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Changes in Chemical Composition of Korean Red Ginseng (*Panax ginseng* C.A. Meyer) Extract With Alcohol Extraction

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Abstract

We extracted red ginseng with various alcohol concentrations and evaluated total carbohydrate, uronic acid, polyphenols compounds and ginsenoside contents, and yields of alcohol extract. The water extraction (0% alcohol extraction) showed a high level of total carbohydrate content. 10% and 20% alcohol extraction showed the highest uronic acid contents (7,978.8 and 7,872.7 μg/mL of extract, respectively). The efficiency order of the red ginseng extract (RGE) preparations in liberating polyphenols was: 0～50% alcohol≥ 60% alcohol > 70～90% alcohol. Solid contents in RGE were decreased with increased alcohol concentration; the same tendency as with the results of total carbohydrate content. Total ginsenoside contents in 20～50% alcohol extracts showed similar levels (42,962.9～47,930.8 μg/mL of extract). Water extraction showed the lowest ginsenoside content (14,509.4 μg/mL of extract). The ginsenoside contents at above 60% alcohol were decreased with increased alcohol concentration. Generally, ginsenoside (Rg2, Rg1, Rf, Re, Rd, Rb2, Rc and Rb1) contents were increased with increased alcohol concentrations. However, Rg3 content was decreased with increases in alcohol concentration.

Key words: red ginseng, ginsenoside, polyphenols, uronic acid, extraction

INTRODUCTION

Ginsenosides are a class of steroid like compounds, particularly triterpenoid saponins, found exclusively in the roots of plant genus *Panax* (ginseng). Ginsenosides are attributed with cardio-protective, immunostimulatory, anti-fatigue, and hepatoprotective physiological and pharmacological effects (1-3). Commercially available ginseng is classified into fresh, white, and red ginseng. White ginseng is made by peeling the fresh ginseng roots and drying them without steaming. To preserve ginseng for an extended period of time, red ginseng is made by steaming and drying the fresh ginseng, suggesting chemical transformation by heat (4).

Recently, ginseng roots and their extracts have also become popular in the US and Europe as dietary health supplements and additives to foods and beverages. Extraction is the important step for the recovery of bioactive compounds from the plant raw materials. Extraction technologies must be versatile, relatively simple and safe for the operating personnel and the consumers and inexpensive to use. Extraction methods are available for the cultivated and cultured cells of ginseng (5,6). An increasingly common method of processing ginseng is to obtain an extract containing the ginsenosides by mixing dried ginseng powder with aqueous ethanolic solution, then removing the solid residue (7). These studies have recommended 40～50% ethanol for optimal extraction but they have only examined the effect on ginsenosides. However, changes of ginsenoside composition during alcoholic extractions from roots are not available currently.

Therefore, we have conducted extraction of various alcoholic concentrations and evaluated total carbohydrate, uronic acid, polyphenol contents and yields of alcohol extract. This paper describes the development of simple, convenient and optimal method of extraction using ethanol as solvent.

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MATERIALS AND METHODS

Materials

Six-year-old red ginseng was purchased at ginseng market in Geumsan, Korea. Standard ginsenosides including compound K, Rh2, Rh1, Rg5, Rk1, Rg2, Rg3, Rg1, Rf, Re, Rd, Rb2, Rc and Rb1 were purchased from Embo Lab. in Daejeon, Korea. All other chemicals were obtained from local suppliers and of reagent grade.

Extraction of ginsenosides

Extraction methods were based on the protocol of Ando et al. (8), but were simplified and modified for smaller samples. Red ginseng material (5.0 g) was placed in a soxhlet extractor and 100 mL of various alcoholic solutions were added into it. The ginseng was extracted twice, with the extract solution under reflux in a water bath at 70°C, for 3 hr each time. The combined extract was evaporated using a rotary evaporator under vacuum at 45°C. The extract was used for the assay of total carbohydrate, uronic acid, polyphenols and dry weight. Ginsenosides were then assayed in the evaporated residue which was dissolved in 100 mL of distilled water and washed with 100 mL of diethyl ether. The aqueous layer was extracted 3 times with 100 mL of water-saturated *n*-butanol. The butanol solution was washed with 100 mL of distilled water to remove impurities, thereby obtaining crude saponins. The remaining butanolic solution was transferred to a tared round bottom flask for evaporation using a rotary evaporator under vacuum at 60°C.

