ELSEVIER
Trends in Food Science & Technology 17 (2006) 300–312
TRENDS IN FOOD SCIENCE & TECHNOLOGY

Review

Recent advances in extraction of nutraceuticals from plants

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Various novel techniques including ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, and accelerated solvent extraction have been developed for the extraction of nutraceuticals from plants in order to shorten the extraction time, decrease the solvent consumption, increase the extraction yield, and enhance the quality of extracts. A critical review was conducted to introduce and compare the conventional Soxhlet extraction and the new alternative methods used for the extraction of nutraceuticals from plants. The practical issues of each extraction method were discussed. Potential uses of those methods for the extraction of nutraceuticals from plant materials was finally summarized.

Introduction

Plants contain a broad range of bioactive compounds such as lipids, phytochemicals, pharmaceutics, flavors, fragrances and pigments. Plant extracts are widely used in the food, pharmaceutical and cosmetics industries. Extraction techniques have been widely investigated to obtain such valuable natural compounds from plants for commercialization.

Traditional methods, such as Soxhlet extraction, which have been used for many decades, are very time-consuming and require relatively large quantities of solvents (Luque de Castro & Garcia-Ayuso, 1998). There is an increasing demand for new extraction techniques with shortened extraction time, reduced organic solvent consumption, and increased pollution prevention. Novel extraction methods including ultrasound-assisted extraction (Vinatoru, 2001), microwave-assisted extraction (Kaufmann & Christen, 2002), supercritical fluid extraction (Marr & Gamse, 2000; Lang & Wai, 2001; Meireles & Angela, 2003), and accelerated solvent extraction (Kaufmann & Christen, 2002; Smith, 2002) are fast and efficient for extracting chemicals from solid plant matrixes. These techniques have the possibility of working at elevated temperatures and/or pressures, greatly decreasing the time of extraction.

The novel extraction techniques have become relatively mature and some potential applications for the extraction of nutraceuticals from solid plant matrices have been reported. This review provides theoretical background on the conventional Soxhlet extraction and on several novel extraction techniques including ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, and accelerated solvent extraction. The practical issues for each extraction method such as matrix characteristics, solvent choice, liquid–solid ratio, temperature, pressure and extraction time are discussed. Finally, potential applications of those extraction methods are reviewed.

Conventional Soxhlet extraction

Principles and mechanisms

Classical techniques for the solvent extraction of nutraceuticals from plant matrices are based on the choice of solvent coupled with the use of heat and/or agitation. Existing classical techniques used to obtain nutraceuticals from plants include: Soxhlet, hydrostillation and maceration with an alcohol–water mixture or hot fat.

Soxhlet, which has been used for a long time, is a standard technique and the main reference for evaluating the performance of other solid–liquid extraction (or leaching) methods. Soxhlet extraction is a general and well-established technique, which surpasses in performance other conventional extraction techniques except for, in limited field of applications, the extraction of thermolabile compounds (Luque de Castro & Garcia-Ayuso, 1998). An overview of Soxhlet extraction of

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0924-2244/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.tifs.2005.12.004

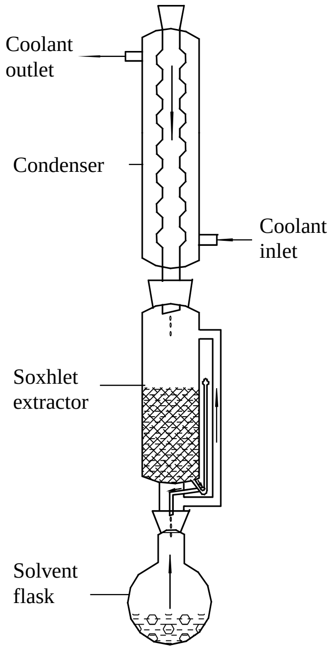

Fig. 1. Experimental Soxhlet extraction apparatus.

solid materials was given by Luque de Castro and Garcia-Ayuso (1998).

In a conventional Soxhlet system as shown in Fig. 1, plant material is placed in a thimble-holder, and filled with condensed fresh solvent from a distillation flask. When the liquid reaches the overflow level, a siphon aspirates the solution of the thimble-holder and unloads it back into the distillation flask, carrying extracted solutes into the bulk liquid. In the solvent flask, solute is separated from the solvent using distillation. Solute is left in the flask and fresh solvent passes back into the plant solid bed. The operation is repeated until complete extraction is achieved.

Practical issues for Soxhlet extraction

Solvent choice

A suitable extracting solvent should be selected for the extraction of targeted nutraceuticals using the Soxhlet extraction method. Different solvents will yield different extracts and extract compositions (Zarnowski & Suzuki, 2004). The most widely-used solvent to extract edible oils from plant sources is hexane. Hexane has a fairly narrow boiling point range of approximately 63–69 °C and it is an excellent oil solvent in terms of oil solubility and ease of recovery. However, *n*-hexane, the main component of commercial hexane, is listed as No. 1 on the list of 189 hazardous air pollutants by the US Environmental Protection Agency (Mamidipally & Liu, 2004).

The use of alternative solvents such as isopropanol, ethanol, hydrocarbons, and even water, has increased due to environmental, health, and safety concerns. Mamidipally and Liu (2004) used *d*-limonene and hexane in the extraction of oil from rice bran. It was observed that *d*-limonene extracted a significantly higher amount of oil than hexane under any given set of conditions. Hanmoungjai, Pyle, and Niranjan (2000) used water to extract rice bran oil at a pH value of 12. The oil extracted by using the aqueous process had a lower content of free fatty acid and lower color imparting components than oil extracted using hexane.

However, alternative solvents often result in less recovery due to a decreased molecular affinity between solvent and solute. The costs of alternative solvents could be higher. A co-solvent is sometimes added in order to increase the polarity of the liquid phase. A mixture of solvents such as isopropanol and hexane has been reported to increase the yield and kinetics of extraction (Li, Pordesimo, & Weiss, 2004).

Matrix characteristics

Soxhlet extraction strongly depends on matrix characteristics and particle size as the internal diffusion may be the limiting step during extraction. For the extraction of total fat from oleaginous seeds, a 2-h extraction obtained 99% extraction efficiency if the particle size was 0.4 mm, while a 12-h extraction was necessary to obtain similar efficiency if the particle size was 2.0 mm (Luque-Garcia & Luque de Castro, 2004).

