Review

DOI: 10.5582/ddt.2018.01010

Increase in the hydroxyl radical-scavenging activity of *Panax ginseng* and ginsenosides by heat-processing

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Summary ***Panax ginseng*** **C.A. Meyer (Araliaceae), mainly cultivated in Korea and Northeast China, is processed before use based on its long history of ethnopharmacological evidence. Ginsenosides have been regarded as the main active components responsible for the pharmacological activities of ginseng. Although the Maillard reaction is known as a major source of compounds related to enhanced antioxidant activity by heat treatment in various crude drugs or foods, the chemical and free radical-scavenging activity changes of ginsenosides brought about by the Maillard reaction have not yet been elucidated. This paper gives a review of our recent findings, with emphasis on the hydroxyl radical (•OH)-scavenging activity changes of ginsengs and ginsenosides by heat-processing using an electron spin resonance spectrometer. 20(*S*)-Rg_3 showed the strongest activity, and the next was in the decreasing order of Rb_1, Rg_1, Rc, Rb_2, and Rd. The •OH-scavenging activities of ginsenosides were related to the ferrous metal ion-chelating activities of their aglycone, 20(*S*)-protopanaxadiol. In addition, the ferrous metal ion-chelating activities of ginsenosides were thought to be influenced by their types of hydrophilic sugar moieties. Moreover, Rb_1 was changed into 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rk_1, and Rg_5 by heat-processing, and the sugar moieties at carbon-20 were separated. The generated amount of 20(*S*)-Rg_3 was higher than when Rb_1 was heat-processed without amino acids, and a significant increase in Maillard reaction products was noted. Based upon chemical and •OH-scavenging activity tests using Maillard reaction model experiments, the scientific evidence underlying the increase in free radical-scavenging activity of ginseng induced by heat-processing was elucidated.**

Keywords: *Panax ginseng*, heat-processing, Maillard reaction, ginsenoside, hydroxyl radical

1. Introduction

Ginseng (*Panax ginseng* C.A. Meyer, Araliaceae) is a medicinal herb that is mainly cultivated in Korea and Northeast China. Considered a valued medicine, it has been used in the Orient for more than 2,000 years. Ginseng and its components have been reported to exhibit a wide range of pharmacological and physiological actions, such as antiaging, antidiabetic, anticarcinogenic, analgesic, antipyretic, antistress, antifatigue, and tranquilizing properties, as well as the stimulation of DNA, RNA, and protein synthesis (*1-9*). These medicinal properties of ginseng have been suggested to be linked, although not totally, to ginseng's action to protect against free radical attack (*10-14*).

Traditionally, the root of ginseng has been processed to make white ginseng (roots air-dried after peeling) and red ginseng (roots steamed at 98-100°C without peeling) to enhance its preservation and efficacy. Red ginseng is more common as an herbal medicine than white ginseng in Asian countries, because steaming induces changes in the chemical constituents and enhances the biological activities of ginseng (*15-17*). A novel heat-processing method of steaming ginseng at a higher temperature than red ginseng was developed to achieve an even stronger efficacy than that of

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red ginseng, and this ginseng product was termed heat-processed ginseng (*18-20*) (Figure 1). Heat-processed ginseng has been reported to exhibit more potent pharmacological effects, such as antioxidant, vasorelaxation, anxiolytic-like, and antitumor activities, than those of conventional white or red ginseng by us and others (*19,21-24*).

