

The Changes of Ginsenoside Patterns in Red Ginseng Processed by Organic Acid Impregnation Pretreatment

Mi Hyun Kim¹, Young Chul Lee¹, Sang Yoon Choi¹, Chang-Won Cho¹, Jeonghae Rho^{1*}, and Kwang-Won Lee²

¹Korea Food Research Institute, Seongnam 463-746, Korea

²Division of Food Bioscience and Technology, Korea University College of Life Science & Biotechnology, Seoul 136-701, Korea

In order to enhance bioactive functionalities of ginseng, an acid impregnation processing was applied as a pre-treatment in producing red ginseng. Acid impregnation studies were conducted, and acids (ascorbic, malic, and citric acid) were selected. The optimal concentration of each acid was investigated in this study in terms of ginsenoside contents. The most concerned ginsenoside, Rg₃ was increased by ascorbic, malic, and citric acid pre-treated red ginseng up to 1 M acid concentration. In the case of ascorbic acid pre-treated red ginseng, Rg₂ concentration was increased depending on acid concentrations. Citric acid pre-treatment enhanced Rg₂, Rg₃, and Rh₁+Rh₂ formation in red ginseng. Therefore, ginsenoside patterns in red ginseng could be changed by acid impregnation pre-treatment depending on acid concentration and acid types. This research is expected to contribute to the development of the ginseng industry via new red ginseng products with selective and intensified functionality.

Keywords: *Panax ginseng*, Ascorbic acid, Malic acid, Citric acid, Ginsenoside Rg₃, Impregnation

INTRODUCTION

Ginseng (*Panax ginseng* Meyer) has been used as a marvelous folk remedy in the East for thousands of years [1,2]. Recent scientific research showed that the major active compounds of ginseng have positive nutraceutical effects on various diseases such as high blood pressure, hyperlipidemia, liver disease, depression, diabetes, cancer, fatigue, stress, and Raynaud's phenomenon, as well as on the improvement of memory, learning, immune system, cardiovascular system, anti-arteriosclerosis and cholesterol metabolism [3-6]. Such nutraceutical effects of ginseng come from ginsenosides, a class of steroid saponins. Saponins are often subdivided into two main classes, the triterpenoid and the steroid saponins by the structure of aglycone (nonsugar moiety). The triterpenoid saponins are further divided into oleanane-triterpenoid

saponins in *Platycodon grandiflorum* and *Polygala tenuifolia*, and dammarane-triterpenoid saponins in ginseng. Representative dammarane-triterpenoid saponins further subdivided into protopanaxadiol, protopanaxatriol and oleanolic acid by the chemical structure [7-10].

More than forty different ginsenosides have been identified so far, and play an important role in the nutraceutical effects of ginseng. Since the amount of an individual ginsenoside is trace, if the amount of a certain type of ginsenoside can be increased to enhance its specific function, it could be a good option for customers. Especially, ginsenoside Rg₃ suppresses cancer metastasis and cancer drug resistance, and functional food such as Rg₃-enriched red ginseng will draw public attention and contribute to expanding the ginseng market [11,12].

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* Corresponding author

E-mail: drno@kfri.re.kr

Tel: +82-31-780-9060, Fax: +82-31-709-9876

Since red ginseng produced by conventional manufacturing processes contains a small amount of ginsenoside Rg₃, many studies focusing on Rg₃ enrichment are being conducted in Korea and other foreign countries. Such functional red ginseng products containing a higher amount of Rg₃ have already been released into the market, and the competitive launches of new products is also expected. However, previously developed Rg₃ enrichment processing methods tend to destruct other functional ginsenosides. Also, the form of previous Rg₃ enriched ginseng products is extract type, not root type and show limitations in developing various products due to excessive heat treatments applied during the extraction of red ginseng [13,14].