Operating conditions

During Soxhlet extraction, the solvent is usually recovered by evaporation. The extraction and evaporation temperatures have a significant effect on the quality of final products. Mamidipally and Liu (2004) found that *d*-limonene extracted rice bran oil was slightly darker compared to hexane extracted oil, probably due to higher extraction and evaporation temperatures used during the *d*-limonene solvent extraction. The high boiling temperature for solvent recovery can be decreased by using vacuum or membrane separation to recover the solvent.

Advantages and disadvantages of Soxhlet extraction

The advantages of conventional Soxhlet extraction include (1) the displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with the solid matrix (2) maintaining a relatively high extraction temperature with heat from the distillation flask, and (3) no filtration requirement after leaching. Also, the Soxhlet method is very simple and cheap (Luque de Castro & Garcia-Ayuso, 1998).

The main disadvantages of conventional Soxhlet extraction include (1) the extraction time is long; (2) a large amount of solvent is used; (3) agitation can not be provided in the Soxhlet device to accelerate the process; (4) the large amount of solvent used requires an evaporation/concentration procedure; and (5) the possibility of thermal decomposition of the target compounds can not be ignored as the extraction usually occurs at the boiling point of the solvent for a long time. The long time requirement and the requirement of large amounts of solvent lead to wide criticism of the conventional Soxhlet extraction method (Luque de Castro & Garcia-Ayuso, 1998).

Envisioned uses of Soxhlet extraction

Soxhlet extraction is a generally well-established technique. Wide industrial applications, better reproducibility and efficiency, and less extract manipulation are the advantages of Soxhlet extraction over other novel extraction methods such as ultrasound-assisted, microwave-assisted, supercritical fluid, or accelerated solvent extractions. However, compared with the novel fast extraction techniques, Soxhlet is an old-fashioned, and time—and solvent—consuming extraction technique. Some solvents used in the conventional Soxhlet have recently been questioned because of their toxicity.

For Soxhlet extraction, the advantages such as sample-fresh solvent contact during the whole, no filtration procedure and simple manipulation should be retained. Meanwhile, auxiliary features such as a vacuum pump, a membrane separation unit, a source of ultrasound and microwave, and supercritical fluids can be incorporated into the conventional Soxhlet method to improve its performance. Use of non-toxic extracting solvents such as supercritical CO_2 and water can be further investigated.

Sonication-assisted extraction

Principles and mechanisms

Sound waves, which have frequencies higher than 20 kHz, are mechanical vibrations in a solid, liquid and gas. Unlike electromagnetic waves, sound waves must travel in a matter and they involve expansion and compression cycles during travel in the medium. Expansion pulls molecules apart and compression pushes them together. The expansion can create bubbles in a liquid and produce negative pressure. The bubbles form, grow and finally collapse. Close to a solid boundary, cavity collapse is asymmetric and produces high-speed jets of liquid. The liquid jets have strong impact on the solid surface (Luque-Garcia & Luque de Castro, 2003).

Two general designs of ultrasound-assisted extractors are ultrasonic baths or closed extractors fitted with an ultrasonic horn transducer. The mechanical effects of ultrasound induce a greater penetration of solvent into cellular materials and improve mass transfer. Ultrasound in extraction can also disrupt biological cell walls, facilitating the release of contents. Therefore, efficient cell disruption and effective mass transfer are cited as two major factors leading to the enhancement of extraction with ultrasonic power (Mason, Paniwnyk, & Lorimer, 1996). Scanning electron micrographs (SEM) have provided evidence of the mechanical effects of ultrasound, mainly shown by the destruction of cell walls and release of cell contents. In contrast to conventional extractions, plant extracts diffuse across cell walls due to ultrasound, causing cell rupture over a shorter period (Chemat, Lagha, AitAmar, Bartels, & Chemat, 2004; Li *et al.*, 2004; Toma, Vinatoru, Paniwnyk, & Mason, 2001; Vinatoru, Toma, & Mason, 1999).

Practical issues for sonication-assisted extraction

It is necessary to take into account plant characteristics such as moisture content and particle size, and solvent used for the extraction in order to obtain an efficient and effective ultrasound-assisted extraction. Furthermore, many factors govern the action of ultrasound including frequency, pressure, temperature and sonication time.

Effects of ultrasound characteristics

Ultrasound frequency has great effects on extraction yield and kinetics. However, the effects of ultrasound on extraction yield and kinetics differ depending on the nature of the plant material to be extracted. A small change in frequency can increase the yield of extract about 32% for ultrasound-assisted solid-hexane extraction of pyrethrines from *pyrethrum* flowers. However, ultrasound has weak effects on both yield and kinetics for the extraction of oil from *woad* seeds (Romdhane & Gourdon, 2002).

The ultrasonic wave distribution inside an extractor is also a key parameter in the design of an ultrasonic extractor. The maximum ultrasound power is observed in the vicinity of the radiating surface of the ultrasonic horn. Ultrasonic intensity decreases rather abruptly as the distance from the radiating surface increases. Also, ultrasound intensity is attenuated with the increase of the presence of solid particles (Romdhane, Gourdon, & Casamatta, 1995). In order to avoid standing waves or the formation of solid free regions for the preferential passage of the ultrasonic waves, additional agitation or shaking is usually used (Vinatoru *et al.*, 1997).

Operating conditions

The use of ultrasound allows changes in the processing condition such as a decrease of temperature and pressure from those used in extractions without ultrasound (Romdhane & Gourdon, 2002; Wu, Lin, & Chau, 2001). For solid-hexane extraction of pyrethrines from *pyrethrum* flowers without ultrasound, extraction yield increases with the extraction temperature and maximum yield is achieved at 66 °C. With ultrasound, the effect of temperature in the range of 40–66 °C on the yield is negligible, such that optimal extraction occurs across the range of temperature from 40 to 66 °C. Therefore, use of ultrasound-assisted extraction is advisable for thermolabile compounds, which may be altered under Soxhlet operating conditions due to the high extraction temperature (Romdhane & Gourdon, 2002). However, it should be noted that since ultrasound generates heat, it is important to accurately control the extraction temperature (Salisova, Toma, & Mason, 1997). The sonication time should also be considered carefully as excess of sonication can damage the quality of extracts.

Advantages and disadvantages of sonication-assisted extraction

Ultrasound-assisted extraction is an inexpensive, simple and efficient alternative to conventional extraction techniques. The main benefits of use of ultrasound in solid–liquid extraction include the increase of extraction yield and faster kinetics. Ultrasound can also reduce the operating temperature allowing the extraction of thermolabile compounds. Compared with other novel extraction techniques such as microwave-assisted extraction, the ultrasound apparatus is cheaper and its operation is easier. Furthermore, the ultrasound-assisted extraction, like Soxhlet extraction, can be used with any solvent for extracting a wide variety of natural compounds.