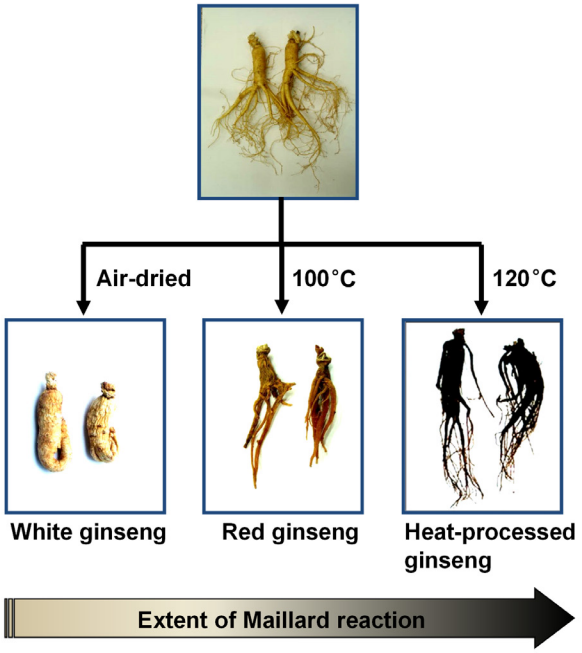

Figure 1. Classification of *Panax ginseng* by heat-processing methods.

The Maillard reaction of amino acids with sugar is a nonenzymatic browning reaction that takes place during the processing, cooking, and storage of foods. It is well-known that Maillard reaction products (MRPs) produced in both heat-treated food systems and in sugar-amino acid model systems exhibit antioxidant activity (*25-27*). The Maillard reaction occurs in the processing of red ginseng (*28*). MRPs in ginseng were reported to increase by heat-processing; these compounds are arginyl-fructosyl-glucose, arginyl-fructose, maltol, maltol-3-*O*-β-D-glucoside, *etc*. (*28,29*). To date, it is not clear what Maillard reaction compounds contribute to the antioxidant activity of MRPs, or how this activity develops over time (*27*). It is possible, therefore, that the formation of heat-processing-induced antioxidants is correlated with the extent of the Maillard reaction in ginseng, and this was experimentally studied by us.

2. Hydroxyl radical (•OH)-scavenging activities of ginseng

Figure 2 shows comparisons of the •OH-scavenging activities and browning levels of white ginseng, red ginseng, and heat-processed ginseng (*30*). White ginseng inhibited •OH production to about 45%, and it was further inhibited to about 40 and 34% by the addition of red ginseng and heat-processed ginseng, respectively, at a concentration of 0.5%. However, none of these effects were stronger than that of thiourea, the •OH-scavenging positive control. In addition, the absorbance value at 420 nm of white ginseng was 0.090 (A.U., arbitrary unit), and