This study aimed to enrich Rg₃ in red ginseng, without destroying the shape of red ginseng, because it will lead to the further development of various red ginseng products. Since previous studies, which aimed to enrich Rg₃ with the acid treatment of red ginseng extracts, did not carefully consider the change of other ginsenosides as well as the change of ginsenoside patterns by various organic acids, this study focused on the change of ginsenoside patterns by impregnating various organic acids in producing red ginseng in order to enrich Rg₃. We herein describe the change of ginsenoside patterns after impregnation pretreatments using three different organic acids (selected in our previous study) and an ideal condition for organic acid impregnation [15-17].

MATERIALS AND METHODS

Organic acid impregnation pretreatments

Four to five-year-old fresh ginseng roots were purchased from Anseong Ginseng Cooperative (Anseong, Korea), and kept at -2°C to 0°C before use. To infiltrate organic acid into fresh ginseng roots, the impregnation processing was performed as follows. Two cleaned fresh ginseng roots (~140 g) were submerged fully in the flask containing 500 mL of acid solution, and the pressure in the flask was reduced by using an aspirator for 30 min. Three different organic acids (malic acid, ascorbic acid, citric acid; Sigma, St. Louis, MO, USA) were selected from previous studies, and various concentrations of the organic acids (0.1 to 2 M) were employed in the pretreatment process [16-19].

Red ginseng manufacturing process

Red ginseng was manufactured as described by Chang *et al.* [19]. The fresh ginseng roots pretreated with organic acid were steamed at 96°C to 98°C using the auto-

clave (MG-6845; Mega Science, Seoul, Korea) in normal pressure for 2.5 h. After cooling to room temperature, the ginseng was dried at 70°C for 24 h and at 50°C for 72 h in Air Flow Oven (WFO-601SD; Eyela, Tokyo, Japan).

Determination of the content of crude saponin

Each 2 g of red ginseng powder was suspended in 100 mL of 80% methanol. Extraction was performed at 80°C for 3 h in water bath, and repeated two times. The extract was concentrated at aspiration pressure, and dissolved in 20 mL of distilled water. The extract solution in 250 mL separation funnel was washed with 20 mL of ethyl ether, and extracted with 20 mL of water-saturated butanol three times. Extracts in water-saturated butanol containing crude saponin were collected in a separation funnel, and washed with 60 mL of distilled water two times. The layer of butanol was concentrated in an evaporator, and dissolved in 2 mL of methanol. The final methanol extract was then filtered through a Whatman 0.45 µm filter (Whatman, Maidstone, England) and used as a source for determining the content of crude saponin.

Determination of total saponin content

Crude saponin in 1 mg of red ginseng powder was dissolved in 1 mL of methanol and filtered using 0.45 µm microfilter kit (Whatman). One hundred microliter of crude saponin solution was mixed with 0.3 mL of 8% vanillin and 4 mL of 75°C sulfuric acid, and incubated at 60°C for 10 min in water bath. The absorbance of the mixture at 545 nm was measured. Standard curves were obtained by using 0.2 to 1.0 mg/mL of ginsenoside Re (Wako, Osaka, Japan) as a reference standard for colorimetric analysis.

Analysis of ginsenoside composition

Crude saponin in 1 mg of red ginseng powder was dissolved in 1 mL of methanol. After filtering crude saponin, solution was analyzed using HPLC (PU-980; Jasco, Tokyo, Japan). Analytic standard solution was prepared using 12 ginsenosides (Rg₁, Re, Rf, Rb₁, Rg₂, Rh₁, Rc, Rb₂, Rb₃, Rd, Rg₂, Rh₂; Fleton Reference Substance Co., Chengdu, China), and the concentration of each individual ginsenoside in standard solution was 0.1 mg/mL. Quantification of individual ginsenoside was performed in the following conditions. As a HPLC column, Lichrosorb-NH₂ (5 µm, 250×4.6 mm; Merck, Darmstadt, Germany) was used. The mobile phase was a solution A (acetonitrile/water/isopropanol:80/5/15) and solution B (acetonitrile/water/isopropanol:80/20/15). Thirty percent to one hundred percent (0 to 20 min), 100% (20 to

55 min) and 100% to 30% (55 to 65 min) of B solution were employed as a gradient system. Mobile phase flow speed was 1.0 mL/min and the detector was ELSD 2000 (Alltech, Deerfield, IL, USA). Analysis was performed at 92°C and nitrogen gas (2 L/min) was used as nebulizing gas [20].

