structures). Flavanones are generally glycosylated by a disaccharide in position 7 (either a neohesperidose, which imparts a bitter taste or a rutinose, which is flavorless). Orange juice contains between 200 and 600 mg hesperidin/L and 15–85 mg narirutin/L, therefore, one glass of orange juice may contain between 40 and 140 mg flavanone glycosides.²⁵ Since the highest flavanone content is found in the solid parts of citrus fruit, the whole fruit may contain up to 5 times more flavanone glycosides. Flavanones may be also found, to a less extent, in aromatic plants such as mint.

Anthocyanins

Anthocyanins are one of the most well studied group of flavonoids; they are pigments found in many foods in which they are responsible for the cyan, pink, red or purple color.²⁶ Structurally, anthocyanins are heterosides of an aglycone unit (anthocyanidin) which is a derivative of the flavylum ion. Main differences among the different anthocyanins are the number of hydroxylated groups in the aglycone, the number and nature of bonded sugars, the aromatic or aliphatic carboxylates bonded to the sugar in

the molecule, and the position of these bonds.²⁷ Figure 5 shows the structure of common food anthocyanins. Their structure influences their chemical properties and therefore, it is crucial to understand stability, color, reactivity, antioxidant ability, etc.

Anthocyanins can be found as aglycones (highly unstable), glycosylated with a glucose at position 3 and esterified with different organic acids (citric and malic acids) and phenolic acids. They can be stabilized by forming complexes with other flavonoids (copigmentation).

< Insert Figure 5 near here >

In the diet, they can be obtained from red wine,²⁸ some cereals,²⁹ and certain leafy and root vegetables such as cabbage, beans, onions, etc.; but they are mainly found in fruits such as red berries (grape berries, elderberries, cranberries, raspberries, etc.), cherries, pomegranates or plums²⁴ (see Table 1 for detailed information).

Flavanols (catechins and proanthocyanidins)

Flavanols exist in the monomeric form (catechins) and in the polymeric form (proanthocyanidins). The monomer has various degrees of hydroxylation on the 5 and 7 position of the A ring and in the 3', 4' and 5' position of the B ring. The 3-position of the C ring usually has a hydroxyl group or is esterified with gallic acid. Catechins are found in many types of fruits (see Table 1), but are mainly obtained from beverages such as fruit juices, green tea and red wine and from chocolate.^{30,31} In contrast to other flavonoids, flavanols are not glycosylated in foods.

Proanthocyanidins, known as condensed tannins, are dimers, trimers, oligomers and polymers of flavanol units linked by C-C bonds either at 4-6 or 4-8 (B-type

proanthocyanidins) (see Figure 6). They are classified as procyanidins (derived from catechin, epicatechin, and their gallic esters) and prodelphinidins (derived from gallocatechin, epigallocatechin and their galloylated derivatives). A-type proanthocyanidins are formed with a second interflavonoid bond by C-O oxidative coupling.

Condensed tannins are found in commonly consumed fruits such as grapes, apples, kiwis and pears and are also obtained from tea, cocoa, etc. Through the formation of complexes with salivary proteins, condensed tannins are responsible for fruit and beverages astringency (for example, beer) and chocolate bitterness.³²

< Insert Figure 6 near here >

Stilbenes

Stilbenes (Figure 1) are found in only small amounts in the human diet; one of them, resveratrol (3,5,4'-trihydroxy-trans-stilbene), has raised a great interest due to its anti-carcinogenic, anti-inflammatory, and cardioprotective properties. Resveratrol has been detected in trees, in a few flowering plants, in peanuts, and in grapevines. The major dietary sources of resveratrol include grapes, wine, peanuts, and peanut products. For example, the content of resveratrol in red wine ranges from 0.3–7 mg aglycones/L and 15 mg glycosides/L.³³⁻³⁵

Lignans

Lignans are phytoestrogens with estrogenic or anti-estrogenic activity and they are formed of 2-phenylpropane units (Figure 1). Dietary lignan compounds are mainly found in linseed³⁶ that is, perhaps, the richest source. Other food sources, that are also a

good source of dietary fiber, protein, antioxidants and other nutritional elements are oilseeds and nuts (sesame, sunflower, cashew, etc.), vegetables (such as curly kale, broccoli and garlic), fruits (apricot, strawberry and peach), olive oil and beverages such as wine, beer, tea and coffee, but only in small amounts.³⁷

Lignans comprise a variety of compounds, both in food sources (secoisolariciresinol, matairesinol) and the human body (enterodiol, enterolactone). Some studies reported that the health effect of the lignans varied depending on the particular lignan type.

5.08.3. FACTORS AFFECTING PHENOLIC'S CONTENT OF FOODS.

Although it is well known that fruits, vegetables and beverages such as red wine and tea are the main source of phenolic compounds, there are many factors that affect their final content and therefore, their final bioactivity and/or possible health promoting effects. Such factors should be considered in order to understand the huge variability that can be encountered and include: species characteristics, environmental factors, ripeness at the time of harvest, processing and storage, etc.

As for species characteristics, the knowledge on composition depends mainly on the interest of the specie; for example, several fruits like apples have been widely studied for their economic impact and therefore, the change in composition among varieties is known³⁸⁻³⁹ while other exotic fruits and cereals have not been studied yet. On the other hand, it is well known that varietal differences may influence in the flavonoids composition of fruits and vegetables,⁴⁰⁻⁴² such as flavones in honey and olives,⁴³⁻⁴⁴ catechins in pears,⁴⁵ procyanidins in apples and berries,⁴⁶⁻⁴⁷ flavanones in citrus fruits,⁴⁸ etc. Differences can be attributed to genetic mutations,⁴⁹ maturity, etc.

Environmental factors undoubtedly influence polyphenol content. Factors such as type of soil, UV radiation, rainfall, infection by pathogens and parasites, wounding, air

pollution, exposure to extreme temperatures⁵⁰ and other more related to agronomy (cultivation techniques, growing conditions such as culture in greenhouses or fields, biological or organic culture, hydroponic culture, etc.) are involved. In general, light exposure has an enormous influence in polyphenols content. The degree of ripeness affects both, the concentration and the relative content of different phenols; for example, in general, phenolic acids concentration decreases during ripening while anthocyanin concentration increases,¹⁹ but the behaviour and the influence of each factor can not be generalized but should be studied independently. For further information refer to some excellent contributions.^{5,22}

In general, processed foods have a significantly different level of phenolics than the original fresh products.⁵¹⁻⁵³ Increased oxidative damage and enzyme action are some of the factors that may contribute to a decrease in phenolics content. Processes such as freezing, canning, heating, irradiation, pasteurization, etc. have been reported to affect, for instance, phenolic content in fruit juices,⁵⁴⁻⁵⁷ grapes,⁵⁸ tomatoes,⁵⁹ tea,⁶⁰ etc. Culinary preparation has also a marked effect on the final content of phenolics in foods; processes such as chopping, shredding, peeling and cooking may loose a significant amount of phenolics; for instance, considering that the outer layers of fruits and vegetables contain higher level of phenolics than inner parts,^{56,61,62} peeling can eliminate a significant amount of phenolics. Cooking also has a major effect, being responsible for important losses of phenolics in consumed vegetables and fruits.^{63,64} A recent review has been published dealing with the influence of postharvest processing and storage on the content of phenolic acids and flavonoids in foods.⁶ In this contribution, a data compilation of papers published since 2002 to 2006 is presented; the main conclusion obtained is that the effect of storage and food production on the phenolic's content is

often negligible in comparison with the differences in content between different varieties of plants.

5.08.4. EXTRACTION OF PHENOLIC COMPOUNDS FROM FOOD.

Figure 7 shows a scheme of the strategies usually employed as well as its work-flow for the analysis of phenolic compounds from food. Although this chapter is focused on the extraction techniques to obtain these compounds from the original matrices, it is also important to briefly consider that previously, some sample treatment may be needed. The sample preparation for the extraction of phenolic compounds from food may vary significantly. Some beverages are directly analyzed even without any extraction procedure, simply filtering the sample. However, other more complex samples may need some processing before extraction. Due to the tremendous complexity and variability among phenolic compounds in terms of polarity, chemical structure, relative amounts in the sample and complexity of the original matrix, there is not any standardized procedure for the sample treatment prior extraction. Instead, each sample has to be separately considered, selecting the most appropriate procedures according to the particular needs. For instance, usually solid samples have to be milled, ground and/or homogenized before being subjected to the extraction procedure. Often, samples are dried before these treatments. The main aim of these steps is to reduce particle size of the sample in order to have a representative sample and to increase the surface contact which will lead, in most cases, to improvements in the extraction procedure thanks to an enhanced mass transfer.

< Insert Figure 7 near here >

Other important parameter to be considered is the final aim of the chemical determination; if the food analysis is focused on the total phenolic compounds as aglycones or if, on the contrary, the aim is to determine the phenolic compounds in their native forms. In the first case, a hydrolysis or digestion step may be needed in order to release all the conjugated compounds to their aglycone forms. Acid as well as basic hydrolysis can be performed. Again, due to the great chemical variability of these components in food, there is not a simple method. In any case, the conditions have to be adjusted according to the type of sample and its expected composition. Generally, HCl is employed to carry out acid hydrolysis of phenolic acids and flavonoids. A treatment with 2 N HCl during 1 h should be strong enough to release all the phenolic compounds to their non-conjugated forms. However, these treatments should not be performed without a proper study of the adequate conditions, because degradations of some of the targeted components may occur under these strong conditions. In some applications, enzymatic digestions could also provide with good results.

Once these steps are performed, the sample is ready to be extracted and the phenolic compounds properly analyzed. To give a clear overview of the techniques that are employed to extract phenolic compounds from food, traditional and advanced extraction techniques are described separately.

5.08.4.1. *Traditional Extraction Processes.*

Although, as mentioned earlier, at present there is a clear trend towards the replacement of traditional extraction techniques for other more efficient advanced extraction techniques, the so-called conventional extraction techniques are still mostly used for the

extraction of phenolic compounds from food. Clearly, the aim of every extraction technique will be the complete and/or partial isolation of the phenolic compounds contained in the food matrix from other interfering compounds, in order to increase the selectivity and sensitivity of the subsequent analytical procedure.

