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Chapter 3

Agrifood By-Products as a Source of Phytochemical Compounds

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Additional information is available at the end of the chapter

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Abstract

In last years, food by-products and waste valorization practices have gained importance because these processes are sustainable and can increase the profit for local economies. Many compound families of phytochemicals like carotenoids, tocopherols, glucosinolates and phenolic compounds can be obtained through plant by-products coming from agroindustries, such as citric peels, tomato wastes or wine pomace. A number of novel methods like pressured liquid, microwaves or supercritical CO_2 are being used for the extraction of compounds, affecting them in different ways. Phytochemicals obtained can be used in cosmetics, medical uses and dietary supplements or reused in agrifood industries among others, as natural pigments, antioxidants or antimicrobials.

Keywords: phytochemical, valorization, by-products, extraction methods, antioxidant, antimicrobial

1. Introduction

It is estimated that one-third of all food produced for human consumption is lost or wasted. The annual global volume of food wastage is estimated to be 1.6 Gtonnes. It seems clear that a reduction of food wastage would have a substantial positive effect on natural and societal resources and that food wastage represents a missed opportunity to improve global food security and to mitigate environmental impact generated by agriculture [1].

Nowadays, the agricultural processing industries produce substantial quantities of by-products, which are currently generally treated as waste of industry. This practice is not only a waste of

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resource, but also causes environmental pollution and they gradually permeant and release odors. The valorization of agricultural and food residues via green chemistry technologies is among the most important objectives of contemporary chemical research. The environmental benefits reduce waste production, reduce energy demand economical grows, thanks to the significant economic value of many bioproducts new revenues made available to farmers and job creation.

2. Classification of phytochemicals and sources

Phytochemicals are natural chemical compounds, which are found in foods derived from plants. In these, phytochemical substances act as systems of natural defenses for their plants, protecting them from infections and microbial invasions and guests giving them color, aroma and flavor. Phytochemicals are not considered essential for our organism; however, most of them have beneficial properties for health. The main sources of these compounds in foods are fruits, vegetables, legumes, whole grains, nuts, seeds, mushrooms, herbs and spices.

There are more than 2000 phytochemicals in plants, which can be grouped according to their structural characteristics in four large groups: terpenoids, phenolic compounds, nitrogen compounds and sulfur compounds.

2.1. Terpenoids

Terpenoids also referred as isoprenoids are a large and diverse family of organic compounds similar to terpenes. Terpenoids are made up of five-carbon isoprene units, assembled and modified in many different ways, always based on the skeleton of the isopentane. Majority of terpenoids have multicyclical structures, which differ from each other not only in functional group but also in their basic carbon skeletons.

These compounds are found in all kinds of living beings, and are biosynthesized in plants, where they are important in numerous biotic interactions [2]. In plants, the terpenoids fulfill many primary functions: some carotenoid pigments are formed by terpenoids; they are also part of the chlorophyll and gibberellin hormones among others. Steroids and sterols are produced from terpenoids precursors.

Terpenoids of the plants are widely used for their aromatic qualities. They play an important role in traditional medicine and herbal remedies, and their possible antibacterial effects and other pharmaceutical uses are being investigated. They are present, for example, in the essences of eucalyptus, the flavors of clove and ginger. The biosynthesis of terpenoids in plants is through the mevalonic acid pathway.

Terpenoids family comprises very different compounds, of which, the most interesting in food can be classified in the following subgroups:

Mono- and sesquiterpenoids: are the chief constituents of the essential oils; these are the volatile oils obtained from the sap and tissues of certain plants and trees. The essential oils

have been used in perfumery from the earliest times. The main terpenoids shown in nature are: myrcene, ocimene, citral, geraniol, eugenol, carvacrol, linanool, citronellal, carvone, limonene, terpinenes, menthol and menthone, the carane group and the pinane group, among others [3]. The main by-product source of essential oils is the citrus juice processing residues.

