Technologies for Extraction and Production of Bioactive Compounds to be Used as Nutraceuticals and Food Ingredients: An Overview

G. Joana Gil-Chávez, José A. Villa, J. Fernando Ayala-Zavala, J. Basilio Heredia, David Sepulveda, Elhadi M. Yahia, and Gustavo A. González-Aguilar

Abstract: Natural bioactive compounds include a broad diversity of structures and functionalities that provide an excellent pool of molecules for the production of nutraceuticals, functional foods, and food additives. Some of those compounds can be found in nature at high concentration such as polyphenols but others can only be found at very low levels, so that massive harvesting is needed to obtain sufficient amounts, and their structural diversity and complexity make chemical synthesis unprofitable. The inherent difficulties in screening and producing these compounds have led to the development of advanced technologies. The commonly used methods for their extraction are the conventional liquid–liquid or solid–liquid extraction and the advanced include pressurized-liquid extraction, subcritical and supercritical extractions, and microwave- and ultrasound-assisted extractions. In addition, these extraction techniques have been improved with previous steps (enzyme–and instant controlled pressure drop-assisted extractions) which help to release the compounds from the matrix. These technologies could provide in the next few years an innovative approach to increase the production of specific compounds for use as nutraceuticals or as ingredients in the design of functional foods.

Introduction

The search for natural bioactive compounds (NBCs) with potential for the treatment and prevention of human diseases and to meet other needs is currently a key topic in many laboratories and industries. These compounds efficiently interact with proteins, DNA, and other biological molecules to produce a desired outcome, which could be exploited for designing natural products-derived therapeutic agents (Ajikumar and others 2008). Nowadays, there is a marked trend in the food industry toward the development and manufacture of functional products. This new class of food products has seen great success in the market due to the growing of consumer interest for "healthy" food. Hence, pharmaceutical and food domains share a similar interest to obtain and characterize new NBCs which can be used as drugs, functional food ingredients, or nutraceuticals. However, supply limitations from natural sources affect the large-scale use of some of these substances.

NBCs are often synthesized in small quantities in nature and are present as conjugates or mixtures in extracts which require labor-intensive and time-consuming purification procedures (Lam 2007). Besides, the structural diversity and complexity of these molecules make their chemical synthesis unprofitable (Lam 2007). In this regard, the inherent difficulties in screening and producing these molecules have led to the development of emerging alternative technologies to address these limitations.

Recent technological advances and the development of new methods to improve the production, detection, separation, and/or characterization have revolutionized the screening of NBCs and provided an opportunity to obtain natural extracts that could be potentially used (Van Lanen and Shen 2006; Wang and Weller 2006). Examples of these technologies include mainly metabolic engineering and methods to optimize the isolation of these molecular species. For example, molecular biological techniques have been employed to use bacteria to produce drugs like isoprenoid compounds, originally isolated from plants (Chang and Keasling 2006), and to produce novel flavanones and dihydroflavonols (Harvey 2008). In addition, extraction processes for the separation of compounds have been developed to obtain highly

purified products, rendering them useful in a wide range of applications. These technologies could provide an innovative approach to increase the production of the desired compounds. The aim of this review is to summarize and give an overview of the sources, technologies, and methods that have been developed to improve the production and isolation of NBCs, with special attention to antioxidants with their possible application in the design of nutraceuticals and functional food products.

Sources of Natural Bioactive Compounds

NBCs have been studied directly in their different natural matrices such as tea, olive oil, exotic fruits, plants, algae, microalgae, bacteria, and fungi. In this regard, an increasing number of scientific studies have been performed on these substances and their sources.

Plant tissues

Plant natural compounds can be divided into primary and secondary metabolites (Wu and Chappell 2008). The primary metabolites are sugars, amino acids, fatty acids, and nucleic acids, as well as the compounds considered ubiquitous to all plants for growth and development, like growth regulators and cell wall components, among others. Secondary metabolism has assorted functions throughout plant's life cycle (Balandrin and others 1985). These functions can be classified as mediating the interaction of the plant with its environment such as plant–microorganism, plant–insect, and plant–plant interactions and pollinating or attracting, among others. Hence, due to the wide range of functions that plant secondary metabolites have in plant cells, these compounds are of special interest to researchers who focus their studies on their bioactivity for useful applications.

Natural biosynthesis of these metabolites depends on the physiology and developmental stage of the plant. Based on their biosynthetic origins, plant secondary metabolites can be divided into 5 groups: polyketides, isoprenoids, alkaloids, phenylpropanoids, and flavonoids (Oksman-Caldentey and Inzé 2004). These compounds are synthesized in specialized cell types and only during a particular growth stage, or under specific seasonal/conditions, making their extraction and purification quite difficult (Verpoorte and others 2002). Examples of commercially useful plant secondary metabolites are carotenoids, terpenoids, alkaloids, phenylpropanoids, and more specific compounds such as corilagin, ellagic acid, vinblastine, vincristine, β- and α-farnesene, among others (Sözke and others 2004; Nobili and others 2009; Yang and others 2010a). These compounds are commonly utilized for the production of pharmaceuticals (Shahidi 2009) and more recently it has been proposed their use as food additives to increase the functionality of foods (Ayala-Zavala and others 2010).

Given the importance and wide biological activities of NBCs in plant tissues, and their bioactive properties, these compounds have come to play a crucial role in the development of new products (Wu and Chappell 2008). In the last 25 y about 60% to 70% of newly approved drugs on cancer and infectious diseases were derived from NBCs (Newman and Cragg 2007). It is important to notice that more than 2/3 of the world population still relies on medicinal plants for their primary pharmaceutical care (McChesney and others 2007). However, although these compounds have been tested empirically by the population obtaining some beneficial effects for human health, more scientific evidence is needed in order to support their efficacy and to ensure their safety.

Microorganisms

Microorganisms are important for many reasons, particularly because they use biological systems for the production of important molecules (Demain 2000; Donnez and others 2009). About 23000 NBCs of microbial origin have been identified, and most of these substances have been isolated from only very few groups of microorganisms (Olano and others 2008).

Fungal microorganisms seem to occupy virtually every living and nonliving niche on earth, including those in deep rock sediments and deserts as well as aqueous environments (Strobel 2003). Like plants and prokaryotes, fungi synthesize bioactive compounds because they have an ecological function. Different compounds have various functions, from protective action against lethal photooxidations to protection against environmental stress, and they can also act as cofactors in enzyme reactions (Mapari and others 2005). There is a wide variety of fungi used in the production of these compounds including *Penicillium, Aspergillus,* and *Streptomyces*, which are also exploited to produce bioactive molecules such as antibiotics, enzymes, and organic acids (Liu and others 2004; Silveira and others 2008).

NBCs produced by microorganism can be incorporated into foods as nutritional supplements, flavor enhancers, texturizers, preservatives, emulsifiers, acidulants, surfactants, or thickeners. Important metabolites that have been isolated from bacteria are isoprenoids like carotenoids (such as β-carotene and lycopene) and phenylpropanoids like stilbene derivatives (resveratrol and others), among others (Chang and Keasling 2006; Klein-Marcuschamer and others 2007; Ajikumar and others 2008; Donnez and others 2009). However, only about 1% of the microbes are cultivated *in vitro*, implying that there is a wide biodiversity and huge numbers of natural compounds in microorganisms that remain unexploited (Van Lanen and Shen 2006). The main reason for using microorganisms to produce compounds from plants and animals (rather than from microbes) is the relative simplicity for obtaining high yields, and the possibility for environmental and genetic manipulation (Demain 2000). Furthermore, microorganisms are excellent to produce many valuable products commonly produced in small quantities that are needed for their own benefit (Demain 2000). However, in this regard, the very low levels of bioactive compounds produced limit their potential use.

Algae and microalgae

Algae are important constituents of many ecosystems ranging from marine and freshwater environments to desert sands (Guschina and Harwood 2006; El Gamal 2010). As many as 30000 distinct microalga species might inhabit the earth and over 15000 novel compounds have been chemically obtained from them (Metting John 1986; Cardozo and others 2007; Rodríguez-Meizoso and others 2010). In addition, their importance as a source of novel compounds is growing rapidly, and researchers have indicated that these compounds exhibit diverse biological activities (Wijesekara and others 2010).

