CHAPTER 5

Extraction and Utilisation of Bioactive Compounds from Agricultural Waste

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Introduction

Production of food waste continues through the entire food life cycle: from agriculture to industrial manufacturing and processing, retail and household consumption. In developed countries, 42 per cent of food waste is produced by households, while 39 per cent of losses occur in the food-manufacturing industry, 14 per cent in food service sector and remaining 5 per cent in retail and distribution. Increasingly, industrial ecology concepts, such as cradle to cradle and circular economy, are considered leading principles for eco-innovation, aimed at 'zero waste economy' in which waste is used as raw material for new products and applications. Many of these residues, however, have the potential to be reused into other production systems, for e.g., biorefineries. The main applications of functional ingredients derived from this transformation are in the nutraceutical and pharmaceutical industry (Fig. 1) (Schieber et al. 2001).

Agro-industrial residues are the most abundant and renewable resource on earth that is poorly valorized or left to decay on the land. Accumulation of this biomass in large quantities every year results not only in deterioration of the environment but also in the loss of potentially valuable material which can be processed to yield a number of value-added products, such as food, fuel, feed and a variety of chemicals. Agro wastes include a wide variety of residues, like molasses, bagasse, oilseed cakes, milling by-products, such as straw, stem, stalk, leaves, husk, shell, peel, lint, seed/stones, pulp, whole pomace, stubble, etc. originating from cereals, pulses, legumes, fruits, vegetables, oil seeds, coffee, tea, etc. (Table 1). Disposal of residue in open spaces or in municipal bins contributes to environmental pollution (Babbar et al. 2011). The best alternative, therefore, is the recovery of phytochemicals/bioactive compounds from such agro-processing residue which can be used in food, cosmetics

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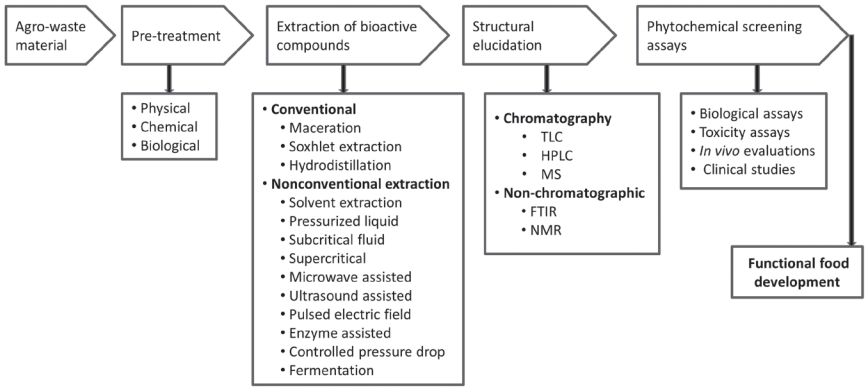

Figure 1. Schematic diagram showing the extraction, isolation and characterization of bioactive compounds from agro-biowaste.

Table 1. By-products/wastes obtained from different crops.

Crop	Waste
Coconut	Fronds, husk, shell
Coffee	Hull, husk, ground
Corn	Cob, stover, stalks, leaves
Cotton	Stalks
Nuts	Hulls
Peanuts	Shells
Rice	Bran, Hull/husk, straw, stalks
Sugarcane	Bagasse
Agricultural crops	Mixed agricultural crops, not limited to crop waste
Mixed type	Agricultural crops and waste including non-organic wastes
Fruits and vegetables	Peel, kernel, rind, stalk, seeds

and the pharmaceutical industry. The recovery of bioactive compounds from such wastes for development of functional/health foods is an efficient way to reuse waste. The agro-industrial residues have alternative uses or markets, though in developing countries they are directly used as fuel along with animal waste and forest litter. Of late, these materials are recycled as a cheap source of renewable feedstock for the production of value-added compounds. They are used as solid substrates in Solid State Fermentation (SSF) processes for the production of different bioactive phenolic compounds (Hernández et al. 2008, Robledo et al. 2008, Vattem and Shetty 2003).

Research on value addition and recycling of the agro- and food industries, localization of bioactive compounds and modification during their processing has received much attention and enormous literature exists on these areas. However, only a few by-products-derived antioxidants have been developed successfully from the vast quantities of plant residues produced by the food processing industry in Europe,

primarily grape seed and olive waste extracts (Alonso et al. 2002, Amro et al. 2002). Potential crop candidates with a high annual production and already confirmed to be of high antioxidant potential include apple (Du Pont et al. 2002), tomato (Fuhrman et al. 2000) and artichoke (Jiménez-Escrig et al. 2003). Recycling of by-products has been justified by the fact that polyphenols are located extensively in the peels (Wolfe et al. 2003) and that processing conditions are known to influence the phenolic content (Wang et al. 2003). Although the antioxidant potential of less important crops, such as strawberry (Kähkönen et al. 2001), pear (Imeh and Khokhar 2002), red beet (Kujala et al. 2001), or broccoli (Kurilich et al. 2002) is known, only scanty literature is available on utilisation of their by-products for phenolic recovery. This is caused by three limiting factors: the effectiveness of recovery and extraction, the marketability of resulting extracts and the practical suitability as food, cosmetic or pharmaceutical products.

Bioactive compounds are extra nutritional constituents that occur naturally in small quantities in plant and food products. Most common bioactive compounds include secondary metabolites, such as antibiotics, mycotoxins, alkaloids, food grade pigments, plant growth factors and phenolic compounds (Kris-Etherton et al. 2002). Among the bioactive compounds, research on few pigments and phenolic compounds due to their therapeutic potential is gaining momentum. Polyphenols and flavonoids are ubiquitous bioactive compounds universally present in higher plants, which belong to a diverse group of secondary metabolites, with significant antioxidant capacities that can protect the human body from reactive free radicals (Robards et al. 1999). Reactive free radicals, such as superoxide anion, hydroxyl radical and peroxy radical may cause the disruption of membrane fluidity, protein denaturation, lipid peroxidation, oxidation of DNA and alteration of platelet functions in the human body (Fridovich 1978), resulting in many chronic health problems, such as cancer, inflammation and atherosclerosis. The search for plant-derived biomaterials has stimulated research interest in extracting polyphenolic compounds from underutilized bulk agro-waste. Some prominent ones are the olive oil industry in Australia, which generates large quantities of olive mill waste rich in biophenols having antioxidant, antimicrobial and molluscicidal activities (Obied et al. 2007); solid by-products from the wine industry are also a potential source of antioxidant phytochemicals (Makris et al. 2007); peels from banana, rambutan and mangosteen are rich in polyphenols. Agro-biowaste must go through pretreatment and extraction processes before valuable bioactive compounds present in them are derived. Thus, the main aim of this chapter is to provide comprehensive information about the nature of residues/by-products, pretreatment methods and extraction of bioactive compounds for futuristic applications in the food and pharmaceutical industry.

