Research Article

Received: 27 September 2011 Revised: 26 February 2012 Accepted article published: 21 March 2012 Published online in Wiley Online Library: 23 April 2012

(wileyonlinelibrary.com) DOI 10.1002/jsfa.5710

Effects of high hydrostatic pressure on structure and colour of red ginseng (*Panax ginseng*)

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Abstract

BACKGROUND: The conventional method of processing ginseng (*Panax ginseng*) roots into red ginseng involves mainly heating and drying processes. In the present study, this method was modified by using high hydrostatic pressure (HHP) to improve the physicochemical characteristics of red ginseng.

RESULTS: The HHP process (600 MPa for 1 min) significantly improved the histological properties of red ginseng by increasing cellular disruption and release of cell contents. The total reducing sugar content was significantly ($P < 0.05$) higher (increased from 10.67 to 15.25 mg g^{-1}) in red ginseng processed at 600 MPa for 1 min. Similarly, the total free amino acid content also increased significantly (from 2.81 to 7.77 mg g^{-1}). The HHP process resulted in superior and more even colouration and gave an attractive visual appearance to red ginseng. The optical density at 420 nm and Hunter's colour *a* value (redness) of extracts prepared from red ginseng increased significantly ($P < 0.05$) with the application of HHP.

CONCLUSION: HHP-processed red ginseng has significantly higher reducing sugar and free amino acid contents together with a more compact cell structure and superior visual quality (brighter red colour). Hence the application of HHP in red ginseng processing can result in ginseng products of improved quality compared with those obtained by the conventional method.

Keywords: red ginseng; high hydrostatic pressure; histological properties; reducing sugars; free amino acids; colour

INTRODUCTION

Ginseng (*Panax ginseng* C.A. Meyer) roots are used extensively in Asia for both culinary and medicinal purposes. Ginseng was reported to have beeen first cultivated around 11 BC and has a medicinal history as a wild herbal plant spanning more than 5000 years.[1] It is a common herb used for its pharmaceutical and nutraceutical properties and reported efficacy in the central nervous and immune systems and antioxidant and anti-stress activities.[2] It is therefore regarded as one of the expensive and highly beneficial herbal medicines. Ginseng produced in Korea is considered a medicinal and functional food in oriental and modern health sciences. As a result of the efficacy and functional properties of Korean ginseng, its consumption in the form of different edible products has been increasing.[3] The biochemical and pharmacological properties of ginseng have been categorised as anticarcinogenic, analgesic, anti-aging, antidiabetic, antipyretic, anti-stress, tranquillising and anti-fatigue activities and promotion of DNA, RNA and protein synthesis activities.[4]

Commercially available ginseng roots are categorised as fresh, white and red ginseng. White ginseng is produced by washing fresh ginseng roots and drying them until the water content is 130 g kg^{-1} or lower. Red ginseng is prepared by steaming and drying fresh ginseng roots. The steaming process gives a glossy reddish-brown colour, which is produced by Maillard reaction between amino acids and reducing sugars in ginseng roots. These processes also prevent spoilage of ginseng, which enables its long-term preservation and decreases its weight and volume, thereby facilitating transport and storage.[3] The quality of red ginseng is based on the hardness of the rhizome, the balance between the main root and lateral roots, colour and inner structure. The reddish-brown colour intensities of inner and outer parts of red ginseng are very important in terms of ginseng quality. Good-quality red ginseng has similar outer and inner reddish-brown colour.[5] In contrast, the presence of inner cavities, 'inner white' and pith in red ginseng renders lower product quality. 'Inner white' is one of the most important parameters in grading red ginseng and can be attributed to white tissues that do not change to reddish-brown after cooking and drying. It is important to reduce inner

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cavities and 'inner white' in order to impart the uniform reddish-brown colour desired for high-quality red ginseng.[6] During heat treatment (<100 °C) of raw ginseng, browning compounds and Maillard reaction products (MRPs) are produced via the reaction of carbonyl groups of reducing sugars with free amino groups of amino acids, giving the typical colouration to red ginseng. MRPs are also regarded as useful antioxidants in foods and herbal medicines.[7]

