Effect of Hot Water Boiling and Autoclaving on Physicochemical Properties of American Ginseng (Panax quinquefolium L.)

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Abstract: This study evaluates changes in the chemical composition and bioactivities of American ginseng (Panax quinquefolium L.) processed by boiling in water, 75°C for 10, 20, 30, and 40 min, and autoclaving at high temperatures, 115°C for 30 and 60 min and 130°C for 90 and 120 min. Total ginsenoside contents of boiled ginseng remained relatively unchanged, whereas the contents of autoclaved ginseng samples significantly decreased with an increase of both time and temperature. Compared to unheated ginseng (control), the color of both boiled and autoclaved ginseng decreased in lightness and increased in redness. The acidic polysaccharide contents, the total phenolic contents and the antioxidant capacity of boiled and autoclaved ginseng were higher than the untreated ginseng, with the highest values being exhibited by the autoclaved samples. In particular, the antioxidant capacity of unheated ginseng increased about 2.5 times (285.7±14.03 mg/100 g to 777.2±26.4 mg/100 g) when ginseng was autoclaved at 130°C for 120 min as compared to the control. It was concluded that as American ginseng was processed at a high temperature, especially steam-heated in an autoclave, its chemical constituents changed and, in particular, acidic polysaccharides, total phenolics and antioxidant capacity were considerably increased.

Key words: American Ginseng, ginsenoside, acidic polysaccharide, total phenolic contents, vitamin C equivalent antioxidant capacity

INTRODUCTION

Ginseng root is a valuable agricultural commodity grown for use in many traditional medicinal therapies. It is known that major biological active components of ginseng include ginsenosides, polyacetylenes, acidic polysaccharides, ginseng proteins, and phenolic compounds. Among the known biochemical and pharmacological activities of ginseng are antiaging, antidiabetic, anticarcinogenic, cardiovascular, endocrine, and immune systems and antistress, antifatigue, antioxidant activities and promoters of DNA, RNA and protein synthesis activities. In general, it is reported that its pharmacological and biological activities are increased after exposure to a heating process. This is because the heating and hydrolysis of raw ginseng generates new ginsenoside constituents which have been reported to possess a medical action. In addition, it is known that heat processing contributes to increases of the total phenolic contents and antioxidant capacity. Also, researchers found that acidic polysaccharide, an important biological active component, is increased when raw ginseng is treated by heating at a high temperature. For these reasons, heat-processed ginseng, in the Hong Kong ginseng market where about 70% of the ginseng is traded worldwide, sells at a much higher price than air-dried ginseng. In Asia, white ginseng is produced by sun dried and red ginseng is produced by steaming process at 98-100°C for 2-3 hr for 6 years aged ginseng. Several studies have identified ginsenosides compounds in red ginseng that are not usually found in fresh ginseng. The chemical and biological differences between white and red ginseng may be result from diverse chemicals produced by the steaming treatment. Asian red ginseng found higher contents of ginsenosides and antioxidant activity with increasing heat temperature and steaming treatment, however, there are the limited information on the antioxidant capacity, phenolic contents, and major individual ginsenosides through heat treatment and high pressure, such as autoclave process.
with fresh America ginseng, convert into red ginseng. Most North American ginseng, produced in the U.S. and Canada, is only dried after the harvest with no prior heat treatment. Therefore, in the present study, we evaluate the effects of both boiling and autoclaving processes on the chemical composition of ginsenosides and acidic polysaccharides, and bioactivities of American ginseng (*Panax quinquefolius* L.). In addition, we conduct bioactivities of antioxidant activities with total phenolics and antioxidant expressed by vitamin C equivalent antioxidiant capacity, which presents tentative vitamin C contents in 100 g sample.

**MATERIALS AND METHODS**

**Sample preparation**

Four-year old ginseng roots were purchased from a Wisconsin ginseng farm in June 2005. After a thorough rinsing in water, ginseng roots were boiled in 75°C water for 10, 20, 30 and 40 min, respectively. Other ginseng roots were steamed in an autoclave at 115°C for 30, 60 minutes, and at 130°C for 90, 120 min. The boiled ginseng, autoclaved ginseng and raw ginseng samples were dried in a 38°C hot air dryer until the moisture contents was reduced to less than 14%. The dried samples were then ground to a 60 mesh powder for analyses.

