Review

The changes in the constituents of American ginseng caused by heat-processing and its antioxidant activity

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Abstract

Panax quinquefolium L. (Araliaceae), which grows in the United States and Canada, is known as American ginseng, and is commonly used as herbal medicine in the United States. The heat-processing method to strengthen the efficacy of Panax ginseng has been well defined in Korea based on the long history of ethnopharmacological evidence. It is possible, therefore, that the efficacy of American ginseng may also increase by heat-processing as in Panax ginseng, and this was experimentally studied by us. Based upon chemical and biological activity tests, the scientific evidence underlying the therapeutic potential of heat-processed American ginseng against oxidative stress and related tissue damage was elucidated. The free radical-scavenging active components such as less-polar ginsenosides and Maillard reaction products in American ginseng were significantly increased in a heat-processing temperature-dependent manner, as in Panax ginseng. From animal experiments related to oxidative tissue damage, heat-processed American ginseng showed a renoprotective effect by ameliorating renal dysfunction and reducing elevated nuclear factor-kappa B, N\textsuperscript{\alpha}-(carboxymethyl)lysine, and receptor for advanced glycation endproducts protein expressions in the diabetic rat kidney. This investigation of specified bioactive constituents is important for the development of scientific ginseng-derived drugs from ethnomedicine. The results of the present study call for further testing involving human subjects to clarify the efficacy of dietary heat-processed American ginseng supplementation in diabetics.

Key words American ginseng, heat-processing, less-polar ginsenosides, Maillard reaction products, oxidative tissue damage.

Abbreviations AGES, advanced glycation endproducts; Ccr, creatinine clearance; CML, N\textsuperscript{\alpha}-(carboxymethyl)lysine; GAE, milligrams of gallic acid equivalent; iNOS, inducible nitric oxide synthase; MRPs, Maillard reaction products; RAGE, receptor for advanced glycation endproduct; STZ, streptozotocin.

General

Panax quinquefolium L. (Araliaceae), which grows in the United States and Canada, is known as American ginseng, and is one of the ten most commonly used herbal medicines in the United States. American ginseng has been widely used in herbal medicine for its antioxidant, anti-lipid peroxidation, anti-hypoxia, and anti-fatigue properties.\textsuperscript{1-4} In addition, American ginseng is known to exhibit a stronger hypoglycemic activity than the other Panax species and directly quenches free radicals, protects low-density lipoproteins from oxidation, and inhibits lipid peroxidation.\textsuperscript{1,4-6} Panax ginseng C. A. Meyer (Araliaceae) being mainly cultivated in Korea and Northeast China, is processed before use based on its long history of ethnopharmacological evidence.\textsuperscript{7,8} Panax ginseng root is air-dried into white ginseng or steamed at 100°C to produce red ginseng.\textsuperscript{9} 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 which is produced by steaming at 98-100°C, and this ginseng product was termed heat-processed ginseng. Heat-processed ginseng has been reported to exhibit more potent pharmacological activities, such as vasorelaxation, anxiolytic-like, antioxidant, and antitumor activities, than those of conventional white ginseng or red ginseng by us and others.  It is possible, therefore, that the efficacy of American ginseng may also increase by heat-processing as in Panax ginseng, and this was experimentally studied by us. The aim of this paper was to review scientific evidence on the changes in constituent of American ginseng brought about by heat-processing and its antioxidant activity.

The changes in constituents of American ginseng brought about by heat-processing

Ginsenosides, unique constituents of ginseng, are glycosides of 30-carbon derivatives of the triterpenoid dammarane, as shown in Fig. 1. 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. Typical ginsenosides of American ginseng are Re, Rb1, Rc, Rb2, and Rd (Figs. 1, 2(A)). After heat-processing at 120°C, the contents of polar ginsenosides (peaks 1-5) were decreased and less-polar ginsenosides (peaks 6-9) became major constituents (Fig. 2(A)).

