Changes in ginsenoside patterns of red ginseng extracts according to manufacturing and storage conditions

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Abstract Although the pharmacological action of red ginseng is mostly generated by ginsenosides, contents of ginsenosides in manufactured extracts are still varying according to processing and storage conditions. Rg1, Rb1, and Rh1 levels significantly decreased overtime under pH adjustment and thermal treatment during storage, and these changes were exacerbated at lower pH and higher storage temperature. However, Rg3 level showed the opposite pattern compared to other ginsenosides. Rg3 level linearly increased at higher temperature and pH while significantly decreased at pH 2. Furthermore, Rg3 level constantly increased during storage. This is the first combined study on the effects of manufacturing and storage conditions on ginsenoside contents of red-ginseng-extract products. To minimize loss of major marker and bioactive compounds of red ginseng products during manufacturing processes and storage, it is recommended that red-ginseng-extracts be maintained at pH 6–8, sterilized at below 105 °C, and stored at below 25 °C.

Keywords Korean red ginseng · Ginsenosides · pH · Thermal processing · Storage conditions

Introduction

Panax ginseng, which originates from the Korea, is a perennial herbaceous plant belonging to the family *Araliaceae* *Panax*. It has long been regarded as a medicine with outstanding abilities to prevent and treat various disease via action of ginseng saponin as known as ginsenoside [1, 2]. More than 40 different ginsenosides have been identified that play an important role in the nutraceutical activity of ginseng. Ginsenosides Rb1, Rb2, Rc, Rd, and Rg3 are protopanaxadiol saponins, whereas ginsenosides Re, Rg1, Rg2, and Rh1 are protopanaxatriol saponins [1, 3]. Ginsenosides have been recognized for their bioactivities, including anti-cancer and neuroprotective properties. Particularly, ginsenoside Rb2 has antagonistic activity towards cancerous toxins as well as an inhibitory effect against tumor angiogenesis [4]. Ginsenoside Rh1 has been shown to have neuroprotective effects as well as stimulating effects on the central nervous system such as anti-fatigue and antihypertensive effects [5, 6]. Rh2 inhibits cancer cell proliferation and induces differentiation into structurally and functionally normal cells [7]. Furthermore, ginsenoside Rg3 suppresses cancer metastasis and cancer drug resistance [4], and functional foods such as Rg3-enriched red ginseng can expand the ginseng market due to recent public attention [8, 9]. Since red ginseng produced by conventional manufacturing processes contains a small amount of ginsenoside Rg3, many studies are focused on Rg3 enrichment via not only general physicochemical treatments but also bioconversion using a microorganism [10, 11]. Among them, heat and acid treatments have been introduced as a straightforward method for Rg3 enrichment during the red ginseng production [12–14].

Current ginseng products are being sold in various forms such as extracts, powders, tea, concentrations, drinks and

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beverages. The Ministry of Food and Drug Safety of Korea distinguishes ginseng products into health functional foods and general ginseng products to regulate the ginsenoside contents of ginseng products. The standard for ginseng products as health functional foods is based on a Rg1 and Rb1 content of 0.8–34 mg/g. However, ginsenoside contents are variable in distributed ginseng products, which is resulted by inconsistency of manufacturing processes and storage conditions [15]. Nevertheless, effects of manufacturing processes and storage conditions on ginsenoside contents of ginseng products are not well-understood. In this study, we investigate the effects of pH treatment (pH 2, 4, 6, and 8) and thermal treatment (95, 105, 115, and 121 °C) during manufacturing as well as storage conditions (5, 25, and 45 °C for 11 weeks) on changes in ginsenoside contents in ginseng extract. Our data reveal that the practical application of pH adjustment and thermal processing can minimize the change of bioactive marker of red ginseng extract.

Materials and methods

Manufacturing of red ginseng aqueous extracts

Six-year-old Korean red ginseng extract was purchased commercially (Korea ginseng corp. Seoul, Korea) and diluted three times in distilled water, resulting in production of red ginseng aqueous extracts. The extract (20 ml aliquots) was then filled into aluminum retort pouches, which were sealed, for commercial sterilization (105 °C, 5 min; VS-1321, Vision, Daejeon, Korea).

pH treatment of red ginseng aqueous extracts

To determine changes in ginsenoside contents according to pH change (2, 4, 6 and 8) of red ginseng extracts (25.5°Bx, pH 4.5), 60%(w/v) citric acid solution (Junsei, Tokyo, Japan) and 20%(w/v) sodium carbonate (Duksan, Seoul, Korea) were added in order to adjust pH to 2, 4, 6, and 8. Citric acid and sodium carbonate are used as a food additive grade.