Tetraterpenoids (carotenoids): the carotenoids are a large group of pigments widely in animal and vegetable kingdoms; they produce colors ranging from yellow to deep red. Chemically, carotenoids are divided into two groups: the carotenes, which are hydrocarbons, and the xanthophylls, their oxygenated derivatives. Among the carotenes, very soluble in no-polar solvents, are α, β and γ-carotenes, and lycopene. The xanthophylls may present as acids, aldehydes or alcohols. Examples of these compounds are the cryptoxanthin, fucoxanthin, lutein and zeaxanthin. Currently, a high proportion of carotenoids is obtained synthetically, since it is cheaper; however, increasingly those of natural origin are used more [4]. Carotenes can be obtained from orange and reddish sources, as carrot waste or tomato pomace, while xanthophylls can be extracted from spinach residues [5].

2.2. Phenolic compounds

Polyphenols or phenol compounds are organic compounds whose molecular structures contain at least one phenol group; an aromatic ring at least joined a hydroxyl group. Many are classified as secondary metabolites of plants, products synthesized in plants, which possess the biological characteristic of being secondary products of their metabolism. They are generally synthesized by one of two biosynthetic pathways: the path of the shikimic acid or via malonic acid (or two, e.g., flavonoids).

Phenolic compounds in plants are a heterogeneous group of products with more than 10,000 compounds. Some are soluble in solvents organic, others are glycosides or carboxylic acids and therefore soluble in water, and others are very large and insoluble polymers.

This group also plays a very heterogeneous range of roles in plants, roles that are generally attributed to the by-products of plants: many are products of defense against herbivores and pathogens; others provide mechanical support to the plant, other they attract pollinators or dispersers of fruits, some of them absorb the ultraviolet radiation or act as allelopathic agents. In humans, these compounds are well known to possess healthy benefits, and the interest in food phenolics has increased due to their antioxidant and free radical-scavenging abilities [6]. Polyphenols can be structurally classified into several groups: flavonoids, lignans, stilbenes, and phenolic acids.

Flavonoids are one of the most studied families, since about 4000 different structures are known. Being a numerous group, they can be subdivided into: flavonols, flavanones, flavanols, flavones, and isoflavones anthocyanidins. Flavonoids have antioxidant and other living-body modulating activities [7]. For its part, phenolic acids can be classified as hydroxybenzoic acids and hydroxycinnamic acids, which are derived from non-phenolic molecules of benzoic and cinnamic acid, respectively. Phenols are present in numerous products, and collection has been studied in by-products of the obtaining of different oils, wine, juices, coffee, as well as saved cereal among others.

Group	Subgroup	Sources
Terpenoids	Mono- and sesquiterpenoid	Citric peels
	Carotenoids	Carrot, tomato and spinach wastes
Phenolic compounds	Flavonoids	Onion skin waste, citric peels, grape skins, olive leaf, soybean flour
	Lignans	Sesame cake
	Stilbenes	Wine pomace
	Phenolic acids	Pomegranate and grape by-products, rice bran
Nitrogen alkaloids	—	Cocoa and coffee wastes
Organosulfur compounds	Glucosinolates	Cauliflower and broccoli by-product
	Allyl sulphides	Garlic

Table 1. Classification of phytochemical and main sources of obtainment in agroindustrial by-products.

2.3. Nitrogen alkaloids

They are a large family of secondary metabolites of plants, which chemically have three characteristics in common: they are soluble in water, contain at least one atom of nitrogen and have biological activity [8]. In plants, alkaloids seem to offer protection from insects and herbivores. In humans, the alkaloids produce physiological and psychological responses, most of them because of their interaction with neurotransmitters. The alkaloids are classified according to the rings present in the molecule. Some of the alkaloids present in foods are of interest as piperine, caffeine and theobromine, from the pepper, coffee and cocoa, respectively.

2.4. Organosulfur compounds

The organosulfur compounds or thiols are chemical compounds containing sulfur in their structure. They are present in garlic and vegetables of genus cruciferous (cabbage, turnips and members of the mustard family). They include the glucosinolates and allyl sulphides.

