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The Chemical and Hydroxyl Radical Scavenging Activity Changes of Ginsenosides Induced by the Maillard Reaction

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ABSTRACT

The root of ginseng, *Panax ginseng*, has been heat-processed to improve its medicinal efficacy in Korea. 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. To demonstrate the Maillard reaction of ginsenosides by heat-processing in ginseng, we heat-processed the two ginsenosides Rb_1 and Rb_2 with glycine as an amino acid, and the hydroxyl radical (•OH) scavenging activity was measured with an electron spin resonance spectrometer. Rb_1 and Rb_2 were gradually 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 •OH scavenging activities of 20(*S*)-Rg_3 and Rg_5 were stronger than that of Rb_1, but 20(*R*)-Rg_3 and Rk_1 showed weak or no •OH scavenging activities. The generation of Maillard reaction products, although limited to the reaction between the glucosyl moiety and glycine, were positively correlated with the •OH scavenging activity. However, certain amino acids such as L-arginine block the structural change of ginsenosides, leading to a stronger •OH scavenging activity. 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, hydroxyl radical

CONTENTS

INTRODUCTION

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 (Park 1996; Yokozawa *et al.* 2007). *P. ginseng* root is air-dried to produce white ginseng or steamed at 98-100°C to yield red ginseng (Kasai *et al.* 1983; Park 1996; Nocerino *et al.* 2000; Yun 2001) (**Fig. 1**). Generally, red ginseng is more commonly used as a medicinal herb than white ginseng in Asian countries, because steaming induces changes in the chemical constituents and enhances the biological activities (Kasai *et al.* 1983; Matsuura *et al.* 1984; Yun 2001; Oh *et al.* 2006; Jia and Zhao 2009).

A few years ago, a novel heat-processing method of autoclaving ginseng at 120°C was developed to achieve an even stronger efficacy than that of red ginseng, and this ginseng product was termed heat-processed ginseng (Park *et al.* 1998; Kim *et al.* 2000; Kwon *et al.* 2001). 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 (Kim *et al.* 1999; Keum *et al.* 2000; Kim *et al.* 2000; Park *et al.* 2005; Kang *et al.* 2006).

The Maillard reaction of amino acids with sugars 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 have antioxidant activity (Wijewickreme *et al.* 1999; Bekedam *et al.* 2008; Chen and Kitts 2008). It is possible, therefore, that the formation of heat-processing-induced antioxidants could be correlated with the extent of the Maillard reaction and melanoidin formation in ginseng, and this interesting idea was experimentally studied by us. The aim of this paper was to review scientific evidence on the changes in constituents of *Panax ginseng* brought about by the Maillard reaction and its antioxidant activity.

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Invited Mini-Review

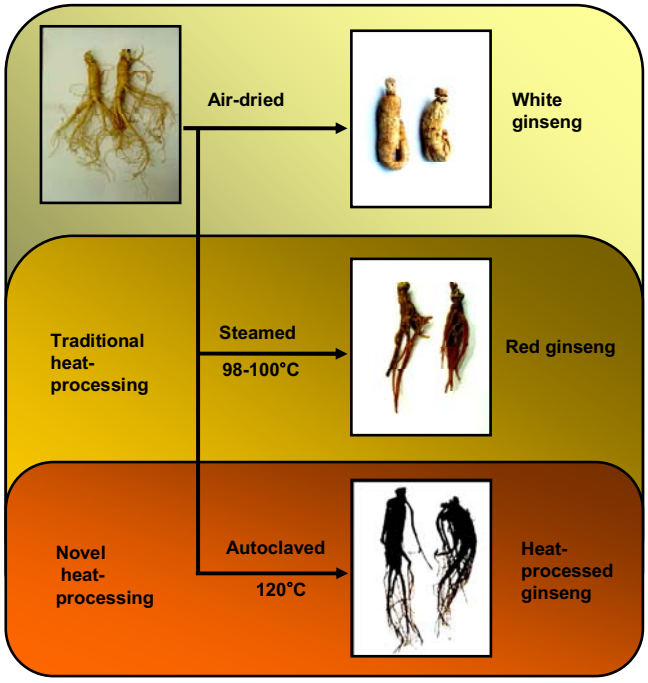

Fig. 1 Classification of *Panax ginseng* by heat-processing methods.

