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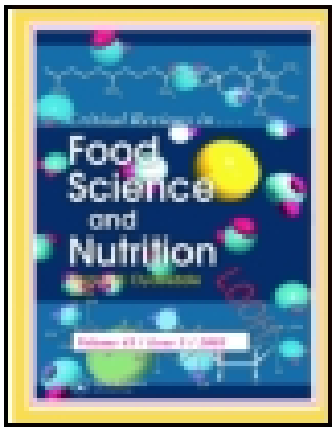

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Ultrahigh Pressure Extraction of Bioactive Compounds from Plants-a Review

Jun Xi[a]
[a] School of Chemical Engineering, Sichuan University, Chengdu 610065, China
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Title: Ultrahigh Pressure Extraction of Bioactive Compounds from Plants-a Review

Author

JUN XI*

Address:

School of Chemical Engineering,

Sichuan University,

Chengdu 610065,

China

***Corresponding author**

JUN XI

Address:

School of Chemical Engineering,

Sichuan University,

Chengdu 610065,

China

Tel: +86 28 85405209

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Fax: +86 28 85405209

E-mail address: xijun@scu.edu.cn (J Xi)

ABSTRACT

Extraction of bioactive compounds from plants is one of the most important research areas for pharmaceutical and food industries. Conventional extraction techniques are usually associated with longer extraction times, lower yields, more organic solvent consumption, and poor extraction efficiency. A novel extraction technique, ultrahigh pressure extraction, has been developed for the extraction of bioactive compounds from plants, in order to shorten the extraction time, decrease the solvent consumption, increase the extraction yields and enhance the quality of extracts. The mild processing temperature of ultrahigh pressure extraction may lead to an enhanced extraction of thermolabile bioactive ingredients. A critical review is conducted to introduce the different aspects of ultrahigh pressure extraction of plants bioactive compounds, including principles and mechanisms, the important parameters influencing its performance, comparison of ultrahigh pressure extraction with other extraction techniques, advantages and disadvantages. The future opportunities of ultrahigh pressure extraction are also discussed.

Keywords: Ultrahigh pressure extraction; Bioactive compounds; Plant; Review article.

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CONTENTS

INTRODUCTION

Plants contain a broad range of bioactive compounds such as polyphenols, ginsenosides, lycopene, caffeine, saliroside and corilagin etc (Abah and Egwari, 2011). 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 (Vilkhu et al., 2008).

Extraction of bioactive compounds from plants is one of the most important research areas for pharmaceutical and food industries (Zhang et al., 2011). Conventional extraction techniques include soaking, maceration, water percolation, Soxhlet extraction, etc. These techniques are usually associated with longer extraction times, lower yields, more organic solvent consumption, and poor extraction efficiency, which incorporate risk of thermal degradation of thermolabile active compounds (Chan et al., 2011). The ideal extraction method should be time-saving, accurate, precise, and little solvent consumption (Chen et al., 2009; Sasidharan et al., 2011). The ultrahigh pressure extraction (UPE) could be the isolation technique capable of meeting these expectations.

The UPE is a novel method to enhance mass transport phenomena, which works under a super-high pressure ranging from 100 to 500 MPa and temperature of 20-50℃, and has been recognized as an environment-friendly technology by the US Food and Drug Administration (Joo et al., 2012; Xi and Wang, 2013). The mild processing temperature of UPE may lead to an

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enhanced extraction of thermolabile bioactive ingredients, being this aspect of paramount importance in the case of heat-sensitive components (Lee et al., 2011; Zhang, et al., 2011). Ultrahigh pressure can be effectively used to improve the extraction rate by increasing the mass transfer rates and possible rupture of cellular wall, membrane and organelles leading to higher product yields with reduced processing time and solvent consumption (Chen et al., 2009; Xi et al., 2011a). The UPE was first reported in 1999 by Knorr for extraction of caffeine from coffee (Knorr, 1999). In 2004, Sanchez-Moreno et al. demonstrated that a higher carotenoid content in tomato puree was extracted by ultrahigh pressure of 100-400 MPa (Sanchez-Moreno et al., 2004). The UPE has received increasing attention in the last two years, as can be deduced from a great number of papers published dealing with this subject based on the publications available in web of knowledge since 2011, as listed in Table 1 (Lee et al., 2011; Xi et al., 2011b; Ji et al., 2011; Joo et al., 2011; Altuner et al., 2012; Guo et al., 2012; Joo, et al., 2012; Xi and Wang, 2013; Zhang et al., 2012; Zhu et al., 2012; Liu et al., 2013a; Liu et al., 2013b). Overall it appears that ultrahigh pressure can be effectively used for intensification of the extraction of important constituents from plants.

The UPE has become relatively mature and some potential applications for the extraction of bioactive compounds from plants have been reported. The present work presents an overview of different aspects of UPE, including principles and mechanisms, the important parameters influencing its performance, comparison of UPE with other extraction techniques, advantages

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and disadvantages. Finally, the future direction of UPE is also discussed.

PRINCIPLES AND MECHANISMS

A laboratory-scale prototype of an ultrahigh pressure extraction system is shown in Figure 1 (Chen et al., 2009; Xi et al., 2011b) which is a batch type for sample operation. The general procedures of UPE are illustrated in Figure 2 and will be discussed systematically. Firstly, the dried plant materials are pulverized and sieved to sizes of 40-60 mesh numbers. Secondly, a suitable solvent is selected according to the similar dissolve mutually theory. Thirdly, the pulverized materials are mixed with the solvent in the sterile polyethylene bag. The bag is sealed after eliminating air from the inside. Then the bag is loaded into a pressure vessel which is equipped with release pressure valves and temperature controllers (thermocouple) at the top and bottom of the vessel to keep desired extraction conditions. The pressure vessel is pressurized with the fluid (usually water) by an ultrahigh pressure booster pump. After extracted in the ultrahigh pressure apparatus for some time at the specified pressure and temperature (Prasad et al., 2009; Lee et al., 2011; Zhang et al., 2012), the mixture is filtered to remove the solid particles. The collected extract is centrifuged at a speed ranging from 4000 up to 8000 rpm for 10-15min. The supernatant liquid is collected and filtered through 0.45μm membrane prior to quantification analysis. The extract is usually evaporated by a rotary evaporator under vacuum at 45-65℃, and then stored at 4℃ in refrigerator. Thus, the plant extracts can be prepared which contains the active compounds that we need.