RESULTS AND DISCUSSION

Contents of crude and total saponin after acid pretreatment

The change of saponin contents in red ginseng by acid pretreatment was summarized in Table 1. The content of crude and total saponin in red ginseng was 3.43% and 1.91%, respectively. Pretreatment of citric acid and ascorbic acid did not produce significant change in crude and total saponin content in red ginseng. Different concentrations of citric acid and ascorbic acid in pretreatment also did not change the crude and total saponin content.

Table 1. The changes of saponin contents in red ginseng by acid pre-treatment

Acid treatment	Concentration (M)	Crude saponin (%)	Total saponin (%)
Control		3.43	1.91
Ascorbic acid	1	3.17	1.60
	0.5	3.02	1.63
	0.1	3.23	1.58
Malic acid	2	2.78	1.34
	1	2.90	1.41
	0.5	2.88	1.44
	0.1	3.37	1.63
Citric acid	2	3.59	1.90
	1	3.24	1.92
	0.5	3.55	1.81
	0.1	3.53	1.91

However, malic acid pretreatment slightly decreased the content of crude and total saponin in red ginseng. Such decrease was already observed in our previous study. It seems that more soluble saponin was released after acid solution was permeated into fresh ginseng roots. The amount of soluble saponin release showed a positive correlation with the concentration of malic acid.

Ginsenoside composition after acid pretreatment

To investigate the effect of acid concentration on ginsenoside compositions in red ginseng, different concentrations of malic acid (0.1 M, 0.5 M, 1 M, 2 M) were employed in pretreatment, and the result was summarized in Table 2. Pretreatment of up to 0.5 M of malic acid did not change the content of Rg₃ in red ginseng. However when 1 M malic acid was used for the pretreatment, the content of Rg₃ (0.185 mg/g) was increased 10 times. Since Rg₃ has nutraceutical functions of anti-metastasis of cancer cells, blood vessel relaxation, and anti-thrombosis, we may expect stronger anti-cancer effects from red ginseng produced by the pretreatment of 1 M malic acid. Ginsenoside Rg₃ was produced by removing glucose-glucose from Rb₁ and arabinose-glucose from Rb₂, respectively (Figs. 1 and 2). Since increased content of Rg₃ by malic acid pretreatment was accompanied with decreased Rb₁ content in red ginseng, Rb₁ is a source for Rg₃ production.

The pattern of ginsenoside composition in red ginseng was summarized in Table 3. The content of Rg₃ became slightly increased by the pretreatment of up to 0.5 M ascorbic acid, but when 1 M ascorbic acid was used in the pretreatment, the content of Rg₃ increased more. That is, the increase of Rg₃ content was proportional to the concentration level of ascorbic acid. The content of Rg₂ was also increased in proportion to ascorbic acid concentration, but the amount of other individual ginsenosides

Table 2. Ginsenosides content depending on malic acid concentrations (mg/g)

Ginsenosides	Control	Malic acid concentration (M)			
		0.1	0.5	1	2
Rh ₁ +Rh ₂	0.018	0.010	0.027	0.049	0.011
Rg ₂	0.043	0.065	0.092	0.080	0.020
Rg ₁	0.037	0.039	0.020	0.019	0.029
Rg ₃	0.011	0.053	0.018	0.185	0.111
Rf	0.035	0.055	0.050	0.027	0.003
Rd	0.040	0.029	0.010	0.004	0.015
Re	0.296	0.215	0.175	0.208	0.111
Rb ₁	0.144	0.205	0.016	0.097	0.081
Rb ₂	0.049	0.055	0.028	0.019	0.043