Pretreatment of Materials

Pretreatment is an important prerequisite for breakdown of the structure of agro-residues, which are mainly composed of cellulose, hemicellulose and lignin (Fig. 2). Lignocellulosic materials, such as agricultural wastes, forestry residues, grasses and woody materials have good potential for bio-fuel production. Typically, agricultural lignocellulosic biomass comprises about 10–25 per cent lignin, 20–30 per cent hemicellulose and 40–50 per cent cellulose (Iqbal et al. 2011). Cellulose is present in

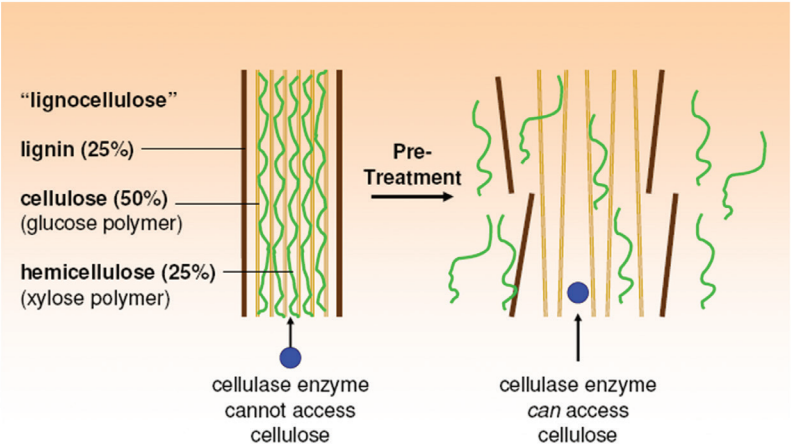

Figure 2. Structural disintegration in the lignocellulosic biomass after pretreatment.
(Percentage of different polymers mentioned in the figure are just indicative and their actual composition differs with the lignocellulosic biomass).

large quantities in agro-industrial residues. As hemicellulose and cellulose are present in the cell wall, they undergo lignification. Hence, there is a need to have an effective and economic method to separate cellulose and hemicellulose from the cell wall. Various pretreatment methods, such as physical, chemical, biological (enzymatic) and their combinations are used. Physical and chemical treatments are used to break down the materials present in the agro-waste residues. Biological pretreatment with microorganisms is also recommended as the glucose present in cellulose is readily used by the microorganisms. Mosier et al. (2005) have provided a comprehensive overview of the promising pretreatment technologies for lignocellulosic biomass. Enzymes like phytase, laccase, lignin peroxidase, manganese peroxidase, produced by micro-organisms help in delignification, bleaching and manufacture of animal feed, etc. (Nigam et al. 2009). Effective pretreatment is characterized by the following parameters: preserving hemicellulose fractions to yield maximum fermentable sugars, limiting the loss of carbohydrate to minimize the formation of inhibitors due to degradation products, minimizing energy input and ensuring that the process is economically efficient and cost-effective (Asgher et al. 2013).

Physical Pretreatment Strategies

Physical pretreatment methods include comminution (mechanical size reduction), steam explosion, hydrothermolysis and microwave treatments where no chemical agents are used. Cellulose fibers absorb water readily and swell, the swelling being limited to the amorphous regions of the fiber, with the crystalline regions counteracting this action. For effective hydrolysis of cellulose, a pretreatment that causes swelling is desirable. Increased swelling can be obtained by physical treatments, such as steam treatment, milling and ultrasonic treatment. After this physical pretreatment, the number of glucosidic bonds available for subsequent chemical and/or biological

pretreatments will substantially increase (Jostein and Jonny 1980). Pretreatment using extrusion processing has been reported for lignocellulosic biomass (Yoo et al. 2011, Zheng and Rehmann 2014). Hydrothermal treatments, like liquid hot water treatment or hot water compression treatments are ideal for low lignin substrates, especially orange peels, banana peels, etc. (Oberoi et al. 2011b, Oberoi et al. 2011c).

Microwaves are radio waves (1 m to 1 mm; 0.3 GHz to 300 GHz frequency) which on interacting with organic matter get absorbed by water, fats and sugars. Their energy gets transferred to organic molecules, generating enormous amount of heat. Microwaves used in pretreatment of lignocellulosic biomass cause localized heating, leading to disruption of lignocelluloses architecture and creating greater accessibility for cellulose and hemicellulose for enzymatic hydrolysis (Ooshima et al. 1984, Sarkar et al. 2012).

Chemical pretreatments

Chemical pretreatment includes alkali, acid, lime (calcium hydroxide) and ammonia treatments. To date chemical pretreatment methods have been most extensively used for delignification of cellulosic materials, for recovery of sugar monomers from cellulose and hemicellulose polymers from lignocellulosic biomass. Acid- and alkali-based hydrolysis are the most commonly used chemical pretreatments (Anwar et al. 2014).

Acid hydrolysis of cellulosic biomass with concentrated hydrochloric acid or sulphuric acid can be performed at very low temperatures as compared to dilute-acid pretreatment. The drawbacks of this process are that it requires acids in higher concentration (30–70 per cent), therefore causing highly corrosive reactions. This increases the infrastructure cost in terms of specialized non-metallic or non-corrosive material (ceramic or carbon-brick lining) and high operating and environmental costs in comparison to pretreatment with dilute-acid hydrolysis (Wyman 1999). Acid pretreatment has been applied on biomass feedstocks, like herbaceous material (grass), hardwoods and agricultural wastes, effectively solubilizing the hemicellulose (Liao et al. 2007). Other factors, including temperature and incubation time during acid pretreatment, also had important impact on alteration of the structure of biomass. Oberoi et al. (2010a) reported significant ethanol production from rice straw through fermentation of hydrolysate obtained through dilute acid hydrolysis. Studies on orange peels using successive acid pretreatments for ethanol production have been reported previously (Oberoi et al. 2010b). A major disadvantage of this process is the formation of secondary products which can lower the ethanol yield due to formation of furfural and hydroxyl-methyl furfural compounds as these compounds interfere in the fermentation process.

Alkali hydrolysis involves pretreatment with sodium, calcium and ammonium hydroxide, resulting in structural alterations inside the lignocellulosic material, such as the depletion of lignin barrier, cellulose swelling and partial decrystallization and solvation of cellulose and hemicellulose, respectively (Sills and Gossett 2011). Alkali pretreatment has been successfully used for corn stover, switch-grass, bagasse, wheat, and rice straw and cotton stalk (Hu et al. 2008, Zhao et al. 2008, Zhu et al. 2010, Oberoi et al. 2011a, Kaur et al. 2012, Rawat et al. 2014). Zhao et al. (2008) reported

that sodium hydroxide pretreatment is effective for hardwood, wheat straw, switch-grass and soft-wood with less than 26 per cent lignin content.

Ammonia pretreatment involves aqueous ammonia treatment at high temperatures, which sufficiently reduces lignin content with loss of some hemicellulose, while cellulose is decrystallized. There are three types of ammonia pretreatment techniques, viz., the Ammonia Recycle Percolation (ARP) (Kim et al. 2003); soaking in Aqueous Ammonia treatments (SAA) (Kim et al. 2008) and Ammonia Fiber Explosion method (AFE) (Teymouri et al. 2005). In ARP treatment, the biomass is pretreated with aqueous ammonia in a flow-through column reactor. In SAA, the process at low temperature removes lignin efficiently by minimizing the interaction with hemicellulose and thereby increasing the surface area and pore size; the retained hemicellulose/cellulose can be hydrolyzed to fermentable sugars by commercial enzymes. Ammonia fiber explosion is a potential technique for pretreatment of lignocellulosic material, wherein the biomass is treated with liquid anhydrous ammonia at 60–100ºC and high pressure (250–300 psi) for 5 min, after which the pressure is released rapidly. The combined effects of ammonia and high pressure lead to swelling of lignocellulose biomass, disruption of lignocellulose architecture leading to hemicellulose hydrolysis and decrystallization of cellulose (Holtzapple et al. 1992).

Organosolv pretreatment process involves extraction of lignin by employing organic solvent or a mixture of solvents, such as ethanol, methanol, acetone and ethylene glycol, in combination with water (Ichwan and Son 2011). Temperature may vary from 100 to 250ºC; catalysts, such as inorganic or organic acids, may be used. This process causes hydrolysis of the internal bonds in lignin and also between lignin and hemicellulose, and hydrolysis of the glycosidic bonds in hemicellulose and to a lesser extent in cellulose. After removal of lignin, the cellulose-rich biomass is used for enzymatic hydrolysis (Zhao et al. 2009).

Delignification of lignocellulose can also be achieved by treatment with oxidizing agents, like hydrogen peroxide, ozone, oxygen or air, where lignin is converted to acids (Hammel et al. 2002, Nakamura et al. 2004). However, these acids can act as inhibitors during the fermentation process and therefore, acids need to be removed (Alvira et al. 2010); further, oxidative treatment also damages the hemicellulose fraction of the lignocellulose complex and a major portion of the hemicellulose gets degraded and becomes unavailable for fermentation (Lucas et al. 2012).