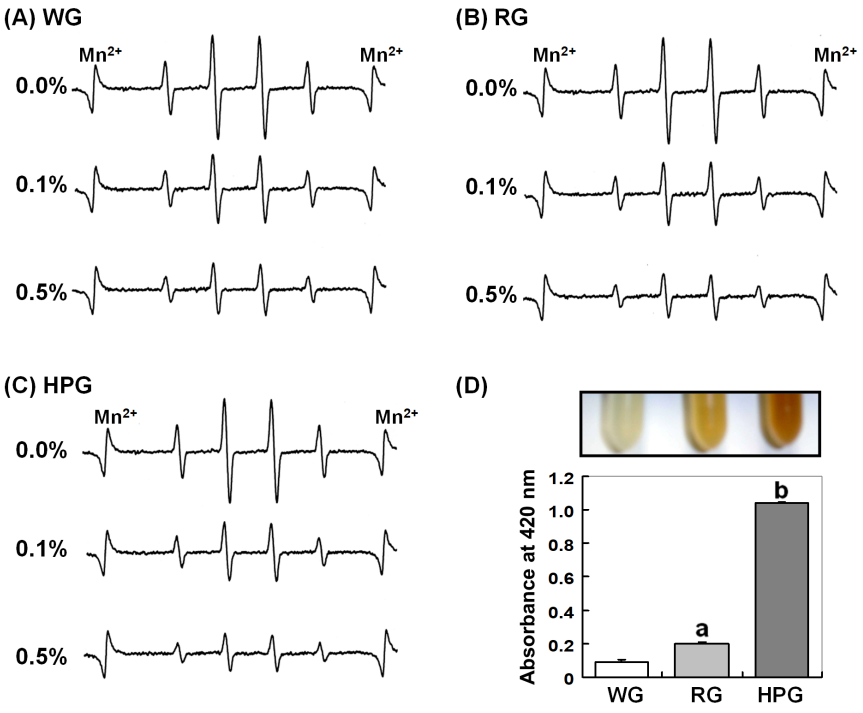

Figure 2. The •OH-scavenging activities of (A) white ginseng (WG), (B) red ginseng (RG), and (C) heat-processed ginseng (HPG). The changes in browning compound levels of *Panax ginseng* brought about by heat-processing **(D)**. $^{a}p < 0.05$, $^{b}p < 0.001$ *vs*. WG. (*30*)

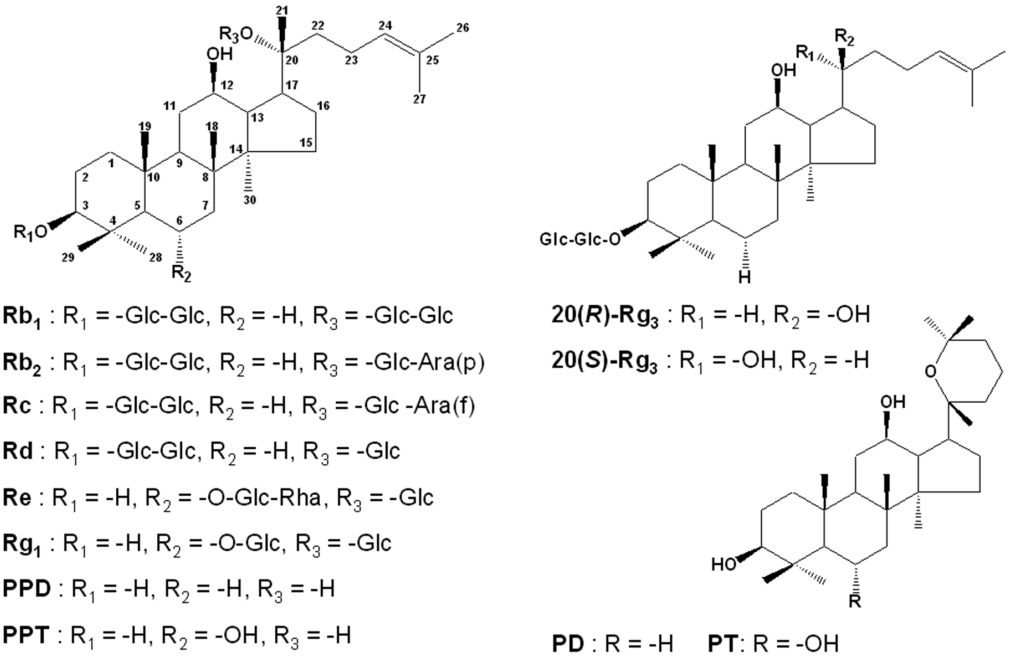

Figure 3. Structures of ginsenosides. -Glc: D-glucopyranosyl, -Rha: L-rhamnopyranosyl, -Ara(p): L-arabinopyranosyl, -Ara(f): L-arabinofuranosyl.

it was increased to 0.198 and 1.043 A.U. in red ginseng and heat-processed ginseng, respectively. Consequently, the •OH-scavenging activities of ginseng extracts were increased by heat-processing in a processing-temperature-dependent manner, but the browning level was more significantly increased by steaming. Therefore, the •OH-scavenging activities of ginsengs were not consistent with the levels of browning, and the effect of MRPs was thought to be minor.