However, the effects of ultrasound on extraction yield and kinetics may be linked to the nature of the plant matrix. The presence of a dispersed phase contributes to the ultrasound wave attenuation and the active part of ultrasound inside the extractor is restricted to a zone located in the vicinity of the ultrasonic emitter. Therefore, those two factors must be considered carefully in the design of ultrasound-assisted extractors.

Potential applications of sonication-assisted extraction

Ultrasound-assisted extraction has been used to extract nutraceuticals from plants such as essential oils and lipids (Chemat *et al.*, 2004; Cravotto, Binello, Merizzi & Avogadro, 2004; Li *et al.*, 2004; Luque-Garcia & Luque de Castro, 2004; Sharma & Gupta, 2004), dietary supplements (Albu, Joyce, Paniwnyk, Lorimer, & Mason, 2004; Bruni, Guerrini, Scalia, Romagnoli, & Sacchetti, 2002; Hui, Etsuzo & Masao, 1994; Melecchi *et al.*, 2002; Salisova *et al.*, 1997; Wu *et al.*, 2001). An overview of the uses of ultrasound in food technology was prepared by Mason *et al.*, (1996). An overview of ultrasound-assisted extraction of bioactive compounds from herbs was drafted by Vinatoru (2001).

Ultrasound can increase extraction yield. Sharma and Gupta (2004) found that ultrasonication was a critical pretreatment to obtain high yields of oils from almond, apricot and rice bran. The yield of oil extracted from soybeans also increased significantly using ultrasound (Li *et al.*, 2004). For ultrasound-assisted extraction of saponin from ginseng, the observed total yield and saponin yield increased by 15 and 30%, respectively (Hui *et al.*, 1994).

Ultrasound can increase extraction kinetics and even improve the quality of extracts. Cravotto *et al.* (2004) found that rice bran oil extraction can be efficiently performed in 30 min under high-intensity ultrasound either using hexane or a basic aqueous solution. Extraction rates of carvone and limonene by ultrasound-assisted extraction with hexane were 1.3–2 times more rapid than those by the conventional extraction depending on temperature (Chemat *et al.*, 2004). Furthermore, the yield and quality of carvone obtained by the ultrasound-assisted extraction were better than those by a conventional method. The ultrasound was also applied to the cartridge of a Soxhlet extraction for the extraction of total fat from oleaginous seeds such as sunflower, rape and soybean seeds. The use of ultrasound reduced the extraction at least to half of the time needed by conventional extraction methods without any change in the composition of extracted oils (Luque-Garcia & Luque de Castro, 2004). Wu *et al.*, (2001) found the ultrasound-assisted extraction of ginseng saponins occurred about three times faster than traditional Soxhlet extraction.

Ultrasound-assisted extraction was considered as an efficient method for extracting bioactive compounds from *Solvia officinalis* (Salisova *et al.*, 1997) and *Hibiscus tiliaceus L.* flowers (Melecchi *et al.*, 2002), antioxidants from *Rosmarinus officinalis* (Albu *et al.*, 2004), and steroids and triterpenoids from *Chresta spp.* (Schinor, Salvador, Turatti, Zucchi, & Dias, 2004). The use of ultrasound as an adjunct to conventional extraction provides qualitatively acceptable tocols from *amaranthus caudatus* seeds but much more quickly, more economically and using equipment commonly available (Bruni *et al.*, 2002).

Microwave-assisted extraction

Principles and mechanisms

Microwaves are electromagnetic radiations with a frequency from 0.3 to 300 GHz. Domestic and industrial microwaves generally operate at 2.45 GHz, and occasionally at 0.915 GHz in the USA and at 0.896 GHz in Europe. Microwaves are transmitted as waves, which can penetrate biomaterials and interact with polar molecules such as water in the biomaterials to create heat. Consequently, microwaves can heat a whole material to penetration depth simultaneously.

Microwave-assisted extraction (MAE) offers a rapid delivery of energy to a total volume of solvent and solid plant matrix with subsequent heating of the solvent and solid matrix, efficiently and homogeneously. Because water within the plant matrix absorbs microwave energy, cell disruption is promoted by internal superheating, which facilitates desorption of chemicals from the matrix, improving the recovery of nutraceuticals (Kaufmann, Christen, & Veuthey, 2001a). Kratchanova, Pavlova, and Panchev (2004) observed using scanning electron micrographs that microwave pretreatment of fresh orange peels led to destructive changes in the plant tissue. These changes in the plant tissue due to microwave heating gave a considerable increase in the yield of extractable pectin. Furthermore, the migration of dissolved ions increased solvent penetration into the matrix and thus facilitated the release of the chemicals. The effect of microwave energy is thus strongly dependent on the dielectric susceptibility of both the solvent and the solid plant matrix.

There are two types of commercially available MAE systems: closed extraction vessels under controlled pressure and temperature, and focused microwave ovens at atmospheric pressure (Kaufmann & Christen, 2002). The closed MAE system is generally used for extraction under drastic conditions such as high extraction temperature. The pressure in the vessel essentially depends on the volume and the boiling point of the solvents. The focused MAE system can be operated at a maximum temperature determined by the boiling point of the solvents at atmospheric pressure. Ericsson and Colmsjo (2000) introduced a dynamic MAE system, which was demonstrated to yield extract equivalent to yield of extract from Soxhlet extraction, but in a much shorter time.

Practical issues for microwave-assisted extraction

Matrix characteristics

As MAE depends on the dielectric susceptibility of solvent and matrix, better recoveries can be obtained by moistening samples with a substance that possesses a relatively high dielectric constant such as water. If a dry biomaterial is re-hydrated before extraction, the matrix itself can thus interact with microwaves and hence facilitate the heating process. The microwave heating leads to the expansion and rupture of cell walls and is followed by the liberation of chemicals into the solvent (Spar Eskilsson & Bjorklund, 2000). In this case, the surrounding solvent can have a low dielectric constant and thus remains cold during extraction. This method can be used to extract thermosensitive compounds such as essential oils (Brachet, Christen, & Veuthey, 2002). However, it was found that it was impossible to perform a good MAE for completely dry as well as for very wet samples when a non-polar solvent such as hexane was used as the extraction solvent (Molins, Hogendoorn, Heusinkveld, Van Zoonen, & Baumann, 1997).