**Extraction and determination of ginsenosides**

The procedure for extraction and determination of ginsenosides was modified from Court et al.11) A 100 mg powdered sample was extracted with 10 mL of 100% HPLC-grade methanol (Fisher Chemicals, Fairlawn, NJ) in a plastic centrifuge tube and placed in a sonicator bath for 15 min at room temperature. The sample tube was centrifuged at 4,500 rpm for 1 min and the supernatant collected. The pellet was re-extracted two additional times in 10 mL of solvent and supernatants combined. This supernatant was reduced to dryness using vacuum rotary evaporator (Buchi 011, Buchi Analytical, Inc., DE) at 38°C and the residue was resolved in 2 mL of 100 % methanol. The extract was dried under a stream of N₂ at 38°C and then dissolved in 500 µL of 73% acetonitrile diluted with HPLC-grade water. A 15 µL sample was injected to HPLC column. The HPLC unit was a Waters Associates (Model 2690 Separations Module, Waters, MD) with a PDA detector (Waters 996 Photodiode Array, Waters, MD) and the absorbance set at 203 nm. An HPLC column (Chrompack Standard Columns, LiChrosorb RP18, 5 µm, 250 x 3 mm, NJ) was used with a guard column (Chromsep Guard R, NJ), and the gradient of two solvents, (A) phosphate buffer (10.3 mM KH₂PO₄ at pH 5.8) and (B) acetonitrile was 0-20 min, 84-82% A and 16-18% B; 20-60 min, 82-60% A and 18-40% B, at a flow rate of 1.15 mL/min. M-cresol was used as an internal standard. Ginsenoside standards included Rb₁, Rb₂, Rc, Rd, Re, and Rg₁ (Indofine Chemical Company, Hillsborough, NJ). Qualitative identification of ginsenoside peaks was determined by cochromatography (equivalent to the retention time) with chemically pure standards, and quantification was based on the integration of the peak area compared with a standard curve. Results are reported in the percentage of ginsenosides on a dry weight basis.

**Acidic polysaccharides**

Ginseng’s acidic polysaccharides were analyzed by the carbazole-sulfuric acid methods.12) One gram of ginseng powder was added to 70 mL of distilled water, stirred for 2 hrs and then centrifuged at 6,000 rpm for 30 minutes. After that, the sample was filtered using Whatman paper No.1. A 0.5 mL aliquot of filtered sample liquid and 0.25 mL of 0.125% carbazole solution were mixed in a test tube followed by the addition of 3 mL of concentrated H₂SO₄. The sample was placed in the 85°C water bath for 5 min and cooled at room temperature for 15 minutes. The absorbance was measured using a spectrophotometer (Model DU 530, Beckman, MD) at 525 nm to determine the acidic polysaccharide contents. The concentration of acidic polysaccharides in each ginseng sample was calculated on a basis a standard curve, which was obtained using various concentrations of galaturonic acid.

**Color**

The color of the ginseng powder was measured for L (lightness degree), a (redness degree), and b (yellowness degree) coordinates using a Hunter UltraScanXE (Reston, VA). The colorimeter was calibrated against a white calibration plate and a white surface with L, a, and b values of 98.74, -0.39 and -0.28, respectively. Measurements were duplicated 4 times for each sample and the averages of L, a, and b values of each sample recorded.

**Extraction and determination of total phenolics**

One gram ginseng powder was directly weighed into a 250 mL evaporating flask and pre-chilled 100 mL of 80% methanol was slowly added with swirling. Samples were prepared in duplicate. Flasks were sonicated under nitrogen gas for 20 min with occasional mixing. Extracts were filtered through chilled Buchner funnels and No. 2 Wha-
man paper using 50 mL 100% methanol to rinse. Precipitates were transferred back into their original flasks using 100 mL of 80% methanol. Samples were re-sonicated another 20 min under nitrogen and refiltered using 50 mL aliquots of 100% methanol. Supernatants were transferred to 1,000 mL liter evaporating flasks with 50 mL of 80% methanol and concentrated to near dryness. Final dilutions of 100 mL 50% methanol were prepared by rinsing evaporating flasks deionized distilled water to a volume of 50mL and continuing rinsing flasks with 100% methanol to final volumes of 100 mL. Samples were centrifuged at 10,000 rpm for 20 minutes. Extracted samples were transferred to storage bottles, purge with nitrogen and frozen until analyzed. The total phenolic contents were measured using the Folin-Ciocalteu method.13) Briefly, 1 mL of appropriately diluted samples or a standard solution of gallic acid was added to a 25 mL volumetric flask containing 9 mL of ddH2O. A reagent blank was prepared using ddH2O. One milliliter of Folin-Ciocalteu phenol reagent was added to the mixture and mixed by shaking. After 5 min, 10 mL of 7% Na2CO3 solution was added with mixing. The solution was then immediately diluted to a volume of 25 mL with ddH2O and mixed thoroughly. After incubation for 90 min at 23°C, the absorbance relative to that of a prepared blank was read at 750 nm using a spectrophotometer (model DU 530, Beckman, MD). The total phenolic contents were expressed in mg of gallic acid equivalents (GAE) per 100 g dried white ginseng weight.