Glucosinolates are metabolites characteristic of the *Brassicales* order. Many of us are familiar with the characteristic pungent flavor of wasabi and mustard. Chemically, glucosinolates are organic anions with an amino acid-derived side chain and oxime moiety with an S-linked thioglucose and an O-linked sulfate group [9]. Allyl sulphides are a very important phytochemical group present in garlic.

Main sources of obtainment of these compounds in agrifood by-products are summarized in **Table 1**.

3. Extraction processes

Numerous methods of extraction have been developed for obtaining phytochemical compounds. The use of one or other will depend on both type of matrix (solid or liquid) and

type of molecule extracted, as well as the use that will be given to extract thus obtained. The conventional techniques used include maceration, infusion, decoction or Soxhlet extraction among others [10]. However, since the main objective of the extraction is the reuse of waste and reduce environmental impact, green extraction techniques are necessary for this purpose. The objective of these techniques is to improve extraction performance, decreasing the use of organic solvents or the energy used. These techniques include:

3.1. Ultrasound-assisted extraction (UAE)

Ultrasound is a mechanical wave that propagates in an elastic medium and their frequency is above the audible sounds, this is more than 20,000 Hz. The ultrasound-assisted extraction is a technique that uses ultrasonic waves to agitate an immersed sample in an organic solvent. The major effects of ultrasound in a liquid medium are attributed to the cavitation phenomena, which comes from the physical processes that create, enlarge, and implode microbubbles of gases dissolved in the solvent [11].

Since the extraction is carried out in a medium, the temperature, time and solvent-type can affect not only the extraction yield but also the composition of the extract and should thus be taken into consideration. Furthermore, after extraction, compounds with organic solvent are separated from the matrix by centrifugation or filtration like in a conventional extraction.

The greatest advantage of using this technique against conventional techniques is that this technology shortens the total time of procedure, together with a decrease of consumed energy and pollution. For that, the procedures using ultrasound assistance have a production cost and a functioning cost much lower than the cost for conventional procedures with a high purity final product. On the other hand, as the temperature reached is not very high, sonication is suitable for extracting thermolabile compounds [12]. However, and despite the fact that this technology can be easily integrated into the industry, its use implies initial costs, which range between 10,000 and 200,000 euros [11].

The application of this technology for the extraction of phytochemicals from by-product has been widely researched, but its application differs according to the various matrices and analytes to extract. Thus, terpenoids have been obtained by UAE from different by-products: The industrial extraction of terpenoids limonin, nomolin and obakunone were performed from lemon seeds with UAE, obtaining high extraction yields of limonoids [13]. In case of lycopene, tomato paste processing wastes are an important source of obtainment; the UAE has demonstrated to decrease the solvent-solid ratio and to require less time and lower temperature than conventional organic solvent extraction [14]. Papaya processing waste is also studied to lycopene extraction. In this case, results indicated that UAE was the most effective extracting method among the conventional and Soxhlet method [15]. In addition, the extraction of carotenoids without solvent also can be improved with the use of ultrasound, as is the case with the extraction of carotenoids from pomegranate peel using sunflower oil [16].

Different wastes have been used for the ultrasound-assisted extraction of phenolic compounds. Mandarin peel has been utilized for the extraction of flavonoids and hesperidin comparing the results with maceration extraction. These showed greater extraction efficiency

and lower times of extraction with UAE method [17]. On the other hand, the pomace formed in the wine or juice making from blueberry contains many phenolic and other bioactive compounds. These can be retrieved through ultrasound-assisted extraction obtaining the same compounds than in a conventional solvent extraction but with higher yields of extraction [18]. Spent coffee grounds are a valuable source of phenolic compounds, of which, the main are chlorogenic and protocatechuic acids. These can be extracted with an ultrasound-assisted solid-liquid extraction with mild temperatures and short times [19].

For its part, isothiocyanates extraction has been optimized from cauliflower by-products using ultrasound-assisted extraction. These extracts can be added to an apple beverage until 10% preserving well the sensorial properties to obtain a new functional drink [20].

