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Review Paper

Antioxidants extraction by supercritical CO_2

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Antioxidants such as phenolics, terpenoids, carotenoids, sterols, VE and alkaloids, are receiving increasing attention due to their demonstrated health benefits. However, conventional technologies used to extract some of these antioxidants may have some limitations. Therefore, there is a need for emerging efficient technologies that are environmentally friendly and that are cost-effective. Supercritical CO_2 extraction, which has already reached commercial application for some products, is one of the most widely studied techniques. This technology is simple and fast and allows selective extraction at moderate pressures and low temperatures. This paper reviewed recent findings about the antioxidants including phenolics, terpenoids, carotenoids, sterols, VE and alkaloids. The impact of important parameters related to sample preparation and extraction process on the efficiency of extraction and on the characteristics of the extracted products is evaluated based on an extensive review of recent literatures. The future of extraction of antioxidants is certainly bright with the use of combined other technologies, such as microwave extraction, pulsed electric field, high-pressure processing, ultrasonic extraction, and ohmic heating, that will allow high-quality antioxidants to be produced.

Key words: Antioxidants; supercritical CO2

INTRODUCTION

According to a widely used definition, an antioxidant is any substance that, when present at lower concentrations than those of an oxidizable substrate can significantly delay or prevent oxidation of that substrate (Halliwell et al., 1989; Halliwell, 1995). Natural antioxidants play a decisive role in different systems such as in plants, in foods and in biological systems (Willcox et al., 2004). They are generally commercialized as nutraceuticals and as ingredients for functional foods. Since considerable evidence indicates that oxidative damage may be reason of the development of age-related and degenerative diseases, the protective effects of beneficial compounds have been ascribed to their antioxidant activity, although many antioxidants *in vivo* probably act by other mechanisms than *in vitro* assays or are unlikely to have such effects at the concentrations available in plasma (Azzi et al., 2004; Halliwell et al., 2005).

Due to an increasing consumer demand to replace controversial synthetic antioxidants, such as butylated hydroxytoluene, butylated hydroxyanisole, tertiary butyl hydroquinone, and gallates, a promising application of natural antioxidants, which could confer additional biological activities to the products, is for the foods preservation. Although natural antioxidants are assumed to be much safer than synthetic antioxidants, lack of toxcity should be confirmed.

Antioxidants have been commonly obtained by conventional extraction methods using organic solvents, requiring their removal by subsequent evaporation. But, the heat applied for solvent removal may be detrimental to heat-labile antioxidants such as lycopene. In addition, government regulations on the use of organic solvents are becoming stricter, and the safety of residual organic solvents in the final product is being questioned. The technology supercritical CO_2 extraction is growing because of the capability of this technology to overcome

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many of the disadvantages associated with conventional technologies. The advantages of SC-CO_2 include its low processing temperature, which minimizes thermal degradation; the ease of separation with no solvent residue left in the final product; and minimization of undesirable oxidation reactions. Supercritical CO_2 extracts are regarded as "natural"; are free from pathogenic and spoilage microorganisms, spores, and enzymes; the absence of light and oxygen prevents oxidation (Pokorny et al., 2001).

The objectives of this paper are to review some of the recent literature findings related to the antioxidants and the use of supercritical CO_2 for the antioxidants extraction from different plant sources. Factors affecting extraction yield such as sample preparation (moisture content and particle size) and extraction parameters (temperature, pressure, flow rate) will be discussed.

TYPES AND BIOLOGICAL PROPERTIES OF ANTIOXIDANTS

Lipid oxidation is important in food deterioration. According to the free radical theory, various oxidative reactions occurring in the organism generate free radicals, which damage nucleic acids, proteins, and lipids and result in aging and age-associated pathologies. The stages of the classical nonenzymatic free radical–mediated chain reactions are: 1) initiation (by heat, light, ionizing radiation, metal ions, or metalloproteins), 2) propagation, 3) branching, and 4) termination. The mechanisms of lipid oxidation and antioxidant action have been detailed in the literature (Gordon, 2001; Frankel et al., 2000; Yanishlieva, 2001; Antolovich, 2002; Roginsky, et al., 2005).