It has been reported that algae are a very interesting natural source of new compounds and many of them possess antioxidant, antimicrobial, and antiviral activities (Onofrejová and others 2010; Plaza and others 2010a; Rodríguez-Meizoso and others 2010). These organisms live in habitats exposed to extreme conditions, and therefore they must adapt rapidly and efficiently, and as a consequence, produce a great variety of biologically active secondary metabolites that participate in the natural defense mechanisms (Rodríguez-Meizoso and others 2010). These defense strategies can result in a high level of structural and chemical
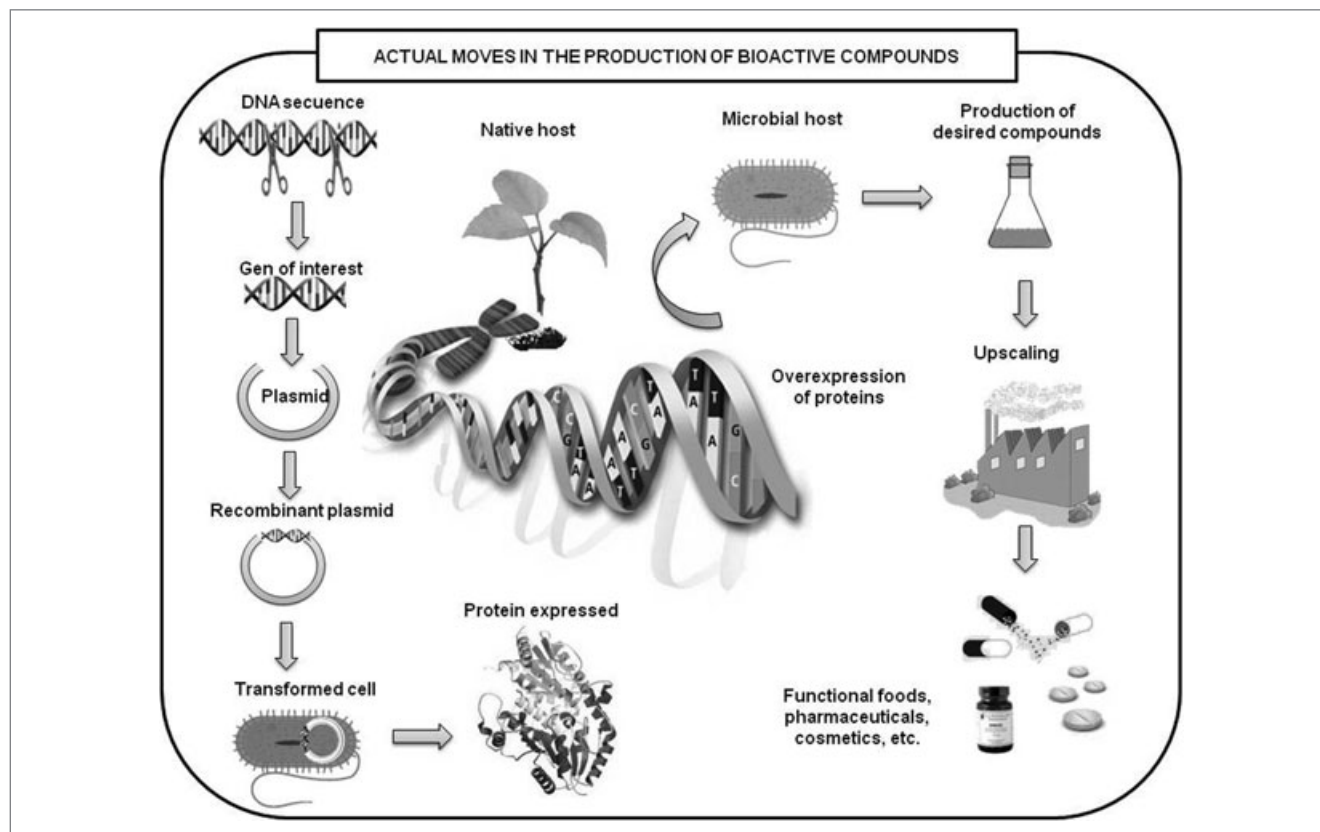
Figure 1–Systematic strategies to achieve high-yield production of natural bioactive compounds utilizing metabolic engineering.

diversity of compounds, originating from different metabolic pathways.

Microalgae have been described to secrete a wide range of compounds that are used or could be potentially employed as functional ingredients including carotenoids, polyphenols, and other antioxidant pigments, flavonoids such as quercetin, catechin, and tiliroside, acid derivates and dipeptides, among others (Lam 2007). Nevertheless, not only the presence of particular bioactive compounds makes algae interesting. They are also characterized by huge diversity and the possibility of harvesting them and also of growing them under different conditions, leading to an enrichment and production of a selection of bioactive compounds which can be useful as part of new drugs or supplements in the pharmaceutical and food industries (El Gamal 2010).

Despite the fact that there are many sources of NBCs, one of the limiting factors in their generation is the low concentration in which these compounds can be obtained. Once again, the development of new strategies and methods to optimize recovery and production of NBCs is an important approach that is being addressed currently by many laboratories around the world.

Metabolic engineering (biotransformation/bioconversion)

Metabolic engineering (ME) is defined as the directed improvement of product formation or cellular properties through the modification of specific biochemical reactions or the introduction of new ones with the use of recombinant DNA technology (Figure 1) (Demain and Adrio 2008). With the recognition that genetic control plays a most crucial role in the production of NBCs, the application of ME has become an intensive area of research in biotechnology to obtain NBCs (Wenzel and Müller

2005), driven by the increasing understanding of NBCs biosynthesis and recent advances in molecular genetics (Wilkinson and Micklefield 2007). The goal is to increase the production of specific NBCs in the normal producing plant species or to transfer a pathway, or part of a pathway, to other plant species and microbes (Verpoorte and others 2002; Crozier and others 2009).

The potential of ME in plants is high, and has been described to modify anthocyanin and flavonoid pathways, such as increased levels of flavonol production in tomato (Mathews and others 2003; Oksman-Caldentey and Saito 2005). However, only a few pathways in plants are well understood such as those for flavonoids, terpenoid indole, and isoquinoline alkaloids (Oksman-Caldentey and Inzé 2004). In this context, ME of secondary metabolic pathways are of great interest and the knowledge of the whole biosynthetic pathway and a well understanding of the regulatory mechanisms controlling the onset and the flux of the pathways still need to be elucidated (Oksman-Caldentey and Saito 2005).

A performance of ME needs to follow several steps. First, partial pathways can be recruited from independent sources and co-localized in a single host (Prather and Martin 2008). An enzymatic step in the pathway can be knocked out, for example, by reducing the level of the corresponding mRNA via antisense or by overexpressing an antibody against the enzyme (Verpoorte and Memelink 2002). Other approaches include change of the flux into a competitive pathway or an increase in the catabolism of the target compound (Verpoorte and Memelink 2002). However, several points must be taken into consideration such as the versatility of expression hosts, availability of biosynthetic precursors, promoter recognition, product toxicity and self-resistance, protein–protein interaction, and expression levels (Wenzel and Müller 2005). An

important feature of the heterologous host is that they must possess the cellular machinery required for successful NBCs production and maintain their activity. In addition, the functionality of regulatory elements and promoter structures as well as mRNA stability when choosing the host organism, needs to be considered (Wenzel and Müller 2005).

Several species have been used as hosts to produce compounds of interest such as *Escherichia coli, Streptomycetes, Pseudomonads,* and *Bacilli,* among others (Wenzel and Müller 2005; Prather and Martin 2008). An impressive example of the use of microorganisms is the production of riboflavin by *Ashbya gossypii* that has been improved 40000 times and the production of a 100000-fold excess of vitamin B12 by *Pseudomonas denitrificans* (Olano and others 2008). Similarly, the entire trans–resveratrol pathway has been introduced in microorganisms such as *Saccharomyces cerevisiae, Lactococcus lactis, Aspergillus niger,* and *Aspergillus oryzae* (Donnez and others 2009). Moreover, recent advances in the large scale production of terpenoids have been achieved in recent years (Ajikumar and others 2008).

One of the main goals of this area is to provide fine chemicals and pharmaceuticals at a significantly lower cost by scalable fermentation processes using microbes to carry out the biosynthesis of valuable small molecules from inexpensive sugar based carbon sources (Chang and Keasling 2006). In order to accomplish this goal, new technologies have been described to improve ME including whole genome scanning, generation of new functional enzymes, *de novo* DNA synthesis for the design and construction of DNA involved in the biosynthetic pathways, among others (Van Lanen and Shen 2006; Ajikumar and others 2008; Prather and Martin 2008). However, limitations including cost, scalability, safety, and compound authenticity with these expression systems have prompted research into alternative platforms such as plant-based "molecular farming" approach with significant advantages in both cost and safety over other eukaryotic expression systems (for a comprehensive review, see Xu and others 2011).

Technological Methods to Optimize the Production of Bioactive Compounds

Several methods have been developed to improve the isolation and production of NBCs which include various extraction techniques and ME. The most used extraction techniques and the main compounds obtained according to current literature information are discussed in the following sections.

Solvent extraction

Solvent extraction (SE) is used to obtain certain compounds from different materials such as sediments, soil, polymers, bacteria, fungi, algae and microalgae, and, more commonly, plants (Hattab and others 2007; Plaza and others 2010c). Basically, pretreated raw material is exposed to different solvents, which takes up compounds of interest and also other agents (flavors and colorings) (Starmans and Nijhuis 1996). Samples are usually centrifuged and filtered to remove solid residue, and the extract could be used as additive, food supplement or be destined for the preparation of functional foods (Starmans and Nijhuis 1996).

A large number of NBCs have been traditionally extracted from natural sources with organic solvents (some examples are showed in Table 1), representing the most important step in optimizing recovery of desirable components by this technique (Li and others 2006; Dunford and others 2010). Some of the most widely used solvents in the extraction procedures are hexane, ether, chloroform, acetonitrile, benzene, and ethanol and are commonly used in different ratios with water (Starmans and Nijhuis 1996; Vatai and others 2009; Plaza and others 2010c; Tokuoka and others 2010). These organic solvents can be employed for the extraction of both polar and nonpolar organic compounds such as alkaloids, organochlorine pesticides, phenols, aromatic hydrocarbons, fatty acids, and oils, among others (Szentmihályi and others 2002; Li and others 2006; Hattab and others 2007; Villa-Rodriguez and others 2010; Plaza and others 2010c). However, various solvents must be used with care as they are toxic for humans and dangerous for the environment; moreover, the extraction conditions are sometimes laborious (Li and others 2006; Miron and others 2010). The solvent must be separated from the final extract, especially if the product is to be used in food applications (Starmans and Nijhuis 1996).

SE is advantageous compared to other methods due to low processing cost and ease of operation. However, this method uses toxic solvents, requires an evaporation/concentration step for recovery, and usually calls for large amounts of solvent and extended time to be carried out. Moreover, the possibility of thermal degradation of NBCs cannot be ignored, due to the high temperatures of the solvents during the long times of extraction. In general, the art of separation is improving, with new methods and procedures rapidly being developed. SE has been improved by other methods such as soxhlet, ultrasound, or microwave extraction and SFE, among others, in order to obtain better yields (Szentmihályi and others 2002).

Pressurized liquid extraction

Recently, new extraction systems have been proposed to replace common SE, and now pressurized liquid extraction (PLE) is gaining importance and has been widely employed for the extraction of NBCs from natural sources (Miron and others 2010; Plaza and others 2010a).

PLE is referred to as accelerated SE and pressurized SE, using organic liquid solvents at high temperature (50 to 200 °C) and pressure (1450 to 2175 psi) to ensure the rapid extraction rate of compounds (Dunford and others 2010). As the temperature increases the dielectric constant of the solvent decreases, consequently lowering the polarity of the solvent (Abboud and Notario 1999). Thus, temperature could be used to match the polarity of a solvent to that of the compounds of interest to be recovered (Dunford and others 2010; Miron and others 2010). The high pressure helps the extraction cells to be filled faster and forces liquid into the solid matrix. These new techniques allow a faster extraction in which less amount of solvents are used and higher yields are obtained in comparison with traditional SE. In addition, the use of PLE allows the attainment of food-grade extracts obtained only when water or other GRAS (generally recognized as safe) solvents, such as ethanol are used (Plaza and others 2010b).