The most common conventional extraction techniques are based on the use of organic solvents to recover the interesting compounds from their original matrices. When the source of these compounds is a liquid, liquid-liquid extraction (LLE) may be employed. This technique is based on the separation of compounds according to their relative solubility in two different immiscible liquids. For instance, traditionally, the phenolic fraction of olive oil has been extracted by using an oil solution together with a lipophilic solvent with several volumes of methanol or methanol/water mixture, followed by solvent evaporation of the aqueous extract and a clean-up of the residue by solvent partition.^{66,67} The non polar solvent most commonly used is hexane, although other organic solvents such as petroleum ether and chloroform have been also employed. Nevertheless, the use of other solvent combinations, like tetrahydrofuran/water or *N-N*-dimethylformamide has been also explored. After extraction, a centrifugation step was considered enough in order to recover the phenolic compounds when using the tetrahydrofuran/water mixture.⁶⁸

The use of LLE to extract phenolic compounds from liquid samples is very common since it provides precise results and simplicity of application. However, LLE processes can be time-consuming as well as relatively dangerous due to the use of harmful organic solvents. For these reasons, there is an increasing trend to replace LLE by solid-phase extraction (SPE) for the isolation of phenolic compounds from liquids. The SPE approach is an alternative to the use of LLE which allows the reduction of the volumes of organic solvents employed as well as the total extraction time. Besides, SPE can be

partially automated, thus, increasing the reproducibility of the procedure. Although being a solvents-based technique, due to these characteristics SPE is widely regarded as an advanced extraction technique, and therefore, it will be described later.

For solid samples, solid-liquid extraction (SLE) has been also widely employed. In this case, the solid sample is homogenized and extracted with a solvent or a mixture of solvents for a given time. For the SLE of phenolic compounds from food, different parameters have to be considered if a complete extraction of all the phenolics is aimed. Regarding the sample itself, the particle size is of great importance since it directly influences the mass transfer of the interesting compounds and their solubilisation in the organic solvents. Clearly, a smaller particle size will increase the contact between sample and solvent providing an increase on the mass transfer rate. Besides, the moisture contained in the sample can also have a significant influence on the outcome of the extraction. Normally, sample with less humidity are preferred. The next parameter of critical importance is the solvent selection. Ideally, the solvent should have a polarity according to the type of phenolic compounds to be extracted and therefore, should be optimized for each application to achieve a complete solubilization and extraction of the key compounds. Depending on the expected chemical composition of the food, different solvents can be used. Ethanol, methanol and their mixtures with different proportions of water have been used to extract phenolic compounds from food. The addition of a small proportion of an acid (0.1 %) has been shown to have a favourable effect in some applications. Once the solvent is selected, other parameters have to be also taken into consideration, such as the possibility of performing the extraction at a higher temperature or the total extraction time. The temperature has to be selected as a compromise; it can be high to increase the mass transfer and the solubility of the analytes in the solvent but low enough to not produce degradation of the phenolic

compounds. Concerning the extraction time, this parameter varies greatly among applications; although in general the extraction time is always more than one hour, extraction times as long as several days have been also employed. When the extraction is finished, a centrifugation or filtration step is included to separate the solid residue. Later on, the solvent is evaporated and the dry extract re-dissolved in an appropriate solvent for its analysis.

Soxhlet extraction is also a solid-liquid extraction technique involving a simple distillation process performed and repeated a number of times. Although this method has provided with good results in some applications, it is characterized for the use of large extraction volumes and long extraction times. The sample is placed inside a thimble made by thick filter paper and loaded into the main chamber of the soxhlet extractor, composed by an extracting tube, a glass balloon and a condenser. Different solvents have been employed for extraction of phenolic compounds from food samples. For instance, ethyl acetate and ethanol were compared for the extraction of phenolics from guava seeds.⁶⁹ In this case, ethanol provided a higher amount of phenolics recovered due to its increased polarity. The total extraction time was 10 h and the volume of solvent used 250 ml. Depending on the application and the amount of sample processed, both extraction times and volumes can be adjusted, extraction volumes described are as low as 80 ml and as high as 500 ml. In terms of extraction time, Figure 8 shows the need for a careful optimization both, to achieve a complete extraction of the interesting compounds, and to avoid possible degradations. As it can be observed in this Figure, independently of the solvent employed, an increase on the extraction time produced firstly an enhancement of the extraction and, later on, a decrease on the amount of phenolics obtained from bitter melon.⁷⁰ This behaviour may be explained through a product degradation during the extended extraction. Moreover, in this Figure

is also possible to observe that water produced better results than methanol in this application, probably due to the chemical phenolic composition of bitter melon.

< Insert Figure 8 near here>

For samples with high amount of lipids, a previous extraction with a highly lipophilic solvent can be performed so that lipids are extracted from the matrix, avoiding their interference during phenolics extraction. Hexane can be employed to this aim.⁷¹ Later on, a phenolic compounds extraction as those above described can be carried out on the residue.

5.08.4.2. *Advanced Extraction Processes*

In contrast to the traditional techniques, the advanced extraction techniques are more selective, faster, more efficient, allowing higher recoveries and better reproducibilities and permit a good degree of automation. Besides, also of great importance at present, these techniques are environmentally cleaner since the amount of organic solvents that are used, if any, are significantly reduced. In this section, a description of the main advanced extraction techniques applied to the extraction of phenolic compounds from foods is given.

5.08.4.2.1. *Supercritical fluid extraction (SFE)*

Among the considered advanced extraction techniques, supercritical fluid extraction (SFE) is widely regarded as one of the most applied techniques for the extraction of

different compounds from food matrices.⁷² The advantages that this technique provides compared to other extraction techniques, have also pushed to its application for the extraction of polyphenols from food. SFE is based on the extraction with solvents at temperature and pressure conditions above their corresponding critical points. For every solvent, a particular critical pressure and a critical temperature are defined. When this solvent is submitted to temperatures and pressures values higher than the critical point, the solvent will be at supercritical conditions. At these conditions the solvents acquire different properties; supercritical fluids typically have diffusivities comparable to those of gases whereas maintaining densities similar to those of liquids. These properties are responsible of some of the interesting characteristics of SFE compared to other extraction techniques. Besides, the selectivity obtained during the SFE procedure can be modulated by operating at different pressures and temperatures. For a more detailed theoretical information on SFE, the reader is referred to other chapters of this Encyclopedia in Comprehensive Sampling and sample preparation (Chapter 2.5). Different supercritical solvents can be employed, although the most used supercritical fluid, even for polyphenols extraction, is carbon dioxide. CO₂ possesses several very interesting characteristics for their use as supercritical fluid: i) its critical conditions are relatively mild and easily attainable (30.9 °C and 73.8 bar); ii) is cheap compared to other possible solvents; iii) is an environmentally friendly solvent since it is produced as sub-product of different technological processes, that is, no extra CO₂ emission is needed; iv) it is considered as a food-grade solvent (GRAS); v) allows the attainment of a solvent-free extract. This last characteristic obviously is very interesting from the analytical point of view. Since CO₂ is a gas at atmospheric conditions, when the supercritical extract is depressurized, the CO₂ is liberated, leaving as a result of the extraction procedure, a completely solvent-free product theoretically ready to be further

analyzed. As it can be inferred, these properties are responsible for the great use of supercritical CO₂ for extraction of bioactive compounds.

However, the use of CO₂ is not free of shortcomings. The most important is related to its polarity. CO₂ is a very low polar solvent, therefore being more appropriate for the extraction of highly hydrophobic compounds. Nevertheless, in order to extend the range of application of this extraction technique, small proportions of more polar organic solvents can be employed together with the CO₂. The addition of this modifier or co-solvent allows the extraction of more polar compounds, as very often are polyphenolic compounds. Different organic solvents can be used as co-solvents for supercritical CO₂ extractions of phenolic compounds; among them, ethanol is the co-solvent more frequently employed. Among other advantages, this organic solvent is a food-grade reagent, being therefore, more adequate for the analysis of food materials. Other solvents, such as ethyl lactate, have been also tested. However, it has been observed that ethanol in combination with supercritical CO₂ was able to extract the phenolic compounds contained in guava seeds with a higher efficiency than ethyl lactate.⁶⁹ To maximize the extraction of these compounds from guava seeds, extractions including supercritical CO₂ with ethanol as co-solvent at 60 °C and 100 bar were found to be the most adequate.⁶⁹ Typical proportions of ethanol usually vary from 5 to 15 % during the extractions. The careful optimization of this parameter is, nevertheless, of utmost importance in order to obtain the maximum recovery of these compounds from food matrices. It was statistically demonstrated that the type and amount of co-solvent was the second most important parameter involved in the extraction of phenolic compounds from pomegranate seeds oil.⁷³ Besides, in that work, three different modifiers were compared, namely, water, ethanol and hexane. In this sense, it was also demonstrated that the polarity of the co-solvent should be high enough to obtain good amounts of

phenolic compounds. In this application, water produced better results than ethanol and hexane.⁷³ Therefore, it can be easily inferred that the selection of the most appropriate co-solvent would be highly dependent on the type and amount of phenolic compounds present in the original sample and that this parameter should be carefully optimized in terms of type and proportion of solvent for each application.

Once the selection of the extraction solvent has been performed, including co-solvent, other experimental parameters would have to be further optimized; among them, extraction pressure and temperature. For instance, for the extraction of resveratrol from grapes pomace, different ranges of pressures (100-400 bar) and temperatures (35-55 °C) were studied using 5% ethanol as modifier.⁷⁴ The best conditions for the complete extraction of this compound were the highest pressures combined with the lowest temperature of extraction. For this kind of applications, it is of particular interest the use of chemometrics and statistics, such as those based on the use of experimental designs. By using these tools, it is possible to cover wide ranges of experimental parameters in order to find the best extraction conditions with a minimum amount of experimental work.