The phenolic contents of plants can be correlated with their antioxidant activities, and Maillard reaction products (MRPs) are known as major contributors to enhanced antioxidant activity brought about by heat treatment in various crude drugs or foods. Figures 2(B) and 2(C) show the changes in total phenolic contents and browning compound levels of ginsengs, respectively, by heat-processing. The total phenolic content of

Fig. 1 Structures of ginsenosides. -Glc: d-glucopyranosyl; -Rha: l-rhamnopyranosyl; -Ara(p): l-arabinopyranosyl; -Ara(f): l-arabinofuranosyl.
American ginseng was 23.1 GAE (milligrams of gallic acid equivalent), and this value was increased to 57.0 GAE in heat-processed American ginseng. Similarly, the browning compound level of American ginseng was 0.10 A.U., and this level was increased to 0.93 A.U. in heat-processed American ginseng. The increase in the total phenolic contents due to heat-processing is believed to be mediated by the increase of free and conjugated phenolic acid contents caused by the release of bound phenolic acids linked with glucosides or amine functionalities by heat treatment.21) In addition, the increase in MRPs in ginseng brought about by heat-processing is well defined,22) and maltol was found to be abundant and a free radical-scavenging component of heat-processed ginseng in our previous study.16,23)

Increase in the free radical-scavenging activity of American ginseng by heat-processing

Reactive oxygen metabolites, including free radicals such as nitric oxide (NO), superoxide anions (O$_2^-$), hydroxyl radicals (·OH), and peroxynitrite (ONOO$^-$) are toxic and play an important role in tissue injury.24-27) O$_2^-$ reacts rapidly with 'NO to produce the more toxic ONOO$. ONOO$^-$ is protonated and forms peroxynitrous acid (ONOOH) under physiological conditions, and ONOOH easily decays to yield strong oxidants such as nitrogen dioxide, nitryl cations, and ·OH. ONOO$^-$ and its decomposition products contribute to antioxidant depletion, alterations of protein structure, and oxidative damage observed in human diseases.28-31)

Ginseng extract is known to exhibit free radical-scavenging activities against radicals such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), carbon-centered radical, O$_2^-$, ONOO$^-$, and ·OH. However, ginseng has
been found to possess no \textsuperscript{11,12,23} \textsuperscript{·}NO-scavenging activity.\textsuperscript{11,23} According to our \textit{in vitro} studies of free radical scavenging activity tests, heat-processed American ginseng more strongly inhibited DPPH, \textsuperscript{·}NO, \textsuperscript{·}O\textsubscript{2}\textsuperscript{*}, and ONOO\textsuperscript{−} scavenging activity than American ginseng.\textsuperscript{41} In the \textsuperscript{·}OH-scavenging activity tests of American ginseng and heat-processed American ginseng (Fig. 3), American ginseng inhibited \textsuperscript{·}OH generation to about 16\% at a concentration of 0.5\%, and heat-processed American ginseng more strongly inhibited \textsuperscript{·}OH generation to about 8\%, being stronger than thiourea, the \textsuperscript{·}OH-scavenging positive control compound (Fig. 3). Therefore, it was verified that heat-processing, as in \textit{Panax ginseng}, is a useful method to increase the free radical-scavenging activities of American ginseng.

The chemical and \textsuperscript{·}OH-scavenging activity changes of ginsenoside-Rb\textsubscript{1} by heat-processing

Ginsenosides have been regarded as the main active components responsible for the pharmacological activities of ginseng,\textsuperscript{7} and the steaming process is known to induce a change in the chemical constituents and enhance the biological activities of ginseng.\textsuperscript{10,24-36} To demonstrate the chemical and biological activity changes of ginsenoside brought about by heat-processing in ginseng, we have performed a Maillard reaction model experiment using ginsenoside-Rb\textsubscript{1} (Rb\textsubscript{1}) and glycine.\textsuperscript{37} Rb\textsubscript{1} is a well-known diol-type triterpene glycoside that exists abundantly in American ginseng and is known to have anti-inflammatory and antioxidant effects.\textsuperscript{38-40} Rb\textsubscript{1} has a glucosyl moiety at carbon-20 which can be easily separated by heat-processing, and glycine is a