The extraction of NBCs by PLE has been demonstrated in numerous studies which have presented several approaches to optimize the extraction conditions or evaluated their efficiency compared with other methods (Sporring and others 2005; Mustafa and Turner 2011; Santos and others 2012). A summary of recent studies on recovery of NBCs using PLE in several matrices is presented in Table 1. Despite the advantages over conventional methods, this method is not found to be suitable for thermolabile compounds as high temperature can have deleterious effects on their structure and functional activity (Ajila and others 2011).

Subcritical fluid extraction

The use of water under high temperature and a pressure below supercritical conditions in extraction processes is generally

Table 1–Studies published on the use of solvent extraction and pressurized liquid solvent extraction for the recovery of natural bioactive compounds.

Methodology / conditions	Raw material	Compounds of interest	Extraction yields/ response variable	Bioactivity/ Possible uses or applications	Reference
PLE Hexane, ethanol, and water, 50–200 °C, 20 min	Algae: *Himanthalia elongata* and *Synechocystis sp.*	Volatiles, fatty acids, and carotenoids (antioxidant extract)	Yield with hexane: 7.59%, ethanol: 36.91% and water: 46.43%	Antimicrobial and antioxidant/ functional food ingredients	(Plaza and others 2010c)
PLE n-hexane, ethanol, petroleum ether, chloroform, 80–125 °C, 3×15 min, 1500 psi	Wheat straw, germ, and bran	Policosanols	1026 ± 18, 27.6 ± 0.04 and 43.2 ± 3.7 mg/100 g, respectively	Lowering LDL and increasing HDL. Dietary supplements for cardiovascular health	(Dunford and others 2010)
SE Methanol*	Five varieties of eggplant	Phenolics and flavonoids	739.36–1116.13 mg GAE/100 g and 1991.29–3954.20 mg CE/100 g, respectively	Antioxidant and hepatoprotective/ nutraceutical ingredients	(Akanitapichat and others 2010)
SE Methanol, acetone, and water	Bunga kantan inflorescence	Phenols, tannis, flavonoids, and anthocyanins	687 mg GAE/100 g, 1431 mg QE/100 g, 5.9 mg c-3-gE/100 g, and 467.8 mg CE/100 g	Antioxidant/ natural antioxidants for food and nutraceutical applications	(Wijekoon and others 2010)
PLE Ethanol and water 25/75-50/50-75/25, 1500 psi, 20 min, 50–200 °C	Oregano, tarragon, and wild thyme	Phenolics	184.9 mg GAE/g in oregano	Antioxidant / functional compounds for the food industry	(Miron and others 2010)
SE Ethanol acidified, tartaric acid and malic acid, 1.25% v/v, 40 °C, 60–80 min	Eggplant peel	Anthocyanins	65.79–76.44 mg/100 g of peel	Antioxidant activity/ natural food colorings	(Todaro and others 2009)
SE Decane and decane 190 mM CH_2Cl_2	microalga: *Dunaliella salina*	β-Carotene	345 ± 5 μg/mL	Not tested/ Food and pharmaceutical additives in cosmetics and body-care products	(Mojaat and others 2008)
SE Diethyl ether	Alga *Dictyopteris membranacea*	Volatiles and essential oil		Flavoring agents in food and perfume industries	(Hattab and others 2007)
SE Ethanol 68%, 55 °C, liquid solid ratio 32.7 mL/g	*Mangifera pajang* peels	Phenolics	12.9 mg GAE/g	Antioxidant/ health benefits: treatment for stomach ache and diarrhea	(Nagendra and others 2011)
SE Acetone: water 50% v/v, 2 h, 60 °C	*Refošk* grape mark	Phenolics and anthocyanins	16.7 mg GAE/g and 0.74 mg/g of dry material	Natural food colorants and nutraceuticals (health benefits)	(Vatai and others 2008)
SE Ethanol, ethyl acetate, and acetone, 20–60 °C, 2 h	Elderberry (EB) and 3 varieties of red grape mark (RGM): Refošk, merlot, and cabernet	Phenolics and anthocyanins	EB: 60.6 mg GAE/g RGM: 17.3–20.2 mg GAE/g of dry material EB: 0.5–1.2 mg/g RGM: 10.5 mg/g of dry material for anthocyanins	Positive health effects and possible use as food colorants	(Vatai and others 2009)
SE Methanol, ethanol, acetone, acidified waters, or mixtures (1:1, v/v). 1 or 120 min, pH 3 or 8, 22 or 55 °C	Banana peels from 2 cultivars (Grande Naine and Gruesa) by-product	Total phenols and anthocyanins	3.1–3.8 GAE/100 g and 434 μg c-3-gE/100 g of dry material	Antioxidant/ compounds with food applications and health benefits	(González-Montelongo and others 2010)
SE Acetone 50%, solvent-to-solid ratio 20 mL/g, 35–36 °C and 100–102 min	*Parkia speciosa* pod agro-waste	Total phenolic and flavonoids	668 mg GAE/ 100 g and 49.6 mg pyrocatechol E/ 100 g	Antioxidant/ nutraceutical agent for protection of human health	(Gan and Latiff 2010)

*Some conditions such as time of extraction, temperature, and percentage of solvent are not mentioned.
mg GAE/100 g: milligram of gallic acid equivalents per 100 g of extract.
mg CE/100 g: milligram of catechin equivalents per 100 g of extract.
PLE, pressurized liquid extraction; SE, solvent extraction.

referred to as subcritical water extraction (SWE). This technique has emerged as a useful tool to replace traditional extraction methods. SWE presents a series of important advantages over the traditional extraction techniques; it is faster, produces high yields, and the use of solvents can be greatly reduced (Plaza and others 2010a). Therefore, this novel extraction technique is gaining increasing attention due to the advantages it provides compared to other traditional extraction approaches, and because it is environmentally friendly compared to conventional organic liquid SE techniques.

SWE is carried out using hot water (from 100 to 374 °C, the latter being the water critical temperature) under high pressure (usually from 10 to 60 bar) to maintain water in the liquid state (Herrero and others 2006). Parameters of the solvent, such as dielectric constant and solubility, along with temperature are affected when the liquid state is maintained. Hence, although the value of the dielectric constant of water at room temperature is nearly 80, this value can be decreased to about 30 at 250 °C. At these conditions a value is obtained similar to that presented by some organic solvents like ethanol or methanol. The same occurs with the solubility parameter, which decreases, approaching the value obtained for less polar compounds (Adil and others 2007). Therefore, this technique can be used for the extraction of nonpolar NBCs and replace organic solvents. However, it is important to consider the variability of dielectric constants for different types of compounds.

SWE has been successfully applied to the extraction of different NBCs (mainly antioxidants, see Table 2) from several vegetable and other matrices. Hassas-Roudsari and others (2009) studied the extraction of antioxidant compounds from canola seed meal using subcritical water and ethanolic and hot water extraction. They found that SWE at 160 °C yielded the highest total phenolic content and antioxidant capacities per gram of meal basis. Similarly, García-Marino and others (2006) studied the recovery of catechins and proanthocyanidins from wine-related products over a different range of temperatures in sequential extractions. The highest recovery was found when the material was submitted to 3 sequential extractions at 50, 100, and 150 °C. But selective extractions of compounds with different degrees of polymerization can be achieved using a one-step extraction at different temperatures. Other vegetable matrices that have been used to extract bioactive compounds by SWE were citrus pomaces (Kim and others 2009a), oregano (Rodriguez-Meizoso and others 2006) and rosemary (Plaza and others 2010c), as well as some microalgae (Herrero and others 2006). Nevertheless, during the extraction procedure, several components may be modified in their own matrix and affect their functionality (Plaza and others 2010a). Under SWE conditions, the cellular structure of matrices containing NBCs can be broken releasing these compounds to the extracellular medium dissolving in the hot liquid water. These compounds may interact during the extraction forming other compounds that could exhibit different structures and properties as compared to the original targets. Examples of this kind of interactions are Maillard reaction or caramelization, which could be favored under the SWE extraction conditions (Plaza and others 2010a).

Plaza and others (2010a) studied neoformation of antioxidants during SWE extraction of different natural compounds, including microalgae (*Chlorella vulgaris*), algae (*Sargassum vulgare, Sargassum muticum, Porphyra* spp., *Cystoseira abiesmarina, Undaria pinnatifida*, and *Halopitys incurvus*), and plants (rosemary, *Rosmarinus officinalis* L.; thyme, *Thymus vulgaris*; and verbena, *Verbena officinalis*). They suggested that neoformed compounds during the extraction process derived from Maillard, caramelization, and thermoxidation reactions affect the overall antioxidant capacity of water subcritical extracts depending on the nature of the sample. Therefore, the bioactive properties of the individual compounds obtained by SWE must be evaluated in order to obtain a better knowledge of the potential application of these extracts.

In general, the use of SWE provides a number of advantages over traditional extraction techniques used, such as extraction times, higher quality of the extracts, lower costs of the extracting agent, and an environmentally compatible technique. In addition, due to the occurrence of modifications and/or interactions between the extracted compounds by SWE, future research must be addressed to identify the structure of newly formed antioxidants and their individual contribution to the overall antioxidant capacity of the extracts. This approach can be used as functional food ingredients or nutraceuticals providing health benefits.

Supercritical extraction

SFE has been described as an environmentally safe technology due to compounds commonly extracted from natural sources (Herrero and others 2006). These natural sources could be plants, food by-products, algae, and microalgae, among others. Moreover, the goal of this technique is the high selectivity, short times of extraction, increased pollution prevention, and the use of nontoxic organic solvents (Wang and Weller 2006).

SFE is based on some properties of the fluids, such as density, diffusivity, dielectric constant, and viscosity, and usually modifies some conditions such as pressure and temperature to reach a supercritical fluid (SF) (Sihvonen and others 1999; Herrero and others 2006). Under these conditions, a fluid is between gas and liquid because the density of an SF is similar to that of liquid and its viscosity is similar to that of a gas (Sihvonen and others 1999; Wang and others 2008). Thus, the supercritical state of a fluid is the state in which liquid and gas are identical from each other (Wang and Weller 2006). In addition, SFs have better transport properties than liquids because it depends on its density which, unlike liquid solvents, is adjustable by changing pressure and temperature (Sihvonen and others 1999; Herrero and others 2006).