Another clear advantage that SFE possesses over other extraction mechanisms is the possibility of performing on-line fractionation of the obtained extract. This can be done by using instruments with different depressurization chambers, so that a cascade depressurization on, at least, two steps can be performed. In practice, the supercritical conditions are not reverted in one step. On the contrary, on a first separator, a partial depressurization takes place in order to precipitate the extracted compounds not anymore soluble at this reduced pressure. However, as complete depressurization is not performed at this point, other compounds contained in the extract still remain soluble on the supercritical fluid. These then move forward to the second separator where complete

depressurization usually takes place, leaving the rest of the extract free of solvent. This advantage can be successfully employed when dealing with complex matrices like food in two main ways: first of all, a fractionation between phenolic compounds and other compounds extracted from the sample can be carried out on-line, or secondly, a fractionation between different phenolic compounds as a first separation step. This property has been used to extract phenolic compounds from different materials, such as propolis extracts⁷⁵ or *Theobroma cacao* hulls.⁷⁶ Another possibility that has been also applied for phenolic compounds extraction from food, is to use SFE to remove from the matrix other less polar interfering compounds. Using this strategy, anthocyanins from elder berries and grapes were analyzed.⁷⁷ After SFE, the phenolic compounds that remained in the unextracted sample were extracted using other solvents and later on analyzed. A similar procedure may also be utilized, by doing firstly the polyphenols extraction using conventional solvents extraction and later on SFE over these dried extracts at very mild conditions (i.e., 150 bar and 45 °C). In this case, a further purification of the antioxidants is produced due to the extraction at these conditions of other interfering compounds without producing thermal degradation of the extracts.⁷⁸

Considering the importance of some of these compounds in food matrices, the particular solubility of some phenolic compounds has been calculated in order to precisely know their properties under supercritical CO₂ conditions. The solubilities of syringic and vanillic acids,⁷⁹ *p*-coumaric, caffeic and ferulic acids⁸⁰ as well as those values corresponding to protocatechuic acid, methyl gallate and protocatechualdehyde⁸¹ have been calculated by using a data modelling through the Peng-Robinson equation of state, in order to describe the behaviour of the supercritical fluid phase. For all these calculations, the extraction temperature was varied from 40 to 60°C whereas the extraction pressure was modified from 100 to 500 bar, always using neat supercritical

CO₂ to carry out the extractions. However, when using this type of calculations and modelling, different approaches can be chosen depending not only on the experimental conditions to be used but also on the model selected to carry out the fitting. Considering the polarity of phenolic compounds, it is possible to find that a co-solvent may significantly increase their solubility on supercritical CO₂. In this sense, two different thermodynamic models have been applied to estimate the solubility of quercetin under SFE conditions, the group contribution equation of state (GC-EoS) and the Soave-Redlich-Kwong EoS.⁸² In this case, the temperature was maintained constant at 40°C while the pressure was modified between 80 and 120 bar. Ethanol was selected as co-solvent, using different proportions from 5 to 30 %. By using this approach, it was found that the solubility of quercetin increased with the amount of ethanol, and that the solvating power of CO₂ become greater and more solute was transferred to the supercritical phase, with increasing pressure.⁸²

Although the use of CO₂ is highly predominant, the employment of other supercritical solvents has been also explored for the extraction of phenolic compounds from food. Specifically, phenolics from hazelnut kernels were obtained by using supercritical methanol at 260 °C and 300 bar.⁸³ At these conditions, 4.82% of phenolic compounds were obtained.

5.08.4.2.2. *Pressurized liquid extraction (PLE) and subcritical water extraction (SWE).*

Pressurized liquid extraction (PLE) is widely considered as an advanced extraction technique based on the extraction of solid materials with solvents at high temperatures and high pressures, enough to maintain the solvents in the liquid state during the whole extraction procedure. The employment of these conditions allows obtaining higher

extraction yields compared to other solvents-based conventional extraction techniques, using less amounts of solvents and reducing the extraction time. Basically, at high temperatures while maintaining the liquid state, it is possible to obtain an enhancement on the mass transfer rate of the analytes contained in the sample as a response of an increase on their solubility. More theoretical information can be found in Chapter 2.13 of this Encyclopedia in Comprehensive Sampling and sample preparation. In principle, comparing to the conventional extraction techniques applied to the extraction of phenolic compounds, in which relatively polar organic solvents or solvent mixtures are employed, this technique will be more adapted to the extraction of most of these compounds than SFE, since PLE may work with any kind of solvent as extraction media. PLE has been directly compared to other conventional techniques for the extraction of phenolic compounds from eggplant⁸⁴ and flavonoids from onion,⁸⁵ providing better results in terms of recovery, solvent consumptions and total process time than reflux, stirring or direct solvent extraction.

In a typical PLE extraction some parameters have to be optimized. Solvent selection can be considered as one of the most important. Although solvent properties can be somewhat modified under high temperature PLE conditions, the nature of the solvent employed will determine which compounds will potentially be extracted. Different solvents and solvent mixtures have been employed to extract and analyze phenolic compounds from food. Among them, ethanol, methanol and mixtures in different proportions of these solvents with water are the extracting solvents more frequently used. For instance solvent mixtures as different as ethanol/water 50:50, methanol/water 80:20 or ethanol/water 30:70 have been successfully employed for phenolic compounds of different nature. This decision has to be taken systematically. It has been shown how, due to the large variability regarding the chemical structures of phenolic compounds,

the solvent selection can influence the extraction of different phenolics even contained in the same sample. For instance, it was demonstrated that for the extraction of phenolic compounds from parsley it was not possible to find the same solvent conditions for all the phenolic compounds found in this matrix. In fact, the proportions of water included on the extraction solvent determined which compounds could be effectively extracted.⁸⁶ Considering the chemical nature of some phenolic compounds, the acidification of the water used for the solvent mixture, may led to improved results. This strategy has been employed for instance for anthocyanin extraction from grape skin. After a careful optimization using different solvents with different proportions of acidified water, it was found that a complex solvent mixture including methanol/acetone/water/HCl 40:40:20:0.1 provided the best results in terms of maximum recovery of phenolic compounds and total acylated anthocyanins.⁸⁷

Next, other important parameters have to be optimized. It has been observed that in this kind of extractions, parameters such as extraction temperature, extraction time, sample particle size, packing of the sample inside the extraction cell as well as the number of static extraction cycles or extraction solvent flow rate, depending if the process is in the static or dynamic mode, respectively, could have a significant influence. However, although the pressure could theoretically have a rupture effect on the extracted matrix allowing a better penetration of the solvent into the sample, in practice, it has been repeatedly observed that this parameter has not a significant effect on the outcome of the extraction once it is a value higher enough to keep the solvent on the liquid state during the whole extraction process. The rest of parameters have to be optimized for each application in order to obtain a complete recovery of the phenolic compounds of interest.

The effect of temperature on the extraction has been above commented. In principle, it can be guessed that the highest the extraction temperature, the highest the recovery of phenolic compounds. However, being most of these compounds highly labile, attention has to be put on the maximum extraction temperature to be used. Typical temperatures employed for phenolic compounds extraction are usually above 100°C. It was demonstrated, using different phenolic standards, that at these temperatures the degradation that could take place was always above 10% for the most labile compounds catechin and epicatechin;⁸⁸ however, most of the studied compounds (phenolic acids) were stable up to the highest temperature assayed (150 °C). Therefore, according to this study, for the extraction of food samples not containing catechins, extraction temperatures as higher as 150°C could be preferably used. Nonetheless, in complex natural samples, such as food, more complex interactions than just degradation may occur. Some reports have pointed out the possibility of some unwanted compounds being formed during PLE extractions at high temperature, such as compounds related to Maillard reaction.^{89,90} In fact, it has been demonstrated that depending on the sample and its chemical composition, different chemical events, including Maillard reaction occur when water is used as extraction solvent, positively influencing the total antioxidant capacity of the collected extracts.⁹¹

Particle size has also great influence on the extraction outcome. It has to be considered that the mass transfer rate can be increased by decreasing the particle size of the sample being extracted as a response of the increment on the contact surface between sample and solvent. Therefore, the particle size of the sample should be maintained as low as possible, although other practical issues have to be also considered. In practice, using some commercial extraction instruments, a very small particle size can produce the clogging of the system. Practical precautions in order to keep the entire sample inside

the extraction cell as well as to maintain the homogeneity of the sample have to be taken. This can imply the use of a packing material in order to maintain the structure within the extraction vessel. Diatomaceous earth, sea sand as well as other supporting materials can be employed to this aim. Besides, these materials can help to the correct dispersion of the sample as well to avoid the formation of preferential paths through the sample. Besides, for static extractions, the amount of sample to be extracted should be also considered. Although the increase on the amount of sample placed into the extraction cell generally increases the total amount of phenolic compounds extracted, normally the efficiency of extraction does not follow the same trend. This means that if fully extraction of the phenolic compounds contained on a particular sample is seek, considering constant the extraction cell volume, the amount of sample introduced has to be optimized in order to have a complete extraction of the material.

The static extraction time has also an important effect. This value is the time the sample remains in contact with the extraction solvent maintaining the system in equilibrium. Static times from 5 to 30 are often employed to extract phenolic compounds. However, it must be considered that from a given time, the increase on the extraction efficiency is not linear and therefore an increase on extraction time may not be translated on a proportional increase on the extraction of phenolic compounds. For this reason, the possibility of doing successive static extraction cycles over the same sample has to be also explored. For instance, two-5 minutes cycles were found more appropriate for the extraction of polyphenols from carob fruits⁹² and from barley.⁹³

Another possibility is to work under dynamic conditions. In this case, the extraction time refers to the time a particular flow rate of extraction solvent is passing through the extraction cell. It has been demonstrated that a mixture ethanol/acidified water (50:50) pumped at a flow rate of 1.2 ml/min for 30 min (maintaining a pressure of 80 bar at 120

°C) allowed the extraction of different phenolic compounds from grapes pomace.⁹⁴ Namely, it was shown how using dynamic PLE, the extraction recovery for anthocyanins, total phenolics and flavonols was 3, 7 and 11 times higher, respectively, than those obtained using conventional dynamic solid-liquid extraction. However, a combined strategy has also shown its potential for the extraction of phenolic compounds from food matrices. That is, the combination of a relatively short first static period together with a longer subsequent dynamic extraction period. This combined approach has been shown to be useful for the extraction of phenolic compounds from olive oil industry by-products.^{95,96}

In order to obtain a better isolation of the interesting phenolic compounds, PLE is often coupled to a later solid phase extraction (SPE) step. The coupling can be off-line or on-line. On-line coupling refers to the placement of the solid phase inside the extraction cell, just in-line with the sample to be extracted, separated by any dispersing agent, such as sea sand. In any case, this step allows for a better clean-up of the sample extracted before its chemical analysis. Different examples of in-line⁹⁷ and off-line⁹⁸ PLE-SPE approaches for the extraction of phenolic compounds can be found in the literature. Other on-line commercial interfaces have been also employed for the determination of proanthocyanins in malt.⁹⁹ In this case, an automated device transfers the extract from the PLE collection vial to the SPE instrument.