Antioxidants have traditionally been divided into two groups: primary and secondary. Primary antioxidants, such as phenolics or vitamin E, are destroyed during the induction period, which can delay or inhibit the initiation step by reacting with radicals. Secondary, antioxidants slow the oxidation rate removing substrate by binding oxygen from air, complexing with transition metal ions (acetates, citrates, tartrates, and phosphates), quenching singlet oxygen, binding certain proteins with prooxidant effects, absorbing ultraviolet radiation or creating a protective layer between oil and air surface(in the case of phospholipids).

Depending on their chemical structure, antioxidants have been grouped into phenolics, quinones (hydroquinone, tocopherols, hydroxychromanes, hydroxycoumarins), organic acids (ascorbic, citric, tartaric, and lactic acids and their salts and ethylenediaminetet raacetic acid and its salts), sulfur compounds (inorganic: sulfites, bisulfites, and metasulfites; organic: methionine, cisteine), and enzymes (catalases, peroxidases, superoxide dismutase). The natural antioxidants found within biological systems include four general groups: enzymes, large molecules (albumin, ceruloplasmin, ferritin, other proteins), small molecules (ascorbic acid, glutathione, uric acid, tocopherol, carotenoids, polyphenols), and some hormones (estrogen, angiotensin, melatonin) (Prior et al., 2005).

Synthetic antioxidants in food utilization is not permitted in the European Union for some special foods, such as those for infants and young children (European Parliament and Council Directive, 1995;Mikova, 2002), and it is generally restricted to levels that depend on the considered application. However, antioxidants from natural origins do not need to be declared and are allowed at higher doses (Yanishlieva, 2001; Mukhopadhyay, 2000).

Phenolics

Phenolic compounds are a heterogeneous class of secondary plant metabolites that can be divided into two main groups: flavonoid compounds, with a C6-C3-C6 structure (anthocyanins, flavan-3-ols, flavonols, flavones, and flavanones) and nonflavonoid compounds, with C6-C1 and C6-C3 structures, (hydroxybenzoic and hydroxycinnamic acids and stilbenes), responsible for color development, pollination, and protection against UV radiation and pathogens. In foods, these compounds contribute to sensory properties (color, astringency).

Phenolics are mainly present in fruits, seeds, and herbs such as elderberry, grape, mat´e tea leaves, rose hip, Rosmarinus officinalis, Origanum dictamnus, Teucrium polium, and Styrax officinalis. The amount and type of phenolics and their conjugates differ markedly even in different tissues of the same species. Most flavonoids present in plants are conjugated with sugars, although occasionally they are found as aglycons (Ross, 2002). More than 4,000 different naturally occurring flavonoids have been discovered, and more than 36,000 different flavonoid structures are possible (Ross, 2002).

Phenolic compounds have powerful antioxidant activities *in vitro* (Miller, 2002), based on their structure, hydrogen-donating potential, and ability to chelate metal ions. They may show higher efficacy than synthetic antioxidants (Soobrattee, 2005). Their antioxidant activity (Meyer et al., 2001; Collins et al., 2003; Mansouri et al., 2005) and their structure-activity relationships have been examined (Mukhopadhyay, 2000; Nenadis et al., 2003; Van Acker et al., 1996; Rice-Evans et al., 1996; Firuzi et al., 2005). A variety of biological effects have been reported for phenolic acids, including alleviation of hyperuricemia and protection against LDL oxidation, anti-inflammatory, antitumor, and autoimmune-related effects (Nakagami et al., 1995; Fernandez et al., 1998; Zang et al., 2000; Cartron et al., 2001; Kweon et al., 2001). Caffeic and ferulic acids provide protection against carcinomas (Krishnaswamy, 2001), ferulic acid esters protect against UV radiation (Taniguchi et al., 1995), and transcinnamic acid can be used in the prevention or treatment of diabetes (Lee, 2002). Flavonoids are excellent candidates as health-promoting,

