Food Sci. Biotechnol. Vol. 18, No. 2, pp. 565 ~ 569 (2009)

RESEARCH NOTE

Food Science and Biotechnology

ⓒ The Korean Society of Food Science and Technology

Comparison of Preparation Methods for the Quantification of Ginsenosides in Raw Korean Ginseng

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Abstract This study was conducted to evaluate the effects of different preparation methods on the recovery and quantification of ginsenosides in raw Korean ginseng (*Panax ginseng* C.A. Meyer). Eight major ginsenosides (Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rf, and Rg_1) were analyzed by high performance liquid chromatography (HPLC), after which the recovery and repeatability of the extraction of those ginsenosides using 3 different preparation methods were compared [A. direct extraction (DE) method, hot MeOH extraction/evaporation/direct dissolution; B. solid phase extraction (SPE) method, hot MeOH extraction/evaporation/dissolution/C_{18} cartridge adsorption/MeOH elution; C. liquid-liquid extraction (LLE) method, hot MeOH extraction/evaporation/dissolution/*n*-BuOH fractionation]. Use of the DE method resulted in a significantly higher recovery of total ginsenosides than other methods and a relatively clear peak resolution. Use of the SPE and LLE methods resulted in clearer peak resolution, but lower ginsenoside recovery than the DE method. The LLE method showed the lowest ginsenoside recovery and repeatability among the 3 methods. Given that the DE method employed only extraction, evaporation, and a dissolution step (avoiding complicate and time consuming purification), this technique may be an effective method for the preparation and quantification of ginsenosides from raw Korean ginseng.

Keywords: Korean ginseng, ginsenoside analysis, extraction, purification, high performance liquid chromatography

Introduction

Ginseng (*Panax ginseng* C.A. Meyer) is a perennial herb that belongs to the family Araliaceae within the genera *Panax* (1). The roots of ginseng have long been used as a traditional medicine in East Asian countries such as Korea, China, and Japan (2). Ginsenosides have been identified as effective biochemical and medicinal compounds produced by ginseng (3). Indeed, several studies have demonstrated the beneficial effects of ginsenosides, which include anti-tumor, anti-diabetic, and anti-oxidant activities, protection against gastric damage, and angiogensis (4-8). Until now, 26 ginsenosides have been isolated and identified from the root of *P. ginseng* (9), of which Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rf, and Rg_1 are the primary constituents of raw Korean ginseng.

The structural similarity of the ginsenosides has made their separation and identification difficult, and therefore, many analytical approaches have been developed to separate and assay these compounds. Because the compositions of herbal extracts are very complex, sample preparation is the most important step in the development of analytical methods for the analysis of herbal preparations (10,11). Accordingly, a variety of preparation procedures have been utilized to obtain and quantify ginsenosides from ginseng. Some of these procedures involve direct injection of the ginseng extract solution for the high performance liquid chromatography (HPLC) assay (12,13). However, liquid-liquid extraction (LLE) is normally used to clarify the extracts prior to HPLC analysis because it can remove compounds that interfere with analysis (14,15). In addition, solid phase extraction (SPE) has recently been used to purify samples of ginseng extract (16,17).

In the present study, the recovery and quantification of major ginsenosides from raw Korean ginseng obtained using 3 different sample preparation methods was compared.

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Received September 4, 2008; November 11, 2008;
Accepted November 12, 2008

Materials and Methods

Plant material The 5-year-old dried *Panax ginseng* C.A. Meyer roots used in this study were obtained from Punggi Ginseng Cooperative Association (Punggi, Gyeongbuk, Korea) and pulverized to be fine enough to pass through an 80-mesh.

Solvents and standards All solvents (HPLC-grade water, acetonitrile, and methanol) used in this study were obtained from SK Chemicals (Ulsan, Korea). The ginsenosides Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_1, and Rh_2 were purchased from Fleton Reference Substance Co., Ltd. (Chengdu, PR China).

Sample preparation The procedure used to prepare the ginseng extract is shown in Fig. 1. One g of the powdered sample was placed in a 250-mL Erlenmeyer flask and then extracted for 1 hr by refluxing it with 70 mL of 70% MeOH at 80°C. The 70% MeOH was then decanted into another 250-mL Erlenmeyer flask, after which the extraction was repeated. The solvent from the 2 extractions was then combined and filtered into a 500-mL evaporation flask using 20-μm filter paper (Whatman International Ltd., Maidstone, Kent, England). The extract solution was then concentrated by evaporation under vacuum. The dried ginseng extract was then subjected to one of 3 different preparation methods.