Biological pretreatment

Biological pretreatment employs wood-degrading microorganisms, such as white rot fungi, brown or soft-rot fungi and bacteria to modify the chemical composition and/or structure of the lignocellulosic biomass. Biodelignification replaces or supplements chemical-based pretreatments detailed above; it is simple, economical, eco-friendly, does not require huge infrastructure and is less health hazardous, compared to physico-chemical or chemical-based pretreatment approaches. Therefore, research on biological pretreatment of lignocellulosic biomass is gaining momentum. Cellulose is commonly degraded by cellulases which are produced by several microorganisms. Many bacteria and fungi produce significant quantities of extracellular enzymes, capable of completely hydrolyzing crystalline cellulose *in vitro*. Fungi are the

main cellulose-producing microorganisms. Cellulase is capable of breaking down a highly ordered cellulose polymer into sufficiently smaller sugars which are able to pass through the microbial cell wall and are expelled out in the medium. Enzymatic degradation of cellulose is a complex process that requires the participation of at least three types of enzymes: Endoglucanases (Cx) (E.C.3.2.1.4), Exoglucanase (C1) (E.C.3.2.1.91) and β-glucosidase (E.C.3.2.2.21) (Bhavsar et al. 2015). Bioconversion of lignocellulosic materials to useful products is normally a multi-step process which includes pretreatment, enzymatic hydrolysis and fermentation (Xiao et al. 2012), so that the modified or pretreated biomass is more amenable to enzyme digestion. The limitations of biological pretreatment are that it is a very slow process, requiring careful control of growth conditions and a large area for treatment. In addition, most lignolytic microorganisms solubilise/consume not only lignin but also hemicellulose and cellulose. Because of these techno-economic barriers, biological pretreatment is less attractive commercially (Eggeman and Elander 2005).

Extraction techniques

Bioactive compounds are extra-nutritional constituents that naturally occur in small quantities in plant and food products. Natural bioactive compounds have diverse structures and functionalities with molecules having enormous potential for the production of nutraceuticals, functional foods and food additives. Some of these compounds can be found in nature in high concentrations, such as polyphenols, but others are found at very low levels so that mass harvesting is required to obtain sufficient amounts, thus making chemical synthesis unprofitable (Joana Gil-Chavez et al. 2013). Phenolic compounds, include flavonoids, phenolic acids and tannins, among others. Flavonoids are the largest group of plant phenolics, comprising over half of the eight thousand naturally-occurring phenolic compounds, and include flavonols, flavones, flavanones, flavanols, isoflavones and anthocyanidins. Phenolic acids are another bioactive group of phenolic compounds found in plant and food products and comprise of the subgroups, i.e., hydroxybenzoic and the hydroxycinnamic acids (Harborne et al. 1999).

Bioactive compounds are generally recovered from natural sources by solid-liquid extraction employing organic solvents in heat-reflux systems. The classical techniques to obtain bioactive compounds from plants are: (1) Soxhlet extraction, (2) Maceration and (3) Hydrodistillation.

Soxhlet extractor was first proposed by the German chemist, Franz Ritter Von Soxhlet (1879), designed initially for extraction of lipids but now applied to other valuable bioactive compounds from various natural sources. In this method, a small amount of the dry sample is placed in a thimble in a distillation flask containing a specific solvent. On heating and condensing to an overflow level, the solution of the thimble-holder is aspirated by a siphon which unloads the solution back into the distillation flask. This solution carries extracted solutes into the bulk liquid. The solute remains in the distillation flask and the process runs repeatedly for exhaustive extraction.

Maceration has been used in homemade preparation of tonics since long. It became a popular and inexpensive way to obtain essential oils and bioactive compounds. For small-scale extraction, maceration generally consists of grinding plant materials to

increase the surface area for proper mixing with an appropriate solvent in a closed vessel. The solvent is strained off but the marc (solid residue of this extraction process) is pressed to recover large amounts of occluded solutions. The strained and the pressed out liquid are filtered for further processing.

Hydrodistillation is a traditional method for extraction of bioactive compounds and essential oils from fresh plant materials using water as a solvent. Water distillation, water and steam distillation and direct steam distillation are the types of hydrodistillation (Vankar 2004). In hydrodistillation, the plant materials packed in a still compartment are boiled in sufficient water. Alternatively, direct steam is injected into the plant sample. Indirect cooling by water condenses the vapour mixture of water and oil. The condensed mixture flows to a separator, where oil and bioactive compounds separate automatically from water (Silva et al. 2005). Hydrodistillation involves three main physicochemical processes; hydrodiffusion, hydrolysis and decomposition by heat. A drawback limiting its use for thermo labile compounds is that at high extraction temperatures some volatile components are lost. Recently, more efficient clean and green techniques have been employed to obtain these compounds, such as the supercritical fluids, high pressure processes, microwave-assisted extraction ultrasound-assisted extraction and fermentation processes (Cortazar et al. 2005, Markom et al. 2007, Wang and Weller 2006, Martins et al. 2011).

Solvent extraction

In solvent extraction (SE) the pretreated raw material is exposed to different solvents which selectively extract compounds of interest and also other agents (flavors and colorings). Samples are usually centrifuged and filtered to remove solid residue and the extract is used as additive, food supplement or for preparation of functional foods (Starmans and Nijhuis 1996). Some of the most commonly used solvents in the extraction process are hexane, ether, chloroform, acetonitrile, benzene, methanol and ethanol in different ratios with water for extraction of both polar and nonpolar organic compounds, such as alkaloids, organochlorine pesticides, phenols, aromatic hydrocarbons, fatty acids and oils, among others (Plaza et al. 2010c) (Table 2). The disadvantages are that large amounts of solvent are required over extended periods, and since most of these solvents are toxic to humans and the environment (and the

Table 2. Bioactive compounds extracted using different solvents.

Water	Ethanol	Methanol	Chloroform	Dichloromethanol	Ether	Acetone
Anthocyanins	Tannins	Anthocyanin	Terpenoids	Terpenoids	Alkaloids	Flavonoids
Tannins	Polyphenols	Terpenoids	Flavonoids		Terpenoids	
Saponins	Flavonol	Saponins				
Terpenoids	Terpenoids	Tannins				
	Alkaloids	Flavones				
		Polyphenols				

Adapted from Cowan (1999)

extraction conditions are sometimes laborious), the solvent must be completely separated from the final extract by evaporation or concentration, before the product is used in food applications (Starmans and Nijhuis 1996). There is also the possibility of thermal degradation of bioactive compounds due to high temperatures and longer extraction time. The advantages are low processing cost and ease of operation. Solvent extraction has been improved by other methods, such as ultrasound or microwave extraction and super critical fluid extraction (SCFE), among others, to obtain better yields (Szentmihalyi et al. 2002).

Aludatt et al. (2016) found that the acetone-water solvent mixture (1:1; v/v) helped in efficient extraction of phenolics from the extracts of *Thymus vulgaris* L., with antioxidant and inhibitory activities on angiotensin-converting enzyme and α-glucosidase. The optimal extraction temperature for maximum phenolic content and antioxidant activity associated with methanol extraction was 60°C, whereas a lower temperature of 40°C was required to maximize activities for antihypertensive (inhibitory activities of ACE) and antidiabetic properties (inhibitory activities on α-glucosidase and α-amylase). Generally, major differences were noticed in phenolic profiles among the tested extraction conditions with thymol as the predominant phenolic seen in most extractions, while gallic acid, rosmarinic acid or diosmin were predominant in other extracts. Extracts with the same predominant phenolic compound and similar phenolic content showed major disparities in their ACE, glucosidase and α-amylase inhibitory activities, indicating that the major phenolic profiles of thyme extracts may not be necessarily related to the degree of inhibition of ACE, glucosidase and α-amylase enzymes. This study highlights the effect of varying extraction conditions, such as solvent type and combination of extraction time and temperature as related to free and bound phenolic content and profiles of the extracts with their therapeutic effects.