A simple explanation of the SFE system is as follows. During the process of SFE, raw material is placed in an extractor vessel, which has temperature and pressure controllers to maintain the desired conditions. Then the extractor vessel is pressurized with the fluid by a pump. Once the fluid and the dissolved compounds are transported to separators, the products are collected through a tap located in the lower part of the separators. Finally, the fluid is regenerated and cycled or released to the environment (Sihvonen and others 1999).

Selection of SFs is very important for the development of a SFE process, and a wide range of compounds can be used as solvents in this technique (Sihvonen and others 1999). However, despite the fact that there are many compounds that can be used as SFs (ethylene, methane, nitrogen, xenon, or fluorocarbons), most separation systems use carbon dioxide due to its safety and low cost (Daintree and others 2008). Carbon dioxide (CO_2) has been described to ensure minimal alteration of the bioactive compounds and to preserve their curative or functional properties (Cavero and others 2006). Supercritical carbon dioxide (SC-CO_2) is an attractive alternative to organic solvents because it is nonexplosive, nontoxic, inexpensive, and possesses the ability to solubilize lipophilic substances, and can be easily removed from the final products (Wang and Weller 2006; Wang and others 2008; Sahena and others 2009). Another advantage is that CO_2 is gaseous at room temperature and pressure, which makes compound recovery

Table 2–Current studies on the recovery of natural bioactive compounds from different sources using subcritical fluids.

Extraction method and conditions	Raw material	Compound	Research	Possible use	Reference
Subcritical water 100, 150, 200, and 250 °C 30 and 300 min	Basil and oregano (plant herbs)	Terpenes; α-pinene, limonene, camphor, citronellol, and carvacrol	Recovery of terpenes with varying temperature and heating times and quantification of compounds by GC-FID. Stability of molecules under extraction conditions.	Anti-inflammatory and antioxidant activities.	(Yang and others 2007)
Subcritical water 40 MPa, 250 °C	Centella asiatica (Plant herb)	Asiatic acid and asiaticoside	Comparison with conventional solvent extraction (ethanol and methanol). Quantification by HPLC. Extracts were filtered using 3 different size membranes.	Antibacterial and fungicidal. Control of cell division in human colon and breast cancer, melanoma, and so on.	(Kim and others 2009b)
Subcritical water 323–423 K 0.7–4.0 MPa 1–7 h	Mahkota dewa fruit meal (Plant fruit)	Mangiferin	Comparison of subcritical water with supercritical CO_2, Soxhlet extraction and others in terms of mangiferin yield. Chemical identification was performed by LC-MS.	Antidiabetic, anti-HIV, anticancer, immunomodulatory and antioxidant activity.	(Kim and others 2010)
Subcritical water 120–200 °C, 4 MPa 1.6, 2, 4 and 3.2 mL/min	Morinda citrifolia dried root (Plant fruit)	Dammacanthal	Quantification of compounds by RP-HPLC. Effects of the flow rate and temperature on the extraction and use of mathematical models.	Anticancer medicinal compound	(Anekpankul and others 2007)
Subcritical water 120–220 °C 2–4 mL/min and 4 MPa	Terminalia chebula Retz. (Plant fruit)	Gallic acid, ellagic acid and corilagin	Total phenolic contents (Folin-Ciocalteu) and antioxidant activity (ABTS) was compared to those extracted with Soxhlet (ethanol) and hot water extraction.	Anticancer, antimicrobial and anti-inflammatory activities	(Rangsriwong and others 2009)
Subcritical carbon dioxide + ethanol 20–60 MPa, 40–60 °C 14–20% EtOH and 10–40 min	Apple and peach pomaces (Plant fruit)	Polyphenols	Determination of the effect of pressure, temperature, exposure time and percentage of co-solvent on yield, antioxidant activity (DPPH), and total phenolic content (TPC) of the extract.	Health promoting effects	(Adil and others 2007)
Subcritical carbon dioxide 9.40 g/min +6, −6, −13, −16 °C 5.6 MPa	Ground red paprika (Plant fruits)	Carotenoids	Composition analysis by HPLC/DAD. Comparison with Soxhlet extraction (hexane/ ethyl-ether). Composition of fatty acids, triacylglycerols, and unsaponifiable matter. Fractionation of carotenoids in the extract.	Protective effects against cancer, heart disease and degenerative eye disease.	(Rutkowska and Stolyhwo 2009)
Subcritical water 3–11 MPa, 323–423 K 0–3.3 m³/s	Olive leaves (Plant)	Mannitol	Effects of pressure, temperature and flow rate on yield of mannitol. Analysis and quantification was performed by HPLC. A model of the extraction was described using mathematical models. Comparison of method with Soxhlet extraction.	Used as chemical, pharmaceutical and as medicine. Used in diabetic food products.	(Ghoreishi and Shahrestani 2009)
Subcritical water 100 and 200 °C, 5–9 min 1500 psi	Rosemary, thyme and verbena leaves. (Plant)	Phenols, protein, amino acids and sugars.	Studies of neoformation of compounds derived from Maillard, caramelization and thermoxidation. Antioxidant activity (TEAC and ORAC_FL) and total phenolic content (Folin-Ciocalteu). Total protein content, amino acids availability (OPA), sugar content (phenol-sulfuric acid) and estimation of melanoidins.	Functional foods, nutraceuticals and antioxidants.	(Plaza and others 2010a)
Subcritical carbon dioxide 25 ± 1 °C, 70 ± 1 bar	Quisqualis indica L. (Plant flower)	Essential oil	Comparison with distillation (water) and solvent extraction (pentane). Identification was determined by GC/MS and composition by GC-FID.	Germicide against skin diseases, commercial fragrance.	(Rout and others 2008)
Subcritical water 50, 100, 150, 200 °C 1500 psi and 20 min	Haematococcus pluvialis (microalga)	Vitamin E, phenolic compounds, products of caramelization and Maillard reactions.	Chemical composition determined by HPLC-DAD, HPLC QqQ-MS and GC-MS. Effect of the extraction temperature on yield and antioxidant activity	Antioxidant and antimicrobial activity.	(Rodríguez-Meizoso and others 2010)
Subcritical Water 100 and 200 °C, 5–9 min 1500 psi	Chlorella vulgaris (microalgae) Sargassum vulgare, Porphyra spp., Cystoseira abies-marina, Sargassum muticum, Undaria pinnatifida, and Halopitys incurvus. (algae)	Phenols, protein, amino acids, and sugars.	Studies of neoformation of compounds derived from Maillard, caramelization, and thermoxidation. Antioxidant activity (TEAC and ORAC_FL) and total phenolic content (Folin-Ciocalteu). Total protein content (Kjeldhal), amino acids availability (OPA), sugar content (phenol-sulfuric acid) and estimation of melanoidins.	Functional foods, nutraceuticals, and antioxidants.	(Plaza and others 2010b)
Subcritical water 50–200 °C 5min 20–260, 5 min and 200 and 260 °C 5–120 min	Rice bran biomass (by-product)	Phenolic compounds	Phenolic compounds, total phenolic content, antioxidant activity, total soluble sugar, pH and electrical conductivity was performed in order to see the effects of temperature and exposure time in this parameters.	Beneficial effect against cancer and, diabetes, among others. Application in food industries, and health and cosmetic markets.	(Pourali and others 2010)
Subcritical water 50, 100, and 150 °C 1500 psi	Winery grape seeds (by-product)	Catechins and proanthocyanidins	Effect of temperature on the extraction process. Composition of extracts was performed by HPLC-DAD-MS. Comparison with solid-liquid extraction.	Could be employed by pharmaceutical and food industries	(García-Marino and others 2006)
Subcritical water 275 °C 1 h	Bovine bones (bio-waste)	Hydroxyapatite	Comparison with thermal decomposition and alkaline hydrothermal process.	Medical application. Utilized as bioceramics due to their biocompatibility, and high osteo conductive, osteo-inductive, and nontoxic properties.	(Barakat and others 2009)
Subcritical water 100–190 °C 5–30 min 90–131 bar	Onion skin (by-product)	Quercetin	Effect of temperature and extraction time on yield extraction. Subcritical water was compared with conventional methods such as ethanol, methanol and water at boiling point. Chemical composition by HPLC.	Anticancer, antivirus and anti-inflammatory activities.	(Ko and others 2011)

very simple and provides solvent-free extracts. In addition, this molecule is environmentally friendly and "generally recognized as safe" (GRAS) by FDA (U.S. Food and Drug Administration) and EFSA (European Food Safety Authority). However, because of its low polarity, CO_2 is less effective in extracting highly polar compounds from their matrices (Herrero and others 2006).

Polar molecules are poorly soluble in SC-CO_2 and hence are not extractable (Daintree and others 2008). For this reason, the use of other solvent compounds is needed in order to enhance solubility and the selectivity of the process and they must be added only in small quantities (Sihvonen and others 1999). This phenomenon is attributed to various components acting as solubility enhancers and is called "co-solvent effect." Co-solvents or modifiers include hexane, methanol, ethanol, isopropanol, acetonitrilo, and dichloromethane, among others. However, ethanol is recommended as a co-solvent in SFE because of its lower toxicity and miscibility in CO_2 (Lemert and Johnston 1990; Liza and others 2010), although its applications is limited due to its unfavorable properties with respect to safety and environmental considerations (Wang and Weller 2006).

Some studies have described different supercritical applications, usually using CO_2 as a SF (see Table 3). Liza and others (2010) studied the feasibility of the SFE method to extract lipid compounds such as tocopherols, phytosterols, policosanols, and free fatty acids from sorghum and the preventive role of these compounds in many diseases. On the other hand, scientific studies indicated possible antioxidant effects of many spices such as rosemary, sage, thyme, and oregano, among others (Cavero and others 2006). For this reason, Cavero and others (2006) assessed the possibility of using oregano leaves as a source of antioxidants under a wide range of extracting conditions (different pressures and temperatures and considering ethanol as a co-solvent). Their results show that the extracts obtained by SC-CO_2 possess high antioxidant capacity against the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, especially when a co-solvent is used. In this context, SF-CO_2 is important for the natural compound extractions and the food industry, because it allows the extraction of thermally labile or easily oxidized compounds (Herrero and others 2010).