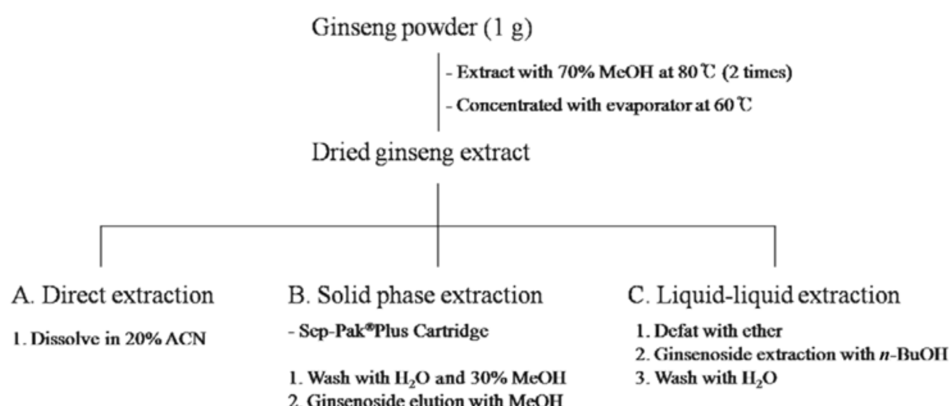

Fig. 1. Procedure for preparation of the ginseng sample from ginseng powder.

***Direct extraction* (*DE*):** The dried ginseng extract was directly dissolved in 25 mL of 20% acetonitrile, after which it was filtered once through a 0.45-μm PVDF syringe filter prior to HPLC analysis.

***Solid phase extraction* (*SPE*):** The dried ginseng extract was dissolved in 25 mL of deinonized water to prepare a sample solution for SPE using a Sep-Pak®Plus C_{18} Cartridge (Waters, Milford, MA, USA). The cartridge was placed in a 10-mL syringe, cleaned with 5 mL of methanol, and then preconditioned with 20 mL of water. Next, 5 mL of the sample solution were applied to the SPE cartridge, which was subsuquently washed with 20 mL of water and then cleaned with 15 mL of 30% MeOH. The ginsenosides were then slowly eluted from the cartridge using 5 mL of methanol. The eluted soltion was then filtered once through a 0.45-μm PVDF syringe filter prior to HPLC analysis.

***Liquid-liquid extraction* (*LLE*):** The dried ginseng extract was dissolved in 20 mL of deionized water and then washed with 20 mL of diethyl ether to remove the fat. The water layer was further extracted with 20 mL of water-saturated *n*-butanol 3 times. Next, the *n*-butanol fraction was combined, cleaned with 20 mL of deionized water twice, and then dried under vaccum. The residue was then dissolved in 25 mL of MeOH and filtered once through a 0.45-μm PVDF syringe filter prior to HPLC analysis.

HPLC analysis Ginsenoside analysis was performed on a Jasco (Tokyo, Japan) HPLC system with a PU-2089 Plus gradient pump equipped with a degasser, an AS-2075 Plus autosampler, and a UV-2075 Plus UV-vis detector. Data were collected using the Jasco chrompass software. Comparative analyses were conducted using a μ-Bondapak (Waters) C_{18} column (3.9×300 mm i.d., 10-μm pore size) with the column temperature set to 35°C. The mobile phase was a binary eluent of (A) water and (B) acetonitrile that was applied under gradient conditions (Table 1) following the method described by Han *et al*. (18). The solvent flow rate was 1 mL/min and the eluted ginsenosides were detected at 203 nm. A stock solution of mixed ginsenoside standards containing the ginsenosides Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rf, Rg_1, Rg_2, Rg_3, Rh_1, and Rh_2 was prepared and diluted to the appropriate concentration for calibration. Four concentrations of the standard solution were then injected into the HPLC, after which calibration curves were prepared by plotting the peak areas versus the concentrations of each standard. Individual ginsenosides from the extracts were then identified and quantified based on the retention time and peak area, respectively, using the calibration curves.

Results and Discussion

HPLC analysis for ginsenosides Usually, buffers are used in elution systems to increase the resolution of ginsenoside separation (13). It has also been reported that the presence of K-phosphate buffer in the mobile phase can produce a satisfactory separation of ginsenosides (19,20). However,