HPLC analysis of ginsenosides

The levels of 14 major ginsenosides were analyzed by an HPLC method developed by Kim et al. (9) and Li et al. (10). The HPLC system used an ACME 9000 HPLC (Young Lin Instrument Co., LTD, Anyang, Korea) with an ELSD detector. A prevail carbohydrate ES column (4.6×250 mm, Alltech Associates, Inc., Illinois, USA) was also used. The solvent flow rate was held constant at 0.8 mL/min. The column temperature was fixed at 35°C using a column oven. The mobile phase used for the separation consisted of solvent A (acetonitrile : water : IPA=80:5:15) and solvent B (acetonitrile : water : IPA=67:21:12). A gradient elution procedure was used as 0～28 min 90% A, 28～35 min 15% A, 35～45 min 20% A, 45～50 min 25% A, 50～51 min 10% A, 51～57 min 0% A, 57～58 min 75% A, and 58～65 min 90% A. The injection volume was 20 μL for analysis. Peak identifications were based on retention times and comparisons with injected standard samples. All solutions were filtered through 0.45 μm membrane syringe filters (Millipore Co.) before analysis. To determine calibration curves, the ginsenoside standards compound K, Rh2, Rh1, Rg5, Rk1, Rg2, Rg3, Rg1, Rf, Re, Rd, Rb2, Rc and Rb1 were dissolved individually in HPLC-grade methanol, whereupon calibration standards were prepared by mixing different concentrations in appropriate quantities. The level of total ginsenosides was determined by summing the levels of the 14 ginsenosides.

Analytical methods

Total polyphenol (TP) content was determined using the Folin-Ciocalteu method (11), adapted to a microscale using gallic acid as the standard (50～800 μg/mL). Total carbohydrate and uronic acid were determined by the phenol-H_2SO_4 (12) and *m*-hydroxydiphenyl (13) method, respectively, using glucose and galacturonic acid as the respective standards. In all cases, analyses were performed in triplicate, unless elsewhere specified, and values averaged. The standard deviation (SD) was also calculated. All data were analyzed by one-way analysis of variance and Duncan's multiple range tests using the SPSS version 10.0 software (SPSS, Chicago, IL). Results were considered significant at $p<0.05$.

Bitterness evaluation

The extract obtained by the above extraction methods were made up to exactly 20 mL with purified water. The suspension was used to evaluate the bitterness. Sensory evaluations of bitterness were conducted in triplicate by the panels, using a five-point hedonic scale method (14). The bitter taste was evaluated as follows: very low (1 point), low (2 point), medium (3 point), strong (4 point), and very strong (5 point). The results of the sensory evaluations were expressed as the means ±standard deviation (SD) of 10 panelists. The significance was verified *via* Duncan's multiple range tests, using the SPSS software package.

RESULTS AND DISCUSSION

Total carbohydrate content of the red ginseng extract (RGE)

Total carbohydrate content of the RGE is shown in Fig. 1. The water extraction (0% alcohol extraction) showed a high level of total carbohydrate content. Total carbohydrate decreased as alcoholic concentrations increased. However, there was not a significant difference in total carbohydrate between 10% and 60% alcohol ($p<0.05$). 70%～90% of alcohol extractions also had similar total carbohydrate concentrations.