3.2. Microwave-assisted extraction (MAE)

Microwave-assisted extraction (MAE) is a relatively new extraction technique that combines microwave irradiation and traditional solvent extraction. Microwave energy can be used to improve the extraction of compounds soluble in specific fluids (liquids or gases) as a result of changes in the cell structure caused by electromagnetic waves. It provides rapid and selective techniques with best recoveries than the obtained in conventional extraction processes [21]. Also offers other advantages over common technologies, such as lower energy consumption, volume of solvent and toxicity of the solvents used and, in general, less waste. Another advantage of MAE is that the quicker heating occurring inside the solids where the dissolution of the extract components takes place.

In general, the use of microwave is widespread at laboratory scale. However, its use in the industry offers some disadvantages such as the high initial cost, maintenance, or safety aspects [22]. Moreover, since its use is based on the warming of the matrix and many of the phytochemicals of interest are thermolabile, its use for this purpose is limited. However, there are numerous studies that have been carried out using MAE, both in the pre-treatment of the sample and to facilitate the extraction. For example, the extraction of β-carotene and other carotenoids contained in carrot residues has been studied using intermittent microwave radiation. The thermal degradation caused by MAE in β-carotene was investigated using for that the measurement of antioxidant activity [23]. For the extraction of phenolic compounds from dried waste grape skins, MAE achieves a savings of 83% with respect to conventional in extraction time [24]. Microwave-assisted extraction has also been investigated for the extraction of phenolics from other different matrix as *Eucalyptus robusta* [25] or green tea leaves [21].

The combined use of microwave-assisted and ultrasound-assisted extraction has also been developed for the extraction of phytochemicals. For example, it has been proposed the extraction of essential oils, polyphenols and pectin from the orange peels waste in a solvent-free process [26].

3.3. Enzymatic-assisted extraction (EAE)

A procedure widely used to improve the efficiency of the extraction of compounds from a plant matrix is based on the enzymatic assisted extraction (EAE) [27]. This procedure is

based on prior treatment of the matrix with the corresponding enzyme followed by a process of extraction solvent [28]. This non-traditional and environmentally friendly technology improve the yield of target compounds while reduces the use of solvents in the process. This is possible because enzymes can catalyze reactions in aqueous solutions under mild conditions. The use of enzymes for the extraction is based on their ability to degrade cell walls and membranes, thereby increasing cell wall permeability and enabling targeted compounds release into the medium.

This involves the use of hydrolytic enzymes to alter the cell, mainly composed of large polymer walls highly complex, such as cellulose, hemicellulose, lignin, and pectin [27]. Specific use of enzymes can increase use pre-treatment effects or decrease the amount of solvent, as well as increase the yield of extracted compounds. Enzymes such as cellulases, pectinases or hemicellulases are widely used in juice processing and clarification of beer for cell wall degradation. When matrix have been previously treated with enzymes, it is gotten the cell walls break, thus increasing the performance of the extraction of bioactive compounds [28].

The use of EAE can be tested and optimized on the laboratory scale, and the use of common food-grade enzymes makes it a low-cost technique for extraction purposes. However, some technical limitations on the industry application are a higher relative cost for processing and the difficulty to optimize the process at industrial scale [10].

EAE has been tested for improve the extractions of carotenoids from tomato peels [29]. Therefore, the use of hydrolytic enzymes can improve the recovery of bound and free phenols from pomegranate peels [30]. EAE has been used successfully for the recovery of polyphenols in citrus peel and ginger [31] winemaking by-products [32]; underutilized watermelon rind [33] or cauliflower outer leaves [34]; among others.

3.4. Supercritical fluid extraction (SFE)

It is a technique that uses a solvent in supercritical conditions. A substance at a pressure higher than its critical pressure and temperature higher than its critical temperature is known as supercritical fluid. Supercritical fluids have properties intermediate between a gas and a liquid, favoring its penetration in different matrices, and therefore the solubilization of solutes, having the possibility of extracting thermolabile compounds. Nowadays, the most important application of SFE to food industry is the extraction of caffeine from coffee.

The supercritical fluid extraction consists of four stages [10]: pressurization step, temperature adjustment stage, extraction stage and separation step.