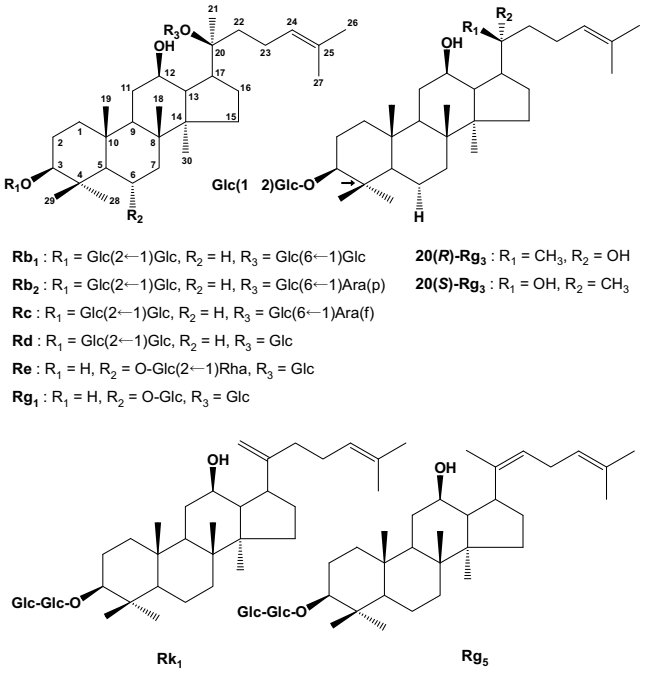

Fig. 3 Structures of ginsenosides. -Glc: D-glucopyranosyl, -Rha: L-rhamnopyranosyl, -Ara(p): L-arabinopyranosyl, -Ara(f): L-arabinofuranosyl.

MAILLARD REACTION OF GINSENG AND ITS ANTIOXIDANT ACTIVITY

The Maillard reaction occurs in the processing of red ginseng (Li *et al.* 1999). 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. (Li *et al.* 1999; Suzuki *et al.* 2004). From the quantitative analysis of contents and free radical scavenging activity tests, maltol was suggested to be the main free radical scavenging component of heat-processed ginseng (Kang *et al.* 2006). However, it is insufficient to explain the free radical scavenging activity of heat-processed ginseng with only maltol because of its relatively low content. In addition, as shown in the comparison of the •OH scavenging activity test using electron spin resonance spectrometer and the contents of MRPs measured based on the browning compound levels, the •OH scavenging activity and MRP levels of *Panax ginseng* were increased in a heat-processing, temperature-dependent manner (**Fig. 2**). White ginseng inhibited •OH production to about 45% (**Fig. 2A**), and it was further inhibited to about 40 and 34% by the addition of red ginseng (**Fig. 2B**) and heat-processed ginseng (**Fig. 2C**), respectively, at a concentration of 0.5%. However, the increase in the •OH scavenging activity of heat-processed ginseng was apparently low when compared with the marked increase in MRP levels from 100 to 120°C.

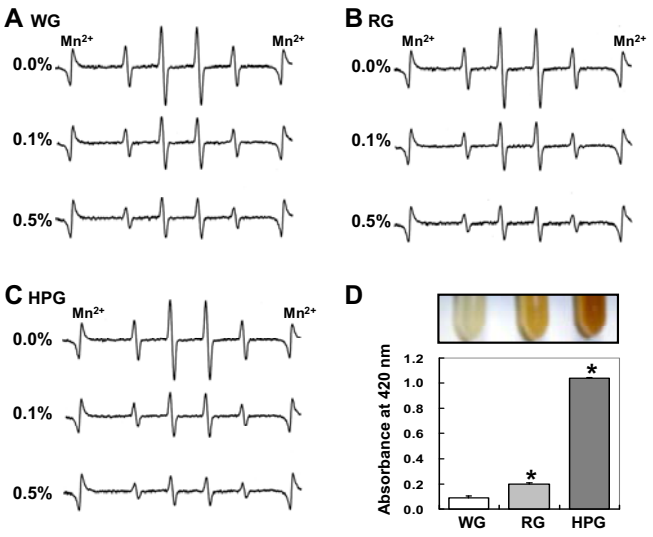

Fig. 2 The •OH scavenging activities of (**A**) white ginseng (WG), (**B**) red ginseng (RG), and (**C**) heat-processed ginseng (HPG). The changes in browning compound levels of *Panax ginseng* brought about by heat-processing (**D**). *$p < 0.05$ compared with white ginseng. (Reproduced with permission from Biological & Pharmaceutical Bulletin Vol. 30. No. 4. Copyright [2007] Pharmaceutical Society of Japan).