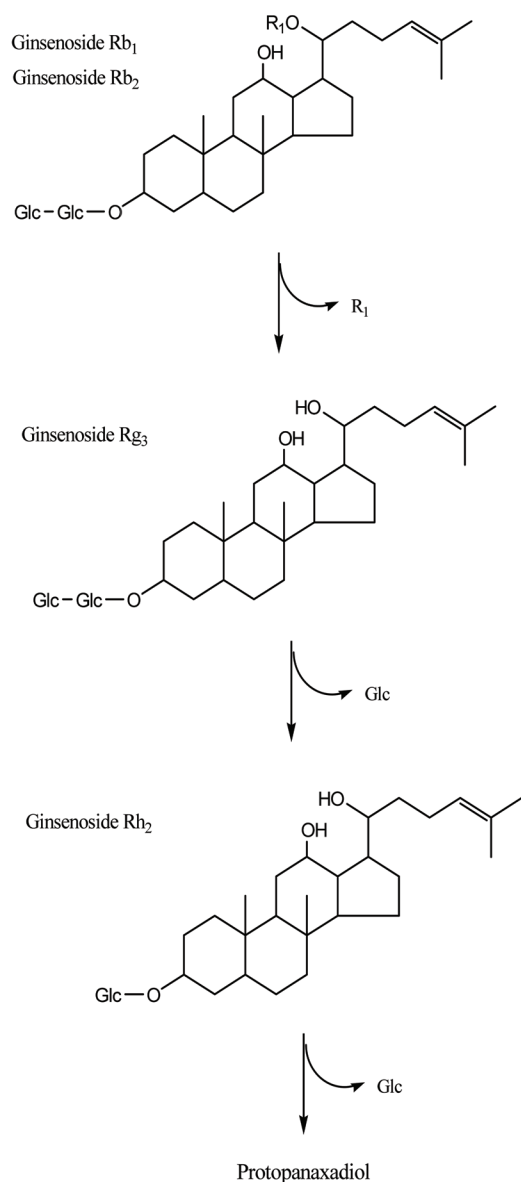


Fig. 1. The chemical pathway of protopanaxadiol ginsenosides.

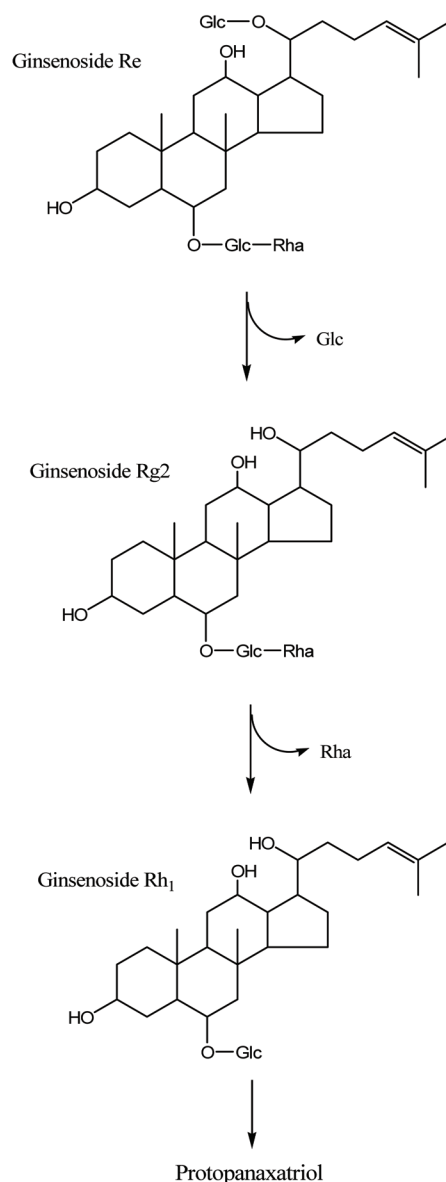


Fig. 2. The chemical pathway of protopanaxatriol ginsenosides.

in red ginseng did not change by ascorbic acid pretreatment. On the contrary of the change by malic acid pretreatment, the pretreatment of ascorbic acid did not lead to a significant decrease in protopanaxadiol ginsenosides of Rb₁ and Rb₂, but also resulted in only a slight increase in Rg₃. Interestingly, the content of protopanaxatriol ginsenoside Re was decreased by ascorbic acid pretreatment. By the ascorbic acid pretreatment, glucose was separated from a hydroxyl group on the 20th carbon of Re, and as the result of this chemical reaction Rg₂ was produced. The increased content of Rg₂ by ascorbic acid pretreatment was shown in Table 4. The functionality of ginsenoside Rg₂ has been reported in cardiovascular dis-

eases, improving memory and anti-cancer. Therefore, the red ginseng pretreated with ascorbic acid would be useful for individuals suffering from these diseases.