disease-preventing, and chemopreventive agents because they are extremely safe and associated with low toxicity (Nijveldt et al., 2001; Marilyn, 2006). Protective action has been postulated for chronic diseases (Choi et al., 2003; Horvathova et al., 2001), cardiovascular diseases (Choi et al., 2003; Spencer, 2005; Ghosh, 2005; Depeint et al., 2002; Erlund, 2004; Van Hoorn et al., 2003), stroke (Van Hoorn et al., 2003), hyperlipidemia (Choi et al., 2003), diabetes (Ghosh, 2005), inflammation (Ghosh, 2005; Das et al., 2006; Hodek et al., 2005; Kim et al., 2004; Kim et al., 2004), allergies (Ghosh, 2005; Das et al., 2006; Hodek et al., 2005), immune system disorders (Horvathova et al., 2001; Strickland, 2001), mutagenesis (Ghosh, 2005; Hoensch et al., 2005), and cataracts (Horvathova et al., 2001) as well as for neurological disorders (Horvathova et al., 2001;Spencer, 2005; Dajas et al., 2005), particularly those related to aging, such as cognitive, motoric, and mood decline (Schroeter et al., 2003). Some of these protective effects have been confirmed by epidemiological studies (Depeint et al., 2002; Erlund, 2004; Hoensch et al., 2005; Choi et al., 2000), but a direct extrapolation to humans cannot be made on the basis of these data. A growing body of *in vivo* studies is beginning to provide insight into the biological mechanisms of flavonoid action (Van Hoorn et al., 2003). The nature of polyphenol conjugates *in vivo* has been identified, showing that the biological fate of flavonoids, including their dietary forms, is highly complex and dependent on a large number of processes (Walle, 2004).

Antioxidant properties alone are not sufficient to explain the biological properties of flavonoids. Within the last decade, reports on flavonoid activities have been largely associated with enzyme inhibition and antiproliferative activity, which are dependent on their particular molecular structures (Depeint et al., 2002). Although the action mechanisms are not fully understood, recent studies have clearly shown that the role of flavonoids as modulators of cell signalling may be attributed to their effects as anticancer agents, cardioprotectants, and inhibitors of neurodegeneration (Schroeter et al., 2002).

Terpenoids

Terpenoids, also known as isoprenoids, are secondary plant metabolites accounting for the largest family of natural compounds, widespread in plants and lower invertebrates. The isoprenoid biosynthetic pathway generates primary and secondary metabolites of ecological relevance to plant growth and survival. These compounds are involved in interactions between plants, between plants and microorganisms, and between plants and insects, acting as allelopathic agents and attractants or repellants in plants (Grassmann, 2005). They are involved in the defense, wound sealing, and thermotolerance of the plants as well as in the pollination of seed crops, the flavor of fruits, and the fragrance of flowers. Some terpenoids or their precursors act as scavengers for external aggressive molecules in the gaseous phase (i.e., ozone). The term terpenes is used for a group of compounds with the basic C5 isoprene unit. According to the number of these units (1 to 6), terpenoids are classified into hemiterpenoids, monoterpenoids (C10) (limonene, carvone, carveol); sesquiterpenoids (C15); diterpenoids (C20) (retinoids); sesterterpenoids (C25); tri- (C30); and tetraterpenoids (carotenoids), having eight isoprenoid C5 residues.

Terpenoid compounds (monoterpenes, sesquiterpenes, and diterpenes) are the main components of essential oils, which also contain oxygenated derivatives and other compounds (including aldehydes, ketones, phenolic, acetates, and oxides). The antioxidant activity of different essential oils in different model systems is well known (Escuder et al., 2002; Grassmann et al., 2002), and synergistic effects with phenolics have been reported (Milde et al., 2004; Nakagami et al., 1995). Essential oils are the commercial sources of terpenoids.

Some terpenoids are the bioactive compounds of traditional herbal remedies used in the treatment of pain, colds, bronchitis, and gastrointestinal diseases. Terpenoids are present in almost every natural food and have been associated with protection from oxidative stress and chronic diseases (Fernandez et al., 1998). Some exhibit cardioprotective action, such as ginkgolides A and B and bilobalide from G. biloba (Zang et al., 2000). Other relevant properties have been reported, including antibacterial (Cartron et al., 2001), anti-inflammatory (Kweon et al., 2001), anticarcinogenic (Milde et al., 2004), antimalarial, antiulcer, antimicrobial, and diuretic activities. Protection against a variety of infectious diseases (viral and bacterial) and acaricidal activity have been reported for monoterpenes (Krishnaswamy, 2001). The present commercial importance of terpene-based pharmaceuticals is expected to play a more significant role in human disease treatment in the future (Taniguchi et al., 1995).