To date, it is not clear what Maillard reaction compounds contribute to the antioxidant activity of MRPs, and how this activity develops over time (Chen and Kitts 2008). Some studies have indicated that the antioxidant capacity is a result of intermediate and low-molecular-weight MRPs (Hofmann *et al.* 2001; Morales and Babbel 2002), but other studies have suggested that high-molecular-weight MRPs exhibit a higher antioxidant activity than low-molecular-weight MRPs (Monti *et al.* 1999; Jing and Kitts 2004). Therefore, we have investigated serial Maillard reaction model experiments using ginsenosides and amino acids to investigate the factors which would lead to an increase in the •OH scavenging activity.

THE CHEMICAL AND •OH SCAVENGING ACTIVITY CHANGES OF GINSENOSIDE-Rb$_2$ BROUGHT ABOUT BY HEAT-PROCESSING

Generally, ginseng root includes organic (80-90%) and inorganic substances (10-20%). 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. (Park 1996). Ginsenosides have been regarded as the main active components responsible for the pharmacological activities of ginseng (Park 1996; Park *et al.* 1998; Wang *et al.* 2006; Yokozawa *et al.* 2007; Jia and Zhao 2009). Ginsenosides are glycosides of 30-carbon derivatives of the triterpenoid dammarane, as shown in **Fig. 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, -

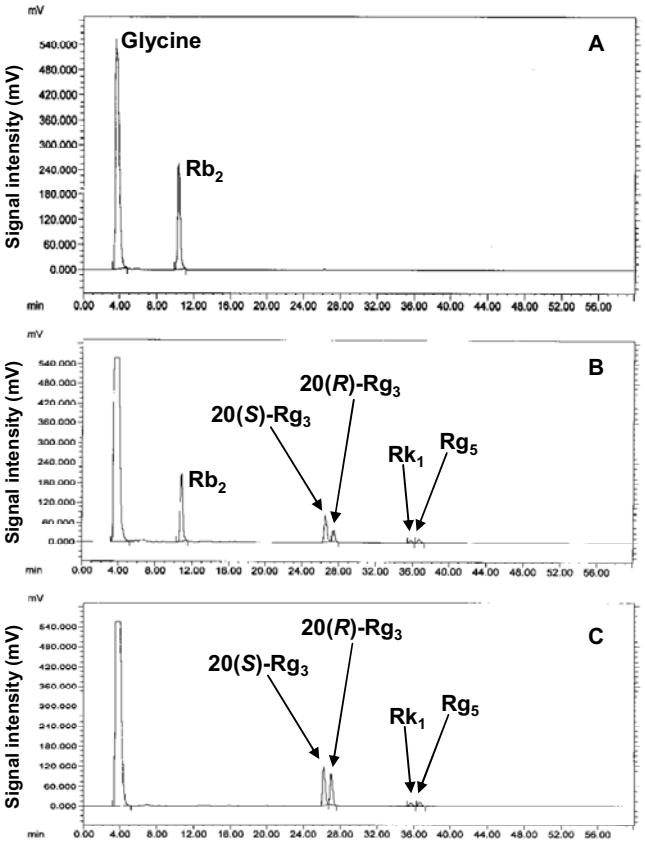

Fig. 4 HPLC chromatograms of the (**A**) glycine-Rb_2 mixture, (**B**) glycine-Rb_2 mixture steamed at 100°C for 3 h, and (**C**) glycine-Rb_2 mixture steamed at 120°C for 3 h. (Reproduced with permission from Biological & Pharmaceutical Bulletin Vol. 30. No. 4. Copyright [2007] Pharmaceutical Society of Japan).