Antioxidant capacity

The antioxidant capacity of ginseng powder was measured and calculated as VCEAC (Vitamin C Equivalent Antioxidant Capacity) according to the method described by Lee et al.14) Briefly, vitamin C standard curves that correlate the concentration of vitamin C with the amount of absorbance reduction caused by vitamin C were obtained using the ABTS radical scavenging assay. The AAPH (1 mM) was mixed with 2.5 mM ABTS as diammonium salt in phosphate buffered saline (PBS) solution (100 mM potassium phosphate buffer (pH 7.4) containing 150 mM NaCl). After the mixture was heated in a water bath at 68°C for 13 min, the blue-green ABTS solution was adjusted with fresh PBS solution to an absorbance of 0.650 at 734 nm. Twenty µL of the sample solution added to 980 µL of the ABTS radical solution was incubated in a water bath at 37°C for 10 min. The decrease of absorbance at 734 nm was measured at 10 min. The control consisted of 20 µL of 50% methanol and 980 µL of ABTS solution. The ABTS radical scavenging capacities of ginseng extracts were expressed on the fresh weight basis as mg of vitamin C equivalent antioxidant capacity/100 g (VCEAC). Samples of each extraction were analyzed in triplicate. Vitamin C standard curves at, 2.5, 5, 10, 20, 40, 60, 80, and 100 mg/L concentrations of L-ascorbic acid, were obtained using the ABTS. The absorbance reduction at 734 nm of ABTS solution by selected pure polyphenol was measured at concentrations of 2.5, 5, 10, and 20 m.

RESULTS AND DISCUSSION

Analysis of ginsenosides

Ginsenoside, also referred to as saponin, is known as glycoside banded with saccharides in aglycone and are believed to play an important role in ginseng's pharmacological action. Of the ginsenosides, Rb1, Rb2, Rc, Rd, Re, and Rg1 have been most popularly studied to determine the variation of ginsenoside contents when ginseng was processed for functional foods. Ginsenoside Rg3 is well known by the major ginsenoside when white ginseng process into red ginseng. However, in this study, we focused on investigating for determination of the relative ginsenoside composition during the process. The six ginsenoside (Rb1, Rb2, Rc, Rd, Re, and Rg1) contents were evaluated as to the effects of the heat treatments. Tables 1 and 2 showed the ginsenoside contents of ginseng boiled in 75°C water and autoclaved at a temperature of 115°C and 130°C. The total ginsenoside contents of ginseng boiled in 75°C water for 10, 20, and 30 min were slightly increased in comparison to that of non-heated ginseng (P < 0.05). The concentrations of total ginsenosides gradually decreased with the boiling time up to 40 min, reaching to the highest concentration and then leveled off thereafter. The decrease of total ginsenosides in boiling for long time compared to fresh ginseng may be due to the degradation of ginsenoside as consequences of heat treatment. On the other hand, ginsenoside contents significantly increased in the autoclaved treatments at 115°C for 30 and 60 min, but rapidly decreased in the autoclaved treatment at 130°C for 90 and 120min. Of the six ginsenosides, Rb2, Rc, and Rd exhibited increased levels in 75°C boiling treatments, while other ginsenoside contents did not change. However, with the autoclaved samples, ginsenoside contentss were dependant on temperatures. In the 115°C treatment, most ginsenoside contents were significantly increased, except for Re and Rg1. In the 130°C treatment, most ginsenoside contents were significantly decreased with the exception of Rb2 and Rd. It has been reported that some
ginsenosides, including Rb1, Rb2, Rc, Rd, Re, and Rg1, were slightly decreased compared to those of raw ginseng when Korean fresh ginseng roots were steamed at 100°C for 2 hours, while the ginsenosides quickly decreased when Korean fresh ginseng roots were steamed at 110°C and 120°C for 2 hours.8) American ginseng have the higher Rb1 ginsenoside contents and lowest contents of Rg1 than found for Rb2, Rc, and Rd. American ginseng root have been consistent with the approximate ginsenosides profile: Rb1 > Re > Rg1 = Rc > Rd.11,20) In this study, contents of the major ginsenosides, such as Rb1 and Re were agreed with previous studies. Rb1, Re among major ginsenosides exhibited about 3.67 and 1.06% which were greater than most of the ginsenoside in American red ginseng and especially Rb1 was mainly increased by the heating process. Asian ginseng had a high Rg1:Rb1 ginsenoside ratio and Rg1 was proved to have a wound healing effect.