\begin{figure}[h]
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\includegraphics[width=\textwidth]{fig3}
\caption{The \textsuperscript{·}OH scavenging activities of American ginseng (A) and heat-processed American ginseng (B) (data derived from reference 4).}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{fig4}
\caption{HPLC chromatograms of the glycine-Rb\textsubscript{1} mixture (A) and glycine-Rb\textsubscript{1} mixture steamed at 120°C for 3 h (B). The graph compares the \textsuperscript{·}OH scavenging activities of Rb\textsubscript{1}, glycine, 20(S)-Rg\textsubscript{3}, 20(R)-Rg\textsubscript{3}, Rk\textsubscript{1}, and Rg\textsubscript{5} (C) (data derived from reference 37).}
\end{figure}
frequently used amino acid in the Maillard reaction model system and also contained in Panax ginseng.\textsuperscript{7,41}

As shown in the HPLC chromatograms of the Rb\textsubscript{1}-glycine mixture, glycine and Rb\textsubscript{1} were detected at about 2.3 and 17.0 min, respectively, when not steamed (Fig. 4(A)). Then, all of the Rb\textsubscript{1} disappeared and the contents of 20(S)-Rg\textsubscript{1}, 20(R)-Rg\textsubscript{3}, Rk\textsubscript{1}, and Rg\textsubscript{5} increased, as shown by the steaming of American ginseng (Figs. 2(A), 4(B)). On the other hand, a change in the content of glycine was not confirmed because its peak overlapped the glucose peak produced by steaming, as shown in Figs. 4(A) and 4(B). When the \textsuperscript{.}OH scavenging activities of Rb\textsubscript{1}, glycine, 20(S)-Rg\textsubscript{1}, 20(R)-Rg\textsubscript{3}, Rk\textsubscript{1}, and Rg\textsubscript{5} were compared, Rb\textsubscript{1} inhibited \textsuperscript{.}OH production to about 47% at a concentration of 0.05%, but glycine showed slight or no \textsuperscript{.}OH scavenging activity (Fig. 4(C)). In the comparison of ginsenosides produced by steaming, 20(S)-Rg\textsubscript{3} and Rg\textsubscript{5} strongly inhibited \textsuperscript{.}OH generation to about 16 and 22%, respectively, at a concentration of 0.05% (Fig. 4(C)). Although strongly \textsuperscript{.}OH scavenging ginsenosides such as 20(S)-Rg\textsubscript{1} and Rg\textsubscript{5} were generated by heat-processing, this accompanied increases in 20(R)-Rg\textsubscript{3} and Rk\textsubscript{1}, weak \textsuperscript{.}OH scavengers. 20(S)-Ginsenosides and 20(R)-ginsenosides are epimers of each other depending on the geometrical position of the OH group on carbon-20. This epimerization is particularly known to occur through the selective attack of the OH group after the elimination of the glycosyl residue at carbon-20 during the steaming process.\textsuperscript{7,17} In addition, more less-polar ginsenosides such as Rk\textsubscript{1} and Rg\textsubscript{5} are known to be easily produced by the elimination of H\textsubscript{2}O at carbon-20 of Rg\textsubscript{3} under high pressure and temperature conditions, such as in autoclaving (Fig. 5).\textsuperscript{10,42}

On the other hand, the development of color is known to be an important and clear feature of the

![Fig. 5 Structural changes in the ginsenoside Rb\textsubscript{1} brought about by heat-processing. \textsuperscript{-}Glc, D-glucopyranosyl.](image-url)
Maillard reaction, and heat-induced antioxidants including melanoids and reductones are known to be produced by this reaction.\textsuperscript{18,43} As mentioned above, the glucosyl moiety at carbon-20 of ginsenoside can be easily separated by heat-processing. Figure 6 shows the changes in \textsuperscript{-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 \textsuperscript{-OH} generation to about 37 and 77\%, respectively, at a concentration of 0.05\% (Fig. 6(A)). In addition, the browning compound levels at a concentration of 0.05\% of MRPs generated from glucose-glycine and maltose-glycine mixtures were 1.019 and 0.117 A.U., respectively. These values were dose-dependently increased as shown in Fig. 6(B), and the brown color of MRPs generated from the glucose-glycine mixture was stronger than that of the maltose-glycine mixture. In addition, the \textsuperscript{-OH} scavenging activity of the glucose-glycine mixture was stronger than that of Rb\textsubscript{1} at the same concentration (from a comparison of Figs. 4(C), 6(A)). Therefore, it is clear that the MRPs generated from the separated sugar moieties of Rb\textsubscript{1} and glycine exhibit \textsuperscript{-OH} scavenging activity, and changes in the browning levels of ginsengs or ginsenosides brought about by heat-processing were thought to be related to the increase in \textsuperscript{-OH} scavenging activity (Fig. 7).