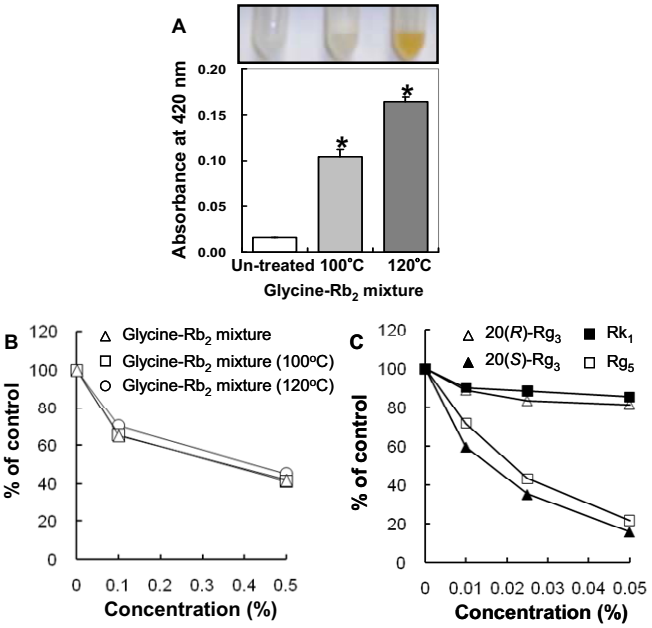

Fig. 5 The graphs compare the (**A**) browning compound levels, (**B**) •OH scavenging activities of the glycine-Rb_2 mixture, and (**C**) •OH scavenging activities of 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rk_1, and Rg_5. *$p < 0.05$ compared with untreated glycine-Rb_2 mixture. (Reproduced with permission from Biological & Pharmaceutical Bulletin Vol. 30. No. 4. Copyright [2007] Pharmaceutical Society of Japan).

6, and -20) hydroxyl groups (-OH), which are free, or bound to monomeric, dimeric, or trimeric sugars (Shoji 1990; Park 1996; Jia and Zhao 2009).

To demonstrate the Maillard reaction of ginsenosides, Rb_2, a well-known diol-type triterpene glycoside that exists abundantly in *P. ginseng*, was steamed with glycine, a frequently used amino acid in the Maillard reaction model system and also contained in *Panax ginseng* (Park 1996; Yoshimura *et al.* 1997). As shown in the HPLC chromatograms of steaming model products using glycine-Rb_2 (**Fig. 4**), glycine and Rb_2 were detected at about 4.0 and 10.5 min, respectively, when not steamed (**Fig. 4A**). Then, about 43% of Rb_2 was changed into 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rk_1, and Rg_5 by heat-processing at 100°C for 3 h (**Fig. 4B**), and all of the Rb_2 disappeared and the contents of Rg_3, Rk_1, and Rg_5 were increased by steaming at 120°C for 3 h (**Fig. 4C**). Rb_2 was gradually changed into 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rk_1, and Rg_5 by heat-processing at 100 and 120°C.

On the other hand, the browning levels of glycine-Rb_2 mixtures were increased by heat-processing, as shown in **Fig. 5A**. Rb_2 generates arabinose and glucose which were separated from carbon-20 during heat-processing, and the MRPs were suggested to be generated from arabinose and/or glucose with the added glycine. However, the effect of MRPs on •OH scavenging activity was not certain because the increase in the •OH scavenging activity of glycine-Rb_2 mixtures induced by heat-processing was not correlated with the changes in MRP levels (from a comparison of **Fig. 5A** and **5B**).

On comparing the •OH scavenging activities of ginsenosides produced by heat-processing, 20(*S*)-Rg_3 and Rg_5 strongly inhibited •OH generation to under 10% at a concentration of 0.5% (**Fig. 5C**), but the effects of 20(*R*)-Rg_3 and Rk_1 were comparably low. Therefore, the generations of 20(*S*)-Rg_3 and Rg_5 from Rb_2 were suggested to be involved in the increased •OH scavenging activity of ginseng brought about by heat-processing.

THE EFFECTS OF GLYCINE OR L-ARGININE ON HEAT STABILITY OF GINSENOSIDE-Rb_1

To ascertain the generation of MRPs from other ginsenosides and amino acids, we have analyzed Maillard reaction model experiments using ginsenoside-Rb_1 and glycine or L-arginine. The sugar moieties of ginsenosides can be a source of MRPs with amino acids contained in ginseng during heat-processing, as shown in a study of Rb_2 (Kang *et al.* 2007). 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 (Yoshimura *et al.* 1997) and L-arginine is the most abundant amino acid contained in *P. ginseng* (Lee and Park 1996).