Acidic polysaccharide contents
Fig. 1 shows the acidic polysaccharide contents of ginseng boiled in 75°C water and ginseng autoclaved at 115°C and 130°C. The acidic polysaccharide contents of ginseng increased from 0.33±0.01 mg/g for the control sample to 0.52±0.01, 0.53±0.01 and 0.53±0.02 0.56±0.01 mg/g when it was boiled for 10, 20, 30 and 40 minutes, respectively. There were no significant differences among the time treatments. However, the acidic polysaccharide contents of the autoclaved ginseng samples were greatly increased compared to the non-heated ginseng. The unheated control sample exhibited an acidic polysaccharide contents of 0.33±0.01 mg/g, but when ginseng was autoclaved at 115°C for 30 and 60 minutes, the acidic polysaccharide contents was 0.74±0.01 and 0.76±0.02 mg/g and at 130°C for 90 and 120 minutes, respectively. This is because additional polysaccharides extracted to a more soluble state when ginseng was heat treated. A previous report showed that the contents of acidic polysaccharides increased by about 120% when Korean ginseng roots were heated due to an increase in extracted polysaccharides during processing.10)
Table 3 shows a change in the color of raw ginseng boiled in 75°C water and ginseng autoclaved at 115 and 130°C. When raw ginseng was boiled at 75°C, the L value (lightness) was decreased and the a (redness) and b values (yellowness) were increased as treatment time increased from 10 to 40 min. Autoclaved samples showed an even greater color change than the boiled samples when compared to the control. The L value was 59.95±0.27 at 115°C for 60 min and 52.85±0.16 at 130°C for 120 min, while the L value of non-heated ginseng was 71.67±0.17. The a value was 4.05±0.08 at 115°C for 60 min and 4.69±0.03 at 130°C for 120 min, while the L value of unheated ginseng was 1.55±0.04. This decrease in lightness and increase in redness occurring when ginseng was autoclaved at a higher temperature for a longer period of time was due to the browning reaction of the constituents of heated ginseng as reported previously.15)

**Total phenolic contents**

Fig. 2 shows the total phenolic contents of various ginseng boiled in 75°C water and ginseng autoclaved at 115°C and 130°C. The total phenolic contents of unheated ginseng was 444.5±25.1 mg GAE/100g. Total phenolic contents were changed to 449.5±13.1, 471.3±6.2, 496.9±10.7 and 514.5±10.9 mg GAE/100g, respectively, when ginseng was boiled at 75°C for 10, 20, 30, and 40 min (p<0.001). As the boiling time increased, the total phenolic contents gradually increased. The total phenolic contents of ginseng autoclaved at 115°C for 30 and 60 min was 529.8±13.1 and 557.8±10.14 mg GAE/100g, respectively and that of ginseng autoclaved at 130°C for 60 and 90 min exhibited much higher amounts, with 787.9±17.43

**Table 3.** Color changes of American ginseng processed by boiling and autoclaving

<table>
<thead>
<tr>
<th>Treatments</th>
<th>L</th>
<th>Hunter color values</th>
<th>a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>71.67±0.17</td>
<td>1.55±0.04</td>
<td>14.29±0.16</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>69.98±0.16</td>
<td>2.12±0.46</td>
<td>15.70±0.12</td>
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</tr>
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<td>20</td>
<td>66.42±0.15</td>
<td>2.44±0.10</td>
<td>15.75±0.15</td>
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</tr>
<tr>
<td>30</td>
<td>65.94±0.25</td>
<td>2.50±0.10</td>
<td>15.79±0.14</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>65.45±0.14</td>
<td>2.54±0.11</td>
<td>15.69±0.16</td>
<td></td>
</tr>
<tr>
<td>Boiling time at 75°C</td>
<td>62.74±0.26</td>
<td>3.72±0.04</td>
<td>16.17±0.14</td>
<td></td>
</tr>
<tr>
<td>(min)</td>
<td>59.95±0.27</td>
<td>4.05±0.08</td>
<td>15.97±0.13</td>
<td></td>
</tr>
<tr>
<td>Autoclaving Treatments</td>
<td>55.86±0.31</td>
<td>4.32±0.06</td>
<td>14.73±0.15</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>52.85±0.16</td>
<td>4.69±0.03</td>
<td>12.88±0.09</td>
<td></td>
</tr>
</tbody>
</table>