Carotenoids

Carotenoids are a group of more than 600 different compounds,synthesized by plants, other photosynthetic organisms, and some nonphotosynthetic bacteria, yeasts, and molds. Carotenoids are formed by C40 polyunsaturated hydrocarbons, which could be considered the backbone of the molecule. This chain may be terminated by cyclic end groups (rings) and may be complemented with oxygen-containing functional groups. They are classified as carotenes (α- and β-carotene, lycopene), composed only of carbon and hydrogen atoms, or xanthophylls (zeaxanthin, lutein, α- and β-cryptoxanthin, canthaxanthin, astaxanthin), with at least one oxygen atom. Carotenoids predominantly occur in their all-trans configuration, although cis-isomers can be formed during

food processing (Wagner et al., 2003). Lycopene exhibits the highest antioxidant activity, and its plasma level is slightly higher than that of β-carotene (Pietri et al., 1997). The results reported for the antioxidant activity of β-carotene differ widely due to the various test systems and the experimental conditions used (Ulubelen, 2003). The conjugated double-bond system is responsible for the antioxidant properties of carotenoids , which can act by quenching singlet oxygen formed due to the effects of UV light, scavenging peroxyl radicals, hydrogen transfer, or electron transfer (De las Heras et al., 2003; Perrucci et al., 1995; Wang et al., 2005).

The major biological functions of carotenoids are related to intercellular gap junction communication, cell differentiation, immunoenhancement, and inhibition of mutagenesis. Some carotenoids (α- and β-carotene, β-cryptoxanthin) are precursors of vitamin A and protect against chemical oxidative damage, several kinds of cancer, and age-related macular degeneration. No convincing evidence exists of their protective action against cardiovascular disease (De las Heras et al., 2003; Perrucci et al., 1995; Deming et al., 2002; Granado et al., 2003; Hix et al., 2004). *In vitro* studies evidenced that carotenoids can interact with several reactive species and can act as prooxidants, although no documented evidence to date indicates true prooxidant activity *in vivo* (Lowe et al., 2003). The maximum antioxidant effectiveness of carotenoids in human cells is related to an optimal dose, because higher doses can be less effective or result in cell damage. The relationship between carotenoid intake and cancer has been evaluated, showing an inverse association for lung, colon, breast, and prostate cancer, although negative effects of supplementations have been found (Wang et al., 2005). Studies on the mechanism of cancer cell growth inhibition by carotenoids at the protein expression level may involve changes in pathways leading to cell growth or cell death, including hormone and growth factor signaling, regulatory mechanisms of cell cycle progression, cell differentiation, and apoptosis (Sharoni et al., 2003).

Sterols

Plant sterols are isoprenoid compounds with an sterol nucleus and an alkyl chain. Most plant sterols have a double bond in position C5 in the nucleus, whereas the stanols are saturated (Moreau et al., 2002). The main sterols in plant materials are sitosterol, campesterol, and stigmasterol (Heinemann et al., 1993). Plant sterols are of interest because of their potential to lower both total serum cholesterol and low-density lipoprotein (LDL) cholesterol in humans by inhibiting the absorption of dietary cholesterol as well as the reabsorption of cholesterol excreted into the bile in the course of the enterohepatic cycle (Piironen et al., 2000; Piironen et al., 2003). A detailed review of plant sterols and their role in health and disease can be found elsewhere (Patel, 2008). Consumption of sitosterol was shown to reduce colon cancer in rats (Raicht et al., 1980). Some studies indicated a beneficial effect of these sterols on immune function (Bouic et al., 1999). However, there is still controversy in the literature about the daily dietary intake of phytosterols. Some researchers reported that a high dietary intake of phytosterols lowers blood cholesterol levels by competing with dietary and biliary cholesterol during intestinal absorption (Piironen et al., 2000;Normen et al., 2000; Jones et al., 1999). But, it has been also hypothesized that because phytosterols are more readily oxidized by free radicals than cholesterol, they could increase the level of oxidized LDL, which is responsible for formation of atherosclerotic plaques in arteries (Plat, et al., 2005). According to the Scientific Committee of Food, the average amount of phytosterols in the Western diet is 150–400 mg/day (Scientific Committee on Food, 2002). However, the recommended dose of phytosterols to reduce LDL–plasma cholesterol level by 5 to 15% is 1.3 to 2 g/day (Hallikainen et al., 2000; Law, 2000).

