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Subcritical water extraction of bioactive components from ginseng roots (*Panax ginseng* C.A. Mey)

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ABSTRACT

The feasibility of applying subcritical water extraction (SWE), which is considered to be an environmentally friendly and efficient extraction technology for the extraction of bioactive components from ginseng roots, was evaluated by comparing it with conventional water (WE) and ethanol (EE) extraction methods SWE was conducted at different temperatures ranging from 120 to 200 °C, and WE and EE were performed by solid–liquid heating extraction methods using water or 70% (v/v) aqueous ethanol as a solvent, respectively. SWE showed significantly and markedly higher extraction yields of total sugar (TS), total protein (TPro), phenolic components (TP), and more potent antioxidant activities than WE and EE. The optimized temperature for TS, TP, and antioxidant activities was 200 °C, and that for TPro was 180 °C. Although vanillin–perchloric acid colorimetric quantification showed that SWE yielded more total ginsenosides (TG) than WE and EE, ultrafast liquid chromatography tandem mass spectroscopy (UFLC–MS/MS) analysis revealed that SWE induced extensive hydrolysis of the ginsenosides, except for Rg_2. At 160 °C, SWE yielded 9.7- and 6.2-fold more Rg_2 than WE and EE, respectively. In comparison, the extraction yields of R_1, Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rg_1, and Ro decreased significantly with the increase of SWE temperature. These findings suggested that SWE is a promising environmentally friendly and efficient technology for extracting bioactive polysaccharides, proteins, TP, and Rg_2 from ginseng roots, but it has potency to induce extensive hydrolysis of ginsenosides, such as Rb_1 and Re.

1. Introduction

Ginseng, the dry root of *Panax ginseng* C.A. Mey. (Araliaceae), is a well-known traditional Chinese medicine with numerous pharmacological effects, including antioxidant (Jung et al., 2006), anti-inflammatory (Lee et al., 2008), antidiabetic (Yun et al., 2004), antineoplastic (Baek et al., 1995), cardiovascular (Chen, 1996), immunoregulatory (Du et al., 2008), and neuroregulatory activities (Tsang et al., 1985; Rausch et al., 2006; Ru et al., 2015). These bioactivities are attributed to various bioactive components in ginseng, such as the ginsenosides, polyphenols, amino acids, and polysaccharides (Lee et al., 2015a, 2015b; Wu and Zhong, 1999). To extract these bioactive components from ginseng, various conventional extraction methods have been developed, among which the conventional solid–liquid heating extraction method using aqueous or organic solvents such as methanol, ethanol, and *n*-butanol (Jung et al., 2006) is widely used. However, most of these conventional methods are time-consuming, energy-inefficient, and involve some organic solvents that are potentially toxic to the environment or human health (Lee et al., 2014; Mlyuka et al., 2016). Therefore, there is a need for new extraction technologies with low processing costs, mild operating conditions, short processing times, and environmentally friendly solvents.

Subcritical water extraction (SWE) is considered to be an environmentally friendly extraction method due to it involving the use of water. Under ambient conditions, water acts as an extremely polar solvent and cannot be used for the extraction of moderately polar and non-polar compounds (Yan et al., 2017). However, subcritical water has a lower dielectric constant (ε) and lower viscosity but higher diffusivity, which promotes diffusion into the plant matrix and the release of moderately polar and non-polar compounds from the solid to the liquid phase (Teo et al., 2010). Therefore, SWE has been used extensively for extracting active ingredients from traditional medicinal plants, with the advantages of a short extraction time, high efficiency, and low energy consumption (Gong et al., 2015; Pavlić et al., 2016). For example, SWE has been successfully used for the extraction of total phenols and total flavonoids from the herbal dust of sage (Pavlić et al., 2016) and flower

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residues of marigold (Xu et al., 2015). Compared with conventional methods, SWE showed significantly higher extraction yields of total phenols and total flavonoids within a very short extraction time. In addition, SWE was used for the extraction of polysaccharides from *Grifola frondosa* (Yang et al., 2013) and triterpenoids from dry loquat leaves of *Eriobotrya japonica* (Mlyuka et al., 2016), and showed higher extraction yields than WE, conventional solid–liquid extraction, and Soxhlet extraction methods.

However, little research has been conducted on the feasibility of SWE as a rapid and efficient method for extracting bioactive components from ginseng. In a recent study conducted by Lee et al. (2014) SWE extracts of ginseng leaves and stems at 190 °C showed much higher cytotoxicity against human cancer cell lines than ethanol extract (Lee et al., 2014). However, the differences in chemical profiles of SWE and ethanol extract were not clearly elucidated in this previous study.

Therefore, the main objective of the present study was to evaluate the feasibility of using SWE for the extraction of bioactive components from ginseng roots. Considering the significant effects of temperature on the ε, viscosity, surface tension, and molecular diffusion rate of water, which would eventually influence the extraction yield of target components (Smith, 2002; Al-Farsi and Lee, 2008), SWE was conducted at different temperatures ranging from 120 to 200 °C. The extraction yields of total sugar (TS), total protein (TPro), total polyphenols (TP), total ginsenosides (TG), and ten major ginsenosides, as well as the antioxidant activities of the extracts, were evaluated and further compared to those of conventional EE and WE methods.