The pattern of ginsenoside composition in red ginseng was also changed depending on the concentration level of citric acid used in the pretreatment, and it was summarized in Table 4. Unlike two other organic acids, the pretreatment of citric acid decreased the content of ginsenosides Rd, Re, Rb₁, and Rb₂ in red ginseng in proportion to the increase of acid concentration. However, the pretreatment of 2 M citric acid significantly increased the Rg₃ content (0.303 mg/g), and it was about 30 times higher than that (0.093 mg/g) of 0.1 M citric acid pre-

Table 3. Ginsenoside contents depending on ascorbic acid concentrations (mg/g)

Ginsenosides	Control	Ascorbic acid concentration (M)		
		0.1	0.5	1
Rh ₁ +Rh ₂	0.018	0.008	0.020	0.046
Rg ₂	0.043	0.099	0.140	0.138
Rg ₁	0.037	0.022	0.033	0.041
Rg ₃	0.011	0.010	0.046	0.078
Rf	0.035	0.043	0.048	0.070
Rd	0.040	0.027	0.019	0.017
Re	0.296	0.198	0.142	0.127
Rb ₁	0.144	0.103	0.149	0.193
Rb ₂	0.049	0.040	0.054	0.069

Table 4. Ginsenoside contents depending on citric acid concentrations (mg/g)

Ginsenosides	Control	Citric acid concentration (M)			
		0.1	0.5	1	2
Rh ₁ +Rh ₂	0.018	0.079	0.155	0.227	0.037
Rg ₂	0.043	0.179	0.261	0.265	0.075
Rg ₁	0.037	0.073	0.022	0.064	0.028
Rg ₃	0.011	0.093	0.346	0.707	0.303
Rf	0.035	0.087	0.071	0.053	0.049
Rd	0.040	0.042	0.020	0.007	0.002
Re	0.296	0.315	0.149	0.055	0.015
Rb ₁	0.144	0.147	0.120	0.012	0.024
Rb ₂	0.049	0.093	0.043	0.025	0.007

treatment. Among the organic acids tested in the present study, citric acid was most effective for increasing Rg₃ content in red ginseng. This may be attributable to the change of ginsenoside Rb₁ and Rb₂ into Rg₃. The content of Rh₁+Rh₂ was also increased up to 0.079 mg/g by the 0.1 M citric acid pretreatment, and such increasing content showed a positive correlation with the concentration of citric acid used in the pretreatment. Rh₂ is produced by removing glucose from Rg₃ and destructed Rg₃ was observed in high citric acid condition. Furthermore, the pretreatment of much higher concentration of citric acid (2 M) rather decreased the content of Rh₁+Rh₂.

Unlike two other acids (malic acid, ascorbic acid) used in the present study, citric acid pretreatment affected the content of both protopanaxadiol and protopanaxatriol ginsenosides in red ginseng. The content of Rg₃ was increased when protopanaxadiol ginsenosides (Rb₁ and Rb₂) were decreased. Citric acid was more powerful than malic acid in increasing the content of Rg₃ in red ginseng (Fig. 3). We may also expect that citric acid pretreatment induced the producing of Rh₂, which needs further

chemical reaction after Rg₃ formation. Unfortunately, our present HPLC analysis could not determine the level of Rh₁ (triol) and Rh₂ (diol) separately. Therefore, further study has to clarify whether Rh₂ is produced from Rg₃ after citric acid pretreatment. Pretreatment of citric acid also led to change of protopanaxatriol ginsenosides in red ginseng. For example, by citric acid pretreatment, the content of Re was reduced, whereas the content of Rg₂ as well as Rh₁+Rh₂ produced by Rg₂ degradation was increased in red ginseng.

