Separation and Purification Technology 66 (2009) 340–346

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

Effects of ultrahigh pressure extraction conditions on yields and antioxidant activity of ginsenoside from ginseng

Ruizhan Chen[a,b,*], Fanlei Meng[a], Shouqin Zhang[c], Zhiqiang Liu[b]

[a] College of Chemistry, Changchun Normal University, Changchun 130032, China
[b] Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China
[c] College of Biological and Agricultural Engineering, Jilin University, Changchun 130025, China

ARTICLE INFO

Article history:
Received 15 September 2008
Received in revised form 1 December 2008
Accepted 17 December 2008

Keywords:
Investigation
Orthogonal experiment optimization
Ginsenoside
Ultrahigh pressure extraction
Antioxidant activity

ABSTRACT

Ultrahigh pressure technique was employed to extract ginsenosides from roots of ginseng (*Panax ginseng* C.A. Meyer). The optimal conditions for ultrahigh pressure extraction (UPE) of total ginsenosides were quantified by UV–vis spectrophotometry with the ginsenoside Re as standard, the signal ginsenosides were quantified by HPLC and ELSD with ginsenosides Re, Rg_1, Rb_1, Rc and Rb_2 as standards. Orthogonal design was applied to evaluate the effects of four independent factors (extraction pressure, extraction temperature, extraction time and ethanol concentration) on the yield and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of ginsenoside, which are based on microwave extraction (ME), ultrasound extraction (UE), soxhlet extraction (SE) and heat reflux extraction (HRE) method. The results showed that UPE method can produce ginsenoside with the highest yield and the best radical scavenging activity compared to other used ones. Scanning electron microscopic (SEM) images of the plant cells after ultrahigh pressure treatment was obtained to provide visual evidence of the disruption effect. The optimal conditions of UPE were obtained at extraction pressure 200 MPa, extraction temperature 60 °C, extraction time 5 min and ethanol concentration 70%. The results of UPE showed obvious advantages in higher efficiency, shorter extraction times, at lower energy costs. In addition, the UPE can be carried out at lower temperatures without any chemical degradation reactions which are very suitable for the thermally unstable active ingredients extraction and separation.

1. Introduction

Ginseng, the root of *Panax ginseng* C.A. Meyer (Araliaceae), is one of the best-known Chinese traditional herbal medicines [1]. It has gained widely attention due to its potential contribution to improve the quality of life in a middle-aged population [2]. The main active ingredients of ginseng are saponins [3], more than 30 of which have been identified. However, six of these (Rg_1, Re, Rb_1, Rc, Rb_2 and Rd) have been reported to account for more than 90% of the saponin content of the root [4], these ginsenosides have been shown to have various therapeutic activities, including antifatigue, anticancer, antidiabetic, antiaging and antioxidant effects [5]. It is normally a time consuming process to extract saponins using traditional techniques. For example, a conventional 3 h reflux at 75 °C using 80% methanol and repeated four times is the current standard extraction method for saponins in the laboratory [6]. However, for the mass production of ginseng extracts, such as in manufacturing various ginseng products, longer periods of more than 40 h extraction with ethanol are being used [7]. Accordingly, the conventional processes are required to be improved from the extraction time and energy consumption points of view.

Extraction is the first important step for the recovery and purification of active ingredients of plant materials. The traditional techniques of solvent extraction from plant materials are mostly based on the correct choice of solvents and the use of heat and/or agitation to increase the solubility of materials and the rate of mass transfer. Usually, the traditional techniques for extraction are time consuming and with low efficiency. Moreover, many natural products with low thermal stability may degrade and lose their biological activities during thermal extraction. On the other hand, the progress in extraction technology has developed newer and simpler sample preparation methods such as ME [8,9], UE [10], supercritical carbon dioxide extraction [11]. For example, pressurized liquid extraction (PLE) [12], and its application in the extraction and analysis of saponins component have been discussed elsewhere. Recently application of ultrahigh pressure method has been widely used in the extraction of various phytochemicals, such as saponins [13,14], flavonoids [15–17], polysaccharides [18], and polyphenols [19], which are from various parts of plant. The extrac-

* Corresponding author at: College of Chemistry, Changchun Normal University, Changchun 130032, China. Tel.: +86 43186168868; fax: +86 43186168868.
E-mail address: ruizhanchen@163.com (R. Chen).

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doi:10.1016/j.seppur.2008.12.026

tion of organic compounds from various plant materials can be significantly improved with the aid of ultrahigh pressure, achieving higher product yields at reduced processing time, energy and solvent consumption. In addition, ultrahigh pressure extraction can be carried out at lower temperatures, avoiding thermal damage to extracts and loss of volatile components. It has been suggested that the improvement of solvent extraction from plant material by ultrahigh pressure is mainly due to the mechanical effects of the rapid pressure changes, which enhances both solvent penetration into the plant material and the intracellular product release by disrupting the cell walls [20].

In this paper, ultrahigh pressure method was first used to extract saponin components from roots of ginseng. The parameters, which might affect the extraction efficiency, including extraction pressure, extraction temperature, extraction time, extraction solvent, ethanol concentration and solvent–sample ratio were studied in detail. The DPPH radical scavenging activity of crude extracts was determined via measuring the antioxidant activity in vitro.