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The mechanical effects of ultrahigh pressure induce a greater penetration of solvent into cellular materials and improve mass transfer rate due to the destruction of biological cell tissue, facilitating the release of cell contents (Chen et al., 2009; Xi et al., 2011a; Lee et al., 2011; Zhang et al., 2012). Therefore, the efficient cell tissue disruption is a major factor leading to the enhancement of extraction. This phenomenon was confirmed by performing scanning electron micrography on ginseng roots (Chen et al., 2009) and *Salvia miltiorrhiza* Bunge (Liu et al., 2013a). Chen et al. (Chen et al., 2009) employed ultrahigh pressure technique to extract ginsenosides from ginseng root. They observed ginseng root particles using scanning electron micrography that ultrahigh pressure treatment would lead to destructive changes in the ginseng cell tissue (Chen et al., 2009). Figure 3 reveals that the ginseng root tissues of the untreated samples (a) were kept more intact, compared with the structures of the samples treated in heat reflux extraction (b) and ultrahigh pressure extraction (c, d). In the case of heat reflux extraction (b) at 80℃ with 70% ethanol for 6 h, puny damage was observed (Chen et al., 2009). While after ultrahigh pressure treatment at 200 MPa and 60℃ for 5min (c, d), hollow openings were generated and smaller particles were developed, which indicated that the cell walls of the plant tissues were broken and all the cell constituent components were washed away by solvent effect. It demonstrated that the ultrahigh pressure induced a subsequent change in the surface tension of the cellulose and the formation of small particles (Chen et al., 2009). The microstructure change of the cellulose could cause the plant to crumble more readily. Furthermore, the smaller particles

could be propitious to diffusion and osmotic process. These results are in good agreement with studies of Liu et al. (Liu et al., 2013a), who reported that ultrahigh pressure could result in the disruption of the *Salvia miltiorrhiza* Bunge tissue, cellular wall, membrane and organelles, which enhanced the mass transfer of the solvents into the leaves materials and the soluble constituents into the solvents, as illustrated in Figure 4.

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Extraction Parameters and Effects

The efficiency of UPE relies on the selection of the operating conditions, which will strongly affect the extraction mechanisms and yields. The factors that may influence the performance of UPE are pressure level, holding time, the ratio of liquid to solid, temperature, moisture content and particle size of plant samples, type and concentration of solvent, etc (Xi, 2009; Xi et al., 2009; Xi et al., 2010; Liu, et al., 2013a). It is important to understand the effects and interactions of these factors on the UPE processes. Of these factors, the solvent type, pressure level and temperature are regarded as the three most important parameters for UPE (Corrales et al., 2009).

Solvent Choice

A suitable extracting solvent should be selected for the extraction of bioactive compounds using UPE method, which affects the solubility of the target components (Guo et al., 2012; Zhang et al., 2011). Different solvents will yield extracts with different compositions. The polarity of the solvent should be close to that of the target compounds, similar to traditional

methods (Xi et al., 2011a; Zhang et al., 2011; Altuner et al., 2012; Guo et al., 2012; Joo et al., 2012; Liu et al., 2013b). Water, hydrophilic and lipophilic organic solvents of different solvent ratios can all be used in UPE. Thus, UPE can be used to extract strong, weak, and non-polar molecules by using different solvents. Glucides, coumarins, lignans, quinines, flavonoids, terpenes, tannins, triterpenoids, cardiac heterosides, glycosides and aglycones, alkaloids, etc. can all be extracted using UPE (Chen et al., 2009; Xi et al., 2011b; Zhang et al., 2011; Altuner et al., 2012; Guo et al., 2012; Joo et al., 2012; Zhang et al., 2012). For example, the yields of polyphenols from green tea leaves were highly dependent on the type of solvent used (acetone, methanol, ethanol or distilled water) (Xi et al., 2009). Furthermore, the amount of water present in solvent (i.e. the concentration of aqueous solution) significantly influenced extraction yields (Joo et al., 2012). In general, the choice of solvent takes into account its affinity with the target compounds. Solvent toxicity is also evaluated in selecting suitable solvent for UPE. In the extraction of total phenolic from longan fruit pericarp, ethanol is non-toxic, thus it was selected in favor of methanol despite the fact that the latter has higher yield (Prasad et al., 2009; Xi et al., 2010). In general, ethanol is by far the most used solvent which is suitable for extracting many active compounds from plants.

Once a suitable solvent has been decided upon, the ratio of liquid to solid has to be determined as it affects the extraction yields in most cases (Zhang et al., 2011; Altuner et al., 2012; Guo et al., 2012). During UPE, the solvent volume must be sufficient to ensure that the

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solid matrix is entirely immersed. A great amount of solvent can generally dissolve the bioactive ingredients of interest more effectively and lead to a higher extraction yields, which has been reported by some authors (Chen et al., 2009; Xi et al., 2010; Altuner et al., 2012; Joo et al., 2012; Zhang et al., 2011).