The *n*-hexane and dichloromethane fractions obtained from sequential extraction of *Lophostemon suaveolens*, a relatively unexplored endemic medicinal plant of Australia, exhibited antibacterial activity against *Streptococcus pyogenes* and methicillin sensitive and resistant strains of *Staphylococcus aureus*. GC-MS analysis of the *n*-hexane fraction by Naz et al. (2016) showed the presence of the antibacterial compounds, aromadendrene, spathulenol, β-caryophyllene, α-humulene and α-pinene and the anti-inflammatory compounds, β-caryophyllene and spathulenol. Fractionation of the dichloromethane extract led to the isolation of eucalyptin and the known anti-inflammatory compound betulinic acid. The profiles of bioactive compounds (including phenolics and flavonoids in free and bound fractions, anthocyanins, proanthocyanidins, vitamin E, and γ-oryzanol) of outer and inner rice bran from six colored rice samples was obtained using 80 per cent ethanol (Huang and Ng 2012). The authors further reported that the free fraction of the extracts dominated the total phenolics (72–92 per cent) and the total flavonoids (72–96 per cent) of colored rice bran.

Pressurized liquid extraction

Pressurized liquid extraction (PLE) is referred to as accelerated SE and pressurized SE. It uses organic liquid solvents at high temperature (50 to 200ºC) and pressure (1450 to 2175 psi) to ensure rapid extraction rate. As the temperature increases, the

dielectric constant of the solvent decreases, lowering the polarity of the solvent. Thus, temperature can be used to match the polarity of a solvent to that of the compounds of interest to be recovered could be extracted (Dunford et al. 2010, Miron et al. 2010). High pressure helps the extraction cells to be filled faster and forces the liquid into the solid matrix. PLE allows a faster extraction, with lesser solvents and higher yields, compared to traditional SE. In addition, using PLE, food-grade extracts are obtained when water or other GRAS solvents, such as ethanol, are used (Plaza et al. 2010a). Despite these advantages, PLE is not suitable for thermolabile compounds, since high temperature damages their structure and functional activity (Ajila et al. 2011).

Subcritical water extraction

The use of water under high temperature and pressure below supercritical conditions in extraction processes is referred to as subcritical water extraction (SWE). Subcritical water extraction is carried out by using hot water (from 100 to 374ºC, the latter being the critical temperature of water) under high pressure (usually from 10 to 60 bar) to maintain water in the liquid state (Herrero et al. 2006). Solvent parameters, such as dielectric constant, solubility and temperature are affected when the liquid state is maintained. The dielectric constant of water at room temperature, which is nearly 80ºC, decreases to about 30 at 250°C, similar to some organic solvents like ethanol or methanol (Adil et al. 2007). Therefore, this technique can be used for the extraction of non-polar phytochemicals and replace organic solvents, with due consideration for the variability of dielectric constants in different types of compounds. This technique has a number of important advantages over the traditional extraction techniques—it is faster, yields higher, facilitates use of lower solvent volumes and is environment-friendly (Plaza et al. 2010b). Subcritical water extraction has been successfully applied to the extraction of different bioactive compounds (mainly antioxidants) from several vegetable and other matrices. Hassas-Roudsari et al. (2009) reported that SWE at 160°C yielded the highest total phenolic content and antioxidant capacity per gram of canola seed meal. Highest recovery of catechins and proanthocyanidins from wine-related products was observed in the material subjected to three sequential extractions at 50, 100, and 150°C. Selective extraction of compounds with different degrees of polymerization can be achieved by using one-step extraction at different temperatures (Garcia-Marino et al. 2006).

Supercritical fluid extraction (SCFE)

Supercritical fluid extraction (SCFE) is an environmentally-safe technology for extraction of bioactive compounds from plants, food by-products, algae and microalgae, etc. The advantages of this technique are high selectivity, faster extraction, less pollution and the use of nontoxic organic solvents. The technique is based on properties of fluids, such as density, diffusivity, dielectric constant and viscosity, and usually involves modifying conditions, such as pressure and temperature to reach a supercritical fluid state (SF). Under these conditions, a fluid is between the gas and liquid states because the density of SF is similar to that of the liquid and its viscosity is similar to that of a gas. Thus the supercritical state of a fluid is the state in which liquid and gas are identical to each other. In addition, SFs have better transport

properties than liquids due to their density which, unlike liquid solvents, is adjustable by changing pressure and temperature (Herrero et al. 2006). In SCFE, the raw material is placed in a temperature-controlled and pressurized extractor vessel, the fluid and the dissolved compounds are transported to separators and the products are collected through a tap located in the lower part of the separator. The fluid is regenerated and cycled or released into the environment (Sihvonen et al. 1999). Though there are many compounds that can be used as SFs (ethylene, methane, nitrogen, xenon, or fluorocarbons), carbon dioxide is the most popular due to its safety and low cost and causes minimal alteration in the bioactive compounds, thus preserving their functional properties (Daintree et al. 2008, Cavero et al. 2006). Supercritical carbon dioxide is an attractive alternative to organic solvents, because it is GRAS, non-explosive, nontoxic, inexpensive and can solubilize lipophilic substances to be easily removed from the final products, since CO_2 is a gas at room temperature and pressure (Wang and Weller 2006). However, because of its low polarity, it is less effective in extracting highly polar compounds from their matrices (Herrero et al. 2006). Therefore, in order to enhance solubility and selectivity of the process, solubility enhancers, called co-solvents or modifiers such as hexane, methanol, ethanol, isopropanol, acetonitrile, or dichloromethane, are added in small quantities (Sihvonen et al. 1999). Of these, ethanol is a recommended co-solvent in SCFE because of its lower toxicity and miscibility in CO_2 (Liza et al. 2010). This method has been applied to extract lipid compounds, such as tocopherols, phytosterols, policosanols and free fatty acids from sorghum (Liza et al. 2010). Cavero et al. (2006) reported that oregano leaf extracts obtained by SC-CO_2 possessed high antioxidant capacity, especially when ethanol was used as a co-solvent. SF-CO_2 is important for the extraction of natural compounds in the food industry because it allows the extraction of thermally labile or easily oxidized compounds (Herrero et al. 2010). SFE is freely used in many industrial applications, including coffee decaffeination, fatty acid refining and the extraction of essential oils and flavors with the potential use in nutraceuticals and functional foods. The conditions for extraction, recovery and characterization of bioactive compounds using supercritical extraction are listed in Table 3. Thus, this method is an important alternative to conventional extraction methods for extracting biologically active compounds and active substances in microparticles as dry powders (Daintree et al. 2008). Subcritical carbon dioxide soxhlet extraction (SCDS) is an innovative

Table 3. Conditions for the extraction, recovery and characterization of natural compounds from plants using supercritical extraction.

Sample	Temperature (ºC)	Pressure (Bar)	Co-solvent (%)	Target compounds
Red grape pomace	45	100–250	Methanol (5)	Pro-anthocyanidins
Spearmint leaves	40–50–60	100–300	Ethanol (10)	Flavonoids
Green tea leaves	60	310	Ethanol (10)	Catechins
Tomato paste waste	880	300	Ethanol (5)	Carotenoids
Tomato skin	75	350	Ethanol (10)	Carotenoids
Grape seed	30–40	130	Ethanol (30)	Anthocyanins

Adapted from Babbar et al. (2015)

green technology with the capacity to extract 10 times more bioactive compounds than hexane, from rice bran oil, viz., total tocopherol, total tocotrienol including α-, β-, γ- and δ-tocopherol; α-, γ- and δ-tocotrienol and γ-oryzanol (Chia et al. 2015).