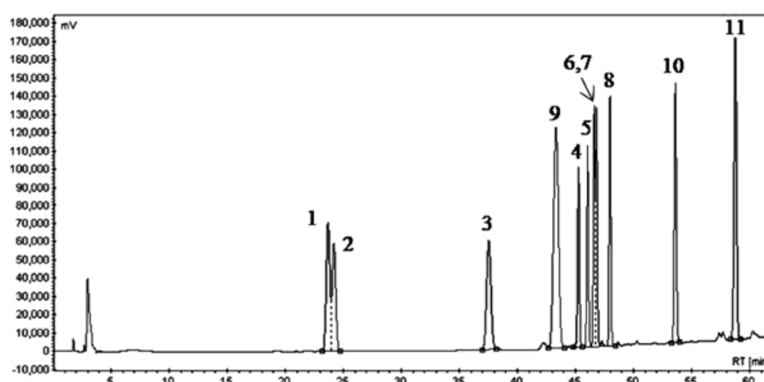

Fig. 2. HPLC chromatogram of the ginsenoside standards. Peaks: 1, Rg_1; 2, Re; 3, Rf; 4, Rb_1; 5, Rc; 6, Rb_2; 7, Rb_3; 8, Rd; 9, Rg_2+Rh_1; 10, Rg_3; 11, Rh_2.

Table 1. Composition of the mobile phase employed in the gradient HPLC system

Time (min)	Composition of mobile phase (%)	
	Water	Acetonitrile
0	80	20
5	80	20
38	67	33
63	20	80
75	20	80
77	80	20
80	80	20

salts containing buffer cannot be stored in the column for a long time; therefore a time-consuming column cleaning process must be performed prior to each assay (16). Accordingly, in this study, acetonitrile and water were used as the mobile phase because they are safe for the column. As shown in Fig. 2, a baseline separation of 12 standard ginsenosides was achieved within 60 min using the gradient system shown in Table 1. This result demonstrates that it is feasible to use the proposed method to separate the 8 major ginsenosides (Rb_1, Rb_2, Rb_3, Rc, Rd, Re, Rf, and Rg_1) found in raw Korean ginseng. In addition, regression analysis of a calibration curve generated using ginsenoside standards revealed that the correlation coefficients (R^2) of all curves were greater than 0.99 when the concentration ranged from 0.04-0.4 mg/mL (data not shown). The chromatograms obtained by HPLC using the 3 different sample preparation methods under the selected chromatographic conditions are shown in Fig. 3. The SPE and LLE methods resulted in better resolution than the DE method. The DE method is considered to be a simple and less time-consuming method than LLE and SPE, however, co-extraction with other components of the matrix can occur during the DE process, thereby lowering its selectivity