\begin{figure}[h]
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\includegraphics[width=\textwidth]{Fig7.png}
\caption{Schematic representation of the heat-processing-induced chemical changes of Rb\textsubscript{1} with glycine.}
\end{figure}

**Comparison of the effects of American and heat-processed American ginseng against streptozotocin (STZ)-induced diabetic renal damage**

Diabetes mellitus is characterized by hyperglycemia. An abnormally elevated blood glucose level causes oxidative stress and the formation of advanced glycation endproducts (AGEs), which have been closely linked to diabetic complications such as neuropathy, retinopathy, and nephropathy.\textsuperscript{44,45} In particular, diabetics are at an increased risk for several types of kidney disease, and the predominant cause of end stage renal disease with this disorder is diabetic nephropathy.\textsuperscript{46,47} Many attempts have been made to improve the treatment of diabetes. Various kinds of hypoglycemic drug or insulin are now available for the control of hyperglycemia, but there is no satisfactory therapy in modern medicine without undesirable side effects or contraindications.\textsuperscript{48} Prevention of the occurrence and progression of diabetic nephropathy has become a very important issue. Therefore, marked effort has been focused on traditional and herbal medicines without toxic effects to identify a novel therapeutic agent for diabetic nephropathy.\textsuperscript{49-52}
Antioxidants are known to protect against glycation-derived free radicals and may have a therapeutic potential.\textsuperscript{44,45} Heat-processed American ginseng showed stronger $\cdot$NO, $\cdot$O$_2$\textsuperscript{•}, ONOO$^-$, and $\cdot$OH-scavenging activities than American ginseng, as described above, and the enhanced free radical scavenging activities of heat-processed American ginseng are thought to be beneficial against diabetic oxidative damage caused by hyperglycemia. Therefore, the protective effect of heat-processed American ginseng against renal damage caused by oxidative stress or the formation of AGEs under diabetes and its molecular biological mechanism were investigated.\textsuperscript{53}

To obtain type 1 (insulin dependent) diabetic rats, STZ (50 mg/kg body weight) was injected intraperitoneally into male Wistar rats. Ten days after the injection, the glucose level of blood from the tail vein was determined and the rats were divided into three groups, avoiding any inter-group differences in blood glucose levels and body weights. The control group was given water, while the other groups were given the American ginseng or heat-processed American ginseng extract orally at a dose of 100 mg/kg body weight daily using a stomach tube. After administration for 20 consecutive days, the urine, blood, and kidneys were collected.

Over the experimental period, the levels of urinary protein excretion were significantly elevated in diabetic rats, indicating the changes in the capillary filtration barrier that result in the increased permeability of the glomerular basement membrane. In addition, this rat model showed a slight decrease in creatinine clearance ($C_Cr$). In patients with diabetes and/or renal failure, $C_Cr$, which is an effective index for expressing the glomerular filtration rate, decreases exponentially, and it eventually causes nephritic syndrome.\textsuperscript{54} However, the present investigation showed that the administration of American ginseng or heat-processed American ginseng for 20 days significantly reduced the levels of urinary protein excretion, and heat-processed American ginseng also significantly increased the $C_Cr$ level (Fig. 8). Therefore, the renal dysfunction of diabetic rats was improved by the administration of American ginseng and more significantly by heat-processed American ginseng.

The protein expressions related to oxidative stress and AGE formation, such as nuclear factor-kappa Bp65 (NF-κBp65), inducible nitric oxide synthase (iNOS), receptor for AGE (RAGE), and $N^\omega$-(carboxymethyl)lysine (CML) protein levels in the kidney, were investigated.