To achieve such high levels and comply with the approved health claim on the role of plant sterols or stanol esters in reducing the risk of coronary heart disease, the food industry has introduced various functional food products with added phytosterols. However, another study demonstrated that daily consumption of 3.8 to 4.0 g/day of plant sterol esters might significantly lower serum concentrations of carotenoids and tocopherols (Plat et al., 2001). Therefore, more research is needed to determine the best dose recommended for daily consumption of plant sterols.

Vitamin E

Vitamin E includes a family of tocopherols (having a phytyl tail attached to their chromanol nuclei), tocotrienols (an unsaturated tail), and some of their ester derivatives (such as succinate and acetate). Vitamin E effectively inhibits the peroxidation of lipids because it can scavenge the peroxyl radicals. The radical-scavenging capacity of α-tocopherol and α-tocotrienol is similar in hexane, but α-tocotrienol is more active in membrane systems and α-tocopherol shows higher bioactivity. The major sources of vitamin E are plant species, and its content varies between tissues, with preferential accumulation in seeds. Due to their amphipathic nature, tocopherols are associated with membrane lipids or lipid storage structures. The actions of tocopherols and tocotrienols have been extensively studied. Vitamin E protects vitamin A, spares selenium and vitamin C, and is the most effective lipidsoluble antioxidant, which protects unsaturated fatty acids in membranes. Other nonantioxidant functions include enhanced immune response and regulation of platelet aggregation (Weber et al., 2002; Landvik et al., 2002). The effects of Vitamin E

have been observed at the level of messenger ribonucleic acid (mRNA) or protein and could be related to regulation of gene transcription, mRNA stability, protein translation, and protein stability. Landvik et al. (2002) published a compilation of human epidemiological studies on vitamin E, carotenoids, and cancer risk. This vitamin also protects against coronary heart disease (Violi et al., 2006), aging, cataracts, UV radiation, air pollution, and lipid peroxidation associated with strenuous exercise. Vitamin E bioavailability and metabolism is influenced by intestinal absorption, plasma lipoprotein transport, and hepatic metabolism (Traber, 2002). Tocotrienols are more effective than tocopherols at inhibiting neuronal cell death. It has been suggested that neither the anticarcinogenic effects of tocotrienols nor the neuroprotection are related to the antioxidant properties of tocopherols and tocotrienols (Weber et al., 2002).

Alkaloids

Alkaloids are compounds that contain nitrogen in a heterocyclic ring and are commonly found in about 15–20% of all vascular plants. Alkaloids are subclassified on the basis of the chemical type of their nitrogen-containing ring. They are formed as secondary metabolites from amino acids and usually present a bitter taste accompanied by toxicity that should help to repel insects and herbivores. Alkaloids are found in seeds, leaves, and roots of plants such as coffee beans, guarana seeds, cocoa beans, peppermint leaves, coca leaves, and many other plant sources. The most common alkaloids are caffeine, theophylline, nicotine, codeine, and indole alkaloids. Research has demonstrated that the consumption of some alkaloids provides health benefits. For example, theobromine has strong diuretic, stimulant, and arterial dilating effects (Li et al., 1991; Sotelo et al., 1991). In the case of indole alkaloids, many studies have shown to have biological activity such as antitumoral, anti-inflammatory, analgesic, antioxidant, and antimycobacterial effects (Rate et al., 1993; Delorenzi et al., 2001; Pereira et al., 2005). Alkaloids can be potentially used in the pharmaceutical industry as drugs, or in a few cases such as caffeine, they find application in beverages.