Effects of Pressure and Holding Time

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Pressure is another important factor contributing to the yield (Xi et al., 2010; Xi et al., 2011b; Zhang et al., 2011; Altuner et al., 2012; Guo, Han et al., 2012; Zhang et al., 2012). Generally, elevated pressure results in improved extraction efficiency. Xi et al. (Xi et al., 2009) used UPE to obtain polyphenols from green tea leaves. Various experimental conditions, such as different solvents (acetone, methanol, ethanol and water), pressure (100, 200, 300, 400, 500, 600 MPa), holding time (1, 4, 7, 10 min), ethanol concentration (0 – 100% mL/mL), and liquid/solid ratio (10:1 to 25:1 mL/g) for the UPE procedure, were investigated to optimize the extraction. Through a study of the effect of pressure on the extraction yields of polyphenols, they found when the pressure increased from 100 to 600 MPa, the extraction yield of polyphenols increased from 15±1.4 to 30±1.3%, which indicated the polyphenols content was greatly influenced by high pressure treatments.

Ultrahigh pressure can increase the mass transfer rate of solutes, enhance dissolving capacity and speed of solvent penetration into the cells by disrupting the cellular walls which may lead to a high permeability during extraction and an efficient extraction of bioactive compounds

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(Corrales et al., 2009). Ultrahigh pressure can also cause deprotonation of charged groups and distraction of salt bridges and hydrophobic bonds, resulting in conformational changes and denaturation of proteins making the cellular membranes less and less selective, thereby rendering the compounds more available to extraction (Butz et al., 1994). Zhang et al. (Zhang et al., 2005) reported that according to the mass transfer theory, the mass transfer rate = pressure/resistance of mass transfer, and based on phase behavior theory, the dissolution is faster at higher pressure (Yan, 2002). Under ultrahigh pressure conditions, the differential pressure between the inner and the exterior of the cell is very large, which causes the solvent to permeate very quickly through the broken membranes into cells, and the mass transfer rate of solute or the rate of dissolution is very large. This could result in a very short extracting time of UPE (Zhang et al., 2011; Zhang et al., 2012). Besides, the pressurized cells show increased permeability (Yan, 2002). The higher the pressure is, the more solvent can enter cells and the more compounds can permeate out to the solvent. The equilibrium of solvent concentration between inner and outer of cells would be established during the pressure holding period. Furthermore, in the extraction process with high pressure, the solubility of solutes is improved as the pressure increases (Noble, 1988; Richard, 1992). If the substrate is dry, ultrahigh pressure may be used to facilitate swelling and cause an enlargement of the pores of the cell wall (Zhang et al., 2011; Altuner et al., 2012; Joo et al., 2012). The holding time should also be considered carefully because overlong pressure maintaining time can damage the biological activity of extracts (Zhang et al., 2011; Altuner et al.,

2012).

Effect of Temperature

Temperature during the extraction is one of the critical factors that affect the efficiency and selectivity in UPE. The use of high temperatures improves the efficiency of the extraction (Lee et al., 2011). An important advantage of using higher temperature of the solvent is the improved diffusion rate, i.e. mass transfer of the molecule in the solvent, which allows faster extractions especially in diffusion-controlled samples. For example, Prasad et al. (Prasad et al., 2009) investigated the effects of different temperature (30℃, 50℃, 70℃) on the extraction yields and total phenolic content from longan fruit pericarp. When extraction temperature increased from 30 to 50 °C, the extraction yield increased from 17.6 to 30%, which indicated the extraction yields increased with increasing temperature. The choice of extraction temperature depends on the stability and extraction yields of the desired active compound. In the extraction of thermolabile compounds, high temperatures may cause the degradation of extracts (Zhang et al., 2011).

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COMPARISON BETWEEN UPE AND OTHER EXTRACTION TECHNIQUES

A comparison between UPE and other extraction techniques in their optimized condition are shown in Table 1. From Table 1, it is obvious that the extraction yield of UPE is higher and the extraction time required is shorter when compared with other extraction techniques such as

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Soxhlet extraction, heat reflux extraction, ultrasonic assisted extraction, microwave assisted extraction and leaching extraction. Guo et al. (Guo et al., 2012) reported that the comparison among UPE, heating reflux extraction and microwave assisted extraction of pectin from navel orange peel was carried out. As shown in Table 1, among these three extraction methods, the extraction yield of UPE (20.44%±0.64) was significantly higher than those of heating reflux extraction ($15.47\% \pm 0.26$) and microwave assisted extraction ($18.13\% \pm 0.23$). While the extraction time of UPE (10 min) was much shorter than the extraction time of heating reflux extraction (60 min) and microwave assisted extraction (21 min) as well. The high efficiency of UPE is further supported by the extraction of flavonoids from Laoying tea, phenolic compounds from *Pinus densiflora* root, phenolic compounds from maclura pomifera, ginsenosides from ginseng, phenolic compound and amino acids from camellia sinensis leaf, and berberine from cortex phellodendri, etc (Ji et al., 2011; Joo et al., 2011; Joo et al., 2012; Lee et al., 2011; Xi et al., 2011b; Zhang et al., 2012; Altuner et al., 2012; Guo et al., 2012; Liu et al., 2013b).

Not only the more bioactive components are obtained, but also the extracts have better antioxidant activity through UPE method. Xi et al. (Xi et al., 2011b) investigated the effect of UPE at pressures of 150 MPa, 250 MPa, 350 MPa and 450 MPa on the total phenolic contents, the extraction yields and the antioxidant activities of green tea. The results showed that the phenolic contents and the antioxidant activities of extracts were greatly influenced by high pressure. The total phenolic contents and the antioxidant activities of UPE at 450 MPa were

higher than those of other UPE and conventional extraction. The high content of phenolic compounds in the green tea leaves could account for the antioxidant activity. Similar results were obtained in ginseng (Chen et al., 2009) and *Camellia sinensis* leaf (Joo et al., 2012) using the UPE technique.

These results definitely demonstrated that UPE as a novel method can be utilized in the extraction of many bioactive compounds from various natural materials with less time consumption, high extraction efficiency and excellent antioxidant activity.