Plant particle size and size distribution usually have a significant influence on the efficiency of MAE. The particle sizes of the extracted materials are usually in the range of 100 μm–2 mm (Spar Eskilsson & Bjorklund, 2000). Fine powder can enhance the extraction because the limiting step of the extraction is often the diffusion of chemicals out of the plant matrix and the larger surface area of a fine powder provides contact between the plant matrix and the solvent. For example, for MAE of cocaine, finely ground coca powder was more easily extracted than large particles (Brachet *et al.*, 2002).

Solvent choice

Solvent choice for MAE is dictated by the solubility of the extracts of interest, by the interaction between solvent and plant matrix, and finally by the microwave absorbing properties of the solvent determined by its dielectric constant. Csiktusnadi Kiss *et al.*, (2000) investigated the efficiency and selectivity of MAE for the extraction of color pigments from *paprika* powders using 30 extracting solvent mixtures. Their results showed that both efficacy and selectivity of MAE depend significantly on the dielectric constant of the extraction solvent mixture. Usually, the chosen solvent should possess a high dielectric constant and strongly absorb microwave energy. Solvents such as ethanol, methanol and water are sufficiently polar to be heated by microwave energy (Brachet *et al.*, 2002). Non-polar solvents with low dielectric constants such as hexane and toluene are not potential solvents for MAE. The extracting selectivity and the ability of the solvent to interact with microwaves can be modulated by using mixtures of solvents (Brachet *et al.*, 2002). One of the most commonly used mixtures is hexane-acetone (Spar Eskilsson & Bjorklund, 2000). A small amount of water (e.g. 10%) can also be incorporated in non-polar solvents such as hexane, xylene, or toluene to improve the heating rate (Spar Eskilsson & Bjorklund, 2000).

Operating conditions

During extraction, the solvent volume must be sufficient to ensure that the solid matrix is entirely immersed. Generally, a higher ratio of solvent volume to solid matrix mass in conventional extraction techniques can increase the recovery. However, in the MAE, a higher ratio may give lower recoveries. This is probably due to inadequate stirring of the solvent by microwaves (Spar Eskilsson, Bjorklund, Mathiasson, Karlsson, & Torstensson, 1999).

Temperature is another important factor contributing to the recovery yield. Generally, elevated temperatures result in improved extraction efficiencies. However, for the extraction of thermolabile compounds, high temperatures may cause the degradation of extracts. In this case, the chosen power during MAE has to be set correctly to avoid excess temperatures, leading to possible solute degradation (Font, Hernandez, Hogendoorn, Baumann, & van Zoonen, 1998).

Advantages and disadvantages of microwave-assisted extraction

MAE has been considered as a potential alternative to traditional solid–liquid extraction for the extraction of metabolites from plants. It has been used to extract nutraceuticals for several reasons: (1) reduced extraction time (2) reduced solvent usage and (3) improved extraction yield. MAE is also comparable to other modern extraction techniques such as supercritical fluid extraction due to its process simplicity and low cost. By considering economical and practical aspects, MAE is a strong novel extraction technique for the extraction of nutraceuticals.

However, compared to SFE, an additional filtration or centrifugation is necessary to remove the solid residue during MAE. Furthermore, the efficiency of microwaves can be very poor when either the target compounds or the solvents are non-polar, or when they are volatile.

Potential applications of microwave-assisted extraction

Although MAE of organic compounds from environmental matrices and microwave-assisted leaching in process metallurgy has widely been investigated (Al-Harahsheh & Kingman, 2004; Barriada-Pereira *et al.*, 2003; Ganzler, Salgo, & Valko, 1986; Lorenzo, Vazquez, Carro, & Cela, 1999; Spar Eskilsson & Bjorklund 2000; Tomaniova *et al.*, 1998), very few applications have been published in the nutraceutical area. An overview of publications on MAE of natural products was prepared by Kaufmann and Christen (2002).

MAE can extract nutraceutical products from plant sources in a faster manner than conventional solid–liquid extractions. MAE of the puerarin from the herb *Radix puerariae* could be completed within 1 min (Guo *et al.*, 2001). MAE (80% methanol) could dramatically reduce the extraction time of *ginseng* saponins from 12 h using conventional extraction methods to a few seconds (Kwon, Belanger, Jocelyn Pare, & Yaylayan, 2003). It took only 30 s to extract cocaine from leaves with the assistance of microwave energy quantitatively similar to those obtained by conventional solid–liquid extraction for several hours (Brachet *et al.*, 2002). For extracting an equivalent amount and quality of tanshinones from *Salvia miltiorrhiza bunge*, MAE only needed 2 min, whereas extraction at room temperature, Soxhlet extraction, ultrasonic extraction, and heat reflux extraction needed 24 h, 90, 75 and 45 min (Pan, Niu, & Liu, 2002). Williams, Raghavan, Orsat, and Dai (2004) found MAE was efficient in recovering approximately 95% of the total capsaicinoid fraction from *capsicum* fruit in 15 min compared with 2 h for the reflux and 24 h for the shaken flask methods.

A higher extraction yield can be achieved in a shorter extraction time using MAE. A 12-min MAE could recover 92.1% of artemisinin from *Artemisia annua L*, while several-hour Soxhlet extraction could only achieve about 60% recovery (Hao, Han, Huang, Xue, & Deng, 2002). A 4–5 min MAE (ethanol–water) of glycyrrhizic acid from *licorice* root achieved a higher extraction yield than extraction (ethanol–water) at room temperature for 20–24 h (Pan, Liu, Jia, & Shu, 2000). For the extraction of tea polyphenols and caffeine from green tea leaves, a 4 min MAE achieved a higher extraction yield than an extraction at room temperature for 20 h, ultrasonic extraction for 90 min and heat reflux extraction for 45 min, respectively, (Pan, Niu, & Liu, 2003). Shu, Ko, and Chang (2003) reported that the extraction yield of ginsenosides from *ginseng* root obtained by a 15-min MAE (ethanol–water) was higher than that obtained by 10-h conventional solvent extraction (ethanol–water).

Microwave-assisted extraction can also reduce solvent consumption. Focused MAE was applied to the extraction of withanolides from air-dried leaves of *Iochroma gesnerioides* (Kaufmann *et al.*, 2001a). The main advantages of MAE over Soxhlet extraction are associated with the drastic reduction in organic solvent consumption (5 vs 100 ml) and extraction time (40 s vs 6 h). It was also found that the presence of water in the solvent of methanol had a beneficial effect and allowed faster extractions than with organic solvent alone.