Up to this point, in this section the extractions described have been referred as PLE. However, when this technique is used only with water as extraction solvent, it is also commonly known as subcritical water extraction (SWE) or superheated water extraction (SHWE). The use of SWE is very interesting since it is widely regarded as an environmentally friendly extraction technique. The fundamentals are obviously the same, the application of high temperatures (typically above 100 °C) in combination with

high pressure in order to maintain the water in the liquid state. Nevertheless, the parameters involved in this type of extraction are slightly different. SWE is characterized by the dielectric constant of water (ϵ), defined as a measure of its polarity. This value also defines the characteristics of water as solvent (in terms of compounds that can be effectively dissolved) and is highly dependent of the temperature. This way, while the dielectric constant of water at room temperature is around 80, when water is heated to 250 °C and is maintained liquid, ϵ decreases to around 25. This value is quite similar to that of some organic solvents such as ethanol or methanol, being, therefore, an alternative to the use of these solvents in some applications. SWE has been also applied to the extraction of phenolic compounds from food either in the static extraction mode or in the dynamic extraction mode. These extraction modes are carried out similarly as in PLE. Usually, after extraction, if the concentration of the extract is not high enough or just to have a precisely known amount, the extract collected is dried, mainly by freeze drying. This drying procedure is quite limiting in terms of total analysis time since freeze-drying often last for at least 24 hours. Nevertheless, a new procedure based on SWE has been tested for the extraction of phenolic compounds from rosemary. This new process, called WEPO (Water Extraction and Particle formation On-line,¹⁰⁰ involves the dynamic subcritical water extraction of the sample and the on-line drying of the obtained extract. To achieve a dried extract, a current of supercritical CO₂ is employed together with hot nitrogen in order to produce a fine spray of the extract and its complete drying, respectively.¹⁰¹ Other strategy employs a sorbent trap to collect the extracted compounds in the hot water. Once the extraction is finished, the trap is directly heated into a liquid chromatograph for the analysis of the extracted compounds.¹⁰²

It has been repeatedly observed that very high temperatures (from 130 to 200 °C) can provide with good results, better to those that can be obtained even using conventional extraction techniques.^{70,103} Nevertheless, the temperature is a parameter which has to be carefully selected, considering that depending on the matrix, it may have a degradation effect over the phenolic compounds present. The success of this type of extraction in which water is used alone will depend on the chemical characteristics of the phenolic compounds included on the sample to be extracted. For phenolic compounds of high polarity, the use of this technique may produce good results. However, in the case of other less polar phenolic compounds, different solvent mixtures can be employed, including a small amount of an organic solvent.

5.08.4.2.3. *Ultrasound assisted extraction (UAE)*

Ultrasound-assisted extraction (UAE) is an advanced extraction technique with great potential to obtain phenolic compounds from food, due to the simple instrumental requirements and easy application. This technique is based on the application of high-frequency sounds and a limited amount of solvent to produce an effective extraction of the compounds contained in a solid matrix. The application of ultrasounds in the food industry can be divided into two distinct categories, low-intensity high frequency ($f > 100$ kHz) and high-intensity low frequency ($20 \text{ kHz} \leq f \leq 100 \text{ kHz}$) ultrasounds. Low-intensity ultrasounds do not alter the physical or chemical properties of the material through which the ultrasonic wave propagates. In contrast, high-intensity shock wave generates intense pressures and temperature gradient due to the bubble cavitation producing a disruption effect within the matrix. This way, the mass transfer rate and the solubility of the analytes are improved, not only by the increasing temperature, that

should be controlled, but also by the increasing contact between the original matrix and the solvent. In fact, UAE has been proven to significantly decrease extraction time and increase extraction yields in many vegetables¹⁰⁴ due to the improved release of phenolic compounds as a result of the cell wall disruption by the application of ultrasounds. The most important parameters involved in the extraction of phenolic compounds from food are extraction time, extraction temperature, power, solvent composition and amount of sample.

The effects of extraction temperature and time have been well observed. At a controlled temperature, the extraction of phenolic compounds will be increased by an increasing extraction time up to complete extraction or solvent saturation. On the other hand, for a given extraction time, the increase on the extraction temperature can produce also an increase on the recovery of the phenolic compounds. Nonetheless, this increase will be limited by possible degradation of these compounds at relatively high temperatures. To be safe and to completely avoid phenolics thermodegradation, temperatures in UAE should not exceed 40 °C. Nevertheless, slightly higher temperatures may be employed depending on the particular application provided that the no degradation of the compounds is assessed. The power of the energy applied as well as its frequency is also an important parameter. This value can vary greatly and will also depend on the instrument employed. Power values from 25 to 200 W have been reported for phenolics extraction. For instance, power and frequency values of 250 W and 40 kHz, respectively, were used to extract anthocyanins from grape seeds by UAE,¹⁰⁵ whereas 150 W and 25 kHz were used to obtain flavanone glycosides from orange peel¹⁰⁶ and 40 W and 20 kHz were employed to extract different phenolic compounds from soybean.¹⁰⁷ The amount of sample and its physical state is an important variable as well. The amount of sample will determine the amount of compounds that could be released to the

solvent. In case of extracting too much sample, the solvent can be readily saturated avoiding the complete phenolics extraction. Besides, the sample particle size is of importance. Smaller particles allow for more drastic effects of ultrasounds in their structure as a result of a better penetration of the solvent. Lastly, the degree of humidity of the sample is also of critical importance. It has been demonstrated how dry samples produce higher yields than samples with a high degree of humidity. This phenomenon was suggested to be due to the higher porosity of the dry material which implies a higher solvent diffusion rate.

In order to successfully optimize all these parameters, the use of chemometric tools is greatly recommended. These statistical approaches allow the simultaneous optimization of several parameters as a response of different variables of interest. Factorial designs, central composite designs as well as surface response methodology are some of the chemometric tools that have been used to optimize the extraction conditions of different phenolic compounds from citrus,¹⁰⁸ grapes¹⁰⁵ and coconut,¹⁰⁹ respectively. Using these tools, even the solvents mixtures to be used can be optimized. The solvent selection for UAE can be considered in the same terms than for the above mentioned classical techniques; according to the chemical composition of the sample to be extracted and the nature of the phenolic compounds, relatively more or less polar solvents or solvent mixtures may be used.

When the total content of phenolics, as aglycones, is to be determined, an interesting UAE approach can be used based on the use of an acid together with the extraction solvent. By using this methodology, a simultaneous extraction and hydrolysis of the phenolic compounds is done. It has been shown that extraction times of only 2 min were enough to achieve the extraction and hydrolysis of the phenolic compounds present in strawberries using an aqueous solution containing 0.4 M hydrochloric acid.¹¹⁰

In some cases, UAE has been directly compared to other conventional extraction mechanisms to obtain phenolics from food. In general, these comparisons with SLE concluded that UAE is able to provide higher extraction efficiencies using less solvent and faster than using the traditional procedures. Besides, the temperature can be maintained at lower values, thus avoiding thermal degradation.

5.08.4.2.4. *Microwave assisted extraction (MAE)*

In microwave assisted extraction (MAE), microwave energy is used to heat solvents in contact with solid samples and to favour the solubilisation of analytes from the sample into the solvent. The highly localized temperature and pressure can cause selective and faster migration of target compounds from the material to the surrounding and with similar or better recoveries compared with conventional extraction. Since the main advantages of MAE are the possibility of reducing both extraction time and solvent consumption, this technique is considered as an advanced extraction technique. Several parameters have to be carefully selected to successfully apply this technique for the extraction of phenolic compounds from food. Among them, solvent composition, solvent volume, extraction temperature, microwave power, extraction time and matrix characteristics (including particle size, amount of sample (sample-to-solvent ratio) and water content) are the most important.

As it has been repeatedly mentioned for solvents-based extraction techniques, the particular composition of the solvent has to be tuned to the sample to be extracted. Generally, ethanol, methanol, water and their mixtures are the most used solvents for the MAE of phenolic compounds. Ethanol/water 50:50 (v/v) has been observed as a good extraction solvent for matrices rich on polyphenols such as tea. As for the rest of

parameters, values can vary somewhat among applications. In general, it can be concluded that the use of chemometrics is of interest in order to optimize these parameters for each application. In this regard, response surface methodology has proven to be successful. In Figure 9, an example of this kind of methodology applied to the optimization of a MAE extraction of phenolic compounds from citrus peels can be observed.¹¹¹ As it can be seen, by using this procedure, the effect of different factors in a particular response variable (yield of phenolic acids) can be simultaneously observed, allowing the selection of the values which maximize this response.

< Insert Figure 9 near here >

Typical MAE extraction times to obtain phenolic compounds are around 2 minutes. Therefore, this technique can be considered as a very fast extraction method when compared not only to the classical and conventional extraction techniques, but also to the other advanced extraction techniques. For instance, 30 s were enough to recover the phenolic compounds contained in peanut skins using 37.5 mL of 30 % ethanol in water.¹¹² Slightly higher extraction times, of ca. 5 min have been also successfully tested. The extraction temperature is a parameter closely correlated to the microwave power used in MAE, since the application of this energy will produce an increase in the temperature of the solvent, and hence, on the sample. Summarizing, it has been observed that high power values for short extraction times are more adequate than the application of low power together with longer extraction times. Extending the irradiation time with high microwave power may lead to thermal degradation of the phenols. Powers up to 600-700 W have been employed for the extraction of phenolic compounds. For example, using 600 W, two different controlled temperatures were

shown to be useful for the extraction of diverse phenolic compounds: 100 °C was more efficient to extract hydroxycinnamic acids from tea, whereas 80 °C was a better temperature in order to preserve the thermally sensitive and tea-specific flavanols.¹¹³ As it can be deduced, the selection of the optimum extraction time will be also closely related to the selection of the appropriate power.