Heat treatment of red ginseng aqueous extracts

To determine the effect of heat treatment on ginsenosides in red ginseng aqueous extracts, ginsenoside (Rg1, Rb1, Rh1, and Rg3) contents were analyzed after heating red ginseng aqueous extracts (25.5°Bx, and pH 4.5) at different temperatures (95, 105, 115, and 121 °C for 5 min).

Storage of red ginseng aqueous extracts

To determine changes in ginsenoside contents (Rg1, Rb1, Rh1, and Rg3) during storage at various temperatures, red

ginseng aqueous extracts (25.5°Bx, and pH 4.5) were heated at 105 °C for 5 min (standard) and then stored in the incubator at 5, 25, and 45 °C for 11 weeks. Changes in ginsenoside contents were analyzed every 2 weeks.

Ginsenosides analysis

Each sample was analyzed for ginsenoside content by HPLC with slight medication described by Choi et al. [16]. The Briefly, after washing three times with 5 ml of distilled water in a Sep-Pack C₁₈ cartridge (Waters, Milford, MA), 5 ml of 20%(v/v) MeOH and 10 ml of 80%(v/v) MeOH were passed in this order. The sample (5 ml) was passed through the washed Sep-Pack C₁₈ cartridge; 5 ml of 20%(v/v) MeOH was passed, and 5 ml of MeOH (Duksan) was eluted. The MeOH eluate (5 ml) was obtained and filtered through a 0.45 µm polytetrafluorethylene (PTFE) syringe filter (Advantec, Tokyo, Japan), after which it was analyzed by HPLC. For the analysis criteria, Alliance 2695 (Waters, Milford, MA, USA) with a UV detector Waters 2487 (Waters) was used. Xterra RP18 (5 µm, 3.9 × 150 mm, Waters) column was used with an oven temperature at 35 °C and analytical wavelength of 203 nm. The mobile phase solvent was analyzed by using 10%(v/v) CH₃CN (Duksan) and 80%(v/v) CH₃CN under gradient conditions at a velocity of 1.6–1.8 ml/min. For the standard ginsenoside compounds, ginsenoside Rg1, Rb1, Rh1, and Rg3 (WAKO, Tokyo, Japan) at >99% purity were used.

Statistical analysis

Values are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and *T* test were used for multiple comparisons. Treatment effects were analyzed using Duncan's multiple range test by SPSS 19.0 software. Differences are considered significant with *p* values < 0.05.

Results and discussion

Effects of pH on ginsenoside contents of red ginseng extracts

As shown in Table 1, ginsenoside contents of red ginseng aqueous extracts (pH 4.5) were significantly affected at pH 2 and 8. The pH adjustment at pH 2 showed higher differences compared to other groups. After pH adjustment of red ginseng aqueous extracts at pH 2, Rg1 and Rb1 contents were reduced by 29 and 23%, respectively, in a comparison of normal group. This pattern is consistent with previous studies indicating Rb1, Re, and Rg1 drastically decreased with increasing acidity [17, 18]. However,

Table 1 Changes in ginsenoside Rg1, Rb1, Rh1, and Rg3 contents of red ginseng aqueous extracts at various pH levels

	Ginsenoside content (mg/g)		
	Normal ¹	pH-treatment	Heat treatment ²
Rg1			
pH 2	0.401 ± 0.009 ^a	0.285 ± 0.007 ^{bC3}	N.D
pH 4		0.398 ± 0.005 ^{aA}	0.368 ± 0.010 ^{cA}
pH 6		0.400 ± 0.011 ^{aA}	0.375 ± 0.005 ^{cA}
pH 8		0.373 ± 0.008 ^{bB}	0.336 ± 0.009 ^{cB}
Rb1			
pH 2	1.819 ± 0.005 ^a	1.393 ± 0.011 ^{bC}	N.D
pH 4		1.802 ± 0.006 ^{bA}	1.697 ± 0.007 ^{cB}
pH 6		1.800 ± 0.005 ^{bA}	1.735 ± 0.007 ^{cA}
pH 8		1.743 ± 0.010 ^{bB}	1.524 ± 0.006 ^{cC}
Rh1			
pH 2	0.496 ± 0.006 ^a	0.507 ± 0.007 ^{aA}	0.280 ± 0.010 ^{bC}
pH 4		0.493 ± 0.007 ^{aA}	0.471 ± 0.007 ^{bA}
pH 6		0.492 ± 0.008 ^{aA}	0.477 ± 0.005 ^{bA}
pH 8		0.471 ± 0.010 ^{bB}	0.421 ± 0.007 ^{cB}
Rg3			
pH 2	0.723 ± 0.005 ^b	1.494 ± 0.013 ^{aA}	0.263 ± 0.006 ^{cD}
pH 4		0.740 ± 0.010 ^{bB}	0.810 ± 0.010 ^{aA}
pH 6		0.713 ± 0.008 ^{bC}	0.773 ± 0.010 ^{aB}
pH 8		0.705 ± 0.009 ^{bC}	0.733 ± 0.006 ^{aC}

¹ Non-treated normal red ginseng aqueous solution (pH 4.5)² Heat treatment (105 °C, and 5 min) after pH treatment³ Means with different superscripts in the same column (A–D) or row (a–c) are significantly different ($p < 0.05$) by Duncan's multiple range test. Each value is mean ± S.D (n = 3)

N.D Not detected

patterns of Rh1 and Rg3 were different from those of Rg1 and Rb1. Rh1 and Rg3 contents increased by 2.2 and 106%, respectively, compared to the normal group after adjustment to pH 2. Similar studies conducted by Choi et al. [14] and Kong et al. [19] reported that pH adjustment of red ginseng extracts with citric acid significantly increased the content of Rg3 through conversion of Rb1 to Rg3. After adjustment to pH 8, Rg1, Rb1, and Rh1 contents were significantly reduced, and Rg3 contents tended to decrease.

Furthermore, heat treatment at 105 °C for 5 min showed dramatic changes in all groups. At pH 2, Rg1 and Rb1 contents significantly decreased to undetectable levels after additional heat treatment, whereas Rh1 and Rg3 contents drastically decreased by 44.8 and 82%, respectively. On the other hand, at pH 8, contents of Rg1, Rb1 and Rh1 were decreased by 9.9, 12.6, and 10.6%, respectively. Based on these results, contents of Rg1 and Rb1, which are marker compounds of red ginseng products, were unstable under acidic conditions (pH 2, 4, and 6), whereas contents of Rh1

and Rg3, which are important bioactive compounds of red ginseng products, increased under acidic conditions. Especially, Rg3 content in red ginseng extract was stable and even dramatically increased at pH 2 but it was unstable upon combined heat treatment.

Heat and acid treatments during production of red ginseng extract have been introduced as Rg3 enrichment processing methods [14]. However, based on our results, low pH (~pH 2) accompanied by heat treatment rather reduced the content of Rg3.

Changes in ginsenoside contents of pH-adjusted red ginseng extracts during storage at room temperature (25 °C)

To investigate the effects of pH on the ginsenoside contents of red ginseng extracts, samples adjusted pH prior to sterilization at 105 °C for 5 min were kept at 25 °C for 11 weeks. As presented in Table 2, Rg1, Rb1, and Rh1 contents consistently decreased at all pH ranges with increasing storage. However, Rg3 content at pH levels of 4, 6, and 8 consistently increased during storage, although it decreased at pH 2. The Rg1 and Rb1 contents of the pH 6 group were 0.375 ± 0.005 mg/g and 1.735 ± 0.007 mg/g at the start and 0.298 ± 0.004 mg/g and 1.172 ± 0.011 mg/g after 11 weeks, and rates of reduction were 20.5 and 32.4%, respectively. However, the reduction rates of Rg1 content at pH 4 and 8 as well as the normal group were 31, 27, and 29.6%, respectively. The reduction rates of Rb1 at pH 4 and 8 as well as the normal group were 34.6, 37, and 34.6%, respectively. This indicates that a pH level of 6 is proper for stability of Rg1 and Rb1 in red ginseng extract products. Changes in Rh1 and Rg3 contents at pH 2 were 0.280 ± 0.010 mg/g and 0.263 ± 0.006 mg/g, respectively, and their contents after 11 weeks were 0.190 ± 0.007 mg/g and 0.123 ± 0.004 mg/g with reduction rates of 32 and 53%, respectively. Rh1 contents at pH levels of 4, 6, and 8, and the normal group after 11 weeks decreased by 14, 15, 12, and 15%, respectively. However, Rg3 contents at pH levels of 4, 6, and 8, and the normal groups significantly increased by 11, 6.5, 10.8, and 9.5%, respectively.

Changes in ginsenoside contents of red ginseng extracts according to heat treatment

Red ginseng extracts were processed via thermal treatment at different temperatures (95, 105, 115, and 121 °C) by using a commercial sterilizer for sterilization, and contents of ginsenosides (Rg1, Rb1, Rh1, and Rg3) were analyzed. As shown in Fig. 1(A, B), thermal processing mediates the reduction of Rg1 and Rb1 contents, in which reduction was significantly increased at higher temperature. Rg1 contents

Table 2 Changes in ginsenoside Rg1, Rb1, Rh1, and Rg3 contents of red ginseng aqueous extracts at various pH levels during storage at 25 °C for 11 weeks

Ginsenoside content (mg/g)		0 week ¹	1 week	3 weeks	5 weeks	7 weeks	9 weeks	11 weeks
Rg1	Normal ²	0.368 ± 0.008 ^{aA3}	0.360 ± 0.006 ^{aA}	0.345 ± 0.008 ^{bA}	0.324 ± 0.005 ^{cB}	0.300 ± 0.006 ^{dB}	0.283 ± 0.007 ^{eB}	0.259 ± 0.009 ^{fB}
	pH 2	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	pH 4	0.368 ± 0.010 ^{aA}	0.363 ± 0.005 ^{aA}	0.346 ± 0.009 ^{bA}	0.326 ± 0.006 ^{cB}	0.303 ± 0.007 ^{dB}	0.280 ± 0.008 ^{eB}	0.253 ± 0.010 ^{fBC}
	pH 6	0.375 ± 0.005 ^{aA}	0.369 ± 0.007 ^{aA}	0.355 ± 0.005 ^{bA}	0.341 ± 0.011 ^{cA}	0.320 ± 0.008 ^{dA}	0.308 ± 0.005 ^{eA}	0.298 ± 0.004 ^{eA}
	pH 8	0.336 ± 0.009 ^{aB}	0.329 ± 0.007 ^{Ab}	0.316 ± 0.006 ^{bB}	0.299 ± 0.009 ^{Cc}	0.285 ± 0.005 ^{dC}	0.261 ± 0.007 ^{eC}	0.245 ± 0.008 ^{fC}
Rb1	Normal	1.732 ± 0.010 ^{aA}	1.695 ± 0.005 ^{bA}	1.589 ± 0.007 ^{cA}	1.505 ± 0.003 ^{dA}	1.386 ± 0.006 ^{eB}	1.255 ± 0.007 ^{fB}	1.132 ± 0.010 ^{gB}
	pH 2	N.D	N.D	N.D	N.D	N.D	N.D	N.D
	pH 4	1.697 ± 0.007 ^{aB}	1.660 ± 0.006 ^{bA}	1.593 ± 0.007 ^{cA}	1.485 ± 0.005 ^{dB}	1.362 ± 0.008 ^{eB}	1.225 ± 0.009 ^{fC}	1.109 ± 0.006 ^{gC}
	pH 6	1.735 ± 0.007 ^{aA}	1.675 ± 0.010 ^{bA}	1.572 ± 0.006 ^{cA}	1.482 ± 0.008 ^{dB}	1.402 ± 0.007 ^{eA}	1.292 ± 0.005 ^{fA}	1.172 ± 0.011 ^{gA}
	pH 8	1.524 ± 0.006 ^{cC}	1.478 ± 0.008 ^{bB}	1.392 ± 0.007 ^{cC}	1.295 ± 0.010 ^{dC}	1.182 ± 0.004 ^{eC}	1.072 ± 0.008 ^{fD}	0.960 ± 0.005 ^{gD}
Rh1	Normal	0.478 ± 0.007 ^{aA}	0.470 ± 0.005 ^{abA}	0.458 ± 0.006 ^{bA}	0.444 ± 0.004 ^{cA}	0.433 ± 0.006 ^{cdA}	0.425 ± 0.009 ^{dA}	0.411 ± 0.010 ^{eA}
	pH 2	0.280 ± 0.010 ^{aC}	0.275 ± 0.005 ^{abC}	0.263 ± 0.007 ^{bc}	0.250 ± 0.004 ^{cC}	0.232 ± 0.008 ^{dC}	0.212 ± 0.006 ^{eC}	0.190 ± 0.007 ^{fC}
	pH 4	0.471 ± 0.007 ^{aA}	0.465 ± 0.008 ^{abA}	0.455 ± 0.005 ^{bA}	0.442 ± 0.008 ^{cA}	0.427 ± 0.007 ^{dA}	0.415 ± 0.005 ^{eA}	0.400 ± 0.004 ^{fA}
	pH 6	0.477 ± 0.005 ^{aA}	0.469 ± 0.009 ^{aA}	0.456 ± 0.006 ^{bA}	0.443 ± 0.005 ^{cA}	0.433 ± 0.006 ^{cA}	0.420 ± 0.005 ^{dA}	0.405 ± 0.007 ^{eA}
	pH 7	0.421 ± 0.007 ^{aB}	0.418 ± 0.005 ^{abB}	0.409 ± 0.007 ^{bcB}	0.400 ± 0.005 ^{cdB}	0.391 ± 0.004 ^{deB}	0.382 ± 0.006 ^{eB}	0.370 ± 0.008 ^{fB}
Rg3	Normal	0.820 ± 0.007 ^{aA}	0.827 ± 0.005 ^{deA}	0.840 ± 0.008 ^{cdA}	0.853 ± 0.006 ^{cA}	0.870 ± 0.010 ^{bA}	0.883 ± 0.008 ^{bA}	0.898 ± 0.008 ^{aA}
	pH 2	0.263 ± 0.006 ^{aD}	0.252 ± 0.007 ^{aE}	0.235 ± 0.008 ^{bD}	0.215 ± 0.005 ^{cD}	0.189 ± 0.008 ^{dD}	0.157 ± 0.007 ^{eC}	0.123 ± 0.004 ^{fC}
	pH 4	0.810 ± 0.010 ^{aA}	0.812 ± 0.005 ^{cB}	0.838 ± 0.006 ^{dA}	0.852 ± 0.008 ^{cA}	0.862 ± 0.008 ^{cA}	0.878 ± 0.007 ^{bA}	0.899 ± 0.007 ^{aA}
	pH 6	0.773 ± 0.010 ^{aB}	0.774 ± 0.007 ^{fC}	0.781 ± 0.006 ^{eB}	0.791 ± 0.005 ^{deB}	0.800 ± 0.008 ^{cdB}	0.811 ± 0.010 ^{abB}	0.823 ± 0.007 ^{aB}
	pH 8	0.733 ± 0.006 ^{fC}	0.737 ± 0.007 ^{fD}	0.750 ± 0.005 ^{eC}	0.767 ± 0.004 ^{dC}	0.785 ± 0.008 ^{cB}	0.798 ± 0.008 ^{bB}	0.812 ± 0.005 ^{aB}

¹ Heat treatment (105 °C, 5 min) after pH treatment² Non-treated normal red ginseng aqueous solution (pH 4.5)³ Means with different superscripts in the same column (A–D) or row (a–g) are significantly different ($p < 0.05$) by Duncan's multiple range test. Each value is mean ± S.D. ($n = 3$)

N.D Not detected

at temperatures of 95, 105, 115, and 121 °C significantly decreased by 6.2, 8.2, 13.4, and 48.8%, respectively, compared to the non-thermal treated sample. Similar results were also observed for Rb1. On the other hand, Rh1 and Rg3 contents showed different patterns as shown in Fig. 1(C, D). Rh1 content slightly but constantly increased with higher temperature. Rh1 contents of 95, 105, 115, and 121 °C significantly increased by 3.6, 4.2, 4.8, and 12.9%, respectively, compared to the normal group. Hydrolysis caused by heating induces structural alteration of Re into Rh1, thereby explaining the higher Rh1 contents in ginseng extracts after heat treatment [20, 21]. The results for Rg3 were similar to those for Rh1, as Rg3 contents at 95, 105, 115, and 121 °C significantly increased by 18.7, 13.4, 32.9, and 131%, respectively, compared to the normal group. Rg3 and Rh1 are usually produced as derivatives hydrolyzed from ginsenosides such as Re and Rb1 in the stomach. Heat treatment also increased the content of Rg3 in red ginseng extract by heat treatment that is consistent with the finding of Kim et al. [22] showing significant enhancement of Rg3 content by steaming at higher temperature.

Changes in ginsenoside contents of red ginseng extracts according to storage conditions

After pH adjustment of red ginseng extract, extracts were processed at optimum temperature at 105 °C for 5 min and then stored in an incubator at different temperatures (5, 25, and 45 °C) for 11 weeks. As presented in Table 2, Rg1, Rb1, and Rh1 contents at all storage temperatures significantly decreased with increasing storage time, and this effect was exacerbated at higher storage temperatures (Fig. 2). However, Rg3 contents at all storage temperatures constantly increased during storage, and this effect was more significant at a high temperature of 45 °C. Rg1 and Rb1 contents were significantly reduced at high temperature (45 °C) compared to those at low temperature (5 °C) (Fig. 2A, B). Contents of Rg1, Rb1, and Rh1 decreased by 15, 18, and 8.4% at 5 °C as well as by 54, 60, and 27% at 45 °C, respectively, compared to the normal group after 11 weeks (Fig. 2A–C). Contents of Rg3 at 5, 25, and 45 °C increased by 6.1, 9.5, and 24.9%, respectively, after 11 weeks (Fig. 2D).

Fig. 1 Changes in ginsenoside Rg1 (A), Rb1 (B), Rh1 (C), and Rg3 (D) contents of red ginseng aqueous extracts after thermal processing. Means with different letters above the bars are significantly different

($p < 0.05$) by Duncan's multiple range test. Each value is mean $\pm$ S.D (n = 3). Red ginseng aqueous extracts: 25.5°Bx, pH 4.5

Fig. 2 Changes in ginsenoside Rg1 (A), Rb1 (B), Rh1 (C), and Rg3 (D) contents of red ginseng aqueous extracts at different storage temperatures (diamond 5 °C, square 25 °C, triangle 45 °C). Each

value is mean $\pm$ S.D (n = 3), red ginseng aqueous extracts: 25.5°Bx, pH 4.5, sterilization 105 °C (5 min)

Taken together, Rg1, Rb1, and Rh1 contents significantly decreased overtime under pH adjustment and thermal treatment (Fig. 2A–C). Reduction of biomarkers of red ginseng extract was more significant at lower pH level and higher storage temperature. However, Rg3 content showed the opposite pattern compared to other ginsenosides. Especially, Rg3 content linearly increased at higher temperature and pH level but significantly decreased at low pH (pH 2). Furthermore, Rg3 content significantly increased with higher storage time, and this effect was more significant at 45 °C. In conclusion, to minimize the loss of major marker compounds and bioactive compounds of red ginseng products during manufacturing and storage, red ginseng extracts should be maintained at pH 6–8, sterilized below a temperature of 105 °C, and stored at a temperature of below 25 °C. Thus, this study provides useful information showing the effects of manufacturing and storage conditions on the contents of ginsenosides in red ginseng extract products.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Park JD, Rhee DK, Lee YH. Biological activities and chemical of saponins from *Panax ginseng* C. A. Meyer. *Phytochem. Rev.* 4: 159–175 (2005)
- Lee SM, Bae B-S, Park H-W, Ahn N-G, Cho B-G, Cho Y-L, Kwak Y-S. Characterization of Korean red ginseng (*Panax ginseng* Meyer): History, preparation method, and chemical composition. *J. Ginseng Res.* 39: 384–391 (2015)
- Leung KW, Wong AS-T. Pharmacology of ginsenosides: a literature review. *Chin. Med.* 5: 20 (2010)
- Nag SA, Qin J-J, Wang W, Wang M-H, Wang H, Zhang R. Ginsenosides as anticancer agents: in vitro and in vivo activities, structure-activity relationship, and molecular mechanisms of action. *Front. Pharmacol.* 3: 25 (2012)
- Li X-F, Lui CN-P, Jiang Z-H, Ken YK-L. Neuroprotective effects of ginsenosides Rh1 and Rg2 on neuronal cells. *Chin. Med.* 6: 1 (2011)
- Yoon J-H, Choi Y-J, Lee S-G. Ginsenoside Rh1 suppresses matrix metalloproteinase-1 expression through inhibition of activator protein-1 and mitogen-activated protein kinase signaling pathway in human hepatocellular carcinoma cells. *Eur. J. Pharmacol.* 679: 24–33 (2012)
- Keum Y-S, Park K-K, Lee J-M, Chun K-S, Park J-H, Lee SK, Kwon H, Surh Y-J. Antioxidant and anti-tumor promoting activities of the methanol extract of heat-processed ginseng. *Cancer Lett.* 150: 41–48 (2000)
- Mochizuki M, Yoo YC, Matsuzawa K, Sato K, Saiki I, Tonooka S, Samukawa K, Azuma I. Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside-Rb2, 20(R)- and 20(S)-ginsenoside-Rg3, of red ginseng. *Biol. Pharm. Bull.* 18: 1197–1202 (1995)

9. Dela Pena I, Yoon SY, Kim HJ, Park S, Hong EY, Ryu JH, Park IH, Cheong JH. Effects of ginsenoside k-g3, an Rg3-enriched fraction, on scopolamine-induced memory impairment and learning deficit in mice. *J. Ginseng Res.* 38: 1–7 (2014)
10. Bae SH, Lee H-S, Kim M-R, Kim SY, Kim J-M, Suh HJ. Changes of ginsenoside content by mushroom mycelial fermentation in red ginseng extract. *J. Ginseng Res.* 35: 235–242 (2011)
11. Kim E-A, Renchinkhand G, Urgamal M, Park YW, Nam MS. Functional characteristics of *Enterococcus faecium* SA5 and its potential in conversion of ginsenoside Rb1 in ginseng. *J. Life Sci.* 27: 172–179 (2017)
12. Han BH, Park MH, Han YN, Woo LK, Sankawa U, Yahara S, Tanaka O. Degradation of ginseng saponins under mild acidic conditions. *Planta Med.* 44: 146–149 (1982)
13. Nam KY. The comparative understanding between red ginseng and white ginsengs, processed ginsengs (*Panax ginseng* C. A. Meyer). *J. Ginseng Res.* 29: 1–18 (2005)
14. Choi K-H, Kwak Y-S, Rhee M-H, Hwang M-S, Kim S-C, Park C-K, Han G-H, Song K-B. Effects of pH and high temperature treatment on the changes of major ginsenosides composition in Korean red ginseng water extract. *J. Ginseng Res.* 32: 127–134 (2008)
15. Choi JE, Han JS, Kang SJ, Kim KH, Kim KH, Yook HS. Saponin contents and physicochemical properties of red ginseng extract pouch products collected from ginseng markets in Korea. *J. Korean Soc. Food Sci. Nutr.* 39: 1660–1665 (2010)
16. Choi JE, Li X, Han YH, Lee KT. Changes of saponin contents of leaves, stems and flower-buds of *Panax ginseng* C. A. Meyer by harvesting days. *Korean J. Med. Crop Sci.* 17: 251–256 (2009)
17. Yang JW, Do JH, Sung HS, Hong SK. Studies on the manufacturing of ginseng soft drink II. Effect of pH and heat treatment on the stability of panaxadiol saponins. *Korean J. Ginseng Sci.* 6: 25–29 (1982)
18. In JG, Lee BS, Kim EJ, Park MH, Yang DC. Increase of functional saponin by acidic treatment and temperature of red ginseng extract. *Korean J. Plant Res.* 19: 139–143 (2006)
19. Kong YH, Rho JH, Cho C-W, Kim MH, Lee YC, Kim SS, Lee P, Choi SY. Variation of phenolic ingredient and ginsenoside content in red ginseng extract by acid treatment. *J. Ginseng Res.* 33: 194–198 (2009)
20. Bae EA, Shin JE, Kim DH. Metabolism of ginsenoside Re by human intestinal microflora and its estrogenic effect. *Biol. Pharm. Bull.* 28: 1903–1908 (2005)
21. Hwang CR, Lee SH, Jang GY, Hwang IG, Kim HY, Woo KS, Lee J, Jeong HS. Changes in ginsenoside compositions and antioxidant activities of hydroponic-cultured ginseng roots and leaves with heating temperature. *J. Ginseng Res.* 38: 180–186 (2014)
22. Kim WY, Kim JM, Han SB, Lee SK, Kim ND, Park MK, Kim CK, Park JH. Steaming of ginseng at high temperature enhances biological activity. *J. Nat. Prod.* 63: 1702–1704 (2000)