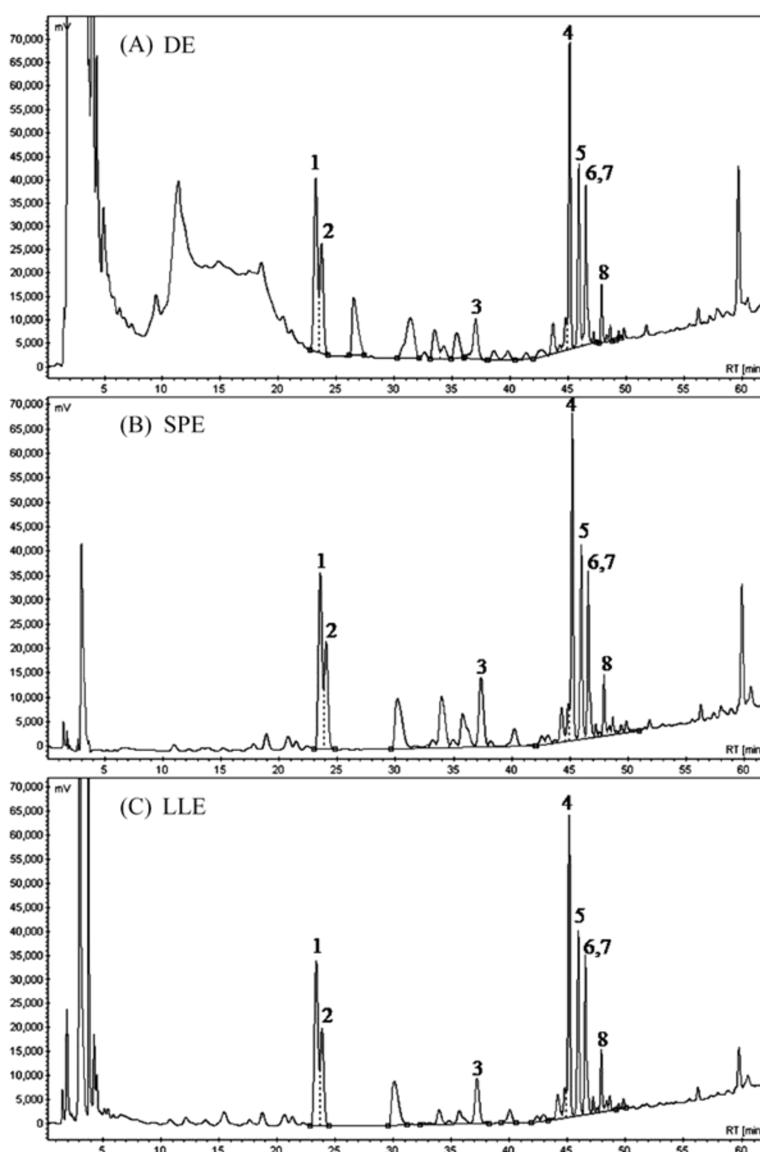

Fig. 3. Elution profiles obtained using 3 different sample preparation methods by HPLC. Peaks: 1, Rg_1; 2, Re; 3, Rf; 4, Rb_1; 5, Rc; 6, Rb_2; 7, Rb_3; 8, Rd.

Table 2. Comparison of the concentration of ginsenoside recovered from raw Korean ginseng using different sample preparation methods[1)]

Ginsenoside	DE		SPE			LLE		
	Conc. (mg/g)	RSD[2)] (%)	Conc. (mg/g)	RSD (%)	Rel. recovery[3)] (%)	Conc. (mg/g)	RSD (%)	Rel. recovery (%)
Rg_1	3.15	1.2	2.75	1.8	87.14	2.51	3.6	79.72
Re	2.28	1.5	1.98	2.4	86.84	1.75	3.3	77.02
Rf	0.88	5.6	1.17	2.5	133.45	0.80	7.5	91.25
Rb_1	4.05	2.7	3.94	1.9	97.22	3.53	4.3	87.11
Rc	2.49	3.9	2.43	2.0	97.84	2.21	4.3	88.92
Rb_2+Rb_3	1.99	1.9	1.81	2.1	91.28	1.66	4.0	83.31
Rd	0.54	4.1	0.55	2.4	101.40	0.50	4.7	92.94
Total	15.37		14.62		95.14	12.96		84.32

[1)]DE, direct extraction; SPE, solid phase extraction; LLE, liquid-liquid extraction.
[2)]Relative standard deviation, n=10.
[3)]Relative recovery expresses the mass recovered using the SPE and LLE methods as a % of the mass recovered using the DE method.

(21). As shown in Fig. 3A, a large amount of co-extracted components (hydrophilic compounds such as saccharides and phenolic acids) were eluted as unbound fractions (retention time 1.5-22 min) when the DE method used. Conversely, hydrophilic compounds and polysaccharides were removed by LLE and SPE, which explains why these methods produced a clearer baseline than DE. However, none of the compounds that were co-eluted during the DE interfered with the 8 major ginsenosides (Fig. 3A). Taken together, these results suggest that the 3 sample preparation methods evaluated here were capable of identifying and quantifying individual ginsenosides without matrix background interference.

Comparison of the 3 different ginsenosides preparation methods The amount of individual ginsenosides extracted using the 3 different sample preparation methods were also evaluated by HPLC. The DE method yielded a significantly higher concentration of 6 of the 8 major ginsenosides (all except Rf and Rd) when compared to the SPE method (Table 2). Previously recovery experiments conducted to determine the accuracy of SPE for the quantification of the 12 major ginsenosides present in American ginseng root extract revealed that the recovery rates ranged from 89.5 to 97.2% (16), which is similar to the results of the present study. Specifically, when compared to the amounts recovered by DE, the relative recovery rates of ginsenosides extracted using SPE ranged from 86.84 to 101.40%, with the exception of Rf, which had a recovery rate of 133.45%. These findings suggest that SPE can purify ginseng extracts without serious ginsenoside loss. The concentration of all 8 major ginsenosides recovered using the LLE method was significantly lower than the concentration recovered using the DE and SPE methods. Specifically, extraction using the LLE method resulted in recovery rates of 77.02 to 92.94% when compared to the concentration of ginsenosides recovered using the DE method (Table 2). Additionally, the LLE method provided the lowest repeatability (RSD) of the 3 methods. Even though the LLE method has been used to purify ginseng extracts prior to HPLC analysis (14,15), many studies have reported problems with this method (16,17). Specifically, the LLE method can be time consuming, is not easy to manipulate, requires large amounts of organic solvent and may have lower extraction efficiencies.

In this comparative study, we compared the efficiency of different methods used to extract raw Korean ginseng. Although the SPE and LLE methods showed clearer peak resolution than the DE method, the highest recovery of total ginsenosides was obtained using the DE method. The LLE method was found to have the lowest ginsenoside recovery and repeatability of the 3 methods. Given that the DE method consisted of only extraction, evaporation, and dissolution, with no additional purification steps, this method can be used to effectively prepare and quantify ginsenosides from raw Korean ginseng.

Acknowledgments

This work was supported by a Korea Food Research Institute grant (No. E080601).

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