The effect of the physical state and morphology of the sample being extracted can not be underestimated. The liquid-to-solid ratio is an important parameter that allows the simultaneous optimization of the amount of sample used for each extraction as well as the volume of solvent needed to carry out the extraction; this volume should be kept as low as possible while maximizing analytes recovery. Solvent-to-sample ratios of 20:1 (mL g⁻¹) have been shown to be adequate for phenolic compounds MAE. Besides, in principle, smaller particle sizes, as it has been already mentioned for other solvents-based extraction techniques, would increase the surface contact between the solvent and the sample improving the mass transfer rate of the analytes from the matrix to the extracting solvent.

The performance of MAE compared to other extraction techniques has been extensively compared, also for the extraction of phenolics from food. In general, MAE has proven to be a more appropriate technique than SLE or Soxhlet extraction in terms of solvent consumption, extraction efficiency and speed.^{111,113}

5.08.4.2.5. *Solid phase extraction (SPE)*

As it was mentioned before, SPE is an advanced alternative to traditional liquid-liquid extractions since is faster, more efficient, can be automated and is more environmentally friendly as solvent consumption is significantly decreased. The applications of SPE to

the extraction of phenolic compounds are divided into two groups, SPE directly applied to liquid samples to extract the phenolics, and the use of SPE as a second step after another extraction procedure. This is the case, for instance, of the application of SPE together with SFE,¹¹⁴ PLE⁹⁸ or MAE.¹¹⁵ More in-deep information on SPE principles and practice can be found in Chapter 2.7 of the present work.

C₁₈ stationary phases are one of the most used for the extraction of phenolic compounds by SPE. SPE cartridges with this kind of stationary phase have been employed to extract a great variety of phenolic compounds, including phenolic acids, flavonoids, catechin or procyanidins, among others. In fact, the retention properties of this material allow also the direct fractionation of the extracts. This is of interest from an analytical point of view, as the extracted compounds can be directly pre-separated into groups of similar polarity. For instance, using a single procedure of extraction, the fractionation of phenolic acids, flavonols, anthocyanin monomers and polymers, procyanidins and catechins contained in red wine was attained using a C₁₈ Sep-Pak cartridge.¹¹⁶

Nevertheless, octadecylsilica-based cartridges are not the only ones that have been used to this aim. In fact, at present, different commercial SPE cartridges are available directed to the successful extraction of phenolic compounds. HLB (Hydrophilic-Lipophilic Balance) cartridges composed of a copolymer formed by divinylbenzene and N-vinylpyrrolidone were tested against the most common C₁₈ SPE cartridges for the extraction and isolation of most of phenolic compounds present in red wine at low concentrations.¹¹⁷ It was demonstrated that this class of stationary phases allowed the removal of interferences by washing the cartridge with water without losing any interesting compound. Besides, this method provided higher sensitivity in the final analytical determination, higher loading capacity than silica-based C₁₈ cartridges and more reproducibility for this application.¹¹⁷ In fact, the chemical nature of the stationary

phase packed on the commercial cartridge may also be optimized to select the one providing the best results for an specific application. It has been observed than comparing the performance of different stationary phases, i.e., HLB, mixed-mode cation exchange (MCX), mixed-mode anion exchange (MAX), mixed-mode weak cation exchange (WCX) and mixed-mode anion exchange (WAX), for the isolation of polymeric polyphenols, different results could be obtained depending on the eluents.¹¹⁸ Hydroalcoholic eluents (methanol and water mixtures) as well as organic eluents (acetonitrile and methanol mixtures) were used, although the combination of organic eluents together with the copolymer stationary phase HLB provided the best results, allowing a correct isolation and fractionation of the phenolic compounds contained in the sample. However, for other food samples, such as beer, mixed-mode anion exchange stationary phases have been proven to be more adequate for the pre-concentration of polyphenols eliminating interfering substances.¹¹⁹

5.08.4.2.6. *Solid-supported liquid-liquid extraction (SS-LLE)*

Solid-supported liquid-liquid extraction (SS-LLE) is a relatively new extraction technique with certain similarities to chromatography: it is based on the immobilization of a liquid in an inert medium packed into a propylene tube, and on the percolation of other immiscible liquid phase through the immobilized liquid. The inert medium most commonly used is a high-purity diatomaceous earth, mainly composed by SiO₂ which can adsorb on its surface large quantities of aqueous samples. When an organic solvent passes trough the wet diatomaceous medium, the enhanced surface area of the aqueous sample allows a higher distribution between fluids, and thus higher analyte recovery than classical liquid-liquid extraction (LLE). At present, there are not too many

applications of SS-LLE for the extraction of phenolic compounds from food samples. Nevertheless, the extraction of polyphenols from wine was achieved using this technique. When comparing this technique to LLE, SS-LLE was proven to be more efficient for most phenolics providing recoveries even higher than those achieved by SPE.¹²⁰ Diatomaceous earth was used as solid support while different solvents were explored such as ethyl acetate, *n*-butanol, isopentyl alcohol and 4-methylpentan-2-one. Among them, *n*-butanol provided the best results in terms of total phenolics extracted from vinegar samples.

5.08.4.2.7. *High hydrostatic pressure extraction (HHPE)*

High hydrostatic pressure extraction (HHPE) is a novel technique that, at present, is used for the high pressure processing of foods in the extraction of functional ingredients from natural products. Super high hydraulic pressures between 1000 and 8000 bar are employed in HHPE. Besides, this high pressure is currently considered as an attractive and innovative non-thermal process that can effectively inactivate microorganisms, therefore, being very useful for preserving fresh food products. Some phenomena occurring at these conditions favour the use of this extraction technique: on one hand, the solubility is greater as the pressure increases, according to the phase behaviour theory. On the other hand, the pressure produces an increase on the permeability of the cells of the food being extracted, enhancing also the values of mass transfer rates.

A typical extraction procedure involves several steps: firstly, the sample is mixed with solvent; then, the mixture is treated with high hydrostatic pressure; and finally, the mixture after processing is filtered to remove the solid particles.

The solvents employed for the extraction of different phenolic compounds from food will depend on the target compounds and their polarity. Water as well as hydrophilic and lipophilic organic solvents have been used. This technique has been already demonstrated as adequate for the extraction of anthocyanins, flavonones and other polyphenols from different food samples. For instance, ethanol has been considered as the most adequate solvent for the extraction of flavonoids from propolis.¹²¹ Nevertheless, after proper optimization, a solvent mixture can be determined as optimum for each application. Ethanol/water mixtures provide good results for a wide range of compounds.

Temperature is another parameter to be controlled in this kind of extractions. Usually, no heating is performed other than that produced as a result of the increase in the compression. In Figure 10, the typical increases in temperature as a result of the application of different pressures can be observed when water is used as extracting solvent. Nevertheless, an initial temperature can be selected. This initial temperature will obviously influence the resulting temperature after compression. Moreover, when using water as extracting solvent, it has to be considered that the increase on temperature while maintaining extremely high temperature will produce a decrease in the dielectric constant of water. Under these conditions, therefore, other less polar compounds that may not be completely soluble in water at room temperature, according to their polarity, could theoretically be extracted.

< Insert Figure 10 near here >

The effect of pressure has been also observed when extracting phenolic from food matrices by HHPE. According to Le Chatelier's theory, the volume of the system tends

to be reduced during the pressure promoting period. That implies that the extracting solvent can enter into the food cells and get directly in contact with their components, among which phenolic compounds are. This way, the solubilisation of these interesting compounds can be enhanced. Therefore, in principle, a higher pressure can effectively provide with higher yields of interesting compounds extracted. In this sense, pressures higher than 5000 bar are usual. Extraction time is another parameter to consider in any extraction procedure. It has been repeatedly observed that in HHPE, the length of the pressure treatment has not a close and direct relationship to the increase in the extraction yield of phenolic compounds. One minute has been considered enough to complete the equilibrium in the extraction of anthocyanins from grape by-products.¹²³ What really seems to be responsible for an easier and effective extraction process is the rate of pressure transfer; in this regard, the increase without stress gradients is preferred.

As in the other solvents-based extraction techniques, the solid-to-liquid ratio can be an important factor in order to increase the extraction yield of phenolic compounds. Since in this extraction the equilibrium is quickly attained, higher solvent volumes can help on increasing the amount of analytes that can be readily solubilised. Typical employed values for extraction of phenolics from food range are around 1:25 (g ml⁻¹).

In summary, compared to other conventional extraction methods, such as SLE, the HHPE procedure allows the attainment of higher extraction yields and selectivity and faster extractions while being a less labour intensive process. However, partially due to the need for a relatively sophisticated instrumentation, HHPE has not still been extensively applied for the extraction of phenolics from foods.

5.08.4.2.8. *Other extraction techniques*

Counter-current chromatography (CCC) has been also applied to the fractionation and isolation of phenolic compounds from food matrices. CCC can be included under the category of liquid-liquid chromatographic techniques. That implies that the stationary phase employed is a liquid, therefore not using particles. Consequently, the fractionation mechanism is based on the partition of the sample and its components between two immiscible solvents. This way, the relative proportion of solute distributing into each of the two phases is determined by the respective partition coefficients. Therefore, CCC benefits from great advantages when compared with the traditional liquid-solid separation methods: it eliminates the complications resulting from the solid support matrix, such as irreversible adsorptive sample loss and deactivation, tailing of solute peaks and contamination. In addition, CCC is a preparative technique with high recovery, acceptable efficiency and ease scaling-up. Regarding the recent literature about CCC development and applications, it is obvious that CCC is a promising preparative separation technique, extremely useful for the extraction, separation and purification of phenolic compounds from foods.¹²⁴ The CCC mode mostly employed in these applications is high speed counter-current chromatography (HSCCC). The liquid samples to be extracted and/or fractionated, can be directly loaded into the system or can be previously extracted using a conventional solvent-based extraction procedure. Once the sample is loaded, the solvent composition is changed in order to achieve the elution of the retained compounds.