Microwave assisted extraction

As detailed in the section on pretreatment, microwave assisted extraction (MAE) is more advanced than the traditional SE methods since it works on the principle of heating the moisture inside the cells and evaporating it to produce 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) by improving the porosity of the biological matrix. It thereby allows better penetration of extracting solvent and improves the yield of the desired compounds (Routray and Orsat 2011). Microwave-assisted extraction is suitable for the recovery of a vast array of bioactive compounds, especially those with antioxidant capacity, such as phenolic compounds (Moreira et al. 2012), carotenoids (Pasquet et al. 2011), terpenoids, alkaloids and saponins (Zhang et al. 2011). Higher antioxidant activity is obtained in peel extracts of citrus mandarin (Hayat et al. 2009), peanut skins (Ballard et al. 2010), tomatoes (Li et al. 2011) and onions (Zill-e-Huma et al. 2011), compared to rotary extraction. Microwave-assisted extraction is affected by a large number of factors, such as power, frequency, exposure time, moisture content and particle size of the sample matrix, type and concentration of solvent, ratio of solid to liquid, extraction temperature, extraction pressure and the number of extraction cycles (Mandal et al. 2007), the solvent being the most critical in terms of its solubility, dielectric constant and dissipation factors. Solvents with high dielectric constant, such as water and polar solvents which can absorb high microwave energy, are usually better than nonpolar solvents (Wang and Weller 2006). In addition, the dissipation factor (the efficiency with which different solvents heat up under microwave) plays an important role. Recovery of phenolic compounds is greater when using solvents, such as ethanol or methanol as compared to water, which is associated with a higher dissipation factor (Ajila et al. 2011). Though water has a higher dielectric constant than ethanol or methanol, since its dissipation factor is low, water is slower in heating the moisture inside the sample matrix and generate the pressure to trigger the leaching out of the phytochemicals. Therefore, it is advisable to use solvents with a high dielectric constant as well as a high dissipation factor which can be achieved by using a mixture of water with other solvents (ethanol or methanol). Microwave-assisted extraction has many advantages over conventional extraction techniques, including lower environmental pollution, higher extraction efficiency and shorter extraction time. However, in order to be considered for industrial applications, some of the important limitations which need to be overcome include recovery of nonpolar compounds and modification of the chemical structure of target compounds which may alter their bioactivity. Carvalho et al. (2016) reported that the quantitative profile of phenolic compounds in goji (*Lycium barbarum*) extracts was strongly dependent on microwave-assisted extraction conditions, with significant correlations found between the presence of several flavonoids and solvent composition, as well as between phenolic acids with methoxy group and the response to DNA-based sensors. The authors suggest that targeted extractions for specific compounds would result in extracts that are richer in antioxidant capacity.

Ultrasonic assisted extraction

Though the ultrasound technology is not new, it has only recently been exploited for the recovery of phytochemicals from natural sources. Ultrasound-assisted extraction (UAE) is a viable alternative to conventional SE, providing higher recovery with better bioactivity and lower solvent consumption. Its extraction efficiency is due to the phenomenon called acoustic cavitation, wherein with sufficient ultrasound intensity the expansion cycle creates cavities or microbubbles in the liquid. Once formed, bubbles will absorb the energy from the sound waves and grow during the expansion cycles to recompress during the compression cycle. Further, bubbles may start another rarefaction cycle or collapse, leading to shock waves of extreme conditions of pressure and temperature (several hundred atmospheres and around 5000 K temperature) (Leighton 2007, Soria and Villamiel 2010, Esclapez et al. 2011). The implosion of cavitation bubbles can hit the surface of the solid matrix and disintegrate the cells, causing the release of the desired compounds. Ultrasound assisted extraction has been used for the extraction of proteins (Qu et al. 2012), sugars (Karki et al. 2010), polysaccharides-protein complex (Cheung et al. 2012) and oil (Adam et al. 2012). Recent studies revealed that with UAE, phenolic compounds were less degraded (Dobias et al. 2010) and sometimes no degradation was observed under optimized conditions (Pingret et al. 2012). However, UAE should be carefully used in the extraction of unstable compounds, such as carotenoids (Zhao et al. 2006).

Pulsed electric field extraction (PEF)

The pulsed electric field (PEF) treatment is useful in improving the pressing, drying, extraction and diffusion processes (Vorobiev and Lebovka 2005). When a living cell is suspended in an electric field, the electric potential passes through the cell membrane and based on the dipole nature of the membrane molecules, electric potential separates molecules according to their charge. After exceeding a critical value of approximately one volt of transmembrane potential, repulsion occurs between the charge carrying molecules that form pores in weak areas of the membrane and cause drastic increase in permeability (Bryant and Wolfe 1987). Usually a simple circuit with exponential decay pulses is used for PEF treatment of plant materials in a chamber consisting of two electrodes in either continuous or batch mode (Puértolas et al. 2010). The effectiveness of PEF treatment strictly depends on the parameters, such as field strength, specific energy input, pulse number, treatment temperature and properties of the materials to be treated (Heinz et al. 2003).

Pulsed electric field can increase mass transfer during extraction by destroying the membrane structure of the plant materials for enhancing extraction and decreasing extraction time. Pulsed electric field treatment at a moderate electric field (500 and 1000 V/cm; for $10^{-4}-10^{-2}$ s) can damage the cell membranes with little temperature increase and minimize the degradation of heat-sensitive compounds (Fincan and Dejmek 2002). Pulsed electric field can also be used as a pretreatment process prior to conventional extraction to lower the extraction effort (López et al. 2009). Pulsed Electric Field treatment (at 1 kV/cm with low energy consumption of 7 kJ/kg) in a solid liquid extraction process for extraction of betanin from beetroot showed maximum extraction as compared with freezing and mechanical pressing (Fincan et al. 2004).

Guderjan et al. (2005) showed that the recovery of phytosterols from maize increased by 32.4 per cent and isoflavonoids (genistein and daidzein) from soybeans increased by 20–21 per cent when PEF was used as pretreatment process. Corralesa et al. (2008) extracted bioactive compounds, such as anthocyanins from grape by-products by using various techniques and found better extraction of anthocyanin monoglucosides by PEF. The application of PEF treatment on grape skin before maceration step can reduce the duration of maceration and improve the stability of bioactives (anthocyanin and polyphenols) during vinification (López et al. 2008).

Enzyme-assisted extraction

Most phytochemicals, such as flavonoids, are present in different forms, interacting with the cell wall components (cellulose, hemicellulose and pectin) (Fu et al. 2008). Enzymes, such as cellulase, β-glucosidase, xylanase, β-gluconase and pectinase help to degrade the cell wall structure and depolymerize the plant cell wall polysaccharides, facilitating the release of these linked compounds, since they can hydrolyze the ester-linked phenolic acids (Chen et al. 2010). There are several reports on enzymatic treatment of plant tissues for the extraction of natural bioactive compounds. Chandini et al. (2011) found that the enzyme tannase was superior to pectinase for improving the quality of black tea extracts. Extraction of luteolin and apigenin from pigeonpea leaves was facilitated by pectinase, cellulase and β-glucosidase. Enzyme-assisted extraction was used to improve the antioxidant composition of black carrot juice and to obtain vegetable oils (Khandare et al. 2010, Szydlowska-Czerniak et al. 2010). Enzyme extraction is used to extract compounds from algae as well, where the structural complexity and rigidity of the algal cell wall prove an obstacle (Wang et al. 2010). Enzyme-assisted extraction is valuable in extracting precious by-products, such as gallic acid from agricultural waste (Curiel et al. 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 antibacterial drug, trimethroprim, by pharmaceutical chemistry (Curiel et al. 2010).

Instant controlled pressure drop-assisted extraction

Instant controlled pressure-drop (DIC) was defined by Allaf and Vidal (1988) and since then, the technology has been refined for the extraction of volatile compounds and antioxidants (Berka-Zougalia et al. 2010). Instant controlled pressure-drop 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 towards a vacuum (Ben Amor and Allaf 2009). The pressure-drop applied provokes the auto-vaporization of volatile compounds, instantaneous cooling of the products which stop thermal degradation and expansion of the cell wall, thus enhancing the mass transfer and improving the recovery of the desired compounds. The auto-vaporization of volatile compounds makes DIC suitable for the recovery of essential oils (Allaf et al. 2012). Kristiawan et al. (2008) extracted 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 after a prolonged 16 hours steam distillation. Allaf et

al. (2012) found that DIC extracts from orange peels had better essential oil quality (major oxygenated compounds) and antioxidant capacity (about 13 per cent) when compared with hydro-distilled extracts—the common way to obtain essential oils. Using DIC, Ben Amor and Allaf (2009) optimized the parameters (vacuum, pressure and temperature) to improve the recovery of anthocyanins from Roselle by up to 135 per cent and by using water as a solvent. Allaf et al. (2012) used DIC and UAE sequentially for the extraction of phenolic compounds (naringin and hesperidin) from orange peels with a very high yield, best kinetics and antioxidant capacity compared to standard SE.