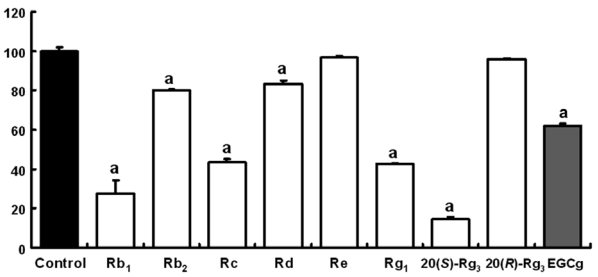

Figure 4. Comparison of the •OH-scavenging activities of ginsenosides at 2 mM when dissolved with distilled water. [a]$p < 0.001$ *vs*. control value. (*34*)

3. The structure and •OH-scavenging activity relationships of ginsenosides

Generally, ginseng root includes organic (80-90%) and inorganic (10-20%) substances. Organic substances contain a number of bio-active constituents, such as saponins (3-6%), carbohydrates (60-70%), nitrogenous substances (9-15%), fat-soluble components (2%), vitamins (0.5%), *etc*. (*31*). Ginsenosides have been regarded as the main active components responsible for the pharmacological activities of ginseng (*17,18,32*). Ginsenosides are glycosides of 30-carbon derivatives of the triterpenoid dammarane, as shown in Figure 3. They have a hydrophobic four-ring steroid-like structure with hydrophilic sugar moieties. About 30 different types of ginsenoside have been isolated and identified from the root of *Panax* species. Each also has at least two (carbon-3 and -20) or three (carbon-3, -6, and -20) hydroxyl groups (-OH), which are free or bound to monomeric, dimeric, or trimeric sugars (*17,33*). Therefore, we investigated the •OH-scavenging and ferrous metal ion-chelating activities of several ginsenosides using electron spin resonance (ESR) for the identification of active ginsenosides and their structure and activity relationships.

When the 8 ginsenosides were determined, 20(*S*)-Rg_3 showed the strongest •OH-scavenging activity, and the next were in the decreasing order of Rb_1, Rg_1, and Rc. These ginsenosides (2 mM) showed more than a 50% inhibitory activity against •OH generation than that of the control. The other ginsenosides such as Rb_2 and Rd showed a comparably lower activity, and Re and 20(*R*)-Rg_3 showed no significant inhibition. (–)-Epigallocatechin 3-*O*-gallate (EGCg) (2 mM), the •OH-scavenging positive control, inhibited •OH generation to about 62% (Figure 4) (*34*). From the comparisons of activities in diol-type ginsenosides, the additional -Glc and -Ara(f) connected to Glc at carbon-20 were thought to increase the •OH-scavenging activities of ginsenosides, as shown by the strong activities of Rb_1 and Rc. However, a low inhibitory activity was observed in diol-type ginsenosides containing additional -Ara(p) and no additional sugar moiety connected to

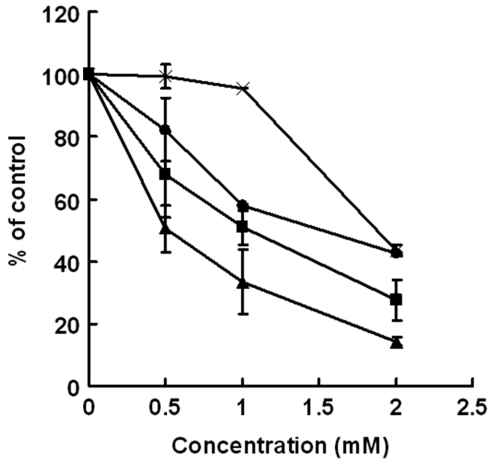

Figure 5. Comparison of the •OH-scavenging activities of Rb_1, Rc, Rg_1, and 20(*S*)-Rg_3. ■: Rb_1, ×: Rc, ●: Rg_1, ▲: 20(*S*)-Rg_3. (*34*)

Glc at carbon-20, as shown by Rb_2 and Rd, respectively (Figures 3 and 4). On the other hand, the effect of -Glc-Glc at carbon-3 was not certain because all diol-type ginsenosides contain this group. In the case of triol-type ginsenosides, the -Glc at carbon-6 was thought to increase the •OH-scavenging activity of ginsenoside, as shown by the strong activity of Rg_1, but an additional -Rha at the carbon-6 position had adverse effects, as shown by the nearly zero activity of Re (Figures 3 and 4).