Nowadays, SFE is much used in many industrial applications including coffee decaffeination, fatty acid refining and the extraction of essential oils and flavors from natural sources with potential use in nutraceuticals and functional foods (Wang and Weller 2006; Daintree and others 2008). This method is an important alternative to conventional extraction methods using organic solvents for extracting biologically active compounds (Wang and Weller 2006). However, to develop a successful SFE, several factors need to be taken in consideration including SFs, raw materials, co-solvents, and extraction conditions for the extraction of a particular compound of interest in order to maximize the extraction. Moreover, it has been proved with good success that SFE can be used to obtain active substances in microparticles as dry powders in which stability and activity are maintained. This approach opens a new opportunity for the use of these compounds in the food and pharmaceutical industries. The application of various supercritical techniques to the preparation of active powders and particles and future challenges has been reviewed recently by Cape and others (2008).

Microwave-assisted extraction

Microwaves are an electromagnetic radiation with a wavelength from 0.001 m to 1 m which can be transmitted as waves. When microwaves pass through the medium, its energy may be absorbed and converted into thermal energy (Zhang and others 2011b). This principle has been the basis for the development of microwave-assisted extraction (MAE) which works heating the moisture inside the cells and evaporates, producing a high pressure on the cell wall. The pressure builds up inside the biomaterial which modifies the physical properties of the biological tissues (cell wall and organelles disrupter) improving the porosity of the biological matrix. This would allow better penetration of extracting solvent through the matrix and improved yield of the desired compounds (for an extensive explanation, see Routray and Orsat 2011). Hence, MAE is more advanced than the traditional SE method since it heats the matrix internally and externally without a thermal gradient in which NBCs can be extracted efficiently and protectively using less energy and solvent volume. These characteristics have recently led MAE to be used as one of the most advanced techniques for the extraction of NBCs from numerous matrices (Table 4).

MAE has been suitable for the recovery of a vast array of NBCs, however, it has been applied mainly for the recovery of NBCs with antioxidant capacity such as phenolic compounds (Li and others 2011b; Moreira and others 2012; Simsek and others 2012) and carotenoids (Zhao and others 2006; Choi and others 2007; Pasquet and others 2011). Other NBCs such as terpenoids, alkaloids, and saponins have also been recovered utilizing MAE (Zhang and others 2011b). Most of these studies concluded that the use of MAE allowed to reduce solvent consumption and extraction times, with better antioxidant capacity and equivalent or higher extraction yields than other methods. For instance, higher yields and higher antioxidant activity were obtained in peel extracts of citrus mandarin (Hayat and others 2009), peanut skins (Ballard and others 2010) tomatoes (Li and others 2011a), and onions (Zill-e-Huma and others 2011) as compared to rotary extraction. Similarly, Xiao and others (2008) and Simsek and others (2012) showed that in Radix Astragali and cherry pomace, respectively, the yield of phenolic compounds obtained by MAE was similar to that of conventional extraction (Soxhlet and rotatory extraction) but significantly in shorten time. Beejmohun and others (2007) found similar results where MAE of the main phenolic compounds in flaxseed gave higher yields as compared to traditional extraction.

MAE of NBCs may be affected by a large variety of factors, such as power, frequency, and time of microwave, moisture content and particle size of sample matrix, type and concentration of solvent, ratio of solid to liquid, extraction temperature, extraction pressure and number of extraction cycles (for a review, see Mandal and others 2007). Nevertheless, between these factors, it seems that solvent is the most critical. There are 3 main physical parameters to select the appropriate solvent i) solubility, ii) dielectric constant, and iii) dissipation factors. Solvents with high dielectric constant such as 'water' and polar solvents which can absorb high microwave energy are usually better solvents than nonpolar solvents (Wang and Weller 2006). In addition, the dissipation factor (the efficiency with which different solvents heat up under microwave) play an important role. For example, it has been observed that the recovery of phenolic compounds is greater using solvents such as ethanol or methanol compared with water which is associated with a higher dissipation factor (Ajila and others 2011). Despite the fact that water has a higher dielectric constant than ethanol or methanol, its dissipation factor is lower making it inefficient to heat up the moisture inside the sample matrix and to generate pressure which triggers the leaching out of the NBCs. Therefore, it is better to use solvents with a high dielectric constant as well as a high dissipation factor which can be achieved using mixtures of water with other

Table 3–Recent studies on the recovery of natural bioactive compounds from different sources using supercritical fluids.

Extraction method and conditions	Raw material	Extracted compound	Research	Possible use/ bioactivity	Reference
Supercritical fluids CO_2 40–60 °C 150–360 bar 0–7% EtOH	Oregano leaves (Plant herb)	Flavone, flavonone, and flavonols	Identification of extracted compounds by HPLC and antioxidant activity by DPPH and b-carotene bleaching.	Food ingredient to flavor sausages, meats, and salads. Antioxidant activity and health benefits.	(Cavero and others 2006)
Supercritical Fluids CO_2 30–40 °C, 100–300 bar 3 h	Rosemary leaves (Plant herb)	Antioxidants Volatile oil	Determination of chemical composition (GC), global yield and antioxidant activity. Determination of mass transfer rate and kinetic parameters. Comparison of 3 techniques used in terms of total content of compounds and yield of the extracts.	Application in functional foods. Antioxidant and anticancer substances.	(Carvalho 2005)
Supercritical Fluids CO_2 100, 200, and 300 bar 30–50°C low-pressure solvent extraction MeOH, EtOH, and hexane	Pfaffia paniculata and Pfaffia glomerata (Plant)	Ginseng	Determination of chemical composition of the extracts by TLC, extraction kinetics and yield. Antioxidant activity of both techniques was compared each other.	Preparation of dermatological and cosmetic compositions.	(Leal and others 2010)
Supercritical fluids CO_2 60 °C 40 MPa 0–60 min	Pitanga leaves (Plant)	Polyphenolic compounds and flavonoids	Evaluation of global extraction yield, concentration, and both polyphenols and flavonoids in the extracts obtained by two-step process. Identification of compounds was performed by GC-MS and antioxidant activity was measured using DPPH and b-carotene bleaching method.	Antibacterial, anti-carcinogenic, antiviral, and anti-inflammatory activities.	(Martinez-Correa and others 2010)
Supercritical fluids CO_2 45, 55, and 65 °C 15, 25, and 35 MPa	Patrinia villosa Juss (Plant herb)	Volatiles	Antioxidant activity (DPPH and ABTS) and chemical composition of extracts.	Antiviral and antibacterial	(Xie and others 2008)
Supercritical fluids CO_2 50 °C, 60 min, 25 MPa	Cherry (Plant fruit)	Phenols perilly alcohol	Quantification and characterization (HPLC) of the extract. Antioxidant capacity (TEAC and ORAC). Cytotoxicity and antiproliferative assay.	Antioxidants Anticancer agent and protector against colon, skin, and lung cancer.	(Serra and others 2010)
Supercritical fluids CO_2 40, 60, and 80 °C 200, 275 and 350 bar	Tomato juice (Plant fruit product)	Lycopene	Effect of temperature and pressure on extraction efficiency. Identification and quantification of lycopene on the active fractions, yield, and antioxidant capacity.	Food coloring.	(Egydio and others 2010)
Supercritical fluids CO_2 35–75 °C, 10 – 40 MPa 60 min Me/H_2O (9:1 v/v) 3%	Sargassum muticum, Sargassum vulgare, Hypnea spinella, Porphyra sp., Undaria pinnatifida, Chondrus crispus and Halopytis incurvus. (algae) Spongiochloris spongiosa, Scenedesmus and Nostoc 7 (Cyanobacteria)	Isoflavones	Combination with sonication pretreatment, fast chromatography analysis and MS/MS determination. Effect of pressure and temperature on yield. Effects of sonication on recovery of compounds.	Component in functional foods and used in pharmaceutical industries.	(Klejdus and others 2010)
Supercritical fluids CO_2 40, 50, 60 °C 100–500 bar 3 h	Synecoccus sp. (microalga)	Carotenoids and chlorophyll	Effect of pressure and temperature on extraction yield. Total concentration of carotenoids and chlorophyll a was measured by spectrophotometric method. Comparison with methanol extraction.	Coloring in some processed food, drinks, and ice creams.	(Macías-Sánchez and others 2007)
Supercritical fluids CO_2 40, 50, and 60 °C 100–500 bar 3 h 3 mmol/min, 5% EtOH	Nannochloropsis gaditana, Dunaliella salina and Synecococcus sp. (microalgae)	Carotenoids	Determination of extraction yield with or without co-solvent. Total concentration of carotenoids and optimization of the conditions for the extraction of carotenoids. Extraction kinetics and mass transfer models were obtained using mathematical models.	Food additives and positive effects in human health.	(Macías-Sánchez and others 2009)

(Continued)

Table 3–(Continued)