Fermentation methods

Extraction/production of bioactive compounds by fermentation is a promising alternative which can provide high quality and high activity extracts, while precluding any toxicity associated with the extractants, especially the organic solvents. During fermentation, bioactive compounds are obtained as secondary metabolites produced by microorganisms after the microbial growth is completed (Nigam 2009). Studies on liquid culture show that the production of these compounds starts when growth is limited by the exhaustion of one key nutrient—carbon, nitrogen or phosphate source (Barrios-González et al. 2005).

Fermentation processes are of two types: submerged fermentation (SmF), where microorganisms are cultivated in a liquid medium containing nutrients and solid state fermentation (SSF), where the microbial growth and product formation occurs on solid particles in the absence (or near absence) of directly available water. However, the substrate contains sufficient moisture to allow growth and metabolism of microorganisms (Pandey 2003). Solid state fermentation has gained more importance since this process may lead to a higher yield and productivity or better product characteristics than SmF. In addition, since low-cost agricultural and agro-industrial residues are utilized as substrates, capital and operating costs are lower as compared to SmF. Low water volume in SSF has also a large impact on the economy of the process due to smaller fermenter-size, reduced downstream processing, reduced stirring and lower sterilization costs (Nigam 2009, Pandey 2003). The main limitation of SSF, however, is the scaling-up of the process, largely due to heat transfer and culture homogeneity problems (Di Luccio et al. 2004, Mitchell et al. 2000). Although many bioactive compounds are still produced by SmF, in the last few decades, the SSF technique is being preferred for its efficiency.

Pomegranate wastes contain phenolic compounds, including anthocyanins (derived from delphinidin, cyanidin and pelargonidin), hydrolysable tannins (catechin, epicatechin, punicalin, pedunculagin, punicalagin, gallic and ellagic acid esters of glucose) (Cuccioloni et al. 2009, Gil et al. 2000), and several lignans (isolariciresinol, medioresinol, matairesinol, pinoresinol, syringaresinol and secoisolariciresinol) (Bonzanini et al. 2009), with antioxidant, anti-mutagenic, anti-inflammatory and anticancer activities (Naveena et al. 2008). Pomegranate husks are successfully used as a matrix to produce almost 8 kg of ellagic acid per ton of waste, by SSF with *Aspergillus niger* GH1. This process is economical and quite profitable from the industrial point of view, considering the commercial price of this acid and the low cost and abundance of

the husks. Elagitannin acyl hydrolase is responsible for bioconversion of elagitannin into ellagic acid during SSF of pomegranate husks (Robledo et al. 2008). Cranberry pomace, the by-product of cranberry juice processing industry, is also a good source of ellagic acid and other phenolic compounds. Bioprocessing of this waste by SSF with *Lentinus edodes*, using its esterase enzyme, was useful to increase the ellagic acid content by being an alternative for the production of bioactive compounds (Vattem and Shetty 2003). In India, Teri pod (*Caesalpinia digyna*) cover, the solid residue obtained during processing of the pod for recovery of oil, contains tannin that can be used as substrate for microbial bioconversion to gallic acid by SSF with *Rhizopus oryzae* (Kar et al. 1999). Green coconut husk, an abundant agro-industrial residue, is a potential source of ferulic acid from which vanillin can be obtained *via* microbial conversion by the basidiomycete *Phanerochaete chrysosporium* under SSF, where the production of lignolytic enzymes released ferulic acid from the coconut husk cell wall and subsequently, vanillin was obtained with a high yield by the ferulic acid conversion (Barbosa et al. 2008). The action of enzymes, such as α-amylase, laccase and β-glucosidase, tannin acyl hydrolase, ellagitanin acyl hydrolase, among others, plays an important role in the mobilization of bioactive phenolic compounds during SSF (Cho et al. 2009, Zheng and Shetty 2000). Lignocellulosic enzymes are mainly produced by fungi, since these microorganisms have two extracellular enzymatic systems—a hydrolytic system that can degrade polysaccharides and an oxidative ligninolytic system, which degrades lignin and opens phenyl rings, increasing the free phenolic content (Sánchez 2009). During SSF of soybean with *Bacillus pumilus* HY1, Cho et al. (2009) reported a significant increase in flavanols and gallic acid content associated with bacterial β-glucosidase and esterase activities. Similarly, improvement in the antioxidant potential of fermented rice is associated with phenolic compound increases by β-glucosidase and α-amylase activities during SSF (Bhanja et al. 2008).

Agricultural or forestry biowastes, such as cereal and vegetable wastes, like straw, bagasse, stover, cobs, husks, among others, are lignocellulosic materials composed mainly of cellulose, hemicellulose and lignin. Lignin contains numerous phenolic components, such as ferulic, *p*-coumaric, syringic, vanillic and *p*-hydroxybenzoic acids (Mussatto et al. 2007), which can be recovered by SSF, using filamentous fungi like the white-rot fungi *Phanerochaete chrysosporium*, *Trametes versicolor*, *Trametes hirsuta* and *Bjerkandera adusta*, which can degrade lignin. These fungi utilize the polysaccharides after lignin degradation to grow and reproduce, thus increasing the nutritional value of the agro-industrial substrates that is generally low. After SSF, the materials can be used as animal feed or soil fertilizer (Nigam et al. 2009, Oberoi et al. 2011b). The main extracellular enzymes participating in lignin degradation are lignin peroxidase, manganese peroxidise and laccase (Philippoussis 2009).

In order to harness the potential of phytochemicals as bioactive compounds, development of better extraction methods using advanced technologies help to obtain the greatest yield in the shortest processing time and at a low cost, but an eye on the environmental cost is imperative. The main differences between these extraction technologies depend on the design of the reactors, solvents used, time and temperature of the processes and yields. The physico-chemical properties of desired compounds and availability of their sources decide the strategy to be adopted. Also, the cost-benefit analysis of the use of a single or combination of extraction technologies should be taken into consideration by the food and pharmaceutical industries.

Functional Food Development

Consumers are increasingly demanding safe and healthy natural food ingredients. There is increasing awareness about the health-promoting benefits of antioxidants in foods, and this in combination with the realization that a number of common synthetic preservatives may have hazardous effects, has led to a vast number of reports on natural antioxidants (Babbar and Oberoi 2013). Functional foods are those foods or ingredients which, when consumed regularly, produce a specific beneficial health effect beyond their basic nutritional properties. These include a variety of bioactive compounds included in food formulations with a specific purpose (Day et al. 2009, Roberfroid 2002, Nobili et al. 2009, Bernal et al. 2010).

From a practical point of view, a functional food can be:

- A natural food
- A food to which a component has been added
- A food from which a component has been removed
- A food in which the bioavailability of one or more components has been modified, or
- A combination of the above possibilities.

All claims for functional foods should be based on the scientific classification of markers (indicators and/or factors) for target functions. The effect must go beyond what is normally the established role of diet, such as a target function or a biological activity or for a disease risk reduction (Diplock et al. 1999). This evidence should rely on three categories of data:

- Biological observations,
- Epidemiological data, and
- Intervention studies, mostly based on markers.

Plaza et al. (2008) emphasized three 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 lead to improvement of physiological function or to reduction of risk of developing a pathological process.