On the other hand, 20(*S*)- and 20(*R*)-Rg_3 are epimers which increase during steaming by the deglycosylation of diol-type ginsenosides, but their •OH-scavenging activities show marked differences. 20(*S*)-Rg_3 showed the strongest activity compared to the other ginsenosides in this study (*34*), and many reports have provided supporting evidence that their antioxidant activity is closely related to the geometrical arrangement of the OH group, especially, at carbon-20. The alkene chain connected to carbon-20 in 20(*S*)-Rg_3 has a stable, fixed orientation and is packed tightly near the terpenoid, while that in 20(*R*)-Rg_3 protrudes further outside and has a flexible structure (*35*). This compact structure of 20(*S*)-Rg_3 is thought to influence the accessibility of water to the OH group of carbon-12 and -20. Therefore, 20(*S*)-Rg_3 is known to be more soluble in water than 20(*R*)-Rg_3. In addition, it was reported that the OH group of 20(*S*)-Rg_3 is better aligned with the OH acceptor group in the ion channels than that of 20(*R*)-Rg_3, and that it was important for Na+ channel regulation (*36*). Moreover, 20(*S*)-Rg_3 has been reported to provide neuroprotection against cerebral ischemia-induced injury in the rat brain through reducing lipid peroxides and scavenging free radicals (*37*). Therefore, it was thought that the strong •OH-scavenging activity of 20(*S*)-Rg_3 in ESR is closely related to the geometrical arrangement of the OH group, especially at cabon-12 and -20.

Figure 5 shows a comparison of the •OH-scavenging activities of ginsenoside Rb_1, Rc, Rg_1, and 20(*S*)-Rg_3,

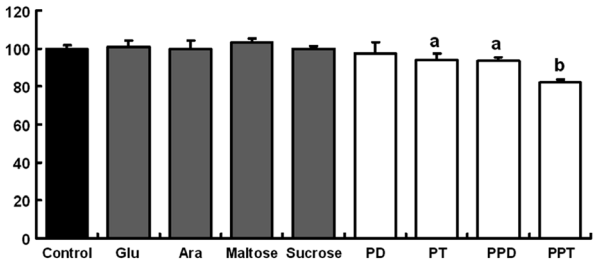

Figure 6. Comparison of the •OH-scavenging activities of sugar moieties and sapogenins at 2 mM when dissolved with distilled water. $^{a}p < 0.01$, $^{b}p < 0.001$ *vs*. control value. (*34*)

in concentrations ranging from 0.5 to 2 mM (*34*). The IC_{50} values of 20(*S*)-Rg_3, Rb_1, Rg_1, and Rc were 0.51, 1.04, 1.51, and 1.87 mM, respectively. EGCg, the •OH-scavenging positive control, showed an IC_{50} value of 3.76 mM (data not shown). From these results, the sugar moieties are thought to have pivotal roles in the •OH-scavenging activity of ginsenosides. The difference in the structures of ginsenosides is only due to the position and type of sugar moieties connected to the ring of the triterpenoid dammarane, and this mutual interaction was suggested to play an important role in the antioxidant effects of ginsenosides.

In the case of sugars and sapogenins, sugars showed no •OH-scavenging activities, and the activities of sapogenins were lower than predicted (Figure 6) (*34*). Although the activity of sapogenins was weak, there was evidence that the number of -OH groups is related to the •OH-scavenging activity of sapogenins. Panaxadiol (PD) and panaxatriol (PT), which have cycled side chains at carbon-20, showed no or weak •OH-scavenging activity, but it was improved in 20(*S*)-protopanaxadiol (PPD) and 20(*S*)-protopanaxatriol (PPT) containing one more -OH group at carbon-20. Moreover, the activity of PT and PPT was slightly higher than in PD and PPD, respectively (Figures 3 and 6). Therefore, it was interpreted that the aglycone of ginsenoside has some •OH-scavenging activity because of its -OH group at carbon-6 and -20. However, the •OH-scavenging mechanism can not be explained with only sapogenins because of their low activity. Consequently, the mechanism was considered to involve an associated function of sapogenins with their sugar moieties.