High hydrostatic pressure (HHP) is a modern food-processing technology wherein foods are subjected to hydrostatic pressures ranging from 100 to 1000 MPa. The HHP process changes the textural characteristics of plant materials and, depending on the type of matrix, can cause either softening or firming of different vegetables and fruits.[8] Zhang *et al.*[9] applied HHP to extract ginsenosides from ginseng and reported higher yields than those obtained by the conventional method. Permeabilisation of plant cells and increased release of intracellular substances after pressure treatment have also been demonstrated.[10] Substantial literature is available on high-pressure processing of various other plant materials. However, there are few reports on the application of HHP for improving the quality characteristics of red ginseng. Therefore we carried out the present research to study the effects of the HHP process, before applying the heating and drying processes during red ginseng manufacture, on the microstructure, reducing sugars, total free amino acids, histological properties, browning intensity and redness of the final product. Our objective was to improve the above-mentioned physicochemical characteristics of red ginseng by modifying its conventional processing method with a more innovative and newer technique such as HHP.

MATERIALS AND METHODS

Sample preparation

Freshly harvested 6-year-old Korean ginseng (*P. ginseng* C.A. Meyer) roots cultivated in Kangwha, South Korea were selected for this study. They were washed and ground to prepare homogeneous samples of 2–3 mm particle size. Ground fresh ginseng was vacuum packaged in polyethylene films of 80 μm thickness using a vacuum packer (Cretel, Eeklo, Belgium). The HHP process was carried out at 200, 400 and 600 MPa for 1 min at 25 °C using a laboratory-scale pressure unit (Frescal MFP-7000, Mitsubishi Heavy Industries, Tokyo, Japan). These conditions were selected based on one of our previous studies in which 400 and 600 MPa pressures gave better results than conventional processing for increasing the concentration of ginsenosides, which are major bioactive components in red ginseng.[11] Samples were analysed for reducing sugars and total free amino acids. Samples for microstructural study were prepared by washing and vertically cutting ginseng roots into two parts. One part was subjected to HHP treatment at 600 MPa for 1 or 10 min and the other part was used as a control. Samples for histological and visual observation of red ginseng were also prepared by cutting the washed roots vertically into two parts. One part was vacuum packed and processed using HHP at 600 MPa for 1 min at 25 °C, followed by steam cooking at 98 ± 1 °C for 3 h and drying at 60 °C for 4 days using a forced convection dryer (OF-22GW, Jeio Tech, Seoul, Korea).[11] Conventional red ginseng was prepared as a control from the other half of each root by following the same steps, except that the HHP process was not applied.

Scanning electron microscopy

Samples of HHP-processed and non-pressurised ginseng root halves were treated for 6 h with Karnovsky's fixative (20 g L^{-1} glutaraldehyde, 20 g L^{-1} paraformaldehyde, 5 g L^{-1} $CaCl_2$), washed in 0.1 mol L^{-1} phosphate buffer solution (pH 7.4), fixed again for 1 h using 10 g L^{-1} osmium tetroxide, dehydrated with ethanol (up to 1000 mL L^{-1}) and finally embedded in iso-amylactate. Samples were dried using a critical point dryer (SCP-2, Hitachi, Tokyo, Japan), coated with 100 nm of gold using an ion coater (E-1010, Eiko Co., Hyogo, Japan) and finally observed under a field emission scanning electron microscope (S-800, Hitachi).

Light microscopy and transmission electron microscopy

HHP-processed (600 MPa for 1 min) and non-pressurised ginseng root halves were analysed for their microstructural characteristics. Tissue samples (1 mm^3) from the roots were fixed overnight in glutaraldehyde in 0.1 mol L^{-1} phosphate buffer (pH 7.4) at 7 °C, rinsed twice with 0.1 mol L^{-1} phosphate buffer and post-fixed for 18 h in 10 g L^{-1} osmium tetroxide solution prepared in the same buffer. Tissues were then dehydrated in a graded series of ethanol and propylene oxide, for 5 min in 500 mL L^{-1} ethanol, 10 min in 800 mL L^{-1} ethanol, 2 × 10 min in 1000 mL L^{-1} ethanol, 5 min in ethanol/propylene oxide (1 : 1 v/v) and 10 min in 1000 mL L^{-1} propylene oxide, and finally embedded in epoxy resin. Samples were cut into 0.5 μm sections using a microtome (EM-UCT, Leica Microsystems, Wetzlar, Germany) and stained with toluidine blue for high-resolution light microscopy analysis. Tissue images were taken using a light microscope (BX-50, Olympus Optical, Tokyo, Japan) at 200× and 400× magnifications.