Another alternative technique for phenolics extraction is matrix solid-phase dispersion (MSPD). This technique consists of different steps in a single process: matrix homogenisation with a dispersant agent, cellular disruption, extraction, and purification. Sample extraction and clean-up are carried out simultaneously with, generally, good

recoveries and precision. Different dispersant agents can be used for phenolic extractions, such as silica-based materials, acid aluminium oxide, florisil, diatomaceous earth or celite, although silica based materials have been pointed out for providing the best results. Influencing also the outcome of the extraction, the elution agent composition has to be studied, both in terms of solvent used as well as in terms of pH or salts composition. MSPD has been demonstrated to be a suitable preparation technique, and a simple possible alternative to liquid-liquid, solid-liquid extraction, SPE, and SFE, for the isolation of phenolics from plant material.⁶⁵

5.08.5. Conclusions.

In this chapter, a revision of the extraction techniques used to determine phenolics content in food matrixes have been discussed. Phenolics composition in different plant-based foods is quite complex in terms of chemical structures and can be affected by many factors that influence its final content (such as specie, sunlight exposure, soil, ripeness, etc). Phenolics characterization and quantification is critical to a better understanding of their role in health and to evidence their mechanisms of action, therefore, care must be taken in carefully selecting not only the most appropriate extraction technique but also the optimal extraction conditions depending on the matrix, the type of phenolics involved, and the objective of the analysis.

Even considering that one of the most important trends in extraction is to lower the consumption of organic solvents, to miniaturize the process and to improve aspects such as selectivity, efficiency and ability to automation, it is true that still most of the literature published on phenolics' determination in foods deals with the use of traditional extraction techniques (such as LLE, SLE, Soxhlet, etc.). Nevertheless, new

advanced extraction techniques are more often used and researchers are more conscious of the advantages that they can offer, thus, a wider use of these new advanced techniques is expected.

It is also clear that due to the above mentioned complexity (in terms of composition, matrix, concentration levels, etc.), no standard procedure can be found common for all types of phenolics (not even for a family of phenolics), thus, extraction conditions should be always optimized. Chemometrics has been suggested as an important tool to perform such optimization since many factors are usually involved such as solvent composition, extraction time and/or extraction temperature and/or extraction pressure, etc. One important aspect that should be considered in the optimization procedure is the maintenance of the integrity of the compounds, thus, undesirable changes in phenolics' chemical structure should be avoided during extraction leading to the determination of complex intact structures.

New trends will be directed towards the development of new strategies including sample pre-treatment such as clean-up, enzymatic or chemical hydrolysis together with extraction (PLE, SFE) in order to minimize sample losses and errors. The use of combined techniques such as PLE-SPE, SFE-SPE, MAE-SPE, PLE-MSPD, etc. will further increase to achieve more selectivity and process efficiency leading to a more correct determination and characterization of phenolic compounds in different complex food matrixes.

5.08.6. References.

1. Clifford, M. N. *J. Sci. Food Agric.* **1999**, 79, 362 – 372.

2. Scalbert, A.; Manach, C.; Morand, C.; Remesy, C.; Jimenez, L. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 287-306.
3. Tucker, G.; Robards, K. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 929-966.
4. Nichenametla, S. N.; Taruscio, T. G.; Barney, D. L.; Exon, J. H. *Crit. Rev. Food Sci. Nutr.* **2006**, *46*, 161-183.
5. Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jimenez, L. *Am. J. Clin. Nutr.* **2004**, *79*, 727– 747.
6. Amarowicz, R.; Carle, R.; Dongowski, G.; Durazzo, A.; Galensa, R.; Kammerer, D.; Maiani, G.; Piskula, M. K. *Mol. Nutr. Food Res.* **2009**, *53*, S151-S183.
7. Selma, M. V.; Eespín, J. C.; Tomas-Barberan, F. J. *J. Agric. Food Chem.* **2009**, *57*, 6485–6501.
8. Harborne, J. B., in: *Introduction to Ecological Biochemistry*, 2nd Ed. Academic Press, New York, NY, **1982**.
9. Harborne, J. B.; Turner, B. L., in: *Plant Chemosystematics*, Academic Press, London, UK, **1984**.
10. Shahidi, F.; Naczk, M. in: *Phenolics in Food and Nutraceuticals: Sources, Applications and Health Effects*, CRC Press, Boca Raton, FL, **2004**.
11. Beckman, C. H. *Physiol. Mol. Plant Pathol.* **2000**, *57*, 101-110.
12. Naczk, M.; Shahidi, F. J. *Pharm. Biomed. Anal.* **2006**, *41*, 1523–1542.
13. Shahidi, F.; Naczk, M. *Food phenolics, sources, chemistry, effects, applications*. Lancaster, PA: Technomic Publishing Co Inc, **1995**.
14. Tomas-Barberan, F. A.; Clifford, M. N. *J. Sci. Food Agric.* **2000**, *80*, 1024-1032.
15. Clifford, M. N.; Scalbert, A. *J. Food Sci. Agric.* **2000**, *80*, 1118-1125.
16. Sosulski, F.; Krygier, K.; Hogge, L. *J. Agric. Food Chem.* **1982**, *30*, 337-340.
17. Lempereur, I.; Rouau, X.; Abecassis, J. *J. Cereal Sci.* **1997**, *25*, 103–110.

18. Crozier, A.; Lean, M. E. J.; McDonald, M. S.; Black, C. J. *J. Agric. Food Chem.* **1997**, *45*, 590–595.
19. Macheix, J. J.; Fleuriet, A.; Billot, J. *Fruit phenolics*. Boca Raton, FL, CRC Press, **1990**.
20. Price, S. F.; Breen, P. J.; Valladao, M.; Watson, B. T. *Am. J. Enol. Vitic.* **1995**, *46*, 187-194.
21. Herrmann, K. J. *J. Food Technol.* **1976**, *11*, 433-448.
22. Kyle J. A. M.; Duthie, G. G., in: *Flavonoids. Chemistry, Biochemistry and Applications*. Andersen, O.; Markham, K. (Eds.), CRC Press, Boca Ratón, **2006**, pp. 219-262.
23. Feng, Y.; McDonald, C. E.; Vick, B. A. *Cereal Chem.* **1988**, *65*, 452-456.
24. Andersen, O. M.; Jordheim, M. in: *Flavonoids. Chemistry, Biochemistry and Applications*. Andersen, O.; Markham, K. (Eds.), CRC Press, Boca Ratón, **2006**, p. 471.
25. Tomas-Barberan, F. A.; Clifford, M. N. *J. Sci. Food Agric.* **2000**, *80*, 1073-1080.
26. Mazza, G.; Maniati, E. *Anthocyanins in fruits, vegetables, and grains*. Boca Raton, FL, CRC Press, **1993**.
27. Kong, J. M.; Chia, L. S.; Goh, N. K.; Chia, T. F.; Brouillard, R. *Phytochemistry*, **2003**, *64*, 923-933.
28. Rolle, L.; Guidoni, S. *J. Int. Sci. Vigne Vin.* **2007**, *41*, 193-198).
29. Escribano-Bailon, M. T.; Santos-Buelga, C.; Rivas-Gonzalo, J. C. *J. Chromatogr. A*, **2004**, *1054*, 129-141.
30. Lakenbrink, C.; Lapczynski, S.; Maiwald, B.; Engelhardt, U. H. *J. Agric. Food Chem.* **2000**, *48*, 2848-2852.
31. Arts, I. C. ; van De Putte, B. ; Hollman, P. C. *J. Agric. Food Chem.* **2000**, *48*, 1752–1757.

32. Santos-Buelga, C.; Scalbert, A. *J. Sci. Food Agric.* **2000**, *80*, 1094-1117.
33. Bertelli, A.; Bertelli, A. A. E.; Gozzini, A.; Giovannini, L. *Drugs Exp. Clin. Res.* **1998**, *24*, 133-138.
34. Bhat, K. P.; Pezzuto, J. M. *Ann. N. Y. Acad. Sci.* **2002**, *957*, 210-229.
35. Vitrac, X.; Moni, J. P.; Vercauteren, J.; Deffieux, G.; Mérillon, J. M. *Anal. Chim. Acta* **2002**, *458*, 103-110.
36. Adlercreutz, H.; Mazur, W. *Ann. Med.* **1997**, *29*, 95-120.
37. Milder, I. E. J.; Arts, I. C. W.; Van de Putte, B.; Venema, D. P.; Hollman, P. C. H. *British J. Nutr.* **2005**, *93*, 393-402.
38. Guyot, S.; Marnet, N.; Laraba, D.; Sanoner, P.; Drilleau, J. F. *J. Agric. Food Chem.* **1998**, *46*, 1698-1705.
39. Sanoner, P.; Guyot, S.; Marnet, N.; Molle, D.; Drilleau, J. F. *J. Agric. Food Chem.* **1999**, *47*, 4847-4853.
40. Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B. *J. Agric. Food Chem.* **1992**, *40*, 2379-2383.
41. Hempel, J.; Bohm, H. *J. Agric. Food Chem.* **1996**, *44*, 2114-2116.
42. Bilyk, A.; Sapers, G. M. *J. Agric. Food Chem.* **1985**, *33*, 226-228.
43. Martos, I.; Ferreres, F.; Yao, L.; D'Arcy, B.; Caffin, N.; Tomás-Barberán, F. A. *J. Agric. Food Chem.* **2000**, *48*, 4744-4748.
44. Romani, A. *J. Agric. Food Chem.* **1999**, *47*, 964-967.
45. Amiot, M. J.; Tacchini, M.; Aubert, S. Y.; Oleszek, W. *J. Agric. Food Chem.* **1995**, *43*, 1132-1137.
46. Hammerstone, J. F.; Lazarus, S. A.; Schmitz, H. H. *J. Nutr.* **2000**, *130*, 2086S-2092S.