Since all bioactive compounds do not cover these aspects, some are not considered functional food ingredients. Food and Drug Administration in the USA has grouped several compounds with potential as new bioactive food ingredients that are considered generally safe (GRAS), including vegetable oil, sterol esters, phytostanol esters, lactoferin, fish oil concentrate, tuna oil, diacylglycerol and inulin, among others (Burdock et al. 2006). There remains a wide range of bioactive compounds with beneficial effects on health but they need government clearance. The most remarkable case among these 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 natural bioactive compounds, like polyphenols and carotenoids. The health benefits include decrease in cholesterol levels, alleviation of lactose intolerance, maintaining remission of Crohn's disease, faster relief from diarrhoea and inhibition of cancer cell proliferation *in vivo* and *in vitro*, antioxidant, antiviral, antihypertensive (Marette et al. 2010).

Until recently, natural antioxidants have found application mostly as food supplements, like green tea extract (Buetler et al. 2002) or as food preservatives obtained from aromatic plants, like rosemary and salvia extracts (Zupko et al. 2001). They also find application in health products in the cosmetic and pharmaceutical industry due to the customer's demand for 'non-chemical' ingredients. The phenolic antioxidants are a substitute for synthetic preservatives or as active ingredients; for example, a skin-protecting additive in dermatology. These three sectors are promoted together as functional foods: food supplements, nutraceuticals or cosmeceuticals. Food-derived extracts as ingredients in food and cosmetic products are perceived as non-toxic antioxidants and find commercial application as radical scavengers and flavonoids with anti-ageing and photoprotection benefits, in both hydrophilic and lipophilic systems (Katiyar and Elmets 2001). Examples of functional food products that are currently in market are drinks, cereals, bakery products, spreads, meat products and eggs, among others (Siro et al. 2008).

Processing of mango fruits generates a significant amount of by-products, such as peels and seeds which represent up to 60 per cent of the fresh fruit. Mango by-products are an important source of bioactive phenolic compounds. Phenolic characterization is an essential step for utilisation of mango by-products as food ingredients, and so provides an added value to mango production. Dorta et al. (2014) have characterized the phenolic compounds from peel and seed extracts of three mango varieties (Keitt, Sensation and Gomera 3) produced in Spain through different microwave-assisted extraction conditions. Thirty compounds were identified that belong to five phenolic families: gallates and gallotannins; flavonoids mainly quercetin derivatives; ellagic acid and derivatives; xanthones principally mangiferin; benzophenones and derivatives of maclurin, variation was attributed to the variety of mango, the part of fruit studied (peel or seed) and the extraction conditions, with the latter having a greater influence on the phenolic content.

Licorice (*Glycyrrhiza glabra*) is a popular herbal supplement used for the treatment of chronic inflammatory conditions and possesses anticancer and antiviral activities. It contains a number of phytochemicals including terpenoids, saponins, flavonoids, polyamines and polysaccharides. There are over 30 species in the Glycyrrhiza genus world-wide, most of which have been little characterized in terms of phytochemical or pharmacological properties to explain their medicinal use. UV, MS and NMR spectral analyses of extracted components from *Glycyrrhiza glabra*, *G. uralensis*, *G. inflata* and *G. echinata* have identified the major components as glycyrrhizin, 4-hydroxyphenyl acetic acid and glycosidic conjugates of liquiritigenin/isoliquiritigenin. Primary metabolites profiling use of GCMS revealed the presence of cadaverine, an amino acid, exclusively found in *G. inflata* roots (Farag et al. 2012).

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 et al. 2008, Lattanzio et al. 2009, Aider and Barbana 2010). Functional foods include probiotic microorganisms, such as lactic acid bacteria *Lactobacillus acidophilus*, *L. johnsonni*, *L. casei shirota*, and also various species of bifidobacterium (Sanders 1998) and prebiotic products mainly presented through dairy products and milk which are good vehicles to deliver the probiotics (Day et al. 2009, Saulnier et al. 2009). Probiotics can

modify the immune system, help in the improvement and maintenance of intestinal flora and prevention of gastrointestinal disorders (Hekmat et al. 2009). However, the bioavailability of bioactive ingredients after consumption, their mechanism and mode of action, biological effects *in vivo* and *in vitro* and structure-to-function relationship and physiological mechanism by which the health benefits manifest are not well understood. So research on these aspects need greater attention.

Functional foods from residues/by-products of food processing

The growing interest in replacement of synthetic food antioxidants by natural ones has fostered research on screening of plant-derived raw materials for identifying new antioxidants. Attention is now focused on inexpensive or residual sources from agricultural industries. Fruit peels, often the waste parts of various fruits from consumption and food industry, are rich sources of powerful natural antioxidants. Table 4 gives the composition and content of bioactive compounds observed in methanolic extracts of various plant wastes. Dehydrated waste grape skins from the juice industry were added to aged and young red wines as an innovative way of compensating for colour loss before bottling and increasing colour intensity of wines by 11 to 31 per cent. Total polyphenols increased by 10 to 20 per cent, which mostly comprised of gallic acid, catechin, epicatechin and resveratrol; its anthocyanins content also increased by 50 mg/l (Pedroza et al. 2013). Among the fruit peels, mangosteen peel is an important source of natural antioxidants, like phenolic acids and flavonoids, which possess biological and medicinal properties (Suttirak and Manurakchinakorn 2014). Schieber et al. (2001) have reviewed the scope for obtaining functional compounds from by-products of plant food-processing industry. Pectin production is an economical use for apple pomace with superior gelling properties compared to citrus pectins; the limitation being the slightly brown hue of apple pectins caused by enzymatic browning (Renard et al. 1997). Apple pomace is a good source of polyphenols which are predominantly localized in the peels, such as catechins, hydroxycinnamates, phloretin glycosides, quercetin glycosides and procyanidins. Apple pomace phenols are rich in antioxidant activity and cariogenicity of *Streptococci.* Therefore they have possible application in dentifrices (Yanagida et al. 2000). Release of phenolics can be enhanced by using pectinases and cellulases.

Apple, golden rod and artichoke by-products were extracted on a pilot plant scale by Peschel et al. (2006) and their antioxidant activity was confirmed to be similar to the established antioxidants (Oxynex® 0.1 per cent, Controx® KS 0.15 per cent and butylated hydroxytoluene (BHT) 0.01 per cent), demonstrating the possibility of recovering high amounts of phenolics with antioxidant properties from fruit and vegetable residuals not only for food but also cosmetic applications. Broccoli by-products—leaves and stalks—are rich in bioactive compounds, including nitrogen-sulphur compounds (glucosinolates and isothiocyanates) and phenolics (chlorogenic and sinapic acid derivatives, and flavonoids), as also essential nutrients (minerals and vitamins). They are of huge interest as a source of health-promoting compounds useful as ingredients for the development of functional foods. Green tea enriched with broccoli concentrates showed improved physical quality, phytochemical composition and antioxidant capacity, encouraging the production of novel products

Table 4. Composition and content of bioactive compounds in methanolic extracts of various plant materials.

Plant material	Compound	Concentration (mg/g)
Tomato peel	Cis-lycopene	22.02
	Beta-carotene	6.87
	Trans-lycopene	36.49
	Lutein	1.08
	Ascorbic acid	12.27
	Quercetin	2.89
	Kaempferol	7.20
Cucumber peel	Chlorophyll	3.46
	Pheophytin	1.95
	Phellandrene	1.21
	Caryophellene	1.49
Water melon peel	Chlorophyll	5.28
	Diosmetin	1.57
	Pheophytin	1.27
	Malvidin 3,5 diglycoside	1.23
Potato peel	Gallic acid	0.16
	Protocatecheic	1.84
	p-Hydroxybenzoic	0.26
	Caffeic acid	0.19
	Vanillic acid	0.04
	Chlorogenic acid	0.28
Orange peel	p-coumaric	1.02
	Ferulic acid	0.91
	Syringic acid	7.71
	Naritutin	1.21
	Nazingin	3.83
	Ascorbic acid	14.9
Olive leaves	Oleuropein	71.61
	Apigenin 7-glucoside	4.10
	Rutin	0.15
	Vanillin	0.15
	Vanillic acid	1.87
	Caffeic acid	1.02
	Hydroxytyrosel	3.29

Adapted from Zeyada et al. (2008)

and applications for nutritional and/or health claims under the EU Regulations (EC) No. 1924/2006 and 834/2007 (Perles et al. 2011).