SUPERCRITICAL-CO_2 EXTRACTION OF ANTIOXIDANTS

Various methods are used to extract antioxidants from different natural sources. One of the widely used is methods supercritical CO_2 extraction. A compound is in its supercritical state when it is heated and compressed above its critical temperature and critical pressure. In the supercritical state, the substance exists as a single fluid phase with properties intermediate between those of liquids and gases: the densities are liquid-like, whereas the diffusivities and viscosities are gas-like (Mchugh et al., 1994). Moreover, supercritical CO_2 has zero surface tension, which allows easy penetration into most matrices. In addition, in the supercritical state, supercritical CO_2 is extremely sensitive to small changes in temperature and pressure such that a compound may be extracted from a matrix at one set of conditions and then separated from supercritical CO_2 in a downstream operation under a slightly different set of conditions. Some of the other advantages of supercritical CO_2 that CO_2 is available in high purity at relatively low cost, it can be easily removed from the matrix after the process, and it can be easily separated from the extracted compounds. It is well documented that CO_2, a nonpolar solvent, is best suited for the extraction of nonpolar organic compounds. Therefore, for the extraction of more polar compounds, the polarity of supercritical CO_2 can be increased by adding modifiers such as ethanol and water, which in turn increases the solubility of more polar compounds in supercritical fluid. One important consideration for the extraction with any solvent is the solubility of the antioxidants in the solvent. For example, when using supercritical fluid extraction, solubility is a strong function of supercritical CO_2 density and the properties of the solute such as molecular weight, polarity and vapor pressure. Antioxidants are soluble in supercritical CO_2 to different extents depending on the temperature and pressure conditions. Solubility behavior of phenolics, carotenoids, sterols, and alkaloids in supercritical CO_2 has been previously reviewed (Choi et al., 1998; Murga et al., 2002; Murga et al., 2003; Gomez-Prieto, et al., 2002; Guclu-Ustundag, et al., 2004; Saldana et al., 2000; Saldana et al., 2006; Saldana et al., 2007). The extraction efficiency of these different extraction techniques is affected by several parameters such as particle size and moisture content of the feed, extraction temperature and pressure, power, solvent flow rate, extraction time, frequency, and the use of a cosolvent or a mixture of solvents. Therefore, the following discussion will focus on the impact of these processing parameters on the yield of antioxidants obtained from herbs. Herbs are plants that have nitrogen organic bases. A few studies on the extraction of antioxidants from herbs are reported in the literature. Other antioxidants such as gonine, cocaine, sitosterol, flavonoids, alkaloids, and quercetin were also extracted from natural sources such as coca leaves, dandelion, ginkgo, mat´e, and Myrica rubra leaves (Chen et al., 2008; Liao et al., 2008; Proestos et al., 2008; Simandi et al., 2002; Saldana et al., 2002; Brachet et al., 2002).

FACTORS AFFECTING EXTRACTION YIELD IN SUPERCRITICAL CO2 EXTRACTION

Sample preparation

Leaves such as mat´e tea, laurel, and coca contain

approximately 80 to 95% moisture content, so drying is needed. Dry leaves are then ground to reduce their particle size and facilitate the extraction. Particle size: Antioxidants yield extraction using supercritical CO_2 increased with a decreased in particle size. In a study by Sim´andi and others (Simandi et al., 2002), the extraction of amyrin and sitosterol was evaluated at different temperatures and pressures as well as with different particle sizes of 0.2 to 0.8 mm. In a study by Salda˜na and others (Saldana et al., 2000), the extraction of caffeine and theobromine were compared using whole and ground leaves at pressures of 13.8 and 25.5 MPa and temperatures of 40 and 70°C. The results clearly revealed higher caffeine extraction rates for ground commercial mat´e tea at the early stages of extraction as expected due to the absence of mass transfer resistance and plant matrix interference. All caffeine was removed from the ground leaves using only 0.5 kg of Supercritical CO_2. But, more than 2.5 kg of SC-CO_2 was required to achieve the same level of removal using whole leaves (Saldana et al., 2000).

Moisture. Moisture increased the extraction yield of some alkaloids as demonstrated for black tea and mat´e tea leaves (Saldana et al., 1999). This can be explained considering that water can act as a cosolvent for the extraction of slightly polar compounds, whereas the presence of water is favorable at about 10 to 15% to increase extraction yield.