47. Prior, R. L.; Lazarus, S. A.; Cao, G.; Muccitelli, H.; Hammerstone, J. F. *J. Agric. Food Chem.* **2001**, *49*, 1270-1276.
48. Rouseff, R. L.; Martin, S. F.; Youtsey, C.O. *J. Agric. Food Chem.* **1987**, *35*, 1027-1030.
49. Harborne, J. B. *The Flavonoids: Advances in Research Since 1986*. Chapman & Hall, London, **1994**.
50. Zobel, A. M. in: *Phytochemistry of Fruit and Vegetables*, Tomás-Barberán, F. A., Robbins, R. J. (Eds.), Clanderon Press, Oxford, **1997**, pp. 173–204.
51. Spanos, G. A.; Wrolstad, R. E. *J. Agric. Food Chem.* **1990**, *38*, 1565-1571.
52. Balentine, D. A.; Wiseman, S. A.; Bouwens, L. C. M. *Crit. Rev. Food Sci. Nutr.* **1997**, *37*, 693-704.
53. Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. *J. Clin. Lab. Anal.* **1997**, *11*, 287-313.
54. Spanos, G. A.; Wrolstad, R. E.; Heatherbell, D. A. *J. Agric. Food Chem.* **1990**, *38*, 1572-1579.
55. Spanos, G. A.; Wrolstad, R. E. *J. Agric. Food Chem.* **1990**, *38*, 817-824.
56. Bengoechea, M. L.; Sancho, A. I.; Bartolomé, B.; Estrella, I.; Gómez-Cordovés, C.; Hernández, M. T. *J. Agric. Food Chem.* **1997**, *45*, 4071-4075.
57. Robards, K.; Prenzler, P. D.; Tucker, G.; Swatsitang, P.; Glover, W. *Food Chem.* **1999**, *66*, 401-436.
58. Karadeniz, F.; Durst, R. W.; Wrolstad, R. E. *J. Agric. Food Chem.* **2000**, *48*, 5343-5350.
59. Re, R.; Bramley, P. M.; Rice-Evans, C. *Free Radical Res.* 2002, *36*, 803-810.
60. Tanaka, T.; Matsuo, Y.; Kouno, I. *Int. J. Mol. Sci.* **2010**, *11*, 14-40.

61. Pérez-Ilzarbe, J.; Hernández, T.; Estrella, I. *Z Lebensm-Unters-Forsch* **1991**, *192*, 551-554.
62. Fernández de Simón, B.; Pérez-Ilzarbe, J.; Hernández, T.; Gómez-Cordovés, C.; Estrella, I. *J. Agric. Food Sci.* **1992**, *40*, 1531–1535.
63. Kamisaka, S.; Takeda, K.; Takahashi, K.; Shibata, K.. *Physiol. Plant.* **1990**, *78*, 1-7.
64. Price, K. R.; Bacon, J. R.; Rhodes, M. J. C. *J. Agric. Food Chem.* **1997**, *45*, 938-942.
65. Stalikas, C. D. *J. Sep. Sci.* **2007**, *30*, 3268-3295.
66. Carrasco-Pancorbo, A.; Cruces-Blanco, C.; Segura-Carretero, A.; Fernández-Gutiérrez, A. *J. Agric. Food Chem.* **2004**, *52*, 6687-6693.
67. Carrasco-Pancorbo, A.; Cerretani, L.; Bendini, A.; Segura-Carretero, A.; Gallina-Toschi, T.; Fernández-Gutiérrez, A. *J. Sep. Sci.* **2005**, *28*, 837-858.
68. Cortesi, N.; Azzolini, M.; Rovellini, P.; Fedeli, E. *Riv. Ital. Sost. Grasse*, **2000**, *72*, 241-251.
69. Castro-Vargas, H.I.; Rodríguez-Varela, L.I.; Ferreira, S.R.S.; Parada-Alfonso, F. *J. Supercrit. Fluids*, **2010**, *51*, 319-324.
70. Budrat, P.; Shotipruk, A. *Sep. Purif. Technol.* **2009**, *66*, 125-129.
71. Sudjaroen, Y.; Haubner, R.M; Würtele, G.; Hull, W.E.; Erben, G.; Spiegelhalder, B.; Cahngbumrung, S.; Bartsch, H.; Owen, R.W. *Food Chem. Toxicol.* **2005**, *43*, 1673-1682.
72. Herrero, M.; Mendiola, J.A.; Cifuentes, A.; Ibáñez, E. *J. Chromatogr. A* **2010**, *1217*, 2495-2511.
73. Abbasi, H.; Rezaei, K.; Emamdjomeh, Z.; Ebrahimzadeh Mousavi, S.M. *Eur. J. Lipid Sci. Technol.* **2008**, *110*, 435-440.

74. Casas, L.; Mantell, C.; Rodriguez, M.; Martinez de la Ossa, E. J.; Roldan, A.; De Ory, I.; Caro, I.; Blandino, A. *J. Food Eng.* **2010**, *96*, 304-308.
75. Paviani, L. C.; Dariva, C.; Marcucci, M. C.; Cabral, F. A. *J. Food Process Eng.* **2010**, *33*, 15-27.
76. Arlorio, M.; Coisson, J. D.; Travaglia, F.; Varsaldi, F.; Miglio, G.; Lombardi, G.; Martelli, A. *Food Res. Int.* **2005**, *38*, 1009-1014.
77. Vatain, T.; Skerget, M.; Knez, Z. *J. Food Eng.* **2009**, *90*, 246-254.
78. Louli, V.; Ragoussis, N.; Magoulas, K. *Biores. Technol.* **2004**, *92*, 201-208.
79. Murga, R.; Sanz, M. T.; Beltran, S.; Cabezas, L. *J. Che. Eng. Data*, **2004**, *49*, 779-782.
80. Murga, R.; Sanz, M. T.; Beltran, S.; Cabezas, L. *J. Supercrit. Fluids*, **2003**, *27*, 239-245.
81. Murga, R.; Sanz, M. T.; Beltran, S.; Cabezas, L. *J. Supercrit. Fluids*, **2002**, *23*, 113-121.
82. Chafer, A.; Fornari, T.; Berna, A.; Stateva, R. P. *J. Supercrit. Fluids*, **2004**, *32*, 89-96.
83. Demirbas, A. *Energy Sources A*, **2007**, *29*, 791-797.
84. Luthria, D. L.; Mukhopadhyay, S. *J. Agric. Food Chem.* **2006**, *54*, 41-47.
85. Soltoft, M.; Christensen, J. H.; Nielsen, J.; Knuthsen, P. *Talanta*, **2009**, *80*, 269-278.
86. Luthria, D. L.; Mokhopadhyay, S.; Kwansa, A. L. *J. Sci. Food Agric.* **2006**, *86*, 1350-1358.
87. Ju, Z. Y.; Howard, L. R. *J. Agric. Food Chem.* **2003**, *51*, 5207-5214.
88. Palma, M.; Piñeiro, Z.; Barroso, C. G. *J. Chromatogr. A*, **2001**, *921*, 169-174.
89. Howard, L.; Pandjaitan, N. *J. Food Sci.* **2008**, *73*, C151-C157.
90. Wijngaard, H.; Brunton, N. *J. Agric. Food Chem.* **2009**, *57*, 10626-10631.

91. Plaza, M.; Amigo-Benavent, M.; del Castillo, M. D.; Ibañez, E. Herrero, M. *Food Res. Int.* **2010**, *43*, 1123-1129.
92. Papagiannopoulos, M.; Wollseifen, H. R.; Mellenthin, A.; Haber, B.; Galensa, R. *J. Agric. Food Chem.* **2004**, *52*, 3784-3791.
93. Bonoli, M.; Marconi, E.; Caboni, M.F. *J. Chromatogr. A* **2004**, *1057*, 1-12.
94. Luque-Rodriguez, J. M.; Luque de Castro, M. D.; Perez-Juan, P. *Bioresource Technol.* **2007**, *98*, 2705-2713.
95. Japon-Lujan, R.; Luque de Castro, M. D. *J. Chromatogr. A*, **2006**, *1136*, 185-191.
96. Japon-Lujan, R.; Luque de Castro, M. D. *J. Agric. Food Chem.* **2007**, *55*, 3629-3634.
97. Palma, M.; Piñeiro, Z.; Barroso, C. G. *J. Chromatogr. A*, **2002**, *968*, 1-6.
98. Onofrejova, L.; Vasickova, J.; Klejdus, B.; Stratil, P.; Misurcova, L.; Karcmar, S.; Kopecky, J.; Vacek, J. *J. Pharm. Biomed. Anal.* **2010**, *51*, 464-470.
99. Papagiannopoulos, M.; Zimmermann, B.; Mellenthin, A.; Krappe, M.; Maio, G.; Galensa, R. *J. Chromatogr. A*, **2002**, *98*, 9-16.
100. Ibañez, E.; Cifuentes, A.; Rodriguez, I.; Mendiola, J. A.; Reglero, G.; Señorans, J.; Turner, C. *Device and process for the on-line extraction and drying of complex extracts*, Spanish Patent No. P200900164, **2009**.
101. Herrero, M.; Plaza, M.; Cifuentes, A.; Ibañez, E. *J. Chromatogr. A*, **2010**, *1217*, 2512-2520.
102. Lamm, L. J.; Yang, Y. *Anal. Chem.* **2003**, *75*, 2237-2242.
103. Khuwijitjaru, P.; Chaloodong, K.; Adachi, S. *Food Sci. Technol. Res.* **2008**, *14*, 1-4.
104. Ma, Y. ; Ye, X. ; Hao, Y. ; Xu, G. ; Xu, G. ; Liu, D. *Ultrasonics Sonochem.* **2008**, *15*, 227-232.

105. Ghafoor, K.; Choi, Y. H.; Jeon, J. Y.; Jo, I. H. *J. Agric. Food Chem.* **2009**, *57*, 4988-4994.
106. Khan, M. K.; Abert-Vian, M.; Fabiano-Tixier, A.S.; Dangles, O.; Chemat, F. *Food Chem.* **2010**, *119*, 851-858.
107. Chung, H.; Ji, X.; Canning, C.; Sun, S.; Zhou, K. *J. Agric. Food Chem.* **2010**, *58*, 4508-4512.
108. Londoño-Londoño, J.; Rodrigues de Lima, V.; Lara, O.; Gil, A.; Crecsynski-Pasa, T. B.; Arango, G. J.; Ramirez-Pineda, J. R. *Food Chem.* **2010**, *119*, 81-87.
109. Rodrigues, S.; Pinto, G. A. S. *J. Food Eng.* **2007**, *80*, 869-872.
110. Herrera, M. C.; Luque de Castro, M. D. *J. Chromatogr. A*, **2005**, *1100*, 1-7.
111. Hayat, K.; Hussain, S.; Abbas, S.; Farooq, U.; Ding, B.; Xia, S.; Jia, C.; Zhang, X.; Xia, W. *Sep. Purif. Technol.* **2009**, *70*, 63-70.
112. Ballard, T. S.; Mallikarjunan, P.; Zhou, K.; O'Keefe, S. *Food Chem.* **2010**, *120*, 1185-1192.
113. Nkhili, E.; Tomao, V.; El Hajji, H.; El Boustani, E.S.; Chemat, F.; Dangles, O. *Phytochem. Anal.* **2009**, *20*, 408-415.
114. Klejdus, B.; Kopecký, J.; Benešová, L.; Vacek, J. *J. Chromatogr. A*, **2009**, *1216*, 763-771.
115. Sterbová, D.; Matejíček, D.; Vlcek, J.; Kubán, V. *Anal. Chim. Acta*, **2004**, *513*, 435-444.
116. Oszmianski, J.; Ramos, T., Bourzeix, M. *Am. J. Vitic.* **1998**, *39*, 259-262.
117. Pérez-Magariño, S.; Ortega-Heras, M.; Cano-Mozo, E. *J. Agric. Food Chem.* **2008**, *56*, 11560-11570.
118. Jeffery, D.; Mercurio, M. D.; Herderich, M. J.; Hayasaka, Y.; Smith, P. A. *J. Agric. Food Chem.* **2008**, *56*, 2571-2580.