Efforts were made to change the pattern of ginsenoside composition in ginseng extract, but there has been no report on the change induced by the pretreatment using different types of organic acids. Our overall findings showed that the pretreatment of malic acid and ascorbic acid more selectively affected the production of protopanaxadiol and protopanaxatriol ginsenosides in red ginseng, respectively. Interestingly, when citric acid was used in the pretreatment, the composition of both protopanaxadiol and protopanaxatriol ginsenosides was changed in red ginseng. Because of the trace amount of

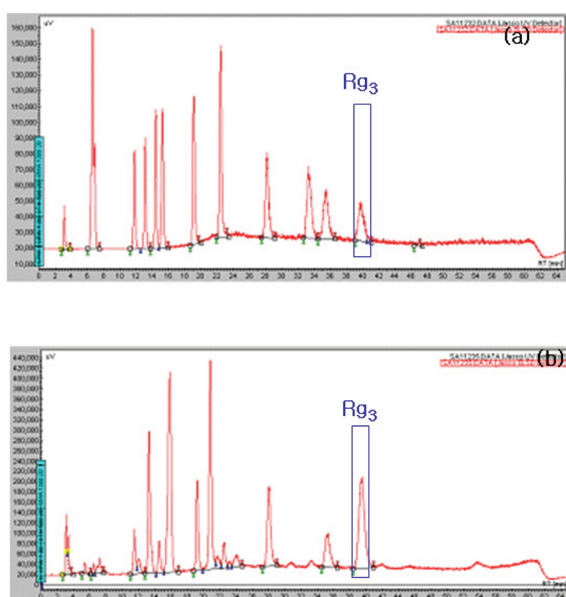


Fig. 3. (A) The patterns of HPLC chromatogram in red ginseng ginsenosids and (B) red ginseng produced by citric acid impregnation pretreatment.

ginsenoside Rg_3 in red ginseng, recent studies have been focused on how to increase Rg_3 during the manufacturing process of red ginseng extract. However, such efforts either required special equipment for the production of raw materials, or had shortcomings of destructing other useful saponin components in red ginseng extract. Our present study suggests a simple and economic organic acid impregnation pretreatment method, which can process root shape red ginseng and significantly increase functionalities of red ginseng without any notable destruction of ginsenosides or any deformation of root shape. Time and temperature to optimize organic acid impregnation pretreatment will be established in further studies.

To develop ginseng products in root shape with useful functionalities, our present study focused on the optimization of organic acid pretreatment and the characteristics of new red ginsengs produced by organic acid pretreatment. The content of crude and total saponin in the red ginsengs was not affected by either different organic acid pretreatments or organic acid concentrations. However, all three organic acids (ascorbic acid, malic acid and citric acid) used in the pretreatment drastically increased Rg_3 content in proportion to the concentration level of organic acid. But a higher concentration of organic acid (2 M) decreased the Rg_3 content in red ginseng. The pretreatment of ascorbic acid increased Rg_2 content in proportion to the concentration level, whereas the content of Rg_3 , Rg_2 , and Rh_1+Rh_2 was increased by citric acid pretreatment. The pattern of ginsenoside composition in red

ginseng was changed depending on type of organic acid employed in the pretreatment, and 1 M of organic acid pretreatment was found to be a suitable concentration level for Rg_3 enriched red ginseng. This study introduces the acid impregnation pretreatment that can increase the content of a certain ginsenoside, and the results of this study will serve as a basis for developing new red ginseng products with enhanced selected functionalities.