4. The chemical changes and Maillard reaction of Rb_1 brought about by heat-processing

The root of ginseng has been heat-processed to improve its medicinal efficacies in Korea based on the long history of ethnopharmacological evidence (*18,31,38*). Although an increasing body of evidence supports MRPs being involved in the increased activity by heat treatment in various crude drugs or foods (*39*), the effect of the Maillard reaction on the active components of ginseng and biological activities have not yet been fully elucidated. Ginsenosides have been regarded

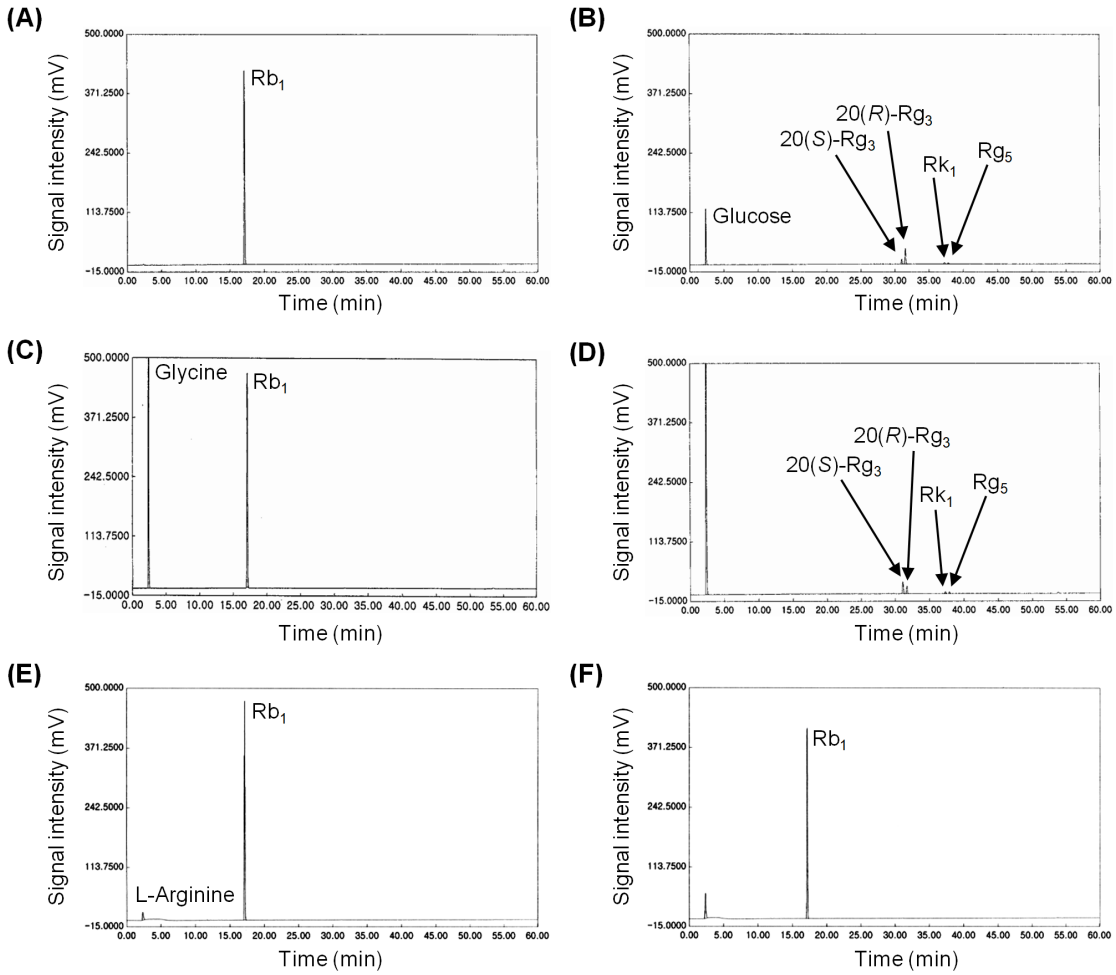

Figure 7. HPLC chromatograms of (A) Rb_1, (B) heat-processed Rb_1, (C) Rb_1-glycine mixture, (D) heat-processed Rb_1-glycine mixture, (E) Rb_1-arginine mixture, and (F) heat-processed Rb_1-arginine mixture. (*42*)