Analysis for reducing sugars

Analysis for reducing sugars was performed using the method described by Chavan *et al.*[12] with some modifications. HHP-processed or non-pressurised ginseng (10 g) was extracted with 800 mL L^{-1} ethanol (100 mL) at 80 °C for 4 h in a round-bottom flask fitted with a cooling condenser. This process was repeated three times. The residue was washed with 800 mL L^{-1} ethanol and filtered through Whatman no. 4 filter paper, then the solvent from the extract was evaporated to a volume of less than 100 mL using a rotary vacuum evaporator (N1000, Eyela, Tokyo, Japan) at 45 °C. This extract was further washed with 100 mL of diethyl ether to remove any fat content. The aqueous layer was washed three times with 100 mL of water-saturated *n*-butanol. The remaining aqueous layer was transferred to a tared round-bottom flask and evaporated under reduced pressure at 60 °C until ethanol vapour was completely removed. The empty flask was dried at 60 °C, cooled in a desiccator and weighed until constant weight. The difference in weights corresponds to the soluble solids of the sample. The residue left after evaporation was dissolved in water and filtered using a 0.45 μm filter. Glucose and maltose in the filtrate were analysed using a high-performance liquid chromatograph (HPLC). The HPLC system consisted of a pump (P680, Dionex, Sunnyvale, CA, USA), autosampler (ASI-100, Dionex), refractive index detector (RI-101, Shodex, Tokyo, Japan), μBondpack NH_2 analytical column (3.9 mm × 300 mm; Waters, Milford, MA, USA) and column oven (TCC100, Dionex). A 20 μL sample was injected and acetonitrile/water (80 : 20 v/v) was used as solvent at a flow rate of 0.8 mL min^{-1} with the column temperature set at 30 °C. Standard grades of sugars were dissolved in analytical-grade water, and different concentrations

were used to obtain calibration curves. Peaks were identified on the basis of retention times by comparison with those of standard samples. Quantification was carried out on a weight basis.

Analysis for free amino acids

HHP-processed or non-pressurised ginseng was extracted with 10 volumes of water at 4 °C for 24 h and centrifuged at 6000 × *g* for 20 min. The supernatant extract was freeze-dried and the resulting powder and amino acid standards were derivatised using a Pico-Tag system (Waters). Analysis was performed by a modified chromatographic procedure[7] using an HPLC system (1100 Series, Hewlett-Packard, Palo Alto, CA, USA) comprising a binary pump and a UV detector. The C18 column (4.5 mm × 250 mm, 5 µm) was maintained at 46 °C using a column heater. Detection was performed at 254 nm. Extracts were eluted for 60 min using buffers 1 and 2 at a maximum flow rate of 1.4 mL min^{-1}. Buffer 1 consisted of 1.4 mmol L^{-1} sodium acetate, 60 mL L^{-1} acetonitrile (pH 6.1) and 1 mL L^{-1} triethylamine. Buffer 2 was 600 mL L^{-1} acetonitrile in water. Total free amino acids were determined by summing the contents of 20 individual amino acids, and values were reported on a wet weight basis.

Measurement of browning intensity and colour

HHP-processed or conventionally prepared red ginseng was extracted with 10 volumes of water at 50 °C for 24 h in a shaking water bath and centrifuged at 6000 × *g* for 20 min. The browning intensity of samples was evaluated by reading the absorbance at 420 nm[13] using a UV–visible spectrophotometer (U-2000, Hitachi, Tokyo, Japan) with a 1 cm path length cell. Appropriate dilutions were made whenever required. Hunter's colour *L* (lightness), *a* (redness) and *b* (yellowness) values were measured using a colorimeter (CR-400, Minolta, Osaka, Japan).