![Graph](image_url)  
**Fig. 8** The changes in renal function parameters. $^a p < 0.05$ compared with normal rats; $^b p < 0.05$ compared with diabetic control rats (data derived from reference 53).
using Western blot analyses. NF-κB is normally present in the cytoplasm of eukaryotic cells as an inactive complex with the inhibitory protein IκB. When cells are exposed to various external stimuli, such as reactive oxygen species or AGEs, IκB undergoes rapid phosphorylation with subsequent ubiquitination, leading to the proteosome-mediated degradation of this inhibitor. The functionally active NF-κB exists mainly as a heterodimer consisting of subunits of the Rel family (e.g., Rel A or p65, p50, p52, c-Rel, v-Rel, and Rel B) and translocates to the nucleus, where it binds to specific consensus sequences in the promoter or enhancer regions of target genes, thereby altering their expression. In addition, NF-κB is involved in the regulation of COX-2 and iNOS expressions, which are known to be involved in the pathogenesis of many chronic diseases associated with oxidative stress. These protein expressions are known to be significantly enhanced in the kidney of STZ-induced diabetic rats or mice. Our results also showed significant increases in NF-κBp65 and iNOS expression levels of the diabetic rat kidney. Although American ginseng or heat-processed American ginseng administration did not reduce these overexpressed NF-κBp65 and iNOS levels significantly (Fig. 9), the

![Fig. 9 The effects of American ginseng and heat-processed American ginseng on NF-κBp65 (A), iNOS (B), CML (C), and RAGE (D) protein expressions. *p < 0.05 compared with normal rats; †p < 0.05 compared with diabetic control rats (data derived from reference 53).](image-url)
NF-κBp65 levels in American ginseng or heat-processed American ginseng-administered groups were not significantly increased compared to those of normal rats. These results imply that American ginseng and heat-processed American ginseng alleviate oxidative stress by preventing NF-κB activation.

On the other hand, CML, one of the major AGEs, is known to be a marker of cumulative oxidative stress and be involved in the development of diabetic nephropathy. Moreover, the activation of RAGE by CML results in the activation of NF-κB and production of proinflammatory cytokines. CML accumulation and RAGE expression in diabetic rats were markedly higher than normal, but they were significantly ameliorated in heat-processed American ginseng-administered groups (Figs. 9(C), 9(D)). These findings imply that heat-processed American ginseng can prevent diabetic nephropathy via inhibiting RAGE activation caused by AGE formation.

This study demonstrated that American ginseng and heat-processed American ginseng ameliorate diabetes-induced renal dysfunction and inhibit AGE-generation through reducing the protein expressions related to oxidative stress and AGE formation under diabetes. Therefore, American ginseng and heat-processed American ginseng may improve diabetic pathological conditions and prevent renal damage associated with diabetic nephropathy, and these preventive effects of American ginseng can be improved by heat-processing.

**Conclusion and perspectives**

Based upon chemical and biological activity tests, scientific evidence supporting the improved therapeutic potential of heat-processed American ginseng than American ginseng against oxidative stress was obtained. The free radical scavenging active components such as less-polar ginsenosides and MRPs in American ginseng were significantly increased in a heat-processing temperature-dependent manner, as in Panax ginseng. From animal experiments related to oxidative tissue damage, heat-processed American ginseng showed a stronger renal protective effect than American ginseng by ameliorating renal dysfunction and reducing elevated NF-κB, CML, and RAGE protein expressions in the diabetic rat kidney (Fig. 10). Moreover, consistent with our observations, the steaming of American ginseng root and berries augments the ginsenoside Rg2 content and increases the antiproliferative effects on HCT-116 and SW-480 human colorectal cancer cell lines.

Therefore, it is clear that heat-processed American ginseng has a therapeutic potential to protect against oxidative tissue damage by inhibiting protein expressions related to oxidative stress and AGEs. This investigation of specified bioactive constituents is important for the development of scientific ginseng-derived drugs from ethnomedicine. The results of our study, along with data from recent studies by others, call for further testing in human subjects to establish the efficacy of dietary heat-processed American ginseng supplementation in diabetics.
References

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