The major components of Korean ginseng are carbohydrates, which include starch, polysaccharides, cellulose, and glycosides. Ginseng mainly consists of carbo-

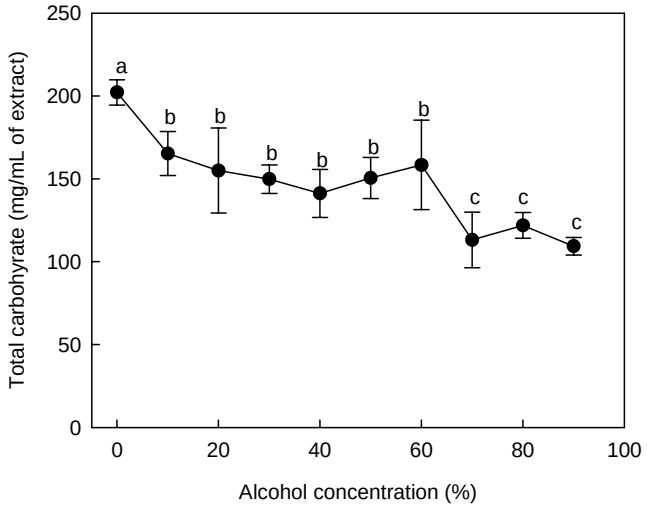

Fig. 1. Changes in total carbohydrate contents of Korean red ginseng extracted with various alcohol concentrations. Different letters at each measurement indicate significant differences among groups ($p<0.05$).

hydrates (60～70 g carbohydrate/100 g solid) and starch is a major component of ginseng carbohydrates (15). Red ginseng with inner white showed not only less dense tissue structure but also relatively small amounts of starch (16). The occurrence of inner hole and inner white in red ginseng increased when the amount of starch in fresh ginseng decreased (17). Therefore, water extraction showed a large amount of total carbohydrate contents because of water solubility.

Uronic acid content of RGE

Uronic acid (as acidic polysaccharide) contents of RGEs are shown in Fig. 2. 10% and 20% alcohol extraction showed the highest uronic acid content (7,978.8 and 7,872.7 μg/mL of exract, respectively). The uronic acid contents were decreased with alcohol concentrations above 30%. Especially, the contents were sharply decreased at alcohol concentration above 50%.

Panax ginseng may have a pectin-type polysaccharide mainly composed of galacturonic and glucuronic acids (93%). This uronic acid content appears to be significantly higher than previously reported (18), and this can probably be attributed to improved techniques for the separation and calculation of neutral and uronic acids. Uronic acids obtained from plant sources have been shown to exhibit a variety of biological activities, including immunostimulatory, antioxidant, antitumor, and antiviral properties (19). A uronic acid with immunomodulating activity, which was obtained from *P. ginseng* leaves, was found to consist of a highly branched glycan structure, composed of arabinose, galactose, rhamnose and galacturonic acid with a β-(1,3)-linked galactan backbone (20).

Polyphenol and solid contents of RGE

Polyphenols are considered secondary metabolites that are synthesized in plants and function as a defense mechanism in response to various stress conditions (21). The observed increase in total polyphenols in roots of *P. ginseng* was accompanied by increased flavonoid content, total proteins and antioxidant activity. Induced polyphenols accumulation may also have an impact on biological activities of ginseng.

As shown in Fig. 3, the efficiency order of the RGE preparations in liberating polyphenols were: 0～50% alcohol≥ 60% alcohol> 70～90% alcohol. There were not a significantly difference of polyphenol contents between

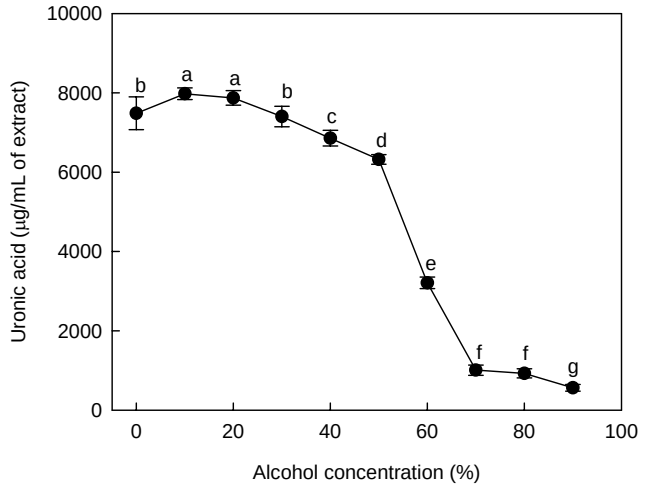

Fig. 2. Changes in uronic acids contents of Korean red ginseng during extracted with various alcohol concentrations. Different letters at each measurement indicate significant differences among groups ($p<0.05$).