Extraction method and conditions	Raw material	Extracted compound	Research	Possible use/ bioactivity	Reference
Supercritical fluids CO_2 32, 46, 60, 39, 53 °C 200–600 bar 300 min	Scenedesmus almeriensis (microalga)	β-Carotene and lutein	Determination of the influence of pressure and temperature on the extraction was measured using a response surface method. Composition analysis by HPLC.	Antioxidant, food coloring agent. Application in human health as preventer of cataracts, atherosclerosis, and some types of cancer.	(Macías-Sánchez and others 2010)
Supercritical fluids CO_2 323 K 350 bar 10, 20 mL/min	Nannochloropsis oculata (microalga)	Carotenoids and lipids	Comparison with Soxhlet (CH_2Cl_2, hexane and EtOH) and ultrasonic extraction. Quantification of carotenoids (HPLC), triglycerides, and total yield of the extraction. Use of co-solvents (EtOH and dichloromethane).	Food supplements and functional foods.	(Liau and others 2010)
Supercritical fluids CO_2 32–55 °C, 25–40 MPa 15–30 L/h, 0–1.5 mL EtOH g^{-1}, 1.5–3 h	Chlorella pyrenoidosa (Alga)	Antioxidants	Optimization of the extraction and comparison of antioxidant activity from extracts with Trolox, BHT and α-tocopherol.	Dietary supplements.	(Hu and others 2007)
Supercritical fluids CO_2 313.15 and 323.15 K 180, 200, and 220 bar 1.7×10^{-4} kg^{-1}	Grape seed oil (by-product)	Triacylglycerides	Oil composition and antioxidant capacity of the extracts using different operating conditions	Antioxidants.	(Passos and others 2010)
Supercritical fluids CO_2 40–100 °C, 20–40 MPa 1.0–2.0 mL/min	Tomato skins (by-product)	Lycopene	Influence of the operating conditions on yield and antioxidant activity of the extracts.	Protective effect against cardiovascular, coronary heart diseases, and cancer.	(Yi and others 2009)
Supercritical fluids CO_2 40–60 °C 148–602 bar 10–60 min	Roasted wheat germ (by-product)	Phenolic compounds and tocopherols	Recovery of phenolic compounds and tocopherols, yields and measurement of antioxidant activity by TPC, TTC, and DPPH.	Pharmaceutical, food and cosmetic formulation. Biological control agents against insects.	(Gelmez and others 2009)
Supercritical fluids CO_2 311–331 K 116–180 bar 0.60 g/mL CO_2	Coriander seeds (by-product)	Antioxidant fractions	Antioxidant activity (DPPH), yield, and effect of the operating conditions. Fractions were compared with commercial antioxidants.	Diet supplements in nutraceuticals industries.	(Yepez and others 2002)
Supercritical fluids CO_2 50 °C and 30 MPa 0, 2 and 3% EtOH	Mangosteen pericarp (by-product)	Xanthones	Characterization of the extracts by HPLC/LC-ESI-MS and antioxidant activity by DPPH.	Inhibition of lipid peroxidation, antioxidant activity, neuroprotective and inhibitor of HIV-1 protease.	(Zarena and Udaya Sankar 2009)
Supercritical fluids CO_2 30 and 50 °C 250, 275, and 300 bar 5 g/min CO_2 5–20% EtOH	Grape seed (by-product)	Proanthocyanidins	Effect of different pressure, temperature, and ethanol percentage. Characterization of the extracts by HPLC.	Anticarcinogenic, antiviral, anticancer and also may act against oxidation of low density lipoproteins.	(Yilmaz and others 2010)
Supercritical fluids CO_2 40, 60, and 80 °C 200, 400, and 600 bar 25 g/min 150 min	Hibiscus cannabinus L. seed (by-product)	Edible oil	Comparison with Soxhlet extraction and ultrasonic-assisted extraction. Antioxidant activity of extract was compared with 7 commercial edible oils.	Functional foods.	(Chan and Ismail 2009)

Table 4–Recent studies on the use of microwave and ultrasonic-assisted extractions from different natural matrices.

Method and conditions	Materials	Bioactive compounds	Applications	Reference
UAE n-heptane, 30 °C, 167 W/cm^2	*Spirulina platensis* Alga	β-carotene	Pharmaceutical industry. It helps to protect against cancer, diabetes, and other chronic diseases.	(Dey and Rathod 2012)
UAE MetOH 20%, 60 °C, 60 min	*Forsythia suspense* Plant	Phillyrin	Used as medicine due to its anti-inflammatory, antioxidant, antiviral and vasorelaxant activities.	(Xia and others 2011)
UAE MetOH, 40 °C, 60 kHz	Penggan peel by-product	Hesperidin	Food and pharmaceutical industry. Posses antioxidant, anti-inflammatory and antiallergic activities.	(Ma and others 2008)
UAE EtOH 41%, 79 °C, 30.5 min	*Prunella vulgaris* L. Plant	Flavonoids	Medical applications. Used for alleviating sore throat, reducing fever and accelerating wound healing.	(Zhang and others 2011)
UAE 5% biomass, 30 min, 1000 W	*Nannochloropis oculata* biomass Alga	Lipids	Feedstock for biodiesel production.	(Adam and others 2012)
UAE EtOH 72%, 65 °C, 37 min, 40 W	Hawthorn seeds by-product	Flavonoids	Health promoting compounds negatively associated with coronary heart diseases.	(Pan and others 2012)
UAE Water, 45 min, 222 W	Litchi seeds by-product	Polysaccharides	Food and biomedical applications. Posse's antitumoral, antioxidant, and hypoglycemic properties.	(Chen and others 2011)
MAE EtOH 80%, 65 °C, 2 cycles of 1 min, 300 W	Pigeonpea leaves Plant	Cajaninstilbene acid and pinostrobin	Medical treatment for postmenopausal osteoporosis and have hypocholesterolemic and hypoglycemic effects, and so on.	(Kong and others 2010)
MAE 1g alga/25 mL water, 1 min at 120 psi	*Fucus vesiculosus* Alga	Sulfated polysaccharides (fucoidan)	Can be used as anticoagulant, antithrombotic, antitumor, antiviral, contraceptive, and so on.	(Rodriguez-Jasso 2011)
MAE EtOH 30%, 1.5 g skins, 30 s at 855 W	Peanut skins by-product	Phenolic compounds	Pharmaceutical applications of health-promoting compounds including cancer prevention.	(Ballard and others 2010)
MAE Water, 50 °C, 5 min at 800 W	Green coffee beans Plant	Chlorogenic acid, caffeine and total polyphenols	Chlorogenic acid-rich conserves for use in functional foods.	(Upadhyay and others 2012)
MAE Acetone, 56 °C, 5 min at 50 W	*Dunaliella tertiolecta (DT)* and *Cylindrotheca closterium (CC)* microalga	Pigments: Chlorophyll a and b, and β,β-carotene for DT. Chlorophyll a and fucoxanthin for CC.	Food, health and biotechnological applications.	(Pasquet and others 2011)
MAE Ultra-pure water, 100 °C, 20 min at 600 W	*Uncaria sinensis* Plant herb	Catechin, caffeic acid, epicatechin and rhynchophylline	Pharmaceutical interest for treatment of fevers and nervous disorders.	(Tan and others 2011)
MAE Ethanol, water, 7 min in cycles of 30 s at 250 W	Rosemary leaves Spice	Total phenolic compounds, rosmarinic and carnosic acids	Natural antioxidants for the food industry.	(Rodriguez-Rojo and others 2012)
MAE EtOH 47.2%, 60 °C, 4.6 min at 150W	Grape seeds of cultivars Cabernet Sauvignon, Shoraz, Sauvignon Blanc and Chardonnay by-product	Polyphenols	Pharmaceutical, cosmetical and food industry.	(Li and others 2011b)

solvents (ethanol or methanol) as was recently demonstrated by Simsek and others (2012).

MAE is a relatively new extraction technology which has been widely applied in a variety of NBCs with many advantages over conventional extraction techniques including lower environmental pollution, higher extraction efficiency and shorter extraction time. However, in order to be considered in industrial applications at least 2 important limitations must be improved including i) the recovery of nonpolar compounds and ii) the modification of the chemical structure of target compounds which may alter their bioactivity and limit their application.

Ultrasonic-assisted extraction

The development of ultrasound technology is not new, however, it is only recently that the main advances in the exploitation of power ultrasound have been achieved (Soria and Villamiel 2010). In this sense, special attention has been paid to its use in the recovery of NBCs from different natural sources.

Ultrasound–assisted extraction (UAE) has been proposed as a timid alternative to conventional SE, providing higher recovery of targeted compounds with lower solvent consumption and/or faster analysis and bioactivity properties. Its better extraction efficiency is related to the phenomenon called acoustic cavitation. When the ultrasound intensity is sufficient, the expansion cycle can create cavities or microbubbles in the liquid. Once formed, bubbles will absorb the energy from the sound waves and grow during the expansion cycles and recompress during the compression cycle. Further, bubbles may start another rarefaction cycle or collapse leading shock waves of extreme conditions of pressure and temperature (several hundred atmospheres and around 5000 K of temperature) (Leighton 2007; Soria and Villamiel 2010; Esclapez and others 2011). Thus, the implosion of cavitation bubbles can hit the surface of the solid matrix and disintegrate the cells causing the release of the desired compounds.

Nowadays, UAE is extensively used for the extraction of valuable molecules. For example, it has been used for the extraction of proteins (Qu and others 2012), sugars (Karki and others 2010), polysaccharides–protein complex (Cheung and others 2012), oil (Adam and others 2012), and so on. However, the extraction of antioxidants such as phenolic compounds has been specially addressed optimizing their recovery based on its yield and antioxidant capacity by means of experimental design (some examples are

presented in Table 4). These studies have showed that the recovery, antioxidant capacity and profile are strongly influenced by extraction variables where extraction time, temperature and frequency are the most important (Chukwumah and others 2009; Ghafoor and others 2009; Hossain and others 2011). Some aspects related to the stability of the compounds extracted has not fully addressed, however, recent studies revealed that UAE of phenolic compounds were less degraded than others (Dobias and others 2010) and even no degradation has been observed under optimized conditions (Pingret and others 2012). Nevertheless, UAE should be carefully used in the extraction of unstable compounds as carotenoids since a major degradation has been observed compared with other technologies (Zhao and others 2006).

Impact of Structural Modification on Natural Compound Extractions

Many extraction processes have been developed to obtain NBCs from a wide range of sources. However, in most cases, remaining compounds are found forming complexes with their matrices after extraction (especially those found in plant tissues) and cannot be easily removed with the conventional methods (Kim and others 2005). Therefore, a large new assisted-extraction methods have been performed by including an initial texturing stage (enzymatical treatment, instant controlled pressure-drop). These novel methods are normally able to increase the extraction yields and improve the process kinetics (Papetti 2012), while preserving the bioactive properties of compounds.

Enzyme-assisted extraction

As described before, one of the main sources for the extraction of antioxidants are plant tissues. Plant cell walls contain polysaccharides such as cellulose, hemicellulose, and pectins which act as barriers to the release of intracellular substances. Some enzymes such as cellulase, β-glucosidase, xylanase, β-gluconase, and pectinase help to degrade cell wall structure and depolymerize plant cell wall polysaccharides, facilitating the release of linked compounds (Moore and others 2006; Chen and others 2010). Hence, these enzymes have been proposed as tools to optimize the extraction of compounds from plant matrix (Kim and others 2005; Wilkins and others 2007; Wang and others 2010).