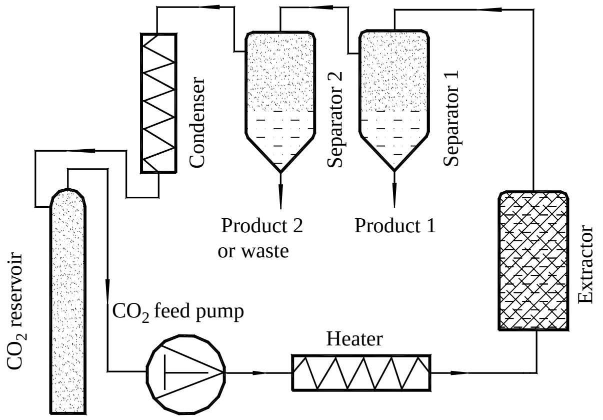

Fig. 2. Schematic diagram of a process-scale supercritical fluid extraction system.

Supercritical fluid extraction

Principles and mechanisms

Supercritical state is achieved when the temperature and the pressure of a substance is raised over its critical value. The supercritical fluid has characteristics of both gases and liquids. Compared with liquid solvents, supercritical fluids have several major advantages: (1) the dissolving power of a supercritical fluid solvent depends on its density, which is highly adjustable by changing the pressure or/and temperature; (2) the supercritical fluid has a higher diffusion coefficient and lower viscosity and surface tension than a liquid solvent, leading to more favorable mass transfer.

A supercritical fluid extraction (SFE) system is shown in Fig. 2. During SFE, raw plant material is loaded into an extraction vessel, which is equipped with temperature controllers and pressure valves at both inlet and outlet to keep desired extraction conditions. The extraction vessel is pressurized with the fluid by a pump. The fluid and the dissolved compounds are transported to separators, where the salvation power of the fluid is decreased by decreasing the pressure or increasing the temperature of the fluid. The product is then collected via a valve located in the lower part of the separators. The fluid is further regenerated and cycled (Sihvonen, Jarvenpaa, Hietaniemi, & Huopalahti, 1999).

Practical issues for supercritical fluid extraction

To develop a successful SFE, several factors must be taken into consideration. These factors include the selection of supercritical fluids, plant material preparation, modifiers and extraction conditions.

Solvent choice

Selection of supercritical fluids is critical for the development of a SFE process. With a reduction in the price of carbon dioxide and restrictions in the use of other organic solvents, carbon dioxide has begun to move from some marginal applications to being the major solvent for supercritical fluid extraction (Hurren, 1999). The critical state of carbon dioxide fluid is at a temperature of only 304 K and pressure of 7.3 MPa. Also, carbon dioxide is non-flammable and non-toxic. Supercritical CO_2 is a good solvent for the extraction of non-polar compounds such as hydrocarbons (Vilegas, de Marchi, & Lancas, 1997). To extract polar compounds, some polar supercritical fluids such as Freon-22, nitrous oxide and hexane have been considered. However, their applications are limited due to their unfavorable properties with respect to safety and environmental considerations (Lang & Wai, 2001). Although supercritical water and superheated water have certain advantages such as higher extraction ability for polar compounds, it is not suitable for thermally labile compounds (Lang & Wai, 2001).

Many nutraceuticals such as phenolics, alkaloids and glycosidic compounds are poorly soluble in carbon dioxide and hence not extractable. Techniques aimed at overcoming the limited solubility of polar substances in supercritical CO_2 have been sought. Addition of polar co-solvents (modifiers) to the supercritical CO_2 is known to significantly increase the solubility of polar compounds. Among all the modifiers including methanol, ethanol, acetonitrile, acetone, water, ethyl ether and dichloromethane, methanol is the most commonly used because it is an effective polar modifier and is up to 20% miscible with CO_2. However, ethanol may be a better choice in SFE of nutraceuticals because of its lower toxicity (Hamburger, Baumann, & Adler, 2004; Lang & Wai, 2001). Furthermore, the use of methanol as a modifier requires a slightly higher temperature to reach the supercritical state and this could be disadvantageous for thermolabile compounds. A mixture of modifiers can also be used in SFE. The best modifier usually can be determined based on preliminary experiments. One disadvantage of using a modifier is that it can cause poor selectivity.

Preparation of plant materials

Preparation of plant materials is another critical factor for SFE of nutraceuticals. Fresh plant materials are frequently used in the SFE of nutraceuticals. When fresh plant materials are extracted, the high moisture content can cause mechanical difficulties such as restrictor clogging due to ice formation. Although water is only about 0.3% soluble in supercritical CO_2, highly water-soluble solutes would prefer to partition into the aqueous phase, resulting in low efficiency of SFE. Some chemicals such as Na_2SO_4, and silica gel are thus mixed with the plant materials to retain the moisture for SFE of fresh materials (Lang & Wai, 2001).

Plant particle size is an also important for a good SFE process. Large particles may result in a long extraction process because the process may be controlled by internal diffusion. However, fine powder can speed up the extraction but may also cause difficulty in maintaining a proper flow rate. Chemat *et al.* (2004) 3 used supercritical CO_2 to extract Foeniculum vulgare volatile oil from fennel fruits with difference mean particle sizes of 0.35, 0.55 and 0.75 mm. They found that the decrease of particle size had a small decrease in the total yield of the extracted oil. Therefore, in this case, some rigid inert materials such as glass beads and sea sand are packed with the fine plant powder to maintain a desired permissibility of the particle bed.

Operating conditions

The solubility of a target compound in a supercritical fluid is a major factor in determining its extraction efficiency. The temperature and density of the fluid control the solubility. The choice of a proper density of a supercritical fluid such as CO_2 is the crucial point influencing solvent power and selectivity, and the main factor determining the extract composition (Cherchi *et al.*, 2001). It is often desirable to extract the compound right above the point where the desired compounds become soluble in the fluid so that the extraction of other compounds can be minimized. For supercritical CO_2 extraction of Chilean hop (*Humulus lupulus*) ecotypes, del Valle, Rivera, Teuber, and Teresa Palma (2003) found that very limited increases in extraction rate were observed when applying pressure >20 MPa at the temperature of 40 °C, rather the increase in pressure increased the co-extraction of undesirable compounds.