Advantages of using this technology are the easy separation between solvent and matrix that avoids to increase the temperature to remove the solvent; the possibility to work with non-toxic solvent and the reuse of this, and a relatively low-cost obtainment of high pressure or temperature. However, its practical application is limited to processes that are not affected by the relatively high temperature necessaries and the initial costs are high.

In the majority of cases, the supercritical fluid used is CO_2, for being generally recognized as safe (GRAS). The application of supercritical CO_2 has high performances for the extraction of nonpolar substances. However, the CO_2 under critical conditions is a poor solvent for polar compounds. This limitation can be solved by adding co-solvents that alter the polarity of the CO_2, but they may become contained, requiring a subsequent separation operation.

SFE has been used for the extraction of lycopene β-carotene from tomato peels [35] and carrot peels [36]. For its part, the recovery of phenol by SFE has been tested in apple pomace [37], cacao pod husk [38], mango by-products [39] or sour cherry pomace [40].

3.5. Pressurized liquid extraction (PLE)

The so-called pressurized solvent extraction is a technique that combines solvent extraction at temperatures (50–200°C) and high pressures (1500–2000 psi) to quickly and efficiently extract compounds from solid matrices. The use of liquid solvents at high temperatures and pressures improves the performance of the extractions since the solubility is increased and the mass transfer is improved and the rupture of the composite-matrix superficial equilibrium is facilitated [41]. PLE can be considered as a green extraction process especially when a nontoxic solvent is used.

A wide range of organic nonpolar to polar solvent and their mixtures have been used in pressurized liquid extraction of phytochemical. However, it is a technology that modifies solvent properties. This fact makes water suitable for the extraction to polar and nonpolar organic compounds.

PLE could be carried out in static and dynamic modes. Static extraction is considered more efficient because of the greater penetration of the solvent into the pores of the source [10]. The advantages of this method with respect to conventional ones are the short extraction times, the yields obtained and the reduced usage of solvents. However, this method is not suitable for thermolabile compounds because the high temperature can alter the structure of these. For that, the use of this technique for the extraction of phytochemical is very limited.

Some researches have been carried out with pressurized liquid for the extraction of carotenoids from shrimp waste [42] or phenolic acid in potato peels [43].

3.6. Other technologies

In addition to the above, other extraction technologies have been developed as the instant controlled pressure drop or pulsed electric field. The latter has been tested for the extraction of phenolic compounds from orange peel [44] or grape pomace [45] and alkaloids form potato peels [46]. Finally, there are numerous investigations in which the combination of these techniques has been used to take advantage of a possible synergy between them. Thus, for example, enzymes-assisted extraction often complemented with ultrasonic extraction or supercritical fluid extraction.

An overview of the improvement of all these methods can be observed in **Table 2**.

Extraction method	Improvement of method
Ultrasound-assisted extraction (UAE)	Cavitation phenomena, which comes from the physical processes that create, enlarge, and implode microbubbles of gases dissolved in the solvent
Microwaves-assisted extraction (MAE)	The quicker heating occurring inside the solids where the dissolution of the extract components takes place
Enzyme-assisted extraction (EAE)	Ability of enzymes to degrade cell walls and membranes, increasing cell wall permeability
Supercritical fluid extraction (SFE)	Supercritical fluids have properties intermediate between a gas and a liquid, favoring its penetration in different matrices
Pressurized liquid extraction (PLE)	The solubility is increased and the mass transfer is improved
Combined extraction processes	The possible synergic effect of techniques

Table 2. Overview of the improvement of the main green extraction techniques.

4. Industry phytochemical residue applications

As stated in previous sections, phytochemicals are present in foods of plant origin. The food industry is considered a source of extraction of phytochemical compounds, which can be obtained from waste generated in the processing of fruit and vegetables. This fact is beneficial for food industry producers, as it generates an economic advantage [47].

It is worth mentioning that in its elaboration process, the less handling and processing of food generate a more retain of these compounds. For this reason, processes like a cereal refining, intense and long heating, cocking in broths which are then discarded and some of its compounds are lost, reduce the food phytochemical content. In nature, phytochemicals are in many foods, but in the future, the development of bioengineering will allow us to create plants with more concentration of these compounds.