119. Dvoráková, M.; Hulín, P.; Karabín, M.; Dostálek, P. *Czech J. Food Sci.* **2007**, *25*, 182-188.
120. Nave, F.; Cabrita, M. J.; Teixeira da Costa, C. *J. Chromatogr. A*, **2007**, *1169*, 23-30.
121. Shouqin, Z.; Jun, X.; Changzheng, W. *J. Chem. Technol. Biotechnol.* **2005**, *80*, 50-54.
122. Xi, J.; Shen, D.; Zhao, S.; Lu, B.; Li, Y.; Zhang, R. *Int. J. Pharm.* **2009**, *382*, 139-143.
123. Corrales, M.; Toepfl, S.; Butz, P.; Knorr, D.; Tauscher, B. *Innov. Food Sci. Emerg. Technol.* **2008**, *9*, 85-91.
124. Schwarz, M.; Hillebrand, S.; Habben, S.; Degenhardt, A.; Winterhalter, P. *Biochem. Engineering J.* **2003**, *14*, 179-189.

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Figure legends.

Figure 1. Chemical structures of different phenolic compounds.

Figure 2. Basic chemical structure of flavonoids.

Figure 3. Chemical structures of the different classes of flavonoids.

Figure 4. Structure of common polymethoxylated flavones found in citrus.

Figure 5. Common anthocyanidins present in food.

Figure 6. Structure of some common flavan-3-ol monomers and proanthocyanidins.

Figure 7. Schematic of strategies for the determination of phenolic acids and flavonoids in beverages, plants, and food. Abbreviations: SFE, supercritical fluid extraction; MSPD, matrix solid-phase dispersion; SPME, solid-phase microextraction; CCC, counter-current chromatography; FL, fluorescence; FID, flame ionisation detection; ECD, electron capture detection. Reproduced with permission.⁶⁵

Figure 8. Total phenolic contents obtained with soxhlet extraction at different conditions. Reproduced with permission.⁷⁰

Figure 9. Response surface plots showing the effects of variables on yield of phenolic acids (liq/sol: liquid to solid ratio) during a MAE extraction of phenolic acids from citrus. Reproduced with permission.¹¹¹

Figure 10. Increase in temperature of water, corn oil and salsa as a result of adiabatic compression in high hydrostatic pressure extraction (HHPE). Note that the increase in temperature upon compression is also a function of the initial temperature. Reproduced with permission.¹²²

Table 1. Polyphenols in foods (according to Manach et al.⁵)

	Source (serving size)	Polyphenol content	
		By wt or vol	By serving
		<i>mg/kg fresh wt (or mg/L)</i>	<i>mg/serving</i>
Hydroxybenzoic acids (2, 6)	Blackberry (100 g)	80–270	8–27
Protocatechuic acid	Raspberry (100 g)	60–100	6–10
Gallic acid	Black currant (100 g)	40–130	4–13
p-Hydroxybenzoic acid	Strawberry (200 g)	20–90	4–18
Hydroxycinnamic acids (2, 5–7)	Blueberry (100 g)	2000–2200	200–220
Caffeic acid	Kiwi (100 g)	600–1000	60–100
Chlorogenic acid	Cherry (200 g)	180–1150	36–230
Coumaric acid	Plum (200 g)	140–1150	28–230
Ferulic acid	Aubergine (200 g)	600–660	120–132
Sinapic acid	Apple (200 g)	50–600	10–120
	Pear (200 g)	15–600	3–120
	Chicory (200 g)	200–500	40–100
	Artichoke (100 g)	450	45
	Potato (200 g)	100–190	20–38
	Corn flour (75 g)	310	23
	Flour: wheat, rice, oat (75 g)	70–90	5–7
	Cider (200 mL)	10–500	2–100
	Coffee (200 mL)	350–1750	70–350
Anthocyanins (8–10)	Aubergine (200 g)	7500	1500
Cyanidin	Blackberry (100 g)	1000–4000	100–400
Pelargonidin	Black currant (100 g)	1300–4000	130–400
Peonidin	Blueberry (100 g)	250–5000	25–500
Delphinidin	Black grape (200 g)	300–7500	60–1500
Malvidin	Cherry (200 g)	350–4500	70–900
	Rhubarb (100 g)	2000	200
	Strawberry (200 g)	150–750	30–150
	Red wine (100 mL)	200–350	20–35
	Plum (200 g)	20–250	4–50
	Red cabbage (200 g)	250	50
Flavonols (11–18)	Yellow onion (100 g)	350–1200	35–120
Quercetin	Curly kale (200 g)	300–600	60–120
Kaempferol	Leek (200 g)	30–225	6–45
Myricetin	Cherry tomato (200 g)	15–200	3–40
	Broccoli (200 g)	40–100	8–20
	Blueberry (100 g)	30–160	3–16
	Black currant (100 g)	30–70	3–7
	Apricot (200 g)	25–50	5–10
	Apple (200 g)	20–40	4–8
	Beans, green or white (200 g)	10–50	2–10
	Black grape (200 g)	15–40	3–8
	Tomato (200 g)	2–15	0.4–3.0
	Black tea infusion (200 mL)	30–45	6–9
	Green tea infusion (200 mL)	20–35	4–7
	Red wine (100 mL)	2–30	0.2–3
Flavones (11–12, 14, 18)	Parsley (5 g)	240–1850	1.2–9.2
Apigenin	Celery (200 g)	20–140	4–28
Luteolin	Capsicum pepper (100 g)	5–10	0.5–1
Flavanones (19–21)	Orange juice (200 mL)	215–685	40–140
Hesperetin	Grapefruit juice (200 mL)	100–650	20–130
Naringenin	Lemon juice (200 mL)	50–300	10–60
Eriodictyol			
Isoflavones (22–25)	Soy flour (75 g)	800–1800	60–135
Daidzein	Soybeans, boiled (200 g)	200–900	40–180
Genistein	Miso (100 g)	250–900	25–90
Glycitein	Tofu (100 g)	80–700	8–70

Monomeric flavanols (6, 17, 26, 27) Catechin Epicatechin	Tempeh (100 g)	430–530	43–53
	Soy milk (200 mL)	30–175	6–35
	Chocolate (50 g)	460–610	23–30
	Beans (200 g)	350–550	70–110
	Apricot (200 g)	100–250	20–50
	Cherry (200 g)	50–220	10–44
	Grape (200 g)	30–175	6–35
	Peach (200 g)	50–140	10–28
	Blackberry (100 g)	130	13
	Apple (200 g)	20–120	4–24
	Green tea (200 mL)	100–800	20–160
	Black tea (200 mL)	60–500	12–100
	Red wine (100 mL)	80–300	8–30
	Cider (200 mL)	40	8

Hydroxybenzoic acids

$R_1 = R_2 = \text{OH}, R_3 = \text{H}$: Protocatechuic acid

$R_1 = R_2 = R_3 = \text{OH}$: Gallic acid

Hydroxycinnamic acids

$R_1 = \text{OH}$: Coumaric acid

$R_1 = R_2 = \text{OH}$: Caffeic acid

$R_1 = \text{OCH}_3, R_2 = \text{OH}$: Ferulic acid

Flavonoids

Stilbenes

Resveratrol

Lignans

Secoisolariciresinol

Figure 1.

Figure 2.

Flavanols

Position	5	7	3'	4'	5'
Compound					
Quercetin	OH	OH	OH	OH	-
Kaempferol	OH	OH	-	OH	-
Galangin	OH	OH	-	-	-
Fisetin	-	OH	OH	OH	-
Myricetin	OH	OH	OH	OH	OH

Flavones

Position	5	7	3'	4'
Compound				
Apigenin	OH	OH	-	OH
Luteolin	OH	OH	OH	OH
Chrysin	OH	OH	-	-

Isoflavones

Position	5	7	4'
Compound			
Genistein	OH	OH	OH
Genistin	OH	O-Glu	OH
Daidzein	-	OH	OH
Daidzin	-	O-Glu	OH
Ononin	OH	O-Glu	CH ₃

Flavanones

Position	5	7	3'	4'
Compound				
Naringenin	OH	OH	-	OH
Naringin	OH	O-Rha-Glu	-	OH
Hesperetin	OH	OH	OH	OCH ₃
Hesperidin	OH	O-Rha-Glu	OH	OCH ₃

Anthocyanins

Position	3	5	7	3'	4'	5'
Compound						
Cyanidin	OH	OH	OH	OH	OH	-
Cyanin	O-Glu	OH	OH	OH	OH	-
Peonidin	OH	OH	OH	OCH ₃	OH	-
Delphinidin	-	OH	OH	OH	-	OH
Pelargonidin	OH	OH	OH	-	OH	-
Malvidin	OH	OH	OH	OCH ₃	OH	OCH ₃

Flavanols

Position	3	5	7	3'	4'	5'
Compound						
(+)-Catechin	βOH	OH	OH	OH	OH	-
(-)-Epicatechin	αOH	OH	OH	OH	OH	-
(-)-Epigallocatechin	αOH	OH	OH	OH	OH	OH

Figure 3.

Figure 4.

Figure 5.

(+)-Catechin

(-)-Epicatechin

(-)-Epigallocatechin

Procyanidin B₁

Procyanidin B₂

**(-)-Epigallocatechin
gallate**

Figure 6.

Figure 7.

Figure 8.

Figure 9.

Figure 10.

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Author of the book	Ibañez, E.; Herrero, M.; Plaza, M.

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