Most of the NBCs, such as flavonoids, are present in different forms, interacting with the cell wall components (cellulose, hemicellulose, and pectin) (Figure 2) (Kim and others 2005; Fu and others 2008). For the release of these compounds, these interactions need to be broken by cell wall-hydrolyzing enzymes. β-Glycosidase breaks the β-1,4 glucosidic linkages in glucosides (flavonoids in conjunction with glucose) (Yang and others 2010b). On the other hand, phenolic compounds are often linked to cell wall polysaccharides and cell wall degradation is a key step in releasing phenols from the cell wall. In this sense, the use of xylanases, β-gluconases, and cellulases has been shown to be effective, since they can hydrolyze the ester-linked phenolic acids (Moore and others 2006). Nevertheless, such release depends on compositional and structural characteristics of compounds (Yang and others 2010b).

There are several reports on enzymatic treatment of plant tissues for the extraction of NBCs (Table 5). Chandini and others (2011) employed the enzymes tannase and pectinase independently to improve the quality of black tea extracts, and the maximum level of polyphenol extraction was observed when tannase was used alone (Chandini and others 2011). On the other hand, luteolin and apigenin were extracted from pigeonpea leaves using pecti-

nase, cellulase, and β-glucosidase. In another study, extraction of flavonoids from *Ginkgo biloba* was performed, not only degrading the cell wall, but also increasing solubility of compounds in the extract (Chen and others 2010). Enzyme-assisted extraction was also used to improve the antioxidant composition of black carrot juice, and more recently, to obtain vegetable oils (Khandare and others 2010; Szydlowska-Czerniak and others 2010b).

Enzyme extraction has not only been used to extract compounds from plants, but also from algae where the structural complexity and rigidity of the algal cell wall represent an obstacle, reducing the extraction efficacy of intracellular bioactive compounds (Wang and others 2010). Moreover, enzyme-assisted extraction could be used to obtain valuable compounds from waste and agricultural byproducts such as gallic acid (Curiel and others 2010) which can be used for the preparation of food additives such as pyrogallol and propyl gallate (Yu and Li 2008) and also to serve as an intermediate for the synthesis of the antibacterial drug trimethroprim by pharmaceutical chemistry (Curiel and others 2010). The versatility of enzymes to catalyze a variety of processes for the production of NBCs represents an interesting approach to be further exploited in terms of its activity, robustness, and efficiency.

Instant controlled pressure drop-assisted extraction

Instant controlled pressure-drop (DIC) was defined by Allaf and Vidal (1988). Since then, this technology has been improved, optimized, and used for various applications being the extraction of volatile compounds (Berka-Zougalia and others 2010) and antioxidants (Allaf and others 2012) the most recent approaches.

DIC consists of thermo-mechanical effects induced by subjecting the raw material for a short period of time to saturated steam followed by an abrupt pressure drop toward a vacuum (Ben Amor and Allaf 2009). The pressure-drop applied provokes the autovaporization of volatile compounds, instantaneous cooling of the products which stop thermal degradation and expansion of the cell wall (Allaf and others 2012), thus enhancing the mass transfer and improving the recovery of the desired compounds. The auto-vaporization of volatile compounds has made this technology very suitable for the recovery of essential oils in terms of process performance and attributes of the final product. A recent study conducted by Kristiawan and others (2008) extracted the essential oils of Indonesian Kananga in less than 6 min with a yield of 2.8 g/100 g dry matter compared with a similar yield (2.5 g/100 g dry matter) but a prolonged 16 h steam. Moreover, Allaf and others (2012) found that DIC extracts from orange peels had better essential oil quality (major oxygenated compounds) and antioxidant capacity (about 13%) compared with hydrodistillated extracts, the common way to obtain essential oils.

As was mentioned above, DIC causes the expansion of the matrix structure. This feature has been recently used for the recovery of antioxidants leading an improved SE. This has been tested by Ben Amor and Allaf (2009) who identifying the effect of various operative parameters (vacuum, pressure and temperature), improved up to 135% the recovery of anthocyanins from Roselle using water as solvent. Similarly, Allaf and others (2012) proved the sequential use of DIC and UAE for the extraction of phenolic compounds (naringin and hesperidin) from orange peels resulting in complementary actions materialized by supplementary effects. They obtained the highest yield with best kinetics and antioxidant capacity by coupling both treatments than standard SE. As far as we know, the studies presented are the only available in which DIC was used as assisted technology for the extraction of NBCs.
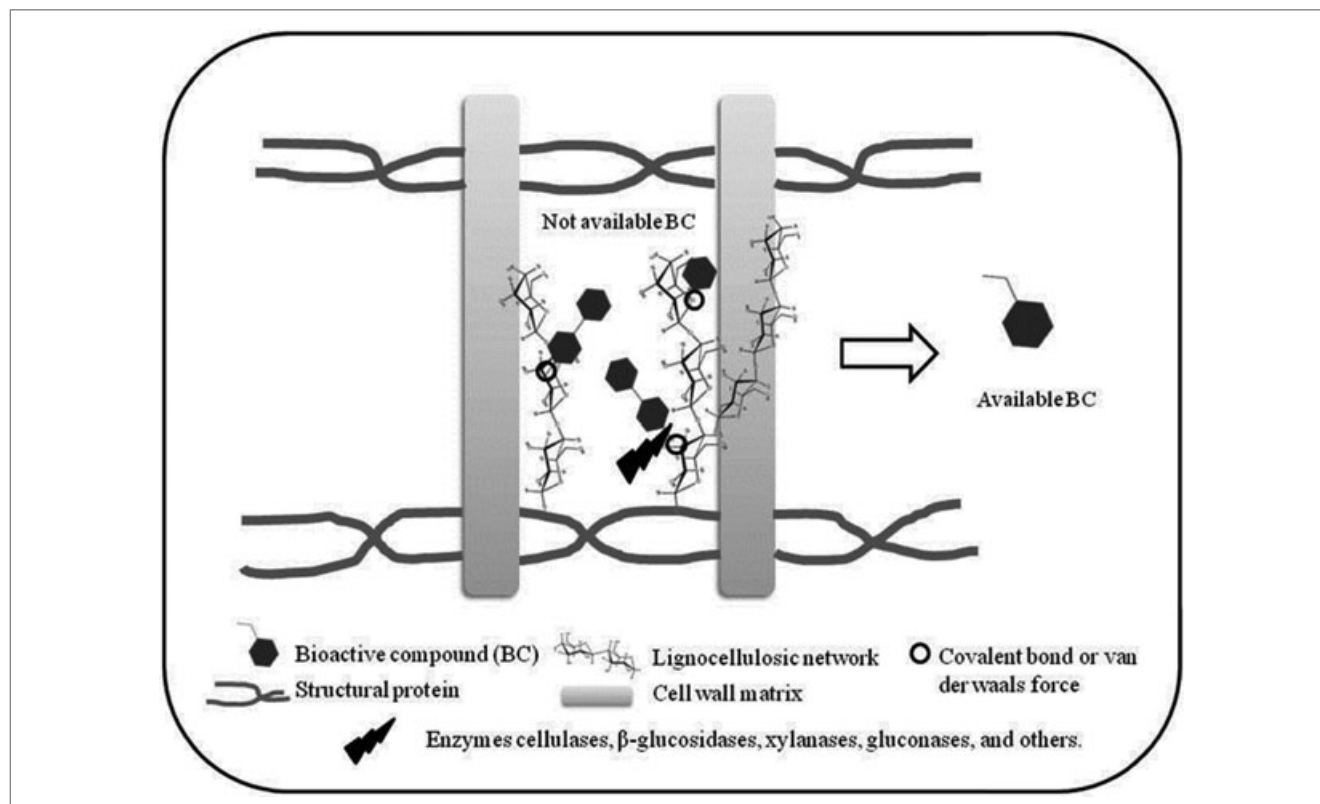
Figure 2–Bioactive compounds found in plant matrices forming interactions with lignocellulosic networks.

The valuable bioactive properties of NBCs has conducted the development for better extraction methods called 'advanced technologies' in order to obtain the greatest possible amount in a shorter processing time and at a low cost. The main differences between these technologies are related to the design of the reactors, the solvents used, the time and temperature of the processes, and yields as well. However, researchers need to take into consideration the knowledge about the entire property spectrum of entire desired compounds and availability of their sources. In addition, the cost benefit analysis of the use of a single or combined extraction technology should be taken in consideration by the food and pharmaceutical industries.

Use of Natural Bioactive Compounds in the Food and Pharmaceutical Industries

Nutraceuticals, functional foods, and other natural health products have been recognized for their health benefits, disease risk reduction, and reduction of health care costs (Bernal and others 2010). Foods, organisms and plants serve as a source of components with health beneficial effects. A large variety of products is currently being developed and is gaining popularity worldwide due to documented health benefits.

Nutraceuticals

One definition, nutraceuticals are dietary supplements that deliver a concentrated form of bioactive compounds from food, present in a nonfood matrix, and used with the purpose of enhancing health in dosages that exceed those that could be obtained from normal foods (Shahidi 2009). Nutraceuticals are usually consumed in pharmaceutical presentations such as pills, capsules, tablets, powder, and vials (Espin and others 2007). Pharmaceuticals are usually

synthesized from pure chemicals or isolated as pure chemicals from natural sources and their efficiency were clinically proved to prevent or cure some diseases. However, synthetic drugs are usually associated with undesirable side effects that can vary from no effects to severe (Lachance and Das 2007). In contrast, nutraceuticals routinely used in the treatment of certain symptoms are expected to be safer and with less and minimal side effects than conventional pharmaceuticals (Lachance and Das 2007; Bernal and others 2010). Hence, nutraceuticals have received recognition for their potential beneficial health effects when consumed as part of the diet.

The interest in nutraceuticals is due to the knowledge generated from epidemiologic studies describing the association between specific diet and/or component of the diet with a lower risk of chronic disease (Biesalski and others 2009). Nutraceuticals from various sources such as plants, fruits and vegetables, fungi, algae, and microalgae have been shown to perform a wide range of biological activities (Nair and others 2010). Some of their activities cover inflammation, chronic and neurodegenerative diseases, and some types of cancer which are attributed to specific NBCs (Lachance and Das 2007; Yang and others 2010a). Health benefits have been associated to higher extent for some, particularly polyphenols and carotenoids (Espin and others 2007; Lachance and Das 2007; Nobili and others 2009; Nair and others 2010).