As shown in the HPLC chromatograms (**Fig. 6A** and **6B**), 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 (**Fig. 6B**). 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 (**Fig. 6C** and **6D**), 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 (**Fig. 7A**). 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-colored nitrogenous polymers, called melanoidins, are known to be formed by this reaction (Adams *et al.* 2003; Samaras *et al.* 2005). When changes in the contents of ginsenosides between heat-processed Rb_1 and a 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 (**Fig. 6B** and **6D**). Therefore, the addition of glycine to Rb_1 for heat-processing was suggested to increase

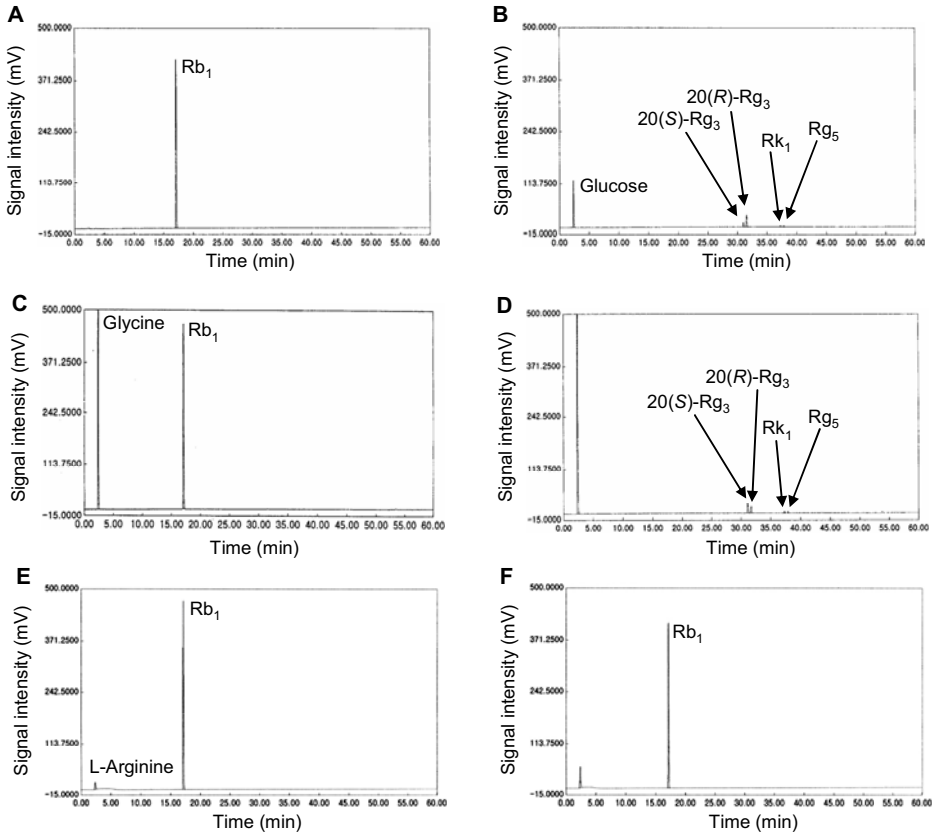

Fig. 6 HPLC chromatograms of (**A**) Rb_1, (**B**) heat-processed Rb_1, (**C**) Rb_1-glycine mixture, (**D**) heat-processed Rb_1-glycine mixture, (**E**) Rb_1-arginine mixture, and (**F**) heat-processed Rb_1-arginine mixture. (Reproduced with permission from Biological & Pharmaceutical Bulletin Vol. 30. No. 10. Copyright [2007] Pharmaceutical Society of Japan).