Guava (*Psidium guajava* L.) seed powder (red guava cv. Paluma) obtained from guava pulp processing contained varying amounts of macronutrients and micronutrients, high content of total dietary fiber (64 g/100 g), protein (11.2 g/100 g), iron (13.8 mg/100 g), zinc (3.3 mg/100 g) and reduced calorie content (182 kcal/100 g). Their lipid profile showed a predominance of unsaturated fatty acids (87.1 per cent), especially linoleic acid and oleic acid. The powder obtained contained significant amounts of bioactive compounds, such as ascorbic acid (87.4 mg/100 g), total carotenoids (1.3 mg/100 g) and insoluble dietary fiber (63.6 g/100 g). With regard to their microbiological quality, the samples were found suitable for consumption. Thus, guava seeds can be a viable alternative to prevent various diseases and malnutrition in our country and to reduce the environmental impact of agricultural waste (Athayde et al. 2014).

Polygonum cuspidatum, used in important traditional Chinese medicine, is widely distributed in the world and many parts of the plant are used to treat hyperlipemia, inflammation, infection, cancer, etc. The roots of this plant are used as an effective agent in pre-clinical and clinical practice for regulating lipids, anti-endotoxic shock, anti-infection, anti-inflammation, anti-cancer and other diseases in China and Japan. Over 67 compounds including quinones, stilbenes, flavonoids, counmarins and ligans have been isolated and identified from this plant (Peng et al. 2013).

Olive mill wastewater is an agricultural waste material produced in high quantities in the Mediterranean basin. It is an inexpensive source of health-promoting phytochemicals with the potential economic value including many low molecular weight compounds, such as the bioaccessible verbascosides (Cardinali et al. 2011). The biophenolic fraction of olive mill waste in Australia contained higher phenol content, higher antioxidant activity and broad spectrum antibacterial activity as seen against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*; molluscicidal activity against *Isidorella newcombi* was also reported (Obied et al. 2007).

Parkia speciosa, known as stink bean, is popular in south-eastern Asia including Malaysia and north-eastern India. The seeds are normally separated from the pods before they are ready to sell. *P. speciosa* is believed by the locals to have medicinal properties; the pods have hypoglycaemic activity (Jamaluddin et al. 1995), seeds contain antibacterial cyclic polysulfides, also responsible for their strong pungent flavour and anticancer activity due to presence of thiazolidine-4-carboxylic acid (Pandeya 1972). The waste of fruits and beans, peels and pods, which were previously used as fertilisers, feeds and landfills, are now considered more precious for their highly antioxidant pectin-like polysaccharide, a functional carbohydrate in food application studies (Gan et al. 2010b).

Controlling the spread of food-borne pathogens in moist environments is an important microbial food safety issue. Isolation of compounds from agricultural waste (such as fruit peels) that would control spread of human pathogens was explored by Mahadwar et al. (2015) by using *Salmonella enterica* serovar *typhimurium* as a model organism. Pomegranate peels were found to have great potential as a bioactive repellent for pathogenic micro-organisms.

Encapsulation

Encapsulation is a process to entrap solid, liquid, or gaseous active agents (or core) within a carrier, wall or coating material (Fang and Bhandari 2010). It is a useful tool to improve delivery of bioactive molecules (e.g., antioxidants, minerals, vitamins, phytosterols, lutein, fatty acids, lycopene) and living cells (e.g., probiotics) into foods (Vos et al. 2010). The materials used for encapsulation must be food-grade, biodegradable and able to form a barrier between the internal phase and its surroundings, the most widely used material being polysaccharides. Among the polysaccharides, starch and their derivates—amylose, amylopectin, dextrins, maltodextrins, polydextrose, syrups and cellulose and their derivatives are commonly used. Plant exudates and extracts—gum Arabic, gum tragacanth, gum karaya, mesquite gum, galactomannans, pectins and soluble soybean polysaccharides are also used. Recently, marine extracts, such as carrageenans and alginate, and microbial and animal polysaccharides like dextran, chitosan, xanthan and gellan have also been exploited. Apart from natural and modified polysaccharides, proteins and lipids are also used as coatings for encapsulation. Examples of the most common milk and whey proteins are caseins, gelatine and gluten and the lipid materials are fatty acids and fatty alcohols, waxes (beeswax, carnauba wax, candellia wax), glycerides and phospholipids. Other materials employed are PVP, paraffin, shellac and inorganic materials (Wandrey et al. 2009).

The most extensively applied encapsulation technique in the food industry is spray drying, because it is economical, flexible and continuous, producing particles of size less than 40 μm (Zuidam and Heinrich 2009). Other encapsulation techniques include spray-chilling, freeze-drying, melt extrusion and melt injection. Encapsulation in cyclodextrins and liposomal vesicles are less preferred since they are more expensive technologies. The advantages of encapsulation of bioactive material include providing barriers between sensitive bioactive materials and the environment (oxygen or water), thus allowing differentiation of taste and aroma, masking bad taste (bitterness and astringency of polyphenols) or smell, stabilizing food ingredients (less evaporation and degradation of volatile actives such as aroma) or increasing their bioavailability. Encapsulation may also be used to immobilize cells or enzymes in food-processing applications, such as fermentation and metabolite production. There is an increasing demand to find suitable solutions that provide high productivity and quality of the final food products (Nedovic et al. 2011). Encapsulation facilitates packaging materials in small capsules that release their contents at controlled rates over prolonged periods and under specific conditions (Desai and Park 2005). Produced particles usually have diameters of a few nm to a few mm (Wandrey et al. 2009). Nanotechnology is the science of the miniature, wherein biological and non-biological structures smaller than 100 nm are now being exploited by agricultural producers and food manufacturers to encapsulate bioactive compounds, to benefit consumers in the long term, to foster a competitive and innovative domestic agricultural and food system and provide new methods to improve safety and nutritional value of food products (Weiss et al. 2006).

Conclusion

To conclude, agricultural wastes have increased in tune with increase in food production, consumption and processing, the sources being the farmer, retailer, food industries,

consumer/household, with each source contributing different quantities, cumulatively adding up to a substantial quantum of waste. This not only adds to the environmental pollution burden, but also results in a massive waste of unutilized wealth. This chapter elaborates on the processes required for an effective utilisation of agro-wastes. Agro-wastes from a variety of residues like molasses, bagasse, oilseed cakes, milling by-products, etc., are a potential source of value-added products, such as food, fuel, feed and a variety of chemicals, nutraceuticals and functional foods. Valorization of the ubiquitous phenolics which are present in the peels and seeds of agri-wastes with diverse medicinal value, for development of nutraceuticals and functional foods is the most effective approach for their utilisation. Utilisation of agro-wastes begins with a process of pretreatment required to break down the structure of agro-residues using physical, chemical, biological and a combination of these methods. This is followed by extraction techniques to harvest the bioactive compounds from the matrix of the plant material adopting classical and/or more advanced non-classical methods. The classical extraction methods include Soxhlet extraction, maceration and hydrodistillation; the more efficient non-classical clean and green extraction methods are solvent extraction, pressurized liquid extraction, subcritical and supercritical fluid extraction, microwave and ultrasonic assisted extractions, pulsed electric field extraction, enzyme assisted extraction, fermentation methods, instant controlled pressure-drop extraction and many more. These extraction methods may be used in combination to increase the efficiency of extraction. The extracted bioactive chemicals must be identified for better understanding of their chemistry and biological activity before being taken forward for the development of functional foods, of which numerous products have already made it to the retail shelves. Encapsulation of bioactives further serves to improve the functionality of the food components. Thus this chapter details the processes by which agricultural waste can be turned to wealth.

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