By controlling the fluid density and temperature, fractionation of the extracts could also be achieved. For supercritical CO_2 extraction of squalene and stigmasterol from the entire plant of *spirodela polyrhiza*, Choi, Kim, Noh, Choi, and Yoo (1997) found the relative extraction yield of squalene was much higher than that of stigmasterol at 10 MPa and 50 or 60 °C. The extraction of squalene was comparable to *n*-hexane extraction but the stigmasterol was not detected under these conditions. Their result confirmed that SFE could selectively extract substances from the plant materials by controlling the conditions such as temperature and pressure (density).

The extraction time has been proven to be another parameter that determines extract composition. Lower molecular weight and less polar compounds are more readily extracted during supercritical CO_2 extraction since the extraction mechanism is usually controlled by internal diffusion (Cherchi *et al.*, 2001; Poiana, Fresa, & Mincione, 1999). Therefore, the extract composition varies with the extraction time. However, Coelho *et al.* (2003) reported that the increase of CO_2 flow rate did not seem to influence the composition for the supercritical CO_2 extraction of *Foeniculum vulgare* volatile oil, although it increased the extraction rate.

Advantages and disadvantages of supercritical fluid extraction

SFE offers unusual possibilities for selective extractions and fractionations because the solubility of a chemical in a supercritical fluid can be manipulated by changing the pressure and/or temperature of the fluid. Furthermore, supercritical fluids have a density of a liquid and can solubilize a solid like a liquid solvent. The solubility of a solid in a supercritical fluid increases with the density of the fluid, which can be achieved at high pressures.

The dissolved nutraceutical compounds can be recovered from the fluid by the reduction of the density of the supercritical fluid, which can usually be reduced by decreasing pressure (Poiana, Sicari, & Mincione, 1998). Therefore, SFE can eliminate the concentration process, which usually is time-consuming. Furthermore, the solutes can be separated from a supercritical solvent without a loss of volatiles due to the extreme volatility of the supercritical fluid.

Additionally, the diffusivity of a supercritical fluid is one to two orders of magnitude higher than that of other liquids, which permits rapid mass transfer, resulting in a larger extraction rate than that obtained by conventional solvent extractions (Roy, Goto, Kodama, & Hirose, 1996). Supercritical CO_2 extraction uses a moderate extraction temperature as low as 30 °C. The low supercritical temperature of CO_2 makes it attractive for the extraction of heat sensible compounds. As SFE uses no or only minimal organic solvent (organic modifiers) in extraction, it is a more environmentally friendly extraction process than conventional solvent-solid extraction. SFE can be directly coupled with a chromatographic method for simultaneously extracting and quantifying highly volatile extracted compounds.

However, the economics and onerous operating conditions of the SFE processes has restricted the applications to some very specialized fields such as essential oil extraction, coffee decaffeination and to university research.

Potential applications of supercritical fluid extractions

SFE is a potential alternative to conventional extraction methods using organic solvents for extracting biologically active components from plants (Andras *et al.*, 2005; Choi *et al.*, 1997; Dean & Liu, 2000; Dean, Liu, & Price, 1998; Ellington, Bastida, Viladomat, & Codina, 2003; Hamburger *et al.*, 2004; Modey, Mulholland, & Raynor, 1996; Szentmihalyi, Vinkler, Lakatos, Illes, & Then, 2002). It has been used to extract plant materials, especially lipids (Bernardo-Gil, Grenha, Santos, & Cardoso, 2002), essential oils (Berna, Tarrega, Blasco, & Subirats, 2000; Coelho *et al.*, 2003; Marongiu, *et al.* 2003) and flavors (Sass-Kiss, Simandi, Gao, Boross, & Vamos-Falusi, 1998; Giannuzzo, Boggetti, Nazareno, & Mishima, 2003). Overviews of fundamentals and applications of supercritical fluids in different processes have been prepared by Hauthal (2001); Marr and Gamse (2000); Sihvonen *et al.* (1999). Turner, King, and Mathiasson (2001) gave a review about supercritical fluids in the extraction and chromatographic separation of fat-soluble vitamins. An overview of published data for the SFE of different materials was given by Marr and Gamse (2000), Lang and Wai (2001), and Meireles and Angela (2003).

SFE can prevent the oxidation of lipids. Bernardo-Gil *et al.* (2002) found that the contents of free fatty acids, sterols, triacylglycerols and tocopherols in the hazelnut oil extracted by SFE were comparable with those obtained with *n*-hexane extraction. However, the SFE-extracted oil was more protected against oxidation of the unstable polyunsaturated fatty acids than the *n*-hexane-extracted oil. Oil extracted with supercritical CO_2 was clearer than the one extracted by *n*-hexane.

SFE can achieve higher yield and quality of essential oils, flavors and natural aromas than conventional steam distillation. The mean percentage yields of cedarwood oil for supercritical CO_2 extraction and steam distillation were 4.4 and 1.3% (Eller & King, 2000). The yield of supercritical CO_2 extraction of essential oil from *juniper* wood at 50 °C and 10 MPa was 14.7% (w/w), while hydrodistillation gave a yield of 11% (w/w) (Marongiu *et al.*, 2003). Coelho *et al.* (2003) found that moderate supercritical CO_2 conditions (9 MPa and 40 °C) could achieve an efficient extraction of *Foeniculum vulgare* volatile oil, enabling about 94% of the oil to be extracted within 150 min. Compared with hydrodistillation, SFE (20 MPa and 50 °C) led to higher concentrations of light oxygenated compounds in the oil extracted from *Egyptain marjoram* leaves, which gave the oil a superior aroma. The antioxidant property of the SFE extract was also markedly higher than that of hydrodistillation extract (El-Ghorab, Mansour, & El-massry, 2004). However, it should be addressed that the composition of the essential oil was determined by two important parameters: CO_2 density (pressure) and extraction time (Cherchi *et al.*, 2001; Roy *et al.*, 1996).

The number of industrial-scale applications of SFE in plant extraction has remained very small due to the lipophilic nature of supercritical CO_2. Comparison of different extraction methods for the extraction of oleoresin from dried onion showed that the yield after supercritical CO_2 extraction was 22 times higher than that after steam distillation, but it was only 7% of the yield achieved by the extraction using a polar solvent of alcohol at 25 °C (Sass-Kiss *et al.*, 1998). However, the flavor and biological activity of onion are attributed mainly to its sulphur-containing compounds. The concentration of sulphur was the highest in steam distilled onion oil while it was the lowest in the extract of alcohol at 25 °C. The oleoresin produced by supercritical CO_2 extraction had the best sensory quality. Many active substances in plants such as phenolics, alkaloids and glycosidic compounds are poorly soluble in CO_2 and hence not extractable (Hamburger *et al.*, 2004).