Statistical analysis

Data were subjected to analysis of variance and Duncan's multiple range test using SAS Version 8.01 (SAS Institute, Cary, NC, USA), with significance defined as $P < 0.05$. The trials for HHP processing of ginseng were properly replicated in order to get optimum results for microstructure and visual quality of the final product. All analytical measurements were carried out in triplicate, and values were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Effects of HHP process on microstructure of ginseng

The effects of the HHP process on the microstructure of ginseng roots were investigated using light microscopy, and the resulting micrographs are shown in Fig. 1. Untreated fresh ginseng (Figs 1A and 1B) showed an organised and compact cell distribution with greater cell-to-cell contact throughout the tissue. Most of the ginseng cells seemed to be filled with starch, with few empty cells. However, HHP processing of ginseng at 600 MPa for 1 min resulted in destruction of cell membranes and some starch gelatinisation due to the physical impact of high pressure (Figs 1C and 1D). Extending the processing time to 10 min caused more extensive structural changes in cell membranes and cell walls (Figs 1E and 1F). During HHP processing, turgor loss and cellular changes take place, including cell conformational changes, cell elongation, cell separation or debonding and cell wall disruption.[14] Cell wall buckling and folding and a reduction in cell-to-cell contact were observed as a result of the HHP process. It thus increased the chances of cell separation, presumably via middle lamella breakdown. Bauer *et al*.[15] explained that most cell wall damage during HHP is generated by excessive stress and strain in cell membranes and cell walls. Similar cell conformational changes have been observed in thermally processed vegetables such as carrots, including cell damage and middle lamella separation.[16,17]

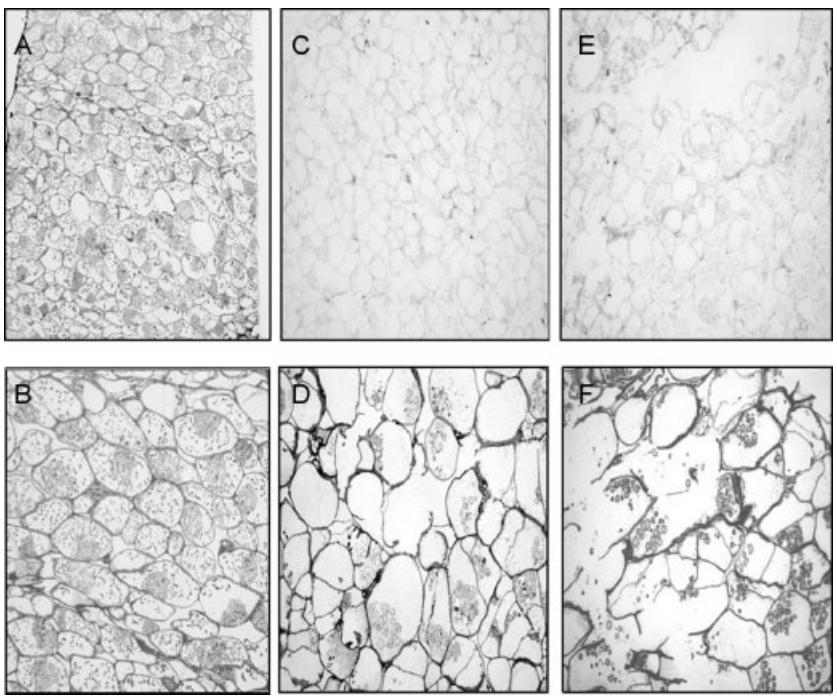

Figure 1. Light micrographs of (A, B) fresh and (C–F) HHP-processed (C and D, 600 MPa for 1 min; E and F, 600 MPa for 10 min) ginseng roots at (A, C, E) 200× and (B, D, F) 400× magnifications.