Date are expressed as mean ± SD. Different letters in the same column are significantly different at p<0.05 by Duncan’s multiple range tests. The color represented for L (lightness degree), a (redness degree), and b (yellowness degree).

* Represented autoclaving treatments 1; autoclaving treatment at 115°C for 30 min, 2; autoclaving treatment at 115°C for 60 min, 3; autoclaving treatment at 130°C for 90 min, 4; autoclaving treatment at 130°C for 120 min.
and 813.8±8.12 mg GAE/100 g, respectively. Overall, total phenolic contents correlated positively with higher temperature and longer time treatments. The increase of total phenolic contents in American ginseng treatments compared to white ginseng may be due to the Maillard browning reactions. Maillard reaction products, which can be formed as consequences of heat treatment, generally exhibit strong antioxidant properties. The antioxidant activities of the Maillard reaction products have been studied on the improvement of the oxidative activity of foods.

VCEAC (Vitamin C Equivalent Antioxidant Capacity)

Fig. 3 shows the antioxidant capacity of heat treated ginseng samples. Antioxidant capacity exhibited a similar result of total phenolic contents, which was positively correlated to time and temperatures. However, the antioxidant capacity was more significantly increased at the highest temperature treatment. The antioxidant capacity of non-heated ginseng was 285.7±14.0 mg/100 g. That of ginseng boiled at 75°C for 10, 20, 30 and 40 minutes was 358.5±33.5, 370.2±22.6, 383.06±8.9 and 401.1±65.8 mg / 100 g, respectively. All values were significantly different from the unheated ginseng (p<0.001). As the boiling time increased, the antioxidant capacity gradually increased. The antioxidant capacity was rapidly increased when ginseng was autoclaved at a temperature of 130°C. The antioxidant capacity of non-heated ginseng was 285.7±14.03
mg/100 g, but that of ginseng autoclaved at 130°C for 90 and 120 minutes was 744.5±36.29 and 777.2 ±26.4 mg/100 g, respectively, and that of ginseng autoclaved at 115°C for 30 and 60 minutes was 383.7±8.1 and 487.5 ±34.3 mg/100 g, respectively. Similar results to our findings, an increase of the total phenolic contents and the antioxidant effect proportional to an increase in temperature and time of the heat-processed ginseng, has been reported that Korean ginseng steamed at 120°C for two hours has stronger radical-scavenging activities than that steamed at 100°C or 110°C for two hours. It has been suggested that the increase of antioxidant capacity in red ginseng as compared to white ginseng may be due to the maillard browning reaction.16,17,18) In this study, the prolonged heating time (120 min) and heating temperature (130°C) significantly enhanced the overall antioxidant activities of American ginseng. This may be explained by the antioxidant activity could be due to releasing of phenolic compounds having antioxidant capacity through thermal treatment and time. As we investigated the color change with boiling and heating treatments (Table 3), non-enzymatic browning reaction products might be formed during prolonged heating process with the improvement of antioxidant activity. For these reasons, we investigated with the heating time (30, 60, 90, and 120) and heating temperature (115 and 130°C) on ginseng treatments. Antioxidant compounds in food are mainly present as a covalently bound form with insoluble polymers,21) but the boiling (heating) and pressure treatments might disrupt the cell wall and liberate free antioxidant compounds from insoluble composition.

In conclusion, this study was conducted to evaluate chemical composition and bioactivities of American ginseng when processed by boiling water and autoclaving treatments at high temperatures for various time intervals. Both modes of heat processing produced a change in the color of American ginseng becoming darker and more red. Depending on the particular time and temperature of the heat process, certain ginsenosides either increased or decreased. Overall, compared to dried unheated ginseng, the contents of such major biologically active substances as acidic polysaccharides, phenolics, and antioxidants were greatly increased which potentially provides a more health beneficial commodity.

REFERENCES