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ADVANTAGES AND DISADVANTAGES

UPE has been considered as a potential alternative to traditional solid-liquid extraction for the extraction of bioactive compounds from plant. The main benefits of UPE include: (1) shorten the extraction time, (2) decreased the solvent consumption, (3) increased the extraction yields, (4) reduced the operating temperature, allowing the extraction of thermolabile compounds, (5) fewer impurities of the extract are noticed in the UPE as compared to the conventional extraction. Furthermore, like conventional extraction techniques (such as soaking, maceration, water percolation, Soxhlet extraction, etc.), the UPE can be used with any solvent for extracting a wide variety of natural compounds (Joo et al., 2012; Lee et al., 2011; Xi et al., 2011b; Zhang et al., 2012; Altuner et al., 2012; Guo et al., 2012; Liu et al., 2013a; Liu et al., 2013b). Thus, the UPE will exhibit great potential for the extraction of bioactive compounds from plant materials.

The UPE, supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) are all

fast and efficient for extracting compounds from plant materials. These techniques have the possibility of working at elevated pressure or/and temperature, greatly decreasing the extraction time (Joo et al., 2012; Lee et al., 2011; Xi et al., 2011b; Zhang et al., 2012; Altuner et al., 2012; Guo et al., 2012; Liu et al., 2013a; Liu et al., 2013b; Ciurlia et al., 2009; Sahena et al., 2009; Saldana et al., 2010; Ong et al., 2006; Carabias-Martínez et al., 2005).

The SFE is a separation technology that uses supercritical fluid as the solvent. A substance is considered to be a supercritical fluid when its temperature and pressure are above its critical values (Ciurlia et al., 2009). Some liquids and gases (ethylene, carbon dioxide, ethane, nitrous oxide, propane, *n*-hexane, acetone, methanol, ethanol, ethyl acetate, water, etc.) are commonly used as supercritical fluids (Sahena et al., 2009). The most commonly utilized gas for SFE is carbon dioxide. The CO_2 is supercritical above 31.1℃ and 7.38 MPa, which makes it an ideal solvent for extracting thermally sensitive compounds (Saldana et al., 2010). Supercritical CO_2 is a good solvent for the extraction of non-polar compounds such as hydrocarbons (Vilegas et al., 1997). Many ingredients such as phenolics, alkaloids and glycosidic compounds are poorly soluble in carbon dioxide and hence not extractable (Hamburger et al., 2004). Therefore, a considerable quantity of polar modifier has to be added to carbon dioxide to extract polar compounds (Luengthanaphol et al., 2004). According to Shi et al. (Shi et al., 2009), non-polar compounds such as hydrocarbons are most efficiently dissolved in relatively non-polar solvents while polar compounds are most efficiently dissolved in relatively polar solvents. Compared

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with SFE, the extracting solvent could be selected to be matched to the polarity of the target components during UPE, as is done in traditional methods (Altuner et al., 2012; Guo et al., 2012). Water, hydrophilic, and lipophilic organic solvents of different solvent ratios can all be used. Thus, UPE can be used to extract strong, weak, and non-polar compounds using different solvents (Xi and Zhang, 2007). Glucides, coumarins, lignans, quinines, flavonoids, terpenes, tannins, triterpenoids, cardiac heterosides, glycosides and aglycones, alkaloids, etc. can all be extracted using UPE (Joo et al., 2012; Lee et al., 2011; Xi, Shen, Li, & Zhang, 2011b; Zhang et al., 2012; Altuner et al., 2012; Guo et al., 2012; Liu et al., 2013a; Liu et al., 2013b). Additionally, apparatuses and equipment of UPE is cheaper and its operation is easier in many cases than SFE. However, the UPE utilizes a volume of organic solvent like methanol, which is probably less environment-friendly than supercritical CO_2 extraction which uses no or only minimal organic solvent (organic modifiers) in extraction (Zhang et al., 2012; Guo et al., 2012). Moreover, compared to SFE, an additional filtration or centrifugation is necessary to remove the solid residue during UPE (Lee et al., 2011; Guo et al., 2012).

The PLE is a technique that involves extraction using liquid solvents at temperature, usually between 50 and 200 ℃, and at pressure between 10 and 15 MPa, which enhance the extraction performance as compared to those techniques carried out at near room temperature and atmospheric pressure (Carabias-Martínez et al., 2005). The solvent is still below its critical condition during PLE (Mustafa and Turner, 2011). This technique is also known as pressurized

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solvent extraction, accelerated solvent extraction and enhanced solvent extraction (Richter et al., 1996). In case when water is used as the extraction solvent, the technique is referred to as sub-critical water extraction, pressurized hot water extraction or superheated water extraction (Ong et al., 2006). Extraction is carried out under pressure to maintain the solvent in its liquid state at high temperature. Increased temperature accelerates the extraction kinetics and elevated pressure keeps the solvent in the liquid state, thus achieving safe and rapid extraction (Carabias-Martínez et al., 2005). But the PLE performs with high extraction temperature, which may lead to degradation of thermolabile compounds (Brachet et al., 2001). So the PLE is not fit for the extraction of the thermally labile compounds. The UPE usually works under the temperature ranging from 20 to 90 ℃. Its low temperature is advantageous, since it allows for extraction at moderate temperatures, which reduces the potential for the heat-sensible compounds degradation (Joo et al., 2012; Xi and Wang, 2013). So the UPE is considered as a potential alternative technique to the PLE for the extraction of thermolabile compounds. However, compared with PLE, the UPE requires the higher investment costs, such as, the elevated pressure needs expensive equipments (Xi et al., 2011b; Zhang et al., 2012; Joo et al., 2012). Nevertheless, the high investment costs could be compensated by the advantages of accomplishing efficient processing of UPE. In addition, particular attention should be paid to the UPE performed with ultrahigh pressure (up to 600 MPa), which may modify the chemical structures of the target compounds (Xi, 2006).