as the main active components responsible for the pharmacological activities of ginseng, and are well-known to be deglycosylated by heat-processing (*18*). The sugar moieties of ginsenosides can be a source of MRPs with amino acids contained in ginseng during heat-processing (*30*), and research on the Maillard reaction of ginsenosides is thought to be beneficial to understand the complex structural changes of ginsenosides brought about during the heat-processing of ginseng.

As one of the major ginsenosides contained in ginseng, Rb_1 is a diol-type triterpene glycoside, and the heat-processing-induced deglycosylation of two glucose molecules at carbon-20 of Rb_1 has been well-documented. Therefore, Rb_1 was used as a target ginsenoside to study a Maillard reaction model experiment in this study. To ascertain the generation of MRPs from ginsenosides and amino acids, we have analyzed Maillard reaction model experiments using Rb_1 and glycine or L-arginine. The sugar moieties of ginsenoside can be a source of MRPs with amino acids contained in ginseng during heat-processing. To identify the effects of amino acids on the heat stability or structural changes of Rb_1, Rb_1 was heat-processed with or without the same amount of glycine or L-arginine, because glycine is a frequently used amino acid in Maillard reaction model experiments (*40*) and L-arginine is the most abundant amino acid contained in *Panax ginseng* (*41*).

As shown in the HPLC chromatograms (Figures 7A and 7B), Rb_1 (1,000 μg) was changed into 20(*S*)-Rg_3 (146 μg), 20(*R*)-Rg_3 (201 μg), Rk_1 (102 μg), and Rg_5 (110 μg) by heat-processing, and the sugar moieties at carbon-20 of Rb_1 were deglycosylated. The separated sugar moiety was determined as glucose based on GC-MS analysis. Then, we added the same amount of glycine to Rb_1 to identify the effect of the Maillard reaction during heat-processing. Rb_1 (1,000 μg) was changed into 20(*S*)-Rg_3 (196 μg), 20(*R*)-Rg_3 (167 μg), Rk_1 (102 μg), and Rg_5 (108 μg) when heat-processed with glycine (Figures 7C and 7D), and the brown color level of heat-processed Rb_1-glycine mixture was significantly higher than that of Rb_1 or heat-processed Rb_1 (Figure 8) (*42*). The Maillard reaction is dependent on several factors such as the pH, time, temperature, concentration of reactants, and reactant type. The development of color is known as an important and clear feature of the Maillard reaction, and brown-

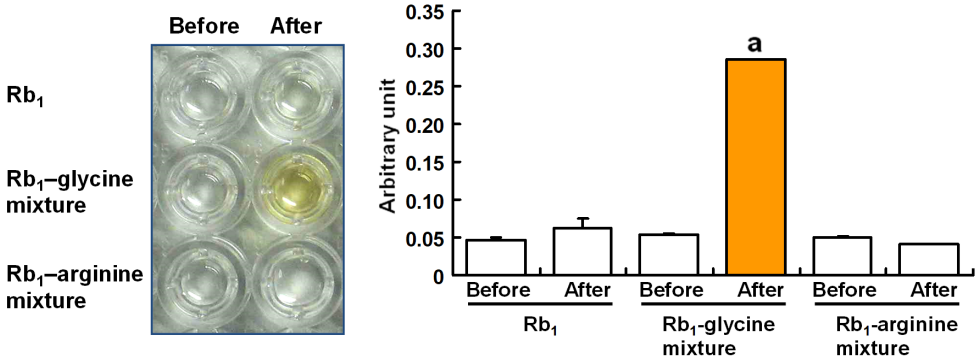

Figure 8. Comparison of MRP levels of samples. [a]$p < 0.001$ *vs*. Rb_1. (*42*)