Oxidative stress has been associated with the occurrence of chronic diseases and is believed to promote cell proliferation and causing cancer and malfunctions in heart disease, diabetes, and autoimmune disease, among others (Yahia 2010). Therefore, selected polyphenols recognized as nutraceuticals are able to alter human cellular signaling and gene expression which include hydroxycinnamates, coumarins, anthocyanins, ellagic acid, lignans, and ellagitannins (Espin and others 2007; Lachance and Das 2007;

Table 5–Studies on the use of enzymatic treatments to release natural bioactive compounds from natural matrices.

Enzyme used for the extraction	Assay conditions	Materials	Bioactive compounds	Applications	Reference
Pectinase, cellulose, and beta-glucosidase	0.4 mg/mL pectinase, 18 h, 30–35 °C, pH 3.5–4.	Pigeonpea leaves Legume	Flavones: Luteolin and apigenin	Pharmacological activities, anti-inflammatory, antiallergic, antiproliferative, and so on.	(Chen and others 2010)
Alpha-amylase and glucoamylase	2% w/w enzyme, 60 min, pH 4.5.	Turmeric (*Curcuma longa L.*) Spice	Oleoresin	Food formulations for the prevention of cancer.	(Fu and others 2008)
Cellulase ® MX, Cellulase ® CL and Kleerase ® AFP	1.5% w/w enzyme, 50 °C, 3 h.	Citrus peels: Yen Ben and Meyer lemon, grapefruit, mandarin, and orange	Total phenolics	Antioxidant and free radical scavenging activities. Implications in human health.	(Kurmudle and others 2010)
ROHALASE ® OS and ROHAPECT ® PTE (Cellulase, glucanase and xylanase activity)	0.05% and 0.1% w/w, 20 min, 70 °C and 50 °C	Rapeseed	Phenolics, tocopherols, and phospholipids	Prevention and treatment of chronic diseases: heart and neurodegenerative diseases, aging, cancer, and rheumatoid arthritis.	(Li and others 2006)
Pectinex XXL and Pectinex Ultra SPL (Pectolytic enzymes)	20 mL/100 kg of mash, 1 h, 20 °C	Pomace	Polyphenols	Effectiveness against colon cancer.	(Munoz and others 2004)
Pectinex B3-L, Vinozym EC, and Vinozym G	1% enzyme, 37–40 °C, 6 h	Grape skin from 3 varieties: Cabernet Sauvignon, Caberménère, and Ribier (industrial waste)	Anthocyanins	Food additives providing health benefits.	(Oszmianski and others 2011)
Cellulase from *Trichoderma reesei*, pectinase from *A. niger* and *P. decumbens* cellulose.	2 mg/mL of enzyme, 30 h, 40 °C	Ginkgo biloba leaves	Flavonoids: Quercetin, kaempferol, and isorhamnetin (predominant)	Physiological activities in therapies for inflammations, heart diseases, and cancer.	(Parrado and others 2006)
Endoprotease mixture	60 °C, pH 8, 40 min	Rice bran	Enzymatic extract with potential use as a functional food as final product.	Prevention of diseases including cancer, fatty liver, hypercalcicuria, kidney stones, and so on.	(Wang and others 2010)

Larrosa and others 2010). Moreover, carotenoids show antioxidant and provide immunomodulatory activities, and they can prevent degenerative diseases and several types of cancer, especially prostate and digestive tract tumors (Bernal and others 2010; Yahia 2010). However, their major benefits have been mainly associated with their high antioxidant capacity that slows down the rate of oxidative stress. Therefore, diets rich in antioxidants appear to be a promising approach to enhance and strengthen the physiological antioxidant defense system, reducing the incidence of chronic diseases (Mandel and others 2005), however, the extend of this statement is uncertain yet. This mainly due to NBCs has not been well studied yet because the low concentrations occurring in nature and because they exert a very rapid metabolism once ingested. Therefore, the design of prodrugs targeting active compounds represents an opportunity to learn more.

Larrosa and others (2010) conducted a study that explored the efficacy of different resveratrol prodrugs and pro-prodrugs to ameliorate colon inflammation in the murine dextran sulfate sodium model. The results showed that mice fed a very low dose (equivalent to 10 mg for a 70 kg/person) of either resveratrol-3-*O*-(6´*O*-butanoyl)-β-D-glucopyranoside or resveratrol-3-*O*-(6-*O*-octanoyl)-β-D-glucopyranoside did not develop colitis symptoms and improved 6-fold the disease activity index compared to resveratrol. In this context, the investigation of potential therapeutic effects of other prodrugs-derived NBCs with important health benefits must be addressed. These could provide an opportunity for the replacement of synthesized pharmaceuticals by nutraceuticals in the near future. In recent years nutraceuticals have been commercialized incorporating food extracts where a beneficial physiological function has been directly or indirectly attributed, and marketing studies have shown increasing demand for these health-promoting food products (Espin and others 2007).

Functional foods

Functional foods are those foods or ingredients that when consumed regularly produce a specific beneficial health effect beyond their basic nutritional properties. Normally, these foods contain different amounts and types of bioactive compounds (Roberfroid 2002; Nobili and others 2009; Bernal and others 2010). When a bioactive compound is included in a food formulation with a specific purpose, the new product could be considered a functional food (Day and others 2009). It is important to point out that the health benefits of the new functional food for one or more physical conditions of the human organism has to be demonstrated. Examples of beneficial effects of bioactive compounds are: decrease in cholesterol levels, alleviation of lactose intolerance, maintaining remission of Crohn's disease, faster relief from diarrhea, and inhibition of cancer cell proliferation *in vivo* and *in vitro* (Hekmat and others 2009; Nobili and others 2009; Marette and others 2010).

Plaza and others (2008) emphasized 3 important aspects of a functional food: 1) the functional effect is different from that of normal nutrition, 2) the functional effect must be demonstrated satisfactorily, and 3) the benefit can consist in an improvement of physiological function or in a reduction of risk of developing a pathological process. For this reason, not all bioactive compounds cover these aspects and, therefore, some are not considered food ingredients. Recently, new natural ingredients that possess biological activity (antioxidant, antiviral, antihypertensive, and so on) have been considered (Plaza and others 2008).

FDA in the USA has grouped several compounds with potential as new bioactive food ingredients (Burdock and others 2006). Examples of this group that are considered as GRAS include vegetable oil, sterol esters, phytostanol esters, lactoferin, fish oil concentrate, tuna oil, diacylglycerol, and inulin, among others (Burdock and others 2006). Nevertheless, there is a wide range

of bioactive compounds with beneficial effects on health which can be incorporated into food which needs further governmental reaction. The most remarkable case that can be considered is the use of polyphenols.

Whole foods such as fruits and vegetables represent the simplest form of functional foods because they are rich in several NBCs. Fruits that contain NBCs such as polyphenols and carotenoids have been described to have antioxidant activity and reduce the risk of developing certain types of cancer (Day and others 2009; Nobili and others 2009). Potential ingredients for use in the food industry such as napin, cruciferon, oleosin, inulin, cynarin, and fiber have been extracted from canola seed meal, artichokes and other important sources such as algae and microalgae (Plaza and others 2008; Lattanzio and others 2009; Aider and Barbana 2010).

Examples of functional food products that are currently on the market are drinks, cereals, bakery products, spreads, meat products and eggs, among others (Siro and others 2008). Beverages are the most popular functional foods because of their relatively easy formulation and processing in comparison with more complex processed foods (Day and others 2009; Hekmat and others 2009). So far, the trend of such studies has been directed toward probiotic and prebiotic products.

Presently, probiotic organisms are mainly administered through functional foods such as dairy products and milk because they are considered a good vehicle to deliver probiotic microorganisms (Day and others 2009; Hekmat and others 2009; Saulnier and others 2009). Commonly, the microorganisms added to the food are lactic acid bacteria including *Lactobacillus acidophilus*, *Lactobacillus johnsonni*, *Lactobacillus casei shirota*, and also various species of bifidobacterium (Sanders 1998). It has been suggested that daily intake of probiotics impart different benefits, such as modification of the immune system, improvement and maintenance of balanced intestinal flora, and prevention of gastrointestinal disorders (Lavermicocca 2006; Hekmat and others 2009).

The new consumer perception is that some food products not only satisfy appetite and provide necessary nutrients for body maintenance, but also prevent several diseases and improve physical and mental well-being (Siro and others 2008). For this reason, consumers have become aware that foods must contribute directly to their health and this trend of thinking and eating is inevitable for the future. Up to now, studies on the bioavailability of bioactive ingredients after consumed, as well as their mechanism and mode of action, and biological effects *in vivo* and *in vitro* models, are not well understood, so are needed. Also, structure-to-function relationship studies, together with more understanding of the physiological mechanism by which functional foods bring about improved health, would provide valuable answers to further enhance the functional food market.

Concluding Remarks

This review focuses on various technologies used for the isolation and production of bioactive compounds from natural sources; moreover, several applications of these molecules in the formulation of nutraceuticals and functional foods are described. Nowadays, people are more aware about the components contained in the foods they consume, preferring those obtained from natural sources due to the often imagined negative effects that some compounds obtained by chemical synthesis provoke. ME is presented as a biotechnological tool to improve the production of NBCs from microorganisms because it has had an actual impact and shows promising future trends. However, some challenges must be ad-

dressed before ME becomes a reliable option, and they include production cost, scalability, safety, and product authenticity.

It is clear that NBCs are the future in the field of functional foods and nutraceuticals. Functional foods are gaining importance due to changes in eating habits and concern about health. Nevertheless, some topics must be addressed prior to successful applications in the food and pharmaceutical industries to replace the common "synthetic pharmaceuticals" by "natural nutraceuticals." More research is needed in the near future to demonstrate the effectiveness with more *in vivo* studies in order to advance more rapidly the design of new functional foods and nutraceuticals. This will allow dealing better with World Health Organization regulations for approval and greater use of these products.

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