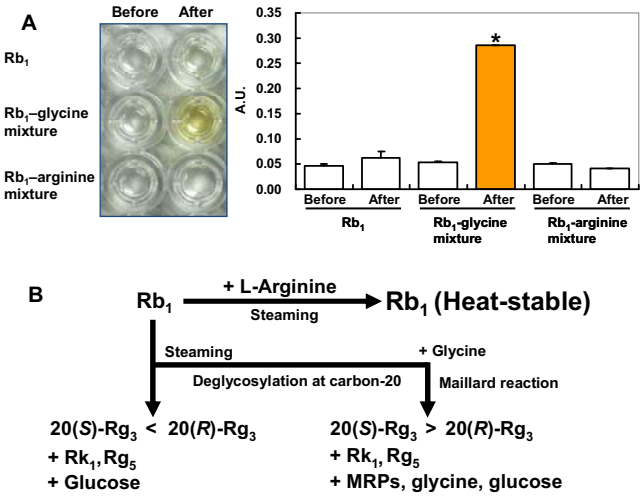

Fig. 7 (**A**) The picture and graph compares the browning compound levels in Rb_1, heat-processed Rb_1, Rb_1-glycine mixture, heat-processed Rb_1-glycine mixture, Rb_1-arginine mixture, and heat-processed Rb_1-arginine mixture at a concentration of 0.05%. (**B**) Schematic representation of the heat-processing-induced chemical changes of Rb_1 with or without glycine. *$p < 0.05$ compared with un-treated glycine-Rb_1 mixture. (Reproduced with permission from Biological & Pharmaceutical Bulletin Vol. 30. No. 10. Copyright [2007] Pharmaceutical Society of Japan).

the generation of 20(*S*)-Rg_3, which has a strong •OH scavenging activity, due to 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 (**Fig. 6E** and **6F**) compared to when Rb_1 was heat-processed with or without the same amount of glycine. In addition, there was no increase in the brown color by heat-processing of the Rb_1-arginine mixture (**Fig. 7A**), and the pH of the Rb_1-arginine mixture was about 10.37. A high temperature and high 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 (Matsuura *et al.* 1994; Li *et al.* 1999; Lertittikul *et al.* 2007). 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 (Mrabet *et al.* 1992). 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 (Cotton *et al.* 1974; Kumar *et al.* 2000). 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 (**Fig. 7B**).

THE CHEMICAL AND •OH SCAVENGING ACTIVITY CHANGES OF GINSENOSIDE-Rb_1 BROUGHT ABOUT BY HEAT-PROCESSING

As shown in the HPLC chromatograms of the Rb_2-glycine mixture (**Fig. 4**), Rb_1 was also changed into 20(*S*)-Rg_3, 20(*R*)-Rg_3, Rk_1, and Rg_5 by heat-processing at 120°C (**Fig. 6C, 6D**). 20(*S*)-Ginsenosides and 20(*R*)-ginsenosides are epimers of each other depending on the geometric position of the OH group on carbon-20. This epimerization is particularly known to occur by the selective attack of the OH group after the elimination of the glycosyl residue at carbon-20 during the steaming process (Shoji 1990; Park 1996). In addition, more less-polar ginsenosides such as Rk_1 and Rg_5 are known to be easily produced by the elimi-

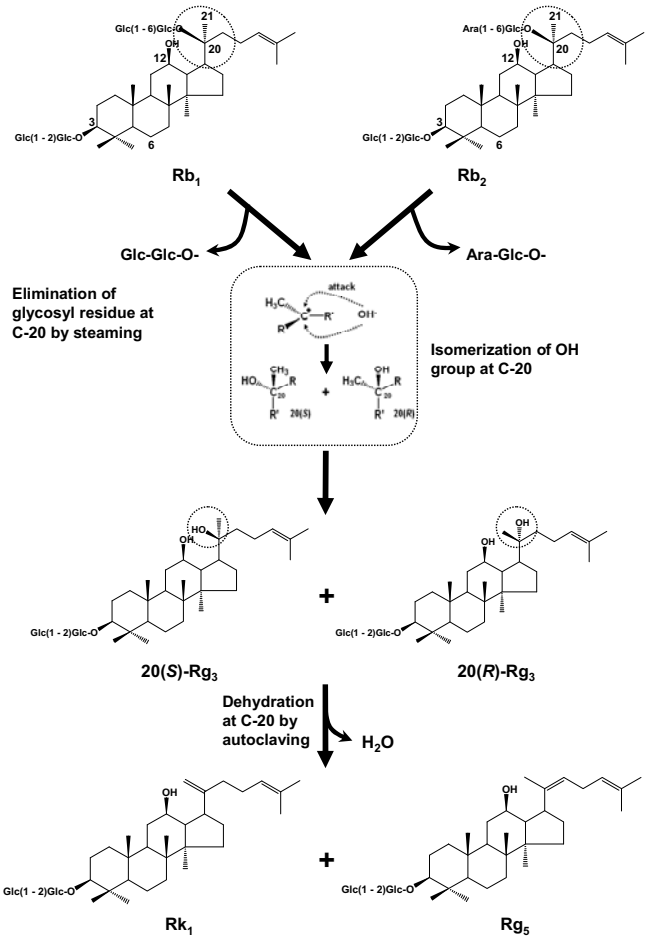

Fig. 8 Structural changes of ginsenosides Rb_1 and Rb_2 brought about by heat-processing.