colored nitrogenous polymers, called melanoidins, are known to be formed by this reaction (*43,44*). When changes in the contents of ginsenosides between heat-processed Rb_1 and heat-processed Rb_1-glycine mixture were compared, the generated amounts of 20(*S*)-Rg_3 and 20(*R*)-Rg_3 were inverse in these samples (Figures 7B and 7D). Therefore, the addition of glycine to Rb_1 for heat-processing was suggested to increase the generation of 20(*S*)-Rg_3, which has a strong •OH-scavenging activity, due to the Maillard reaction. However, the pH values of Rb_1 and Rb_1-glycine mixture were about 4.51 and 5.28, respectively. The pH value of the same amount of ginseng extract was about 5.35. The exact mechanism to explain the effect of glycine on the epimerization of ginsenoside is not certain at present, but the involvement of the Maillard reaction is certain. The addition of glycine to Rb_1 in heat-processing was thought to increase the generation of 20(*S*)-ginsenoside by the Maillard reaction.

At the same time, when Rb_1 was steamed with the same amount of L-arginine, about 0.5% of Rb_1 was lost during heat-processing, but the heat stability of Rb_1 was significantly improved (Figures 7E and 7F) compared to when Rb_1 was heat-processed with or without the same amount of glycine (*42*). In addition, there was no increase in the brown color by heat-processing of the Rb_1-arginine mixture (Figure 8) (*42*), and the pH value of the Rb_1-arginine mixture was about 10.37. High temperature and pH are known to promote the Maillard reaction, and L-arginine is the most abundant amino acid in *Panax ginseng* to generate MRPs such as arginyl-fructose and arginyl-fructosyl-glucose (*41,45,46*). However, the Maillard reaction did not occur when Rb_1 was steamed with L-arginine, and we paid attention to the structural characteristics of L-arginine. The substitution of L-arginine in protein is known to lead to significant heat stability enhancement in the presence of sugar substrates, most probably by interfering with nonenzymatic glycation (*47*). In addition, the guanidyl groups of L-arginine generally form long-range hydrogen bonds or electrostatic interactions with negatively charged groups, and this increased hydrogen bonding is one of the factors enhancing protein thermostability (*48,49*). Therefore, the improved heat stability of Rb_1 brought about by the addition of L-arginine was also thought to be closely related to its characteristics of interfering with nonenzymatic glycation and forming hydrogen bonds with Rb_1. However, we still have unanswered questions and need to conduct more precisely controlled examinations using other amino acids or using similar pH conditions with ginseng to elucidate the detailed mechanism behind the increase or decrease in the heat stability of Rb_1.

5. Conclusion

The recent introductions of various analytical methods with high sensitivity and specificity have been enriching our knowledge of ginseng, helping to identify new chemical entities from various ginseng species, and improving our understanding of this millennium herbal medicine (*17*). Based upon the chemical and •OH-scavenging activity tests using Maillard reaction model experiments, scientific evidence to explain the increase in the free radical-scavenging activity of ginseng induced by heat-processing was obtained. The •OH-scavenging active components such as 20(*S*)-Rg_3, Rg_5, and MRPs in *Panax ginseng* were significantly increased on heat-processing. The critical roles of the Maillard reaction were confirmed and supported by the following lines of observations: firstly, the generated amount of 20(*S*)-Rg_3 from Rb_1 was increased when heat-processed with glycine. Secondly, the generation of MRPs was positively correlated with the •OH-scavenging activity. Finally, certain amino acids such as L-arginine blocked the structural change of ginsenoside, leading to them having a stronger •OH-scavenging activity. Therefore, it is clear that the Maillard reaction is involved in the chemical and antioxidant activity changes of ginsenoside.

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(Received February 13, 2018; Revised April 23, 2018; Re-revised April 29, 2018; Accepted June 17, 2018)