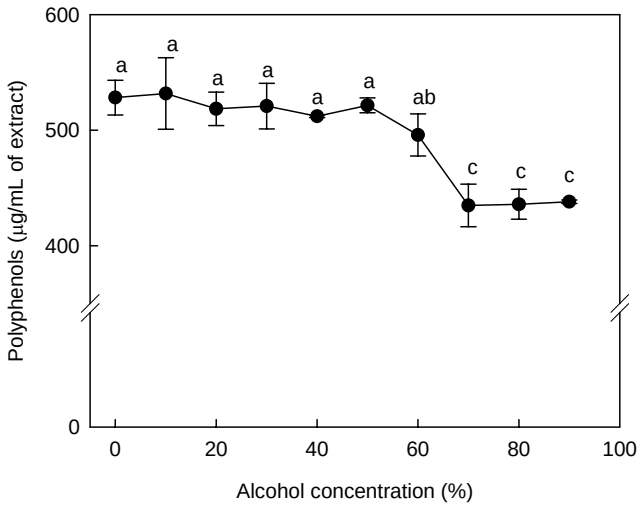

Fig. 3. Changes in polyphenols contents after extraction of Korean red ginseng with various alcohol concentrations. Different letters at each measurement indicate significant differences among groups ($p<0.05$).

0～50% and 60% alcohol extraction. Polyphenols contents tended to decrease with increased alcohol concentration. In general, polyphenols were extracted very well with about 70% alcohol, but alcohol extraction below 50% showed a higher level of polyphenols than alcohol extraction at above 50%. The polyphenols in ginseng were assumed to have high contents of glycoside type polyphenols on the basis of high extraction level by alcohol extraction below 50%. The major components of ginseng are ginsenosides, which are glycosides with a dammarane skeleton aglycone (22).

Besides saponin, *P. ginseng* also accumulates other secondary metabolites (phenolic compounds), and the growth of 4～6 years is needed for proper accumulation of secondary metabolites. Tissue culture is an important tool of plant biotechnology and one of its potential applications is for the production of valuable plant secondary metabolites (21,23). And therefore, development of an efficient root culture system for commercial production of ginseng root requires integrated enhancement strategies and increased extraction yields of polyphenols.

As shown in Fig. 4, solid contents also showed the same tendency with total carbohydrate contents. The contents showed a decreasing tendency with increases in alcohol concentration. However, there were not a significant difference of solid contents between 20% and 60% alcohol. Above results indicate that water extraction increases the solid content due to increases of total carbohydrate, acid polysaccharide and polyphenols contents.

Ginsenoside composition of RGE

Ginseng saponins (ginsenosides) are the principal components having pharmacological and biological activities, such as antidiabetic, and anti-tumor activities (24). More than 30 different ginsenosides so far have been isolated and identified from ginseng saponins.

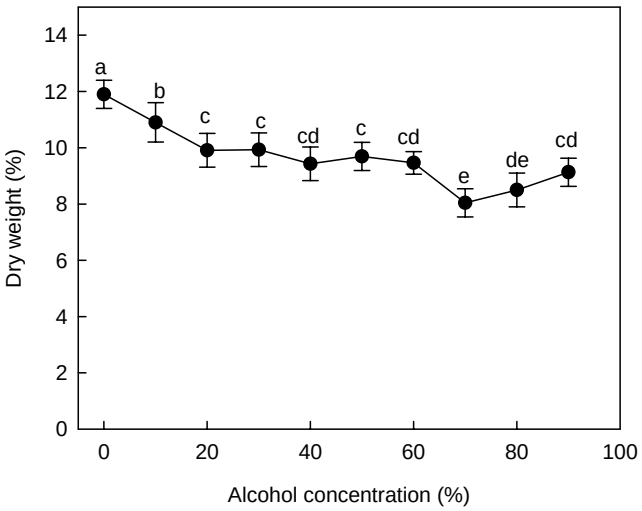

Fig. 4. Changes in solid contents after extraction of Korean red ginseng with various alcoholi concentrations. Different letters at each measurement indicate significant differences among groups ($p<0.05$).