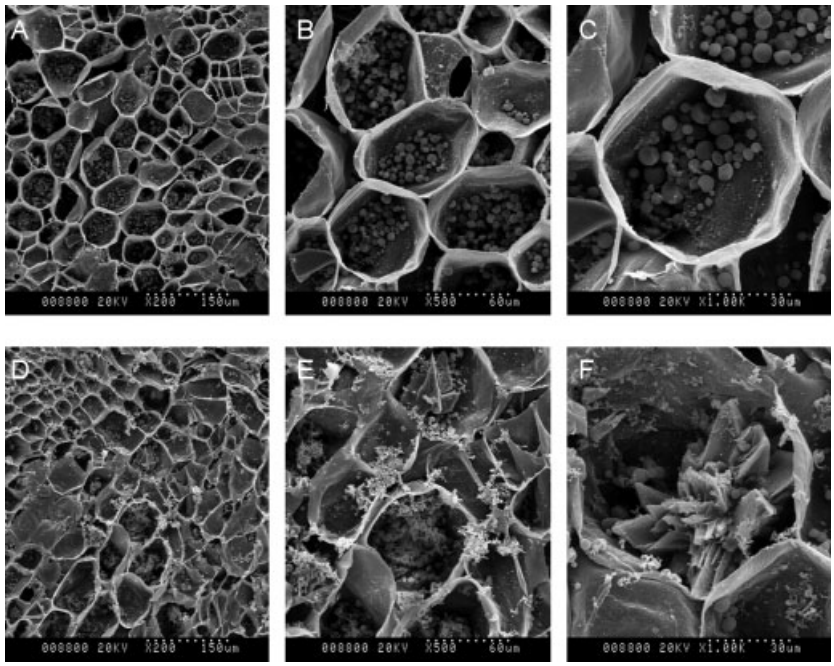

Figure 2. Scanning electron micrographs of (A–C) fresh and (D–F) HHP-processed (600 MPa for 1 min) ginseng at (A, D) 200×, (B, E) 500× and (C, F) 1000× magnifications.

For more detailed observations, scanning electron micrographs of fresh and HHP-processed ginseng root samples were also taken and are presented in Fig. 2. Untreated fresh ginseng cells looked glossy and elastic in nature and were filled with starch granules (Figs 2A–2C). Ginseng samples pressurised at 600 MPa for 1 min showed cell structural changes and gelatinisation of starch (Figs 2D–2F). The HHP process destroyed some parts of cell membranes and cell walls, causing cellular components to burst out. It was also evident that starch gelatinisation had already started in HHP-processed ginseng prior to heat processing.

Effects of HHP process on reducing sugars and free amino acids in ginseng

The non-enzymatic interaction between a reducing sugar and an amino acid, peptide or protein is known as Maillard reaction. This reaction produces a variety of intermediate products, and finally brown pigments (melanoidins) are formed.[18] Both glucose and maltose contents of ginseng were affected by the HHP process (600 MPa, 1 min), more significantly ($P < 0.05$) the increase in glucose content (Fig. 3A). However, the increase in maltose content with increasing pressure was not significant (Fig. 3B). The glucose content was 1.30 mg g^{-1} in non-pressurised ginseng, increasing to 2.78 mg g^{-1} when ginseng was pressurised at 600 MPa. The maltose content was 9.37 mg g^{-1} in non-pressurised ginseng and increased to 12.49 mg g^{-1} in ginseng pressurised at 600 MPa for 1 min. The contents of both sugars increased with increasing pressure to 400 and 600 MPa. The overall effects of the HHP process on total reducing sugars in ginseng at up to 600 MPa for 1 min are shown in Fig. 3C. The HHP process at 600 MPa for 1 min significantly ($P < 0.05$) increased the total reducing sugar content from 10.67 to 15.25 mg g^{-1}.

The effect of the HHP process on individual free amino acid contents of ginseng is shown in Table 1. Arginine, representing more than 50% of total free amino acids, increased from 1.79 to 4.45 mg g^{-1} in HHP-processed (600 MPa) ginseng. This amino acid is widely recognised to have beneficial effects on the immune system through improving lymphocyte activity. Nitric oxides produced from arginine can maintain tissue integrity during liver inflammation and also protect the liver during endotoxaemia and chronic inflammation.[19] Similarly, tryptophan also increased in ginseng from 0.22 to 1.88 mg g^{-1} after processing at 600 MPa. Besides these significant increases, other amino acids such as asparagine, glutamic acid, glutamine, glycine, histidine, threonine, alanine, proline and leucine also showed considerable increases with increasing pressure from 200 to 600 MPa, which indicates that HHP can affect the free amino acids in red ginseng. This effect, however, was not uniform on all amino acids, so it might also depend on the structure and conformation of individual amino acid molecules. The HHP process resulted in an increase in total free amino acids in ginseng (Fig. 4). Total free amino acids were significantly higher ($P < 0.05$) in HHP-processed ginseng. The total free amino acid content in non-pressurised ginseng was 2.81 mg g^{-1}, whereas ginseng processed at 200, 400 and 600 MPa had total free amino acid contents of 5.95, 6.86 and 7.77 mg g^{-1} respectively. This shows a gradual overall increase in total amino acid availability in red ginseng at higher pressures. which is perhaps due to greater cellular disruption and release of cell contents at higher pressures. Cho *et al.*[7] observed increments in the availability of free amino acids in ginseng by raising the steaming temperature to 120 °C, but similar increases can also be achieved by using the HHP process. Like lysosomes, vacuoles function as a digestive compartment but also serve as a storage compartment in which the bulk of basic amino acids are localised.[20] The HHP process might change the cell structure and vacuolar membrane permeability, as we discussed earlier. In fact, the availability of free amino acids from ginseng tissues was increased owing to cellular disruption as a result of HHP.