In reference to its possible applications, the great phytochemicals properties allow us to open a wide range of possibilities. For example, due to its antioxidant activity, these could be used in creams, functional foods and fortified foods formulation, or due to its antimicrobial activity, these could be used in food conservation and medicine. In the following sections, we see some of the most important applications in detail.

4.1. Phytochemicals in medicine and pharmacy

The phytochemical application in medicine and pharmacy is directly related with the phytotherapy (use of plant-derived medications in the treatment and prevention of disease). The large quantity of properties that the plants has allowed us to apply them for treatments of diseases, and this is due to their phytochemical content. Numerous trials and pharmacological studies of specific phytotherapeutic preparations exist but, in some countries, phytotherapy is viewed as a form of traditional medicine.

For example, against respiratory diseases are effective the consumption of essentials oils of *Eucalyptus globulus Labill.* [48], *Origanum vulgare* L. [49], *Pinus* sp. [50] or *Thymus vulgaris* [51], which due to its high concentration of tannins, phenolic acids, terpenes and flavonoids, among others, cause an antiseptic and expectorant action.

Against circulatory diseases, the application of *Vitis vinifera* L. due to its high concentration of polyphenols, mainly resveratrol, in its fruits generate a lot of antioxidant activity and its anthocyanins do a veinotonic and vasoprotective action [52].

Other plant species which are of great importance is *Camellia sinensis* from which green tea is obtained. Some studies have reported the use of this specie rich in catechins against cancer chemoprevention, hypercholesterolemia, atherosclerosis, Parkinson's disease, Alzheimer's disease, and other aging-related disorders [53].

Concretely, an agroindustry residue, olive leaf, is a great source of phytochemicals. These contain a high concentration of oleuropein, tyrosol and hydroxytyrosol, three important phenolic compounds which have bioactive properties like antioxidant capacity [54]. The European Food Safety Association (EFSA) determined officially that oleuropein present in olive leaf extract generate a better glucose tolerance in humans [55]. These are some applications and uses in medicine and pharmacy of phytochemical compounds present in some plants but the number or these is increased day by day.

4.2. Phytochemicals in food industry

The food and agricultural products industries are an important source of phenolics-rich by-products, which have been a good source of natural antioxidant. The application of these by-products in other foods like oil, fish and meat has shown antioxidant values similar to synthetic antioxidant, particularly the flavonoids and hydroxycinnamic acids [56]. The way to consume these by-products and add to our diet is directly, for example, the consumption of plants in tea form, or indirectly, for example, like an alimentary additive or supplement.

Another application of phytochemicals in food is the creation of active packaging for them. The films and coatings of different origin (proteins, polysaccharides, etc.) incorporate a wide variety of essential oils or plant extracts with the aim of creating active packaging with antimicrobial/antioxidant properties that improve the conservation of various types of food, for example, for fruit conservation [57], meat [58] and fish [59].

As we have already mentioned, the consumption of phytochemicals and adding these to our diet generate a lot of benefits to our health due to the large number of biological activities that possess.

4.3. Other phytochemical application fields

A great number of phytochemicals bioactivities open a wide range of possibilities and applications of these important food plants compounds. Other field is the cosmetic industry due to the antioxidant properties of phytochemical compounds. The reactive oxygen species generate

cell damage, and this can generate to appear early signs of aging. If plant extract or directly phytochemicals compounds are adding to cosmetic products these can combat these toxic substances to cells and prevent the appearance of wrinkles, skin blemishes, and so on [60].

Currently, plant extracts rich in phytochemicals are also being evaluated in the oral cavity to prevent the development of microorganisms and prevent the appearance of dental caries and plaque. Specifically, the green tea extract has been evaluated against one of the main microorganisms that develop oral infections, Streptococcus mutants, obtaining great antimicrobial activity when applying the ethanolic extract of *C. sinensis* [61]. These results are of great importance because they show us that plant extracts rich in phytochemical compounds can be applied, for example, to dental prosthesis or toothpaste to prevent buccal infections.

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