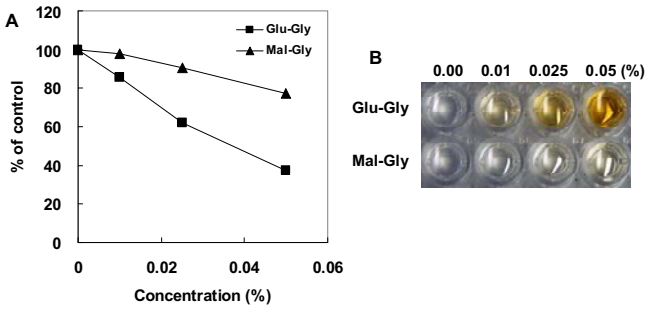

Fig. 9 The graphs compare the (**A**) •OH scavenging activities and (**B**) browning compound levels of MRPs generated from glucose-glycine and maltose-glycine mixtures. Glu: glucose, Mal: maltose, Gly: glycine. (Reproduced with permission from Biological & Pharmaceutical Bulletin Vol. 30. No. 4. Copyright [2007] Pharmaceutical Society of Japan).

nation of H_2O at carbon-20 of Rg_3 under high pressure and temperature conditions, such as in autoclaving (Park *et al.* 1998; Kang *et al.* 2007) (**Fig. 8**). The generation of 20(*S*)-Rg_3 and Rg_5 from Rb_1 were suggested to be involved in the increased •OH scavenging activity of Rb_1 by heat-processing, as in Rb_2.

However, the major difference was shown in MRPs. Rb_1 generates glucose, but Rb_2 generates arabinose and/or glucose during heat-processing (**Fig. 8**). **Fig. 9** shows the changes in •OH scavenging activities and browning compound levels of MRPs generated from glucose-glycine and maltose-glycine mixtures. MRPs generated from glucose-glycine and maltose-glycine mixtures inhibited •OH generation to about 37 and 77%, respectively, at a concentration of 0.05% (**Fig. 9A**). In addition, the browning compound levels at the concentration of 0.05% of MRPs generated

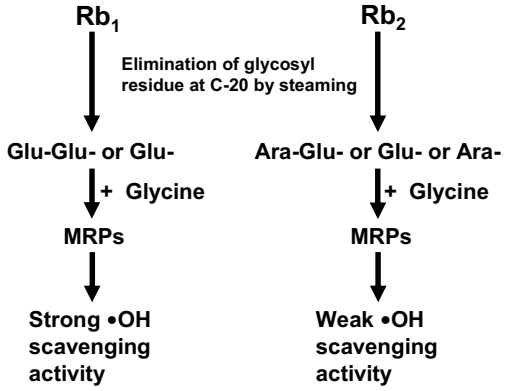

Fig. 10 Schematic representation of the generations of strong or weak •OH scavenging MRPs from ginsenosides Rb_1 or Rb_2, respectively, by heat-processing.

from glucose-glycine and maltose-glycine mixtures were 1.019 and 0.117 A.U., respectively (**Fig. 9B**). These values were dose-dependently increased, and the brown color of MRPs generated from glucose-glycine mixtures was stronger than that of maltose-glycine mixtures (**Fig. 9B**). Therefore, it is apparent that the MRPs generated from the separated sugar moieties of Rb_1 and glycine have a strong •OH scavenging activity, but MRPs from Rb_2 and glycine do not. The major difference in the •OH scavenging activities of Rb_1 and Rb_2 resulted from the difference in separated sugar moieties during heat-processing (**Fig. 10**).

CONCLUSION

The recent introduction 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 (Jia and Zhao 2009). Based upon 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 in a heat-processing, temperature-dependent manner. 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, although limited to the reaction between the glucosyl moiety and glycine, was positively correlated with the •OH scavenging activity. Finally, certain amino acids such as L-arginine block the structural change of ginsenosides, 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 ginsenosides. This investigation of specified bioactive constituents is important for the development of scientific ginseng-derived drugs with standardized ginsenosides.

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