REFERENCES

1. Park JD. Recent studies on the chemical constituents of Korean ginseng (*Panax ginseng* C.A. Meyer). Korean J Ginseng Sci 1996;20:389-415.
2. Ando T, Tanaka O, Shibata S. Chemical studies on the oriental plant drugs (XXV). Comparative studies on the saponins and sapogenins of ginseng and related crude drugs. Syoyakugaku Zasshi 1971;25:28-32.
3. Hwang YK, Lee SD. Inhibitory activity of acidic polysaccharides of Korean red ginseng on lipolytic action of toxohormone-L from cancerous ascites fluid. Korean J Food Nutr 1992;5:7-12.
4. Lee SD, Hawang WI. Effect of Korean red ginseng component on lipolytic action of toxohormone-L from cancerous ascites fluid. Korean J Food Sci Nutr 1995;8:105-109.
5. Kwak YS, Wee JJ, Hwang SY, Kyung JS, Kim SK. Effect of crude saponin fraction from Korean red ginseng on physiological functions of old female rat. J Korean Soc Food Sci Nutr 2000;29:460-465.
6. Takagi K, Saito H, Nabata H. Pharmacological studies of *Panax ginseng* root: estimation of pharmacological actions of *Panax ginseng* root. Jpn J Pharmacol 1972;22:245-249.
7. Shibata S, Fujita M, Itokawa H, Tanaka O, Ishii T. Studies on the constituents of Japanese and Chinese crude drugs. XI. Panaxadiol, a sapogenin of ginseng roots. Chem Pharm Bull (Tokyo) 1963;11:759-761.
8. Okuda H. Medicinal Korean ginseng 95. Tokyo: Kyoritsu Pub. Co., 1955.
9. Horhanmer L, Wagner H, Lay B. Contents of *Panax ginseng* root. Preliminary report. Pharm Ztg 1961;106:1307-1311.
10. Zhang S, Takeda T, Zhu T, Chen Y, Yao X, Tanaka O, Ogiyama Y. A new minor saponin from the leaves of *Panax ginseng*. Planta Med 1990;56:298-300.
11. Mochizuki M, Yoo YC, Matsuzawa K, Sato K, Saiki I, Tono-oka S, Samukawa K, Azuma I. Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside-Rb2, 20(R)- and 20(S)-ginsenoside- Rg_3 , of red ginseng. Biol Pharm Bull 1995;18:1197-1202.

12. Nam KY. The comparative understanding between red ginseng and white ginsengs, processed ginsengs (*Panax ginseng* C.A. Meyer). J Ginseng Res 2005;29:1-18.
13. In JG, Lee BS, Kim EJ, Park MH, Yang DC. Increase of functional saponin by acidic treatment and temperature of red ginseng extract. Korea J Plant Res 2006;19:139-143.
14. Han BH, Park MH, Han YN, Woo LK, Sankawa U, Yahara S, Tanaka O. Degradation of ginseng saponins under mild acidic conditions. Planta Med 1982;44:146-149.
15. Kong YH, Rho JH, Cho CW, Kim MH, Lee YC, Kim SS, Lee PJ, Choi SY. Variation of phenolic ingredient and ginsenoside content in red ginseng extract by acid treatment. J Ginseng Res 2009;33:194-198.
16. Kim MH, Lee YC, Choi SY, Cho CW, Rho JH. Characteristics of acid pre-treated red ginseng and its decoction. J Ginseng Res 2009;33:343-348.
17. Kim MH, Hong HD, Kim YC, Rhee YK, Kim KT, Rho JH. Ginsenoside changes in red ginseng manufactured by acid impregnation treatment. J Ginseng Res 2010;34:93-97.
18. Lee MR, Yun BS, Sun BS, Liu L, Zhang DL, Wang CY, Wang Z, Ly SY, Mo EK, Sung CK. Change of ginsenoside Rg₃ and acetylcholinesterase inhibition of black ginseng manufactured by grape juice soaking. J Ginseng Res 2009;33:349-354.
19. Chang JK, Park CK, Shim KH. Changes in chemical components of red ginseng processed from the fresh ginseng stored at low temperature. Korean J Food Preserv 2003;10:158-161.
20. Hong HD, Sim EM, Kim KT, Rho JH, Rhee YK, Cho CW. Comparison of preparation methods for the quantification of ginsenosides in raw Korean ginseng. Food Sci Biotechnol 2009;18:565-569.