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The comparison regarding the extraction yield, solvent consumption, time and temperature are made among UPE, SFE and PLE extraction of *Schisandrin A* from *Schisandra chinensis* fruits is shown in Table 2. Liu er al. (Liu et al., 2006), Choi et al. (Choi et al., 1998), and Lee et al. (Lee and Kim, 2010) reported that the UPE, SFE and PLE was used to extract *Schisandrin A* from *Schisandra chinensis* fruits, respectively. The UPE optimum extraction conditions were as follows: 50 ml solvent consumption, 5 min extraction time, 20℃ temperature, the extraction yield of *Schisandrin A* was 0.313 %. The extraction yields of *Schisandrin A* with SFE and PLE were 0.134% and 0.128%, the temperature were 60 ℃ and 125℃, and the extraction time were 30 min and 5 min, respectively. UPE had the highest extraction yield with the lowest temperature of the three extraction methods.

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CONCLUSIONS AND FUTURE RESEARCH

This review covers the aspects of UPE of plants bioactive compounds, including principles and mechanisms, the important parameters influencing its performance, comparison of UPE with other extraction techniques, advantages and disadvantages, etc. A lot of examples suggested that UPE had some considerable merits such as shorter extraction time, higher extraction yield and less solvent consumption compared to conventional extraction methods. The low extraction temperature of UPE makes it attractive for the extraction of heat-sensible compounds. The food and medicinal industries will benefit from this emerging technology.

Due to the growing interest in the extraction of bioactive compounds from plants, as well as

the concern of using technologies that are more “green”, the UPE is becoming a more promising extraction technology to fulfill these demands. However, there are still many challenges in the area of UPE extraction of plant bioactive ingredients.

(1) It is necessary to further explore the possibility of extraction of bioactive compounds from some high-cost raw materials by UPE, which is an economical alternative to traditional extraction processes. This is an industry demand for a sustainable development.

(2) The UPE coupled with other extraction technique possibly results in a cleaner and more energy efficient technology. Combining UPE with ultrasound could cause significant degree of intensification and seems to be a promising technique.

(3) Although the UPE have not yet been very widely used in industry, we believe that it could be a useful tool in extracting of bioactive ingredients in the future. Special efforts are needed to unveil the complex physicochemical mechanism, reduce the capital and operating costs, improve the design and scale up of the extraction systems, in order to facilitate the application of UPE to food and drug industries. For one thing, it is necessary to research an effective kinetics model of the UPE system for determining the basic mass transfer data for scale-up procedures. For another, as the extraction process is often constrained by certain key parameters, it is not sufficient to scale everything up at the same rate. Therefore the appropriate scale-up factors must be determined.

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Table 1. List of ultrahigh pressure extraction of bioactive compounds, and a comparison between UPE and other extraction techniques, based on the publications available in Web of Knowledge since 2011.

Plant materials	Target compound	Methods	Operating conditions	Yield	Reference
Laoying tea	Flavonoids	UPE	70 % ethanol, 41 ml/g, 9min, 25℃, 427 MPa	6.26%	Ji et al., 2011
		Reflux	Water, 35 ml/g, 90min, 60℃	6.19%	
Pinus densiflora root	Phenolic compounds	UPE	70% ethanol, 9ml/g, 1h, 25℃, 100MPa	18.5±0.47 %	Joo et al., 2011
		Ultrasonic	70% ethanol, 9ml/g, 1h, 25℃, 20kHz, 750 W	14.66±0.54 %	
		Leaching	70% ethanol, 9ml/g, 12h, 25℃	13.96±0.73 %	
Ginseng	Ginsenosides	UPE	Water, 20ml/g, 12h, 30℃, 80MPa	1106.3 ± 35.5μg/mL	Lee et al., 2011
		Reflux	Water, 20ml/g, 1h, 95℃	1086.7±15.7μg/mL	
Scutellaria barbata	Scutellarin Luteolin Apigenin	UPE	61.4 % ethanol, 78ml/g, 1.5min, 25℃, 200 MPa	1.235 % 0.177 % 0.228 %	Zhang et al., 2011
		Reflux	69.1% ethanol, 78ml/g, 4h, 60℃	0.756 % 0.114 % 0.149 %	
		Ultrasonic	69.1% ethanol, 78ml/g, 40min, 25℃, 40kHz, 100 W	0.913 % 0.128 % 0.196 %	
		Microwave	69.1% ethanol, 78ml/g, 3min, 80℃, 700W	0.926 % 0.136 % 0.185 %	
Maclura pomifera	Phenolic compounds	UPE	Solvent cocktail, 5ml/g, 10min, 25℃, 500MPa	913.17 ug GAE/mL	Altuner et al., 2012
		Leaching	Solvent cocktail, 5ml/g, 2 h, 25℃		