2. Materials and methods

2.1. Materials, reagents and standards

Ginseng samples were purchased from Beijing Tong Ren Tang (Group) Lit. Corp (Beijing, China). Dried samples were ground into powder (60 mesh) using a pulverizer, sealed and stored at −20 °C until analysis. Ten reference standards of ginsenosides, including 20(*S*)-quinquenoside R_1 (R_1), 20(*S*)-ginsenoside Rb_1 (Rb_1), 20(*S*)-ginsenoside Rb_2 (Rb_2), 20(*S*)-ginsenoside Rb_3 (Rb_3), 20(*S*)-ginsenoside Rc (Rc), 20(*S*)-ginsenoside Rd (Rd), 20(*S*)-ginsenoside Re (Re), 20(*S*)-ginsenoside Rg_1 (Rg_1), 20(*S*)-ginsenoside Rg_2 (Rg_2) and ginsenoside Ro (Ro), were obtained from National Institute for Pharmaceutical and Biological Products (Beijing, China). Coomassie Brilliant Blue G-250, bovine serum albumin (BSA), Folin-Ciocalteu's phenol reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-Azinobis(3-ethyl- benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile, methanol, and formic acid were obtained from Fisher Scientific Co. (Waltham, USA). Other chemicals and reagents (analytical grade) were purchased from Beijing Chemical Co. (Beijing, China). Water was generated by a Synergy 185 Ultrapure Water System (Millipore, MA, USA).

2.2. Conventional water/ethanol extraction

Comparative extraction was conducted using conventional solid–liquid heating extraction method with pure water or 70% (v/v) aqueous ethanol as a solvent, which has been extensively used in the extraction of ginseng (Jung et al., 2006). For both ethanol and water extraction, 5 g of ginseng powder was mixed with 150 mL of extraction solvents, and extracted for 3 h at 100 °C and 60 °C, respectively. Then, the slurry was filtered, and the solid residue was extracted twice under the same conditions. After extraction, all extracts were collected, emerged and evaporated using a rotary evaporator (Rotavapor® R-300, Flawil, Switzerland), respectively. Finally, resulting extracts were transferred to freeze-drying tubes and lyophilized, after which the dried samples were then weighed and stored at −20 °C until further analysis.

The total extraction yield (*Ye*) was calculated by using the following equation:

$$Ye = W_1/W_0 \times 100\%$$

Where W_1 and W_0 are weights of extract and ginseng samples in gram on dried basis, respectively.

2.3. Subcritical water extraction

SWE was carried out using an SWE apparatus (Jiangsu Huaan Scientific Research Devices Co., Ltd., Nantong, Jiangsu, China) according to a previous report (Yan et al., 2017). 5 g of ginseng powder was loaded into the extraction chamber (stainless steel vessel with an inner volume of 600 mL) packed in the oven with a heating jacket. The oven was connected with pressure and temperature sensors, and the parameters were monitored and shown on digital displays. After closing the stainless steel cap, 150 mL of deionized water was injected into the chamber, and nitrogen was employed to remove dissolved oxygen before extraction. The extraction pressure was set at 6.0 MPa and the temperature at 120, 140, 160, 180, and 200 °C, respectively. All samples were extracted twice, 20 min for each time, after which all resulting extracts were filtered under vacuum, lyophilized and finally stored at −20 °C until analysis. The yield of the extract was calculated as described above.

2.4. Determination of fourier-transform infrared spectroscopy

The Fourier-transform infrared (FT-IR) spectroscopy of ginseng extracts were recorded on a Spectrum GX FT-IR spectrometer (Perkin Elmer, Inc). Briefly, ginseng extracts were incorporated with KBr powders (spectroscopic grade), and then pressed into pellets and scanned with a blank KBr as background. The FT-IR spectra were recorded using a DTGS detector in a transmittance mode over the range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1} and 32 scans per sample.

2.5. Determination of total ginsenosides extraction yields

The TG content in extracts were estimated according to the method described in the Pharmacopeia of People's Republic of China (2015 Edition). Briefly, 50 mg extract was dissolved in 20 mL distilled water and extracted with 20 mL water-saturated *n*-butanol for 4 times in a separatory funnel. The upper liquid was evaporated to dryness and redissolved in 10 mL of methanol. Then, 50 μL of the extracts were transferred to glass tubes and evaporated to dryness. After which, 0.5 mL of 1% vanillin-perchloric acid solution (w/v) was added and incubated at 60 °C for 15 min after fully mixed. After incubation, the mixture was cooled down immediately in an ice bath for 2 min, and 5 mL of 77% sulfuric acid solution was added with vigorous shaking. Finally, the absorbance at 540 nm was measured using a UV spectrophotometer (TU 1810, Beijing, China). The TG content (*C*) in extracts was calculated using an external calibration curve plotted using ginsenoside Re as standard (0.01–0.2 mg/g, $r = 0.9989$), and expressed as mg Re equivalents (mg ReE)/g extract. The extraction yield (*Y*) of TG was calculated as $Y = C/Ye$ and expressed as mg ReE/g ginseng roots.

2.6. Determination of total sugar extraction yields

The TS content in freeze–dried ginseng extract was quantified by a slightly modified phenol-sulfuric acid method (Dubois et al., 1956; Lee et al., 2015a, 2015b). In brief, 1.0 mL of water solution of ginseng extracts (0.1 mg/mL) was mixed with 1.0 mL of phenol solution (6%, w/v), after which, 5.0 mL of concentrated sulfuric acid was added dropwise. Then, the mixture was fully mixed and left for reaction for 20 min. Finally, the absorbance at 490 nm was recorded against a blank using a UV spectrophotometer (TU 1810, Beijing, China) at ambient conditions. The content of TS in ginseng extracts (*C*) was calculated based on an