Ginsenoside compositions of RGEs are shown in Table 1. Total ginsenoside contents in 20～50% alcohol extracts showed similar levels (42,962.9～47,930.8 μg/mL of extract). Water extraction showed the lowest ginsenoside content (14,509.4 μg/ mL of extract). The contents at above 60% alcohol were decreased with increase of alcohol concentration. Almost all ginsenoside (Rg2, Rg1, Rf, Re, Rd, Rb2, Rc and Rb1) contents were increased with increased alcohol concentration. However, Rg3 content was decreased with increases of alcohol concentration.

Extraction trials were carried out at room temperature or using heat (25) or sonication (26) to enhance recovery of ginsenosides. The use of heat in the extraction procedure was proven to degrade the thermally unstable malonyl-ginsenosides into the corresponding neutral ginse-

Table 1. Changes of ginsenoside compositions during extraction with various alcohol concentration from Korean red ginseng

Ginsenoside	Concentration (μg/mL of extract)									
	Water	10% EtOH	20% EtOH	30% EtOH	40% EtOH	50% EtOH	60% EtOH	70% EtOH	80% EtOH	90% EtOH
Compd K	0	0	0	0	0	0	0	0	0	0
Rh2	0	0	0	0	0	0	0	0	0	0
Rh1	118.8	115.1	113.4	116.4	118.4	112.1	122.3	0	0	0
Rg5+RK1	1850.7	2293.9	1336.8	407.5	410.4	1607.8	1245.8	389.3	585.6	701.5
Rg2	340.2	538.1	582.5	538.1	521.8	521.6	429.1	304.2	280.0	359.2
Rg3	453.6	495.8	317.4	219.5	196.6	314.6	245.6	176.4	163.4	206.4
Rg1	348.5	1063.3	1359.6	1241.4	1232.3	1120.2	861.8	509.6	419.1	684.7
Rf	282.5	697.6	895.6	790.3	766.2	770.7	563.4	402.1	291.6	469.6
Re	1576.0	4961.1	6717.5	6085.8	6155.4	5464.7	4241.4	2704.5	2076.7	3422.5
Rd	1457.7	4434.5	5894.5	5617	5945.9	5636.2	3810.3	2492.4	1882.4	3059.2
Rb2	1192.3	3000.5	3829.3	3459	3474.9	3613.6	2821.9	2066.4	1445.0	2358.5
Rc	3122.8	8502.6	10967.6	10195.9	10168.3	10323.5	7802.5	5048.2	3746.2	6544.0
Rb1	3766.3	12577.8	15916.6	14292.0	14226.3	14382.0	11164.1	6839.3	4660.5	8742.2
Total	14509.4	38680.3	47930.8	42962.9	43216.5	43867.0	33308.2	20932.4	15550.5	26547.8

nosides. Court et al. (27) showed that, while partial degradation (50%) occurred after 5 hr of extraction using methanol in a soxhlet apparatus, a minimum of 20 hr were necessary to achieve total conversion. Preparation of Korean red ginseng relies on steam processing to ensure preservation which may alter the ginsenoside composition. Steaming ginseng has been shown to produce ginsenosides that are not present in raw ginseng (25). Differently from white ginseng, which is obtained from the dried roots of *P. ginseng*, red ginseng is produced by steaming and drying up the roots of *P. ginseng*. This heating procedure causes degradation of the malonyl ginsenosides m-Rb1, m-Rb2 and m-Rc and m-Rd in the ginsenosides Rb1, Rb2, Rc and Rd, respectively. Ginseng root and leaf hot water reflux extract were found to have 23 and 19 different ion fragments, respectively, whereas, the 80% aqueous ethanol extract contained 14 fragments. Furthermore, the 80% aqueous ethanol extract contained a malonyl ginsenoside that was not present in the hot water reflux extract. Malonyl ginsenosides are heat-labile and readily demalonylate (28).

However, studies on the effect of drying of American ginseng, based on changes in the neutral ginsenosides, resulted in recommended drying temperatures in the range 20～50°C (29).