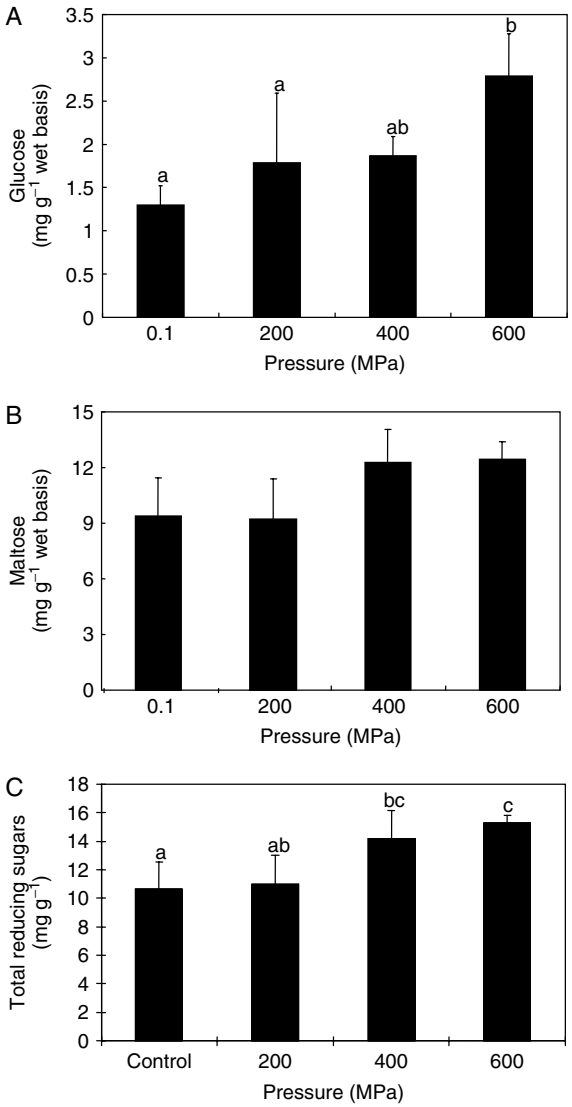

Figure 3. (A) Glucose, (B) maltose and (C) total reducing sugar contents of non-pressurised and HHP-processed (200, 400 and 600 MPa for 1 min) ginseng. Bars represent standard error of mean ($n = 3$). Means with different letters are significantly ($P < 0.05$) different.

Effects of HHP process on visual and histological properties of red ginseng

Non-pressurised fresh ginseng and HHP-processed ginseng were subjected to further thermal treatment by steaming at 98 °C for 3 h followed by drying at 60 °C for 4 days. These treatments are frequently used in ginseng processing to increase the shelf life, and this type of dried ginseng has a typical reddish-brown colour and is referred to as red ginseng.[3] The initial judgement about the quality of a food is influenced by its visual appearance and/or physical quality.[21] HHP has also been applied to improve the physical characteristics of various foods,[14] while its application for microbial and enzyme inactivation has been widely investigated in different foods to increase their shelf life.[21] The shelf life of red ginseng can also be improved using the HHP process through inactivation of microbes and quality-deteriorating enzymes; however, in this study we focused on other valuable attributes of red ginseng relating to its physicochemical quality. Figure 5 shows the visual appearance of conventionally prepared (left) and HHP-processed (right) ginseng roots that were cut vertