Original paper

Changes in Ginsenoside Compositions by High Temperature Processing under Various Soaking Conditions

Gwi Yeong Jang1, Yoon Jeong Lee1, Meishan Li1, Min Young Kim1, Sang Hoon Lee2, In Guk Hwang2, Junsoo Lee1 and Heon Sang Jeong1*

1Department of Food Science and Biotechnology, Chungbuk National University, Cheongju 28644, Korea.
2Functional Food & Nutrition Division, Department of Agrofood Resources, National Academy of Agricultural Science, Rural Development Administration, Wanju 55365, Korea.

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Changes in ginsenoside composition of ginseng at high temperatures (110, 130, and 150°C) under various soaking conditions (0 – 100% ethanol solutions) were determined. The total ginsenoside contents (20.57 – 27.99 mg/g) of heat-processed ginsengs (HG) at 110 and 130°C were higher than raw ginseng (16.96 mg/g). Group A’s ginsenosides (Rg1, Re, Rf, Rb1, Rc, Rg2(S), Rb2, Rb3, and Rd) decreased with increasing temperatures and decreasing ethanol concentrations, whereas those of group B (Rh1, Rg2(R), Rg6, F2, F4, Rk3, Rh4, Rg6(S), Rg3(R), Rk1, Rg5, and Rh2) showed the opposite trend. Significant correlations were observed among groups A and B, ethanol concentration, and the processing temperature. This study suggests that the composition and content of ginsenosides are dependent on the processing temperatures and ethanol concentrations, and ginsenoside composition can be altered and its content can be manipulated by adjusting the processing temperatures and ethanol concentrations of soaking solutions.

Keywords: ginseng, ginsenoside composition, high temperature processing, soaking condition, ethanol concentration

Introduction

Panax ginseng Meyer, which contains various bioactive components, is traditionally used as a medicine and consumed as a health food in East Asian countries. Major bioactive components in ginseng include ginsenosides, acidic polysaccharides, and phenolic compounds (Park et al., 2003). Ginsenosides are triterpenoid dammarane glycosides (Karikura et al., 1991) and more than 80 types of ginsenosides have been isolated from ginseng species (Duo et al., 2002). Major saponins identified in fresh ginseng include ginsenoside Rb1, Rb2, Rd, Rg1, Re, and Rf (Kim et al., 2008); however, Rg3, Rh2, Rho, Rs1, Rs2, and Rg5 are characteristic components in heat-processed ginsengs, such as the red and black ginsengs (An et al., 2001; Lee et al., 2013). Ginsenosides have been shown to boost the immune system and have anti-tumor and cardio-protective properties (Wu and Zhong, 1999). More specifically, ginsenoside Rg1, from red ginseng has the abilities to inhibit hemagglutination, anti-tumor, and vasorelaxation properties (Nam, 2005); whereas ginsenoside Re has demonstrated anti-diabetic effect in ob/ob mice (Xie et al., 2005). In general, the ginsenosides have various bioactivities and bioavailability, depending on chemical structures, and low molecular weight saponins, such as ginsenoside Rd, F2, and compound K are known to be more bioavailable than large ginsenosides, such as Rb1, Rb2, and Re (Tao et al., 2013). For this reason, many researchers are working on improving the bioavailability of ginsenosides using various conversion methods. There are three different ginsenoside conversion strategies (i.e., physical, chemical, and biological treatments) and physicochemical methods are commonly used in the industry for economic reasons. Various methods such as hot drying (Yoon et al., 2005), puffing (An et al., 2011), gamma

*To whom correspondence should be addressed. E-mail: hsjeong@chungbuk.ac.kr
irradiation (Kim et al., 2012), enzymatic treatment (Kim et al., 2013), and hydrolysis by organic acids (Sun et al., 2013) have been used to convert ginsenosides. However, the current methods such as steaming, boiling, and hot drying usually require much time to processing ginseng.

Therefore, this study was performed to determine the changes in ginsenoside composition when ginseng is heated under various soaking conditions (0 – 100% ethanol solutions as the soaking solution) at high temperatures for a short time. In addition, the present study aimed to provide a basis for effect of ethanol as the soaking solution on ginsenoside composition in ginseng at high temperatures.

Materials and Methods

Materials and chemicals A four-year old ginseng was purchased from a ginseng market (Jeungpyeong, Korea) in 2013. Ginsenoside standards (Rg1, Re, Rf, Rb1, Rc, Rg2(S), Rh1, Rg2(R), Rb2, Rb1, F1, Fd, Rd, Rg2, F2, F3, Rk1, Rh2, Rg3(S), Rg3(R), Rk2, Rg5, and Rb3) were purchased from Ambo Institute (Seoul, South Korea). HPLC-grade water and acetonitrile (ACN) were purchased from J.T. Baker (Phillipsburg, NJ, USA). All other chemicals used were of the reagent grade.

Sample preparation Heat-processed ginseng (HG) with various soaking conditions was prepared according to the following procedure. Fresh ginseng was rinsed with tap water and frozen at −18°C for 24 h, and subsequently dried using a freeze dryer (Ilshin Biobase FD5508, Kyunggi-do, Korea). The dried ginseng was then ground using a hammer mill (Micro hammer cutter mill type-3, Culatti AG, Zurich, Switzerland). An equal amount of ginseng powder (0.5 g) was first weighed in their respective capped glass vials, before adding 3.0 mL of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% (v/v) of ethanol-water solutions as the soaking solution.

In order to induce more changes than the general process (below 100°C), these ginsengs with different ethanol concentrations were then heated in a temperature-controlled autoclave apparatus (Jisico, Seoul, South Korea) at 110, 130, and 150°C for 3 h, as determined in a previous study (Yang et al., 2006). These ginsengs with different ethanol concentrations were then heated in a temperature-controlled autoclave apparatus (Jisico, Seoul, South Korea) at 110, 130, and 150°C for 3 h, as determined in a previous study (Yang et al., 2006).

Extraction and analysis of ginsenosides The extraction and analysis of ginsenosides were performed according to the method reported by Hwang et al. (2014). A 40 mL volume of 80% (v/v) ethanol-water solution was added into flasks containing HG and the flasks were sonicated at room temperature for 1 h using an ultra sonicator (SD-350H, Seongdong, Seoul, South Korea). Three replicate extracts were combined and the solvent was evaporated using an evaporator (N-1000, Eyela, Tokyo, Japan) at 40°C. The residue was dissolved in 40 mL of water and defatted with diethyl ether using an extraction funnel. The defatted aqueous layer was extracted three times with 40 mL of water-saturated n-butanol. The n-butanol layer was then evaporated at 50°C and dissolved in 4 mL of methanol, before being filtered through a 0.2 µm membrane filter. The ginsenoside compositions in HG and raw ginseng were analyzed using high performance liquid chromatography (HPLC) with a UV-visible detector (HPLC system: ACME 9000 system, Younglin, Anyang, South Korea; column: Mightysil RP-18 GP, 250 × 4.6 mm, 5 µm i.d., Kanto Chemical Co., Tokyo, Japan). The mobile phase of the analytical system consisted of ACN (A) and water (B), operating under the following gradient: 0 min (18% A), 0 – 42 min (18 – 24% A), 42 – 46 min (24 – 29% A), 46 – 75 min (29 – 40% A), 75 – 100 min (40 – 65% A), 100 – 135 min (65 – 85% A), and 135 – 150 min (85% A). The flow rate, detection wavelength, and injection volume were set at 0.6 mL/min, 203 nm, and 20 μL, respectively.

Statistical analysis The ginsenoside contents were measured in triplicates and expressed as mean and standard deviation values. Correlation coefficients between ginsenoside contents and processing temperature, as well as the ethanol concentration were analyzed using the SPSS statistics software version 12.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Ginsenoside composition of raw ginseng The contents of ginsenoside Rg1, Re, Rf, Rb1, Rc, Rg2(S), Rb2, Rb1, and Rd were 3.29, 4.31, 1.21, 4.56, 0.63, 1.32, 0.88, 0.17, and 0.59 mg/g, respectively, and minor ginsenosides such as F1, Rg5, F2, Rk1, Rh1, Rg3(S), Rg3(R), Rk2, and Rh2, were not detected in raw ginseng. The major ginsenoside species in raw ginseng were ginsenoside Rg1, Re, and Rb1, and the total ginsenoside content was 16.96 mg/g. Hong et al. (2007), and Liu et al. (2012) have previously reported that ginsenoside Rg1, Re, and Rb1 are the major saponins in fresh ginseng, and their contents were the main yardstick for evaluating the quality of ginseng and ginseng-derived products. These results are in close agreement with ginsenoside composition of ginseng roots (Hwang et al., 2014).

Ginsenoside compositions of heat-processed ginsengs The ginsenoside composition of HG with different soaking conditions (0 – 100% ethanol solutions) was arbitrarily categorized into two groups. Group A consisted of neutral ginsenosides in fresh ginseng, including Rg2, Re, Rf, Rb1, Rc, Rg2(S), Rb2, Rb1, and Rd, whereas group B included ginsenosides that have been converted by heat processing such as Rh1, Rg2(R), Rg3(S), Rg3(R), Rh1, Rh2, Rg5, and Rh2. A ginsenoside F1 was not detected in all samples. HPLC chromatograms for ginsenoside standards, raw ginseng, and HG are shown in Fig. 2, and ginsenoside composition varied with heat processing and ethanol concentration.

The data shown in Fig. 3 refer to ginsenoside composition (group A and B) of HG, obtained via heat processing (110°C) at different ethanol concentrations. The contents of ginsenoside Rg1, Re, Rb1, and Rc decreased in HG with 0% ethanol, however these
Contents increased with increasing ethanol concentration. Ginsenoside Rb₁ increased from 4.03 mg/g to 8.96 mg/g when the ethanol concentration was increased from 0% to 60% in HG, whereas dropped to 6.03 mg/g at 100% ethanol. When the ethanol concentration was increased from 0% to 60% in HG, whereas dropped to 6.03 mg/g at 100% ethanol. When the ethanol concentration was increased from 0% to 60% in HG, whereas dropped to 6.03 mg/g at 100% ethanol. When the ethanol concentration was increased from 0% to 60% in HG, whereas dropped to 6.03 mg/g at 100% ethanol. When the ethanol concentration was increased from 0% to 60% in HG, whereas dropped to 6.03 mg/g at 100% ethanol. When the ethanol concentration was increased from 0% to 60% in HG, whereas dropped to 6.03 mg/g at 100% ethanol. When the ethanol concentra

**Fig. 1.** Chemical structures of ginsenosides (Lee, 2007; Park et al., 2010; Park et al., 2014; Samukawa et al., 1995; Liu et al., 2012).
concentration was increased from 0% to 60%, the respective amounts of ginsenoside Rg1, Rc, and Re increased from 0.75, 1.71, and 1.06 mg/g to 3.68, 3.80, and 4.97 mg/g, before gradually declining as the ethanol concentration to 100%. At 0% ethanol, ginsenoside Rg6, F4, Rg3(S), Rg3(R), Rk1, and Rg5 from group B were measured at 1.32, 1.19, 3.24, 1.62, 1.95, and 1.45 mg/g, respectively. The amounts of these ginsenoside species decreased with an increasing ethanol concentration. The suppression of thermal degradation for group A’s ginsenosides by ethanol was postulated as the cause for the reduction of group B’s ginsenoside contents as the ethanol concentration was increased.

The ginsenoside compositions (group A and B) of HG, obtained via heat processing (130°C) at different ethanol concentrations are shown in Fig. 4. The contents of group A’s ginsenosides were less than 1 mg/g in HG at 0 – 30% ethanol, with the sole exception being the Rc species. At 0% ethanol, ginsenoside Rg1, Re, and Rb1 were not detected in HG. At 30% ethanol, the amounts of ginsenoside Rg1, Re, Rb1, Rc, Rg3(S), Rb2, Rb3,
and Rd were measured at 0.28, 0.44, 0.79, 1.66, 0.94, 0.82, 0.20, and 0.14 mg/g in HG treated at 130℃, respectively; and these values were lower than those at 110℃ with the exception of ginsenoside Rc and Rb₂, the contents of other ginsenosides in group A significantly increased with higher ethanol concentrations. However, the opposite phenomenon was observed for the contents of group B’s ginsenosides. The contents of ginsenoside Rg₃(S), Rk₁, Rg₅, and Rg₆ from group B were 5.57, 4.17, 3.07, 2.94, and 2.26 mg/g in HG at 0% ethanol, respectively. As the ethanol concentration was increased, the contents of group B’s ginsenosides declined. This was clearly indicated by the measured values of 0.13, 0.06, 0.53, 0.06, 0.40, 0.19, 0.12, 0.95, 0.40, 1.04, 0.78, and 0.10 mg/g, corresponding to ginsenoside Rh₁, Rg₂(R), Rg₆, F₂, F₄, Rk₁, Rg₅, and Rh₂ in HG at 70% ethanol, respectively.

The ginsenoside compositions (group A and B) of HG, obtained via heat processing (150℃) at different ethanol concentrations are shown in Fig. 5. Most of group A’s ginsenosides were converted to group B’s ginsenosides in HG at 0 – 70% ethanol. The respective contents of ginsenoside Rg₆, Re, Rf, Rb₁, and Rg₃(S) were established at 0.02, 0.26, 0.72, 0.38, 0.21, and 0.86 mg/g, whereas Rb₂, Rb₃, and Rd were not detected in HG at 70% ethanol. Compared to HG treated at 110 and 130℃, group B’s ginsenoside contents, obtained via heat processing (150℃) with 0% ethanol, were affected by the excessive heat treatments. Ginsenosides Rc and Rg₂(R) were not detected in HG at 0% ethanol, while the levels of ginsenoside Rg₆, F₂, F₄, Rk₁, Rh₁, Rg₅, Rg₆(R), Rg₆(S), Rg₆(R), Rk₁, Rg₅, and Rh₂ were measured at 0.21, 0.07, 0.09, 1.59, 2.00, 0.61, 0.96, 3.63, 3.56, and 0.05 mg/g, respectively. These values were lower than those obtained at 130℃ except for ginsenoside Rg₅. Group B’s ginsenoside contents were relatively higher in HG at 30 – 60% ethanol than other concentrations used but they quickly decreased when the ethanol concentration exceeded 80%. The major ginsenosides were Rg₆(S), Rg₆(R), Rk₁, Rg₅, Rg₆, F₂, and Rh₂ in HG at 0% ethanol; however, at 100% ethanol, the major species identified in the temperature range of 110, 130, and 150℃ were Rb₁, Re, Rg₅(S), and Rb₁. These results are in close agreement with other studies on the composition of ginsenoside in HG such as red ginseng, black ginseng, and steamed ginseng (Hong et al., 2007; Liu et al., 2012; Nam et al., 2012; Jo et al., 2011). Changes in ginsenoside composition are found to take place in HG owing to the conversion of high molecular weight ginsenosides to low molecular weight ginsenosides via heat processing, but an excessive heat treatment typically causes the loss of ginsenoside content. A previous study on the changes of ginseng’s physicochemical properties during
repeated steaming processes reported that the levels of ginsenoside Rg1, Re, and Rb1 in steamed ginseng decreased whereas ginsenoside Rg3 increased, as the duration of the steaming processes was prolonged (Hong et al., 2007). Choi et al. (2012) have reported that the content of ginsenoside Rg3 increased from 0.32 to 5.79 mg/g after three steaming processes, before decreasing with further steam processing. Thermal treatment-induced elevation of ginsenoside Rg3(S), Rg3(R), and Rk1 has also been found to take place in black ginseng that undergoes the steaming process (Nam et al., 2012). The contents of major ginsenosides such as Rb1, Re, and Rg1 were higher in red ginseng than black ginseng, whereas ginsenoside Rg5, Rg5, F4, and Rk1 were higher in black ginseng than red ginseng owing to a more intensive heat treatment (Jo et al., 2011). It has been reported that ginsenoside Rg5, Rg5, and Rk1 can be converted from major saponins, such as ginsenoside Rb1, Rb2, Rc, and Rd owing to a dehydration at the C-20 position and the hydrolysis of a glycosyl bond by thermal processing and acid treatment (Han et al., 1982).

Changes in the ginsenoside contents of group A and B, as well as the total ginsenoside content in HG, obtained via heat processing at 110, 130, and 150 °C are shown in Fig. 6. Group A’s contents decreased with an increasing heating temperature, but increased with a higher ethanol concentration. More specifically, the levels of ginsenoside decreased at 110 °C and at ethanol concentrations above 70%. These results are attributed to the degradation of the thermally unstable malonyl ginsenosides. It has been reported that malonyl ginsenosides can degrade into neutral ginsenosides such as Rb1, Rb2, and Rb3 (Sun et al., 2005).

At 60% ethanol, group A’s ginsenoside contents at 150 °C was 1.31 mg/g, but those values were lower than those obtained at 110, and 130 °C. Meanwhile, group A’s ginsenoside contents in HG at 110, 130, and 150 °C (at 100% ethanol concentration) were 20.43, 21.00, and 14.87 mg/g, respectively. At all tested temperatures (except for 150 °C), Group B’s ginsenoside contents in HG
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The total ginsenoside contents of HG at all tested temperatures (except for 150°C) were higher than that of the non-heat processed ginseng. The total ginsenoside contents of HG at 110 and 130°C ranged from 20.57 to 27.99 mg/g, and the corresponding value in raw ginseng was 16.96 mg/g. However, the total ginsenoside content of HG with 0% ethanol at 150°C was only 12.93 mg/g. The increase in the ginsenoside content after undergoing a heat processing is primarily caused by the conversion of malonyl ginsenosides into neutral ginsenosides such as Rb₁, Rb₂, Rc, and Rd. In general, fresh ginseng contains malonyl ginsenosides and chemical species containing the malonyl residue can attach to a glucose unit of the neutral ginsenosides (Yamaguchi et al., 1988). In a previous study by Liu et al. (2012), malonyl ginsenosides constituted 45% of the total ginsenoside contents; however, malonyl ginsenosides were not detected in red ginseng but elevated levels of ginsenoside Rb₁, Rb₂, Rc, and Rd were found in red ginseng. At different storage temperatures, the contents of malonyl and neutral ginsenosides were 38.1 and 46.0 mg/g, and 13.3 and 58.0 mg/g at 40 and 70°C, respectively (Du et al., 2004). Conversion of ginsenosides was related to hydrolysis by organic acids in heat-processed ginseng (Kim et al., 1998), and the differences for ginsenoside conversion with levels of ethanol concentration are occurred by difference for pKa values of organic acids with different solvents. It is reported that the pKa values of organic acids, which have carboxyl group are higher in all non-aqueous solvents, such as methanol, ethanol, and propanol, compared to water (Sarmini and Kenndler, 1999), and these differences was related to solvent hydrogen bond donor capability, solvent hydrogen bond acceptor capability, solvent dipolarity, and polarizability (Rived et al., 1998).

Correlation for factors affecting ginsenoside contents

The correlation coefficients for factors (i.e., temperature and ethanol concentration) affecting ginsenoside contents are shown in Table 1. The heating temperature was significantly correlated to the total contents of group A (r = –0.748, p < 0.001) and B’s ginsenosides (r = 0.603, p < 0.001), as well as the total ginsenoside contents (r = –0.446, p < 0.001). Meanwhile, the ethanol concentration was also highly correlated to the total contents of group A (r = 0.544, p < 0.001) and B’s ginsenosides (r = –0.600, p < 0.001). A significant correlation was observed between groups A and B (r = –0.932, p < 0.001). Nonetheless, the total ginsenoside content was not correlated to the total contents of group A (r = 0.261, p < 0.055) and B’s ginsenosides (r = 0.106, p < 0.443).

In conclusion, the total ginsenoside contents (20.57 – 27.99 mg/g) of HG with different ethanol concentrations at 110 and 130°C were higher than that of raw ginseng (16.96 mg/g), and various ginsenoside composition were observed under different processing conditions. Furthermore, the contents of group A’s ginsenosides (Rg₁, Re, Rf, Rb₁, Rc, Rg₂(S), Rb₂, Rb₁, and Rd) decreased with increasing temperatures and decreasing ethanol concentrations. However, those of group B’s ginsenosides (Rf₁, Rg₂(R), Rg₂(S), F₁, F₄, Rk₁, Rk₂, Rg₂(S), Rg(R), Rk₁, Rg₄, and Rb₂) increased with increasing temperature and decreasing ethanol concentration. Evidently, excessive heat treatment for ginseng may be inappropriate owing to the loss of ginsenoside content. The appropriate conditions (heating temperature and ethanol concentration) for increasing a specific ginsenoside were 110°C and 60% ethanol for Rg₁, Re, Rf, Rb₁, Rc, Rb₂, and Rd, and 130°C and 0% ethanol for Rg₂(S), F₁, Rg₂(S), and Rg(R), and 150°C and 30% ethanol for Rk₂ and Rf₂, and 150°C and 40% for Rg₂(S) and 150°C and 60% ethanol for Rk₁ in ginseng by heat processing, respectively. The results from this study suggest that ginsenoside composition can be altered and its content can be manipulated by adjusting the processing temperatures and ethanol concentrations. Therefore, ginseng processing using high temperature and ethanol solution as the soaking solution might also consider an application for ginseng products with various ginsenoside compositions in ginseng processing industry.

References