Zhang et al. (30) reported the effect of ethanol concentration on the extraction yield of ginsenoside from *P. quinquefolium* L. root (American ginseng). The extraction yield of ginsenoside was improved by increasing ethanol concentration in the range of 10～70%. When the ethanol concentration is higher than 70%, the extraction yield of ginsenoside decreased slowly with increasing of ethanol concentration. It is known that the solubility of neutral and malonyl ginsenosides are varied in different concentrations of ethanol. Therefore, the breakage degree of the cell membrane is different in different concentration ethanol. The protein could be coagulated in higher concentrations of ethanol, making larger diffusion resistance. It was reported (31) that the maximum extraction of neutral, malonyl, and total ginsenosides was obtained with 70%, 40%, and 60% ethanol, respectively.

However, our results showed 20～50% ethanol as optimal extraction concentrations. This discrepancy could be due to the quality variation of products by manufacturer, different drying methods of red ginseng and American ginseng, and genotype-dependent variability in different species of ginseng.

Bitterness evaluation of RGE

Sensory scores of the RGE extracted with various alcohol concentrations are shown in Fig. 5. The bitterness

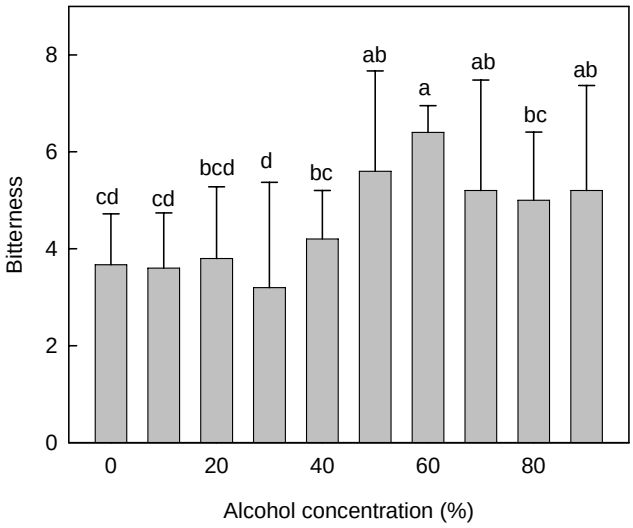

Fig. 5. Changes in bitterness of Korean red ginseng following extraction with various alcoholic concentration. Different letters at each measurement indicate significant differences among groups ($p<0.05$).

values of RGE extracted between 0 and 30% alcohol showed 3.2～3.8, and those of RGE extracted with alcohols above 50% showed 5.0～6.4. Although ginsenoside contents of RGE extracted between 0 and 30% alcohol showed high levels, the bitterness of the RGE extract might be decreased due to increase of total carbohydrate contents.

Saponins are non-volatile, amphiphilic compounds that occur in a wide variety of legume seeds such as peas, soybeans, lentils and lupins (32). They are chemically referred to as triterpene glycosides, and consist of non-polar aglycones coupled with one or more sugar chains (33). A large number of different saponins may occur within a single plant species (34). Ginsenosides are triterpenes saponins considered to be the main bioactive principles of the most important Oriental herbal medicine "ginseng" derived from the roots and rhizomes of different *Panax* species. The bitterness of peas, as well as that of soybeans, has been ascribed to the presence of saponins (35).

Sweetness suppression of bitterness has been demonstrated for sucrose and quinine mixtures and for sucrose and caffeine mixtures (36). An increase in solution viscosity decreased the bitterness of quinine and other solutions (37). Because isoflavones and saponins bind to soy proteins through hydrophobic interactions (38), it seems likely that these phytochemicals bind to milk proteins, and a reduction in bitterness may result. Robinson and others (39) reported using starch to suspend isoflavone aglycons in water with resulting thresholds in the mM range, but not much higher than our thresholds for isoflavones in milk, also supporting the hydrophobic

binding of the saponins to the hydrophobic regions of native starch.

Therefore, RGE extracted between with alcohol concentrations between 0 and 30% alcohol might have high contents of starch, and the bitterness might be reduced owing to the binding of the saponins to the hydrophobic regions of native starch.

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