Extraction parameters

Temperature and pressure: Most of the studies for the extraction of antioxidants from herbs using Supercitical CO2 were performed at temperatures of 40-70°C and pressures of 14 to 40 MPa. The influence of temperature and pressure using supercritical CO_2 was reported for the extraction of sitosterol from dandelion leaves (Simandi et al., 2002); flavonoids from ginkgo leaves (Chiu et al., 2002); and alkaloids from mat´e tea leaves (Saldana et al., 2000; Saldana et al., 2002; Saldana et al., 1999)

Flow rate and extraction time. No report was found for the effect of flow rate in the extraction of antioxidants from herbs. Extractions at lower pressures and/or temperatures required prolonged time and large amounts of CO_2 to achieve the same yield as reported for the extraction of caffeine, theophylline, and theobromine from mat´e tea leaves (Saldana et al., 2002; Saldana et al., 1999).

Use of cosolvent. Pure CO_2 under supercritical conditions is a good solvent for lipophilic compounds but is poor for phenolics. Extraction can be enhanced using a modifier able to interact with the target compounds, possibly improving yield and selectivity. However, high modifier concentrations may decrease selectivity depending on the size of phenolics (Scalia et al., 1999). Alcohols are widely used as modifiers, ethanol being the most recommended one on the basis of toxicological and environmental considerations. Ethanol has been employed to increase the solubility of ginsenoids (Wang et al., 2001), phenols (Vaher et al., 2003), flavonoids (Yang et al., 2002), terpenoids (Dauksas et al., 2001), and carotenoids (Lopez et al., 2004; Baysal et al., 2000; Lim et al., 2002; Suto et al., 1997; Moraes et al., 1997). Modification of the solvent with ethanol resulted in an increase in the quantity of caffeine extracted, from approximately 41 to 75% of caffeine using 448 kg of solvent/kg mat´e leaves. Whereas only 30% of the original caffeine was extracted using water-saturated supercritical CO2, doubling the ethanol concentration resulted in an increase in caffeine extraction that is almost proportional to the increase in the ethanol concentration. Methanol has been also used for extracting phenolics (Goli et al., 2005), flavonoids (Palma et al., 1999; Murga et al., 2000; Louli et al., 2004), and isoflavones (Rostago et al., 2002).

Future trends

The literature reviewed in this paper demonstrates the feasibility of using supercritical CO_2 to extract antioxidants from a variety of biological plant sources. However, it is essential to study each plant composition individually because the pretreatment of material and optimum extraction conditions will depend on the structure and on the composition of specific plant source. Nowadays, the majority of studies are carried out at laboratory scale. There are few pilot-scale studies using supercritical CO_2. In addition, supercritical fluid technology allows combination of extraction with fractionation to further separate bioactive components of interest. However, more research is needed to investigate the quality attributes of extracted antioxidants, such as oxidative stability, chemical composition, and stability of antioxidants throughout extraction and storage by using the emerging extraction technologies. Finally, it is also necessary to better communicate to consumers the advantages of these technologies compared to conventional extraction technologies (Phelps et al., 1996).

CONCLUSIONS

Although for many years very few new methods to extract antioxidants were developed, recently there has been a boom in emerging extraction technologies. For a long time traditional methods using organic solvents were utilized to extract antioxidants. These methods present some disadvantages such as residues of solvent that are left in the final product, high processing temperatures, and emissions of volatile organic compounds into the atmosphere. Some promising emerging extraction technologies that can overcome these disadvantages are supercritical CO_2. These methods are able to recover antioxidants such as phenolics,carotenoids, sterols, and

alkaloids from a wide variety of agricultural plant sources. Extensive research within a large variety of plant materials such as fruits, vegetables, nuts and seeds, and herbs have shown that supercritical CO_2 is the most utilized emerging extraction method for the recovery of phytochemicals. There are many factors that affect extraction efficiency, such as sample preparation, moisture content, and the extraction parameters of temperature, pressure, solvent flow rate, extraction time, and use of a cosolvent. These parameters also have an impact on the various quality attributes such as color characteristics, flavor, and oxidative stability of the extracted antioxidants and residual product.

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