Modifiers such as methanol and ethanol are widely used in the supercritical CO_2 extraction of polar substances. Supercritical CO_2 modified with 15% ethanol gave higher yields than pure supercritical CO_2 to extract naringin (a glycosylated falvonoid) from *Citrus paradise* at 9.5 MPa and 58.6 °C (Giannuzzo *et al.*, 2003). The use of a 10% ethanol co-solvent resulted in a much higher yield of *epicatechin* (13 mg/100 g seed coat) than that with pure CO_2 (22 μg/100 g seed coat) from sweet Thai *tamarind* seed coat (Luengthanaphol *et al.*, 2004). Supercritical CO_2 extraction with methanol in the range of 3–7% as modifier was proven to be a very efficient and fast method to recover higher than 98% of colchicines and colchicoside, and 97% of 3-demethylcolchicine from seeds of *colchicum autumnale* (Ellington *et al.*, 2003).

Accelerated solvent extraction

Principles and mechanisms

Accelerated solvent extraction (ASE) is a solid–liquid extraction process performed at elevated temperatures, usually between 50 and 200 °C and at pressures between 10 and 15 MPa. Therefore, accelerated solvent extraction is a form of pressurized solvent extraction that is quite similar to SFE. Extraction is carried out under pressure to maintain the solvent in its liquid state at high temperature. The solvent is still below its critical condition during ASE. Increased temperature accelerates the extraction kinetics and elevated pressure keeps the solvent in the liquid state, thus achieving safe and rapid extraction. Also, pressure allows the extraction cell to be filled faster and helps to force liquid into the solid matrix. Elevated temperatures enhance diffusivity of the solvent resulting in increased extraction kinetics (Brachet, Rudaz, Mateus, Christen, & Veuthey, 2001; Kaufmann & Christen, 2002; Richter *et al.*, 1996). A typical schematic diagram of an accelerated solvent extraction system is given in Fig. 3.

Although the solvent used in ASE is usually organic solvents. Pressurized hot water, or subcritical water can also be used in an ASE apparatus, which is usually called pressurized hot water extraction or subcritical water extraction (Eskilsson, Hartonen, Mathiasson, & Riekkola, 2004).

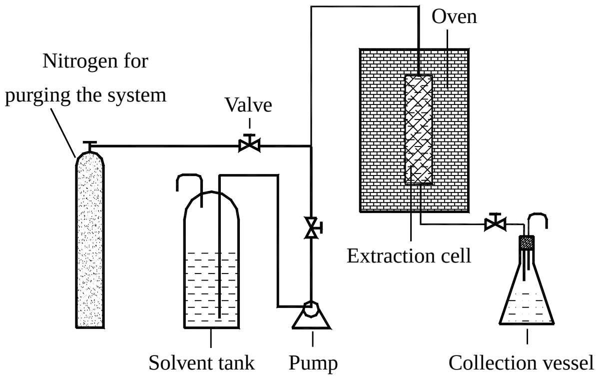

Fig. 3. Schematic diagram of an accelerated solvent extraction system.

Advantages and disadvantages of accelerated solvent extraction

Use of non-toxic extracting solvents such as carbon dioxide and water has economic and environmental benefits. Supercritical CO_2 extraction has been reported to be a valuable novel extraction technique for the extraction of nutraceuticals. However, a considerable quantity of polar modifier has to be added to carbon dioxide to extract polar compounds. Accelerated solvent extraction is considered as a potential alternative technique to SFE for the extraction of polar compounds (Brachet *et al.*, 2001). Compared with traditional Soxhlet extraction, there is a dramatic decrease in the amount of solvent and the extraction time for ASE (Richter *et al.*, 1996).

Particular attention should be paid to the accelerated solvent extraction performed with high extraction temperature, which may lead to degradation of thermolabile compounds.

Potential applications of accelerated solvent extraction

Accelerated solvent extraction is usually used for the extraction of high-temperature stable organic pollutants from environmental matrices. Very few applications of accelerated solvent extraction have been published in the field of nutraceuticals. Kaufmann and Christen (2002) reviewed recent developments in accelerated solvent extraction for natural products. A review of fundamentals and practical use of pressurized hot water extraction was given by Smith (2002).

Accelerated solvent extraction was developed for the rapid extraction of cocaine and benzoylecgonine from *coca* leaves using methanol as solvent. The optimal pressure, temperature, extraction time and particle size were found to be 20 MPa, 80 °C, 10 min and 90–150 μm (Brachet *et al.*, 2001). Their result showed there was a substantial reduction in extraction time, compared with several hour conventional extraction methods. Kaufmann, Christen, and Veuthey (2001b) compared the performance of ASE with traditional Soxhlet extraction for the recovery of steroids from the leaves of *Iochroma gesnerioides*. They found ASE produced similar results to Soxhlet in terms of recovery, repeatability and selectivity. However, both extraction time and solvent consumption were dramatically reduced with ASE. More applications of ASE for natural products can be found in the literature (Kaufmann & Christen, 2002).

Comparison of different extraction methods for selected nutraceuticals

A comparison of different extraction methods for selected nutraceuticals is given in Table 1. To obtain the most effective and potential extract, it is necessary to take into account the characteristics of plant materials and