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		Soxhlet	Solvent cocktail, 5ml/g, 14 h, 85℃	363.45 ug GAE/mL 326.87 ug GAE/mL	
Navel orange peel	Pectin	UPE Microwave Reflux	95% ethanol, 50ml/g, 10min, 55℃, 500MPa 95% ethanol, 50ml/g, 21min, 80℃, 500 W 95% ethanol, 50ml/g, 60min, 80℃	20.44%±0.64 18.13%±0.23 15.47%±0.26	Guo et al., 2012
Camellia sinensis leaf	Phenolic compound Amino acids	UPE Ultrasonic Leaching	70% ethanol, 9ml/g, 1h, 25℃, 100MPa 70% ethanol, 9ml/g, 1h, 25℃, 20kHz, 750 W 70% ethanol, 9ml/g, 12h, 25℃	38.13±1.40 % 9.523±0.141 % 30.19±1.20 % 7.350±0.214 % 26.77±1.04 % 6.377±0.187%	Joo et al., 2012
Cortex Phellodendri	Berberine	UPE Ultrasonic Reflux Microwave	69.1% ethanol, 31.3ml/g, 2min, 25℃, 243.30 MPa 69.1% ethanol, 31.3ml/g, 1h, 25℃, 40kHz, 100 W 69.1% ethanol, 31.3ml/g, 2h, 80℃ 69.1% ethanol, 31.3ml/g, 15min, 80℃, 700W	0.77 % 0.561 % 0.535 % 0.6 %	Liao et al., 2012
Scutellaria baicalensis	Baicalin Baicalein	UPE Reflux Ultrasonic	60.54 % ethanol, 35.6 ml/g, 2min, 25℃, 150 MPa 60.54 % ethanol, 35.6 ml/g, 5h, 60℃ 60.54 % ethanol, 35.6 ml/g, 60min, 25℃, 60kHz	15.35% 1.47% 8.56% 1.04% 10.38% 1.13%	Zhang et al., 2012
Dysosma	Podophyllotoxin	UPE	80% methanol, 12ml/g, 1min, 25℃, 200 MPa	2.19 %	Zhu et al.,

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versipellis	4′-demethylpodophyllotoxin	Reflux	80% methanol, 12ml/g, 1h, 75℃	0.51 % 2.11 % 0.42%	2012
Green tea	Polyphenolic antioxidants	UPE Reflux	50 % ethanol, 20 ml/g, 15min, 25℃, 490 MPa 50 % ethanol, 20 ml/g, 4h, 85℃	58.38 % 44.02 %	Xi and Wang, 2013
Salvia miltiorrhiza Bunge	Dihydrotanshinone Cryptotanshinone Tanshinone I Tanshinone IIA Miltirone	UPE	0.5M[C_8MM][PF_6] in ethanol solvent, 20 ml/g, 2min, 40℃, 300 MPa	4.04±0.099mg/g 9.30±0.125mg/g 20.3±0.453mg/g 37.4±0.659mg/g 0.593±0.010mg/g	Liu et al., 2013a
		Reflux	0.5M[C_8MM][PF_6] in ethanol solvent, 20 ml/g, 120min, 60℃,	3.52±0.281mg/g 8.73±0.531mg/g 15.7±0.323mg/g 30.2±0.439mg/g 0.38±0.034mg/g	
		Ultrasonic	0.5M[C_8MM][PF_6] in ethanol solvent, 20 ml/g, 30min, 40℃, 250 W, 30kHz	4.23±0.082mg/g 9.22±0.113mg/g 21.5±0.336mg/g 37.6±0.541mg/g 0.592±0.031mg/g	
Cortex Phellodendri amurensis.	Palmatine Berberine	UPE	70 % ethanol, 40 ml/g, 4min, 40℃, 300 MPa	0.78±0.02% 1.93±0.06%	Liu et al., 2013b
		Reflux	70 % ethanol, 40 ml/g, 4h, 80℃	0.48±0.01% 1.28±0.02%	
		Soxhlet	70 % ethanol, 40 ml/g, 6min, 90℃	0.58±0.01% 1.51±0.03%	
		Ultrasonic	70 % ethanol, 40 ml/g, 60min, 30℃, 250 W, 42kHz	0.56±0.01% 1.49±0.03%	

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UPE: Ultrahigh pressure extraction, Leaching: Leaching extraction, Microwave: Microwave assisted extraction, Ultrasonic: ultrasonic assisted extraction. Reflux: Heating reflux extraction.

Solvent cocktail: (dH_2O: ethanol: methanol: acetone: CH_2Cl_2 - 1: 2.5: 2.5: 2.2).

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Table 2.The comparison of operating parameter of UPE, SFE and PLE extraction of *Schisandrin A* from *Schisandra chinensis* Fruits.

Method	Solvent consumption	Time	Temperature	The extraction yield of Schisandrin A (%)	Reference
UPE	50 ml ethanol	5 min	20 ℃	0.313±0.018	Liu et al. 2006
SFE	1 mL/min CO_2	30 min	60 ℃	0.134±0.015	Choi et al. 1998
PLE	40 ml methanol	5 min	125 ℃	0.128±0.031	Lee and Kim, 2010

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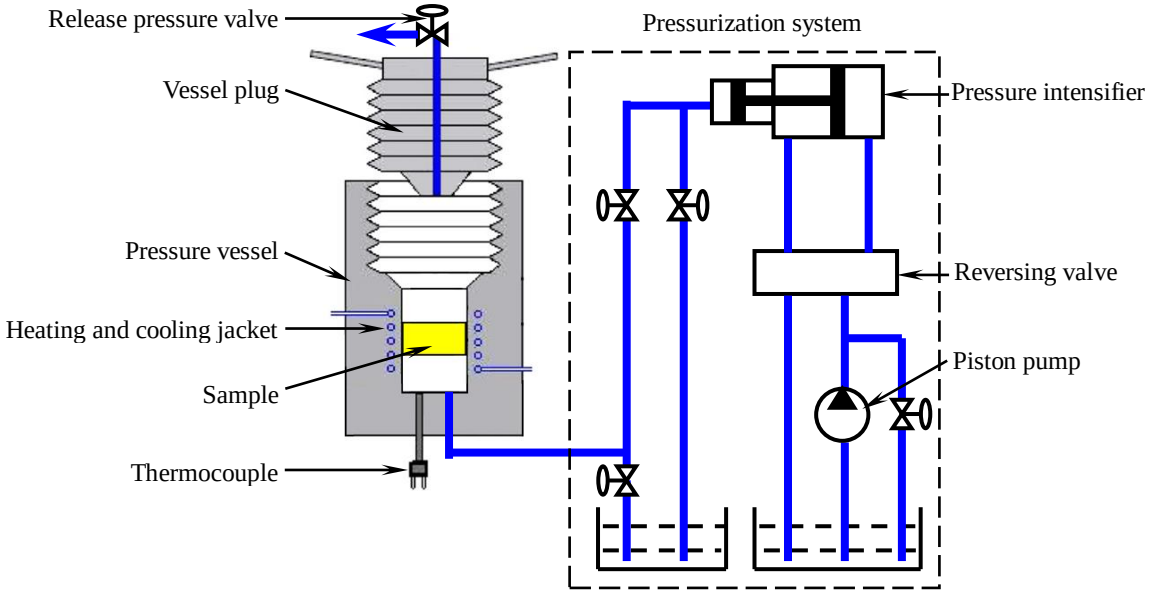