2. Experimental

2.1. Materials

Freshly harvested 4-year-old ginseng roots that had been grown in Fusong County in the northeast of China, were washed several times with water, dried at 50 °C in an oven until a constant weight was obtained before use. The reference standards ginsenoside (Rg_1, Re, Rb_1, Rc and Rb_2) were obtained from the Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). DPPH (Sigma chemical company, St. Louis, MO, USA), HPLC-grade methanol, acetonitrile and acetic acid (Fisher Scientific, USA) were used as-received. All other chemicals with analytical grade were obtained from Beijing Reagent Company (Beijing, PR China). All solvents and non-preconcentrated sample solutions were filtered through 0.45 μm nylon membrane filters. All aqueous solutions (doubly de-ionized distilled water) were prepared from a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. HPLC–ELSD analysis

HPLC system Agilent1100 (Agilent, USA) equipped with quaternary pump, vacuum degasser, autosampler, evaporative light-scattering detector (Alltech ELSD 2000, USA), column heater–cooler and ChemStation system was used in HPLC–ELSD analysis. The chromatographic separation was performed on a Agilent Zorbax Extend-C_{18} column (5 mm, 4.6 mm × 150 mm) from Agilent Company (Beijing, PR China). A linear gradient elution of A (CH_3COOH:H_2O—0.02:100) and B (CH_3CN) was used. The gradient is presented in Table 1. The solvent flow rate was 0.5 mL/min and column temperature was set at 35 °C. The ELSD conditions were drift tube temperature 110 °C, gas flow (N_2) 2.7 L/min, gain 1, and impactor off.

2.3. Determination of total ginsenosides contents

The content of total ginsenosides was determined by the colorimetric method. The ginsenoside standard (Re) was used to construct a standard curve. The sample solution was evaporated by heating at 50 °C, and mixed with 0.2 mL of acetic acid containing 5% (wt.%) vanillin and 0.8 mL perchloric acid at 60 °C for 15 min. The concentration of total ginsenosides was determined using a spectrophotometer (Beckman U700, US) at 548 nm with solvent as blank, against a calibration curve established with a panaxtriol standard. According to $y = 4.0367x - 0.0383$ ($r^2 = 0.9986$) (y (mg) is the content of ginsenoside Re of solution for colorimetric analysis, and x is the absorbance at Vis 548 nm). The estimation of total ginsenosides in the fractions was carried out in triplicate and the results were averaged.

Table 1
Solvent composition of the gradient of the HPLC analysis.

Time (min)	A (%)	B (%)	Curve
0	75	25	–
2	75	25	Linear
60	50	50	Linear

A, 0.2% CH_3COOH (%) aqueous; B, acetonitrile.

2.4. Ultrahigh pressure extraction

The roots of ginseng were powdered and sieved to produce samples with particle sizes in the range between 60 mesh and 80 mesh. The dried ginseng powder (1 g) was mixed with 50 mL 70% ethanol, and soaked for 24 h, then was subjected to an ultrahigh pressure inside a ultrahigh pressure machine (Da Long machine factory, Shanghai, China) with a pressure and temperature control for a given period, afterwards rapid release of pressure. The extracts were prepared and determined as 2.10, 2.2 and 2.3. The schematic diagram of ultrahigh pressure extraction systems was as shown in Fig. 1.

2.5. Microwave extraction

A microwave extractor (MSP-100D, Beijing Rayme Sci. & Technology Co. Ltd., Beijing, PR China) equipped with a pressure control and a second time-base design. Sample was weighed exactly (1.0 g) and soaked for 24 h, then placed in a 250 mL quartz extraction vessel equipped with reflux system. All the microwave extractions were performed under a set microwave irradiation power (1000 W) for a certain period of time (10 min) in 50.0 mL of 70% ethanol. After extraction, the vessels were allowed to cool to room temperature before opening. The extracts were prepared and determined as 2.10, 2.2 and 2.3.

2.6. Ultrasonic extraction

Sample was weighed exactly (1.0 g), and mixed with 50 mL of the extraction 70% ethanol, and soaked for 24 h in a 250 mL reagent

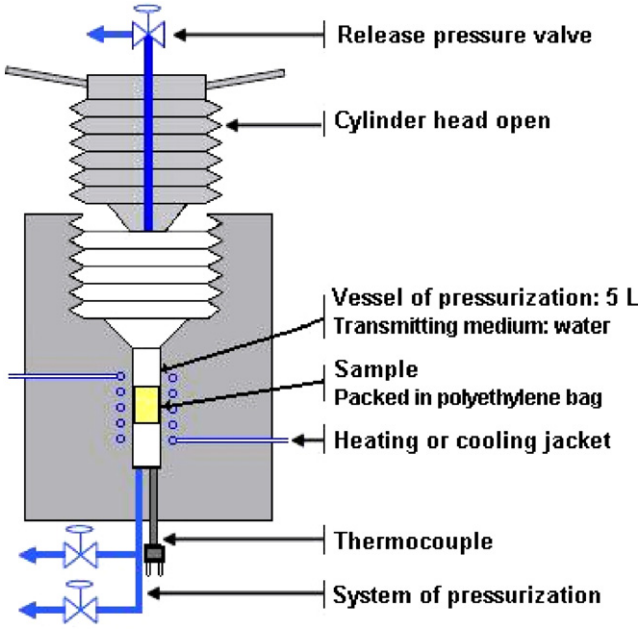

Fig. 1. Schematic diagram of ultra high pressure extraction systems.