Table 1. Comparison of different extraction methods for selected nutraceuticals

Phytochemicals	Plants	Solvent	Extraction method[a]	Extraction time (min)	Yield (mg/kg matrix)	Reference
Tocols	*Amaranthus caudatus*	Methanol	CSE (R: 1/20, 25 °C)	1440	76.32 (mg/kg)	Bruni *et al.* (2002)
		Methanol	UAE (R: 1/20, 25 °C)	60	63.7 (mg/kg)	
		CO_2	SFE (R: 1/30, 25 °C, 400 atm)	15	129.27 (mg/kg)	
β-Sitosterol/ α-Tocopherol/ γ-Tocopherol	Okra seed	*n*-Hexane	Sox	–	2010/127/380 (mg/kg)	Andras *et al.* (2005)
		Ethanol	Sox	–	2680/129/494 (mg/kg)	
		CO_2	SFE (R: 1/24–1/80, 50 °C, 450 bar)	240–800	2390/148/407 (mg/kg)	
Sapoinins	Ginseng	80% methanol	CSE (R: 1/10, 75 °C)	180	5.24 (g/100 g)	Kwon *et al.* (2003)
		80% methanol	MAE(R: 1/10, 75 °C)	0.5	5.31 (g/100 g)	
Sulphur/Oleoresin	Onion	Alcohol	Sox (R: 1/20)	240	3.78/350 (g/kg)	Sass-Kiss *et al.* (1998)
		Steam	Distillation (R: 7/120)	300	0.167/0.4 (g/kg)	
		n-Hexane	CSE (R: 1/20, 25 °C)	120	0.087/11 (g/kg)	
		Alcohol	CSE (R: 1/20, 25 °C)	120	0.895/126 (g/kg)	
		CO_2	SFE (R: 1/14, 65 °C, 300 bar)	180	0.208/9 (g/kg)	
Naringin	*Citrus paradisi*	Ethanol/water (70:30)	Sox (R: 1/10)	480	15.2 (g/kg)	Giannuzzo *et al.* (2003)
		Ethanol/water (70:30)	CSE (R:1/5, 22–25 °C)	180	13.5 (g/kg)	
		CO_2: Ethanol (85:15)	SFE (R: –, 58.6 °C, 95 bar)	45	14.4 (g/kg)	
Carvone/limone	Caraway seeds	*n*-Hexane	Sox (R: 1/20)	300	16.28/15.15 (mg/g)	Chemat *et al.* (2004)
		n-Hexane	CSE (R: 1/20, 69 °C)	60	13.38/12.63 (mg/g)	
		n-Hexane	UAE (R: 1/20, 69 °C)	60	14.45/14.27 (mg/g)	
		n-Hexane	UAE (R: 1/20, 20–38 °C)	60	17.16/16.16 (mg/g)	
Oil	Rose hip seeds	*n*-Hexane	Sox (R: 1/25)	180	48.5 (g/kg)	Szentmihalyi *et al.* (2002)
		n-Hexane	UAE (R: 1/25, 69 °C)	60	32.5 (g/kg)	
		n-Hexane	MAE (R: 1/3.5; 40 °C)	30	52.6 (g/kg)	
		CO_2	SFE (35 °C, 250 bar)	80	57.2 (g/kg)	
		CO_2: propane	SFE (28 °C, 100 bar)	35	66.8 (g/kg)	

[a] CSE, conventional solvent extraction; Sox, Soxhlet; UAE, ultrasound-assisted extraction; MAE, microwave-assisted extraction; SFE, supercritical fluid extraction; and *R*, ratio of solid to solvent (g/ml).

nutraceutical products, the nature of solvents used for extraction and the extraction procedure employed. Sometimes, the high yield of extract will not ensure a high yield of bioactive components in the extract. Some bioactive components such as free fatty acids and tocopherols are very sensitive to oxygen and heat. In this case, more care should be taken to prevent the oxidation and thermal degradation of those components. Therefore, the yield and quality of bioactive components should also be considered when an extraction method is selected.

Future research topics

Scaling up of novel extraction techniques

Ultrasound-assisted, microwave-assisted, supercritical fluid and accelerated solvent extractions are very promising for the extraction of nutraceuticals from plants. However, most of these novel extraction techniques are still conducted successfully at the laboratory or bench-scale although several industrial applications of supercritical fluid extraction can be found. More research is needed to exploit industrial applications of the novel extraction techniques.

Novel extraction processes are complex thermodynamic systems with higher capital costs. The engineering design of novel extraction systems requires knowledge of the thermodynamic constraints of solubility and selectivity, and kinetic constraints of mass transfer rate. Modeling of novel extraction processes can provide a better understanding of the extraction mechanisms and be used to quickly optimize extraction conditions and scale-up any design.

Technical barriers of novel extraction techniques

Efficient cell disruption and effective mass transfer are two major factors leading to the enhancement of extraction using ultrasound. However, the presence of a dispersed phase contributes to ultrasound wave attenuation and the active part of ultrasound inside the extractor is restricted to a zone located in the vicinity of the emitter. Therefore, these two factors must be carefully considered in the design of ultrasound-assisted extractors.

The high temperature and homogeneous temperature distribution over penetration depth reached by microwave heating reduces dramatically both the extraction time and solvent consumption. However, the efficiency of microwaves can be very poor when either the target compounds or the solvents are non-polar, or when they are volatile. Furthermore, many nutraceuticals are thermally unstable and may degrade during extraction. More research is needed to investigate the interaction between microwaves, and plant materials and solvents.

Supercritical fluid extraction is one of the most successful and recent contributions to extraction techniques of nutraceuticals from plants. However, supercritical fluid extraction, which exclusively uses CO_2 as the extracting solvent, is also restricted to non-polar extracts. Accelerated solvent extraction under elevated temperature and high pressure can be supplementary to SFE for extraction of polar compounds. More research is needed to reduce the capital and operating costs of SFE and ASE.

Improvement in design of novel extraction systems

High-pressure and microwave energy can be combined with conventional Soxhlet extraction leading to a new design of high-pressure and microwave-assisted Soxhlet extractors. Also supercritical CO_2 solvent can be used in the Soxhlet extraction. Ultrasound can be used to enhance the mass transfer in supercritical fluid extraction (Riera *et al.*, 2004). Supercritical fluid extractions can be coupled with silica gels to improve the overall extraction selectivity and on-line fractionations. These combined techniques retain the advantages of conventional Soxhlet, while overcoming the limitations of the novel extraction techniques such as ultrasound-assisted, microwave-assisted, supercritical fluid and accelerated solvent extractions.

Conclusions

The need to extract nutraceuticals from plant materials prompts continued searching for economically and ecologically feasible extraction technologies. Traditional solid–liquid extraction methods require a large quantity of solvent and are time consuming. The large amount of solvent used not only increases operating costs but also causes additional environmental problems. Several novel extraction techniques have been developed as an alternative to conventional extraction methods, offering advantages with respect to extraction time, solvent consumption, extraction yields and reproducibility. However, novel extraction techniques have only been found in a limited field of applications. More research is needed to improve the understanding of extraction mechanism, remove technical barriers, improve the design and scale up of the novel extraction systems for their industrial applications.

Acknowedgements

A contribution of the University of Nebraska Agricultural Research Division, Lincoln, NE. Journal Series No. 14920. This research was supported in part by funds provided by the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service (Grant No. 2004-35503-14824) and through the Hatch Act.

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