Figure 1. Schematic representation of a laboratory-scale prototype of an ultrahigh pressure extraction system

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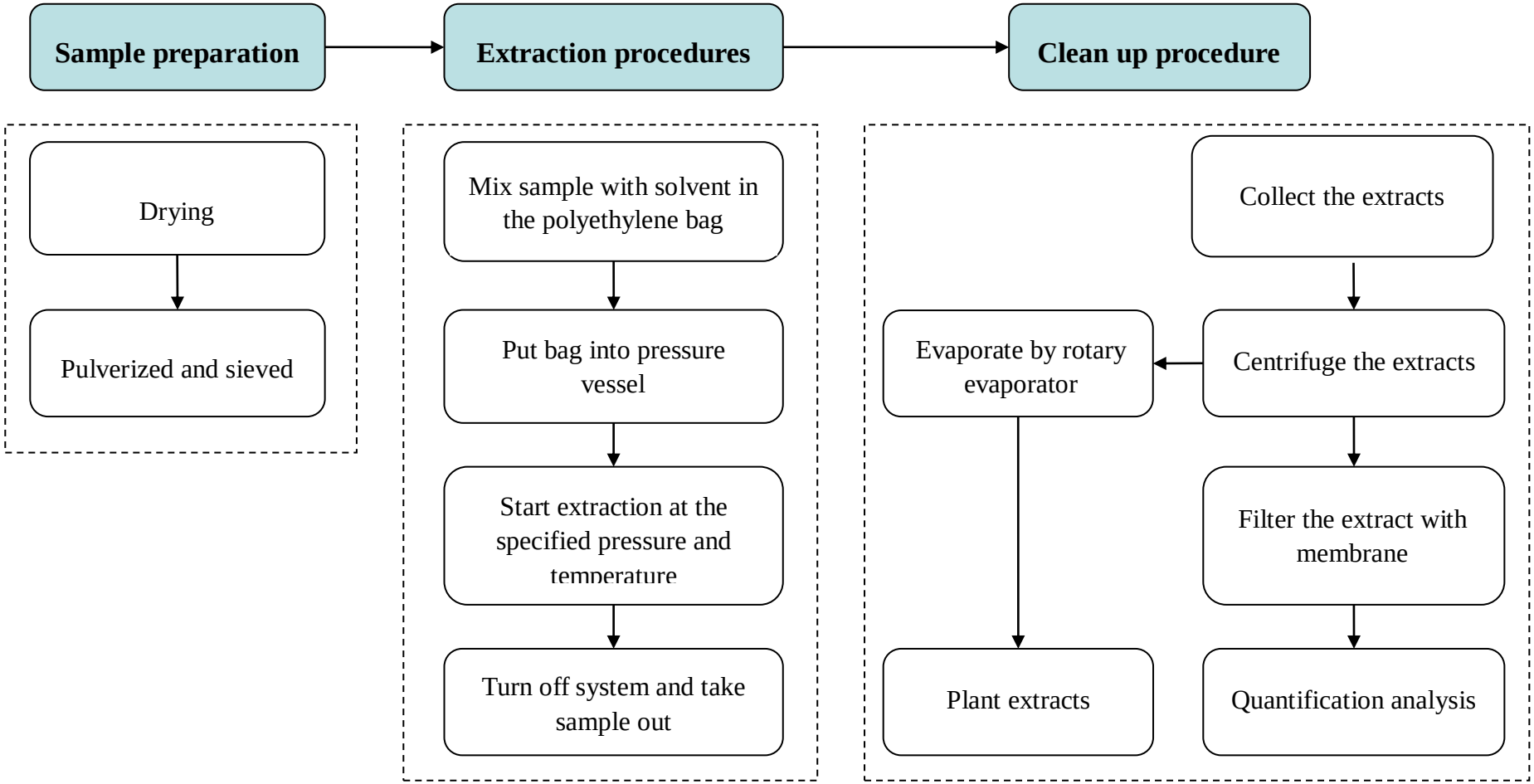

Figure 2. Schematic procedures of UPE processing.

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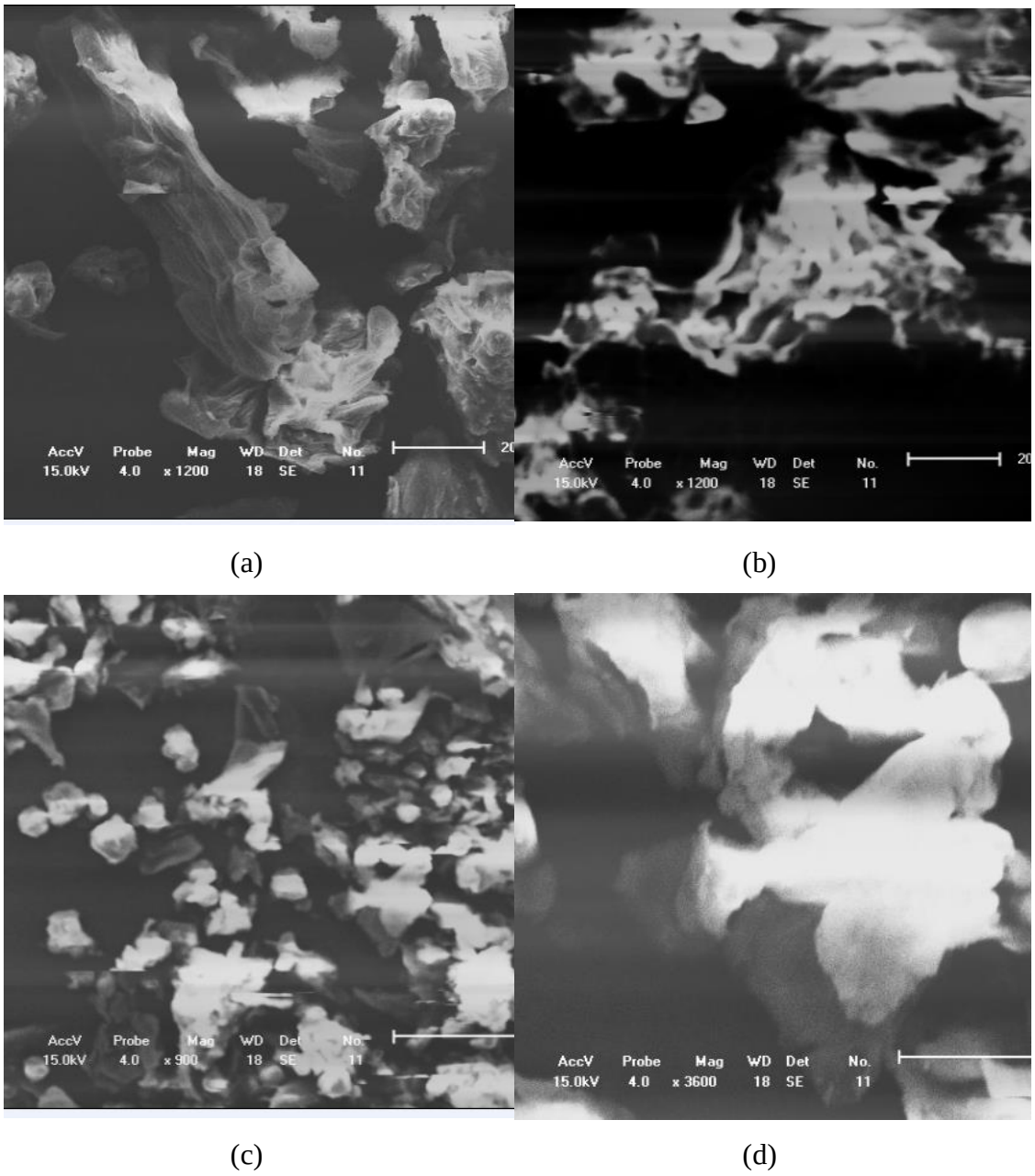

Figure 3. Electron micrograph of ginseng sample: untreated (a, 1200×magnification); after reflux extraction (70% ethanol, 80℃, 6 h) (b, 1200×magnification); after ultrahigh pressure extraction (70% ethanol, 200 MPa, 60℃, 5 min) (c, 900×magnification) and (d, 3600×magnification).

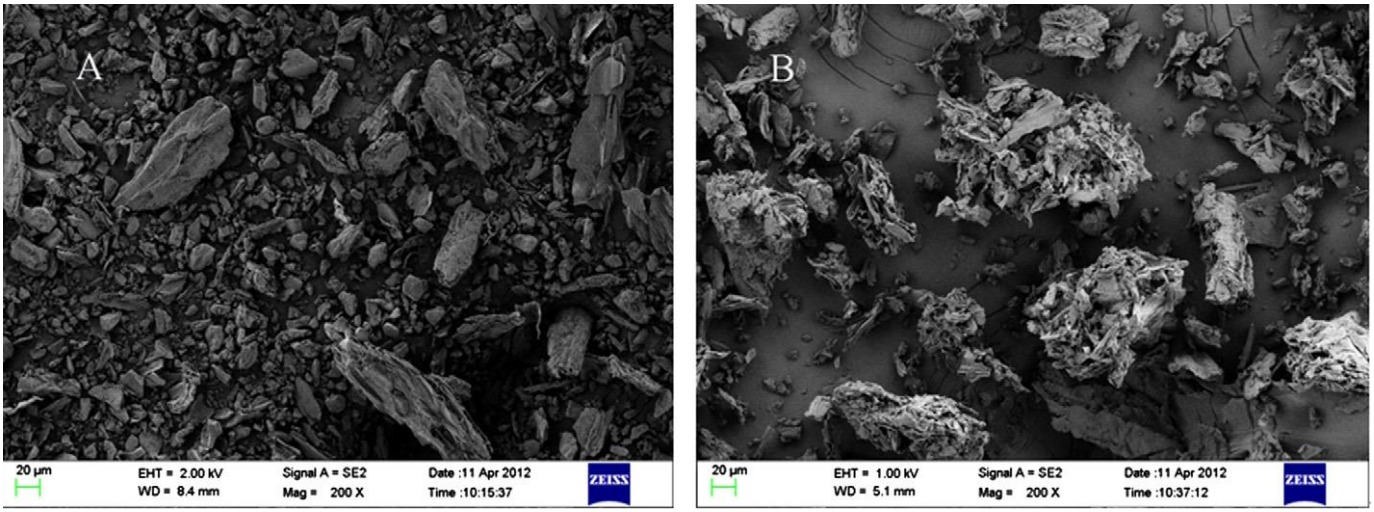

Figure 4. Scanning electron micrographs of the control samples and UPE samples of *Salvia miltiorrhiza* Bunge. A, untreated leaves; B, after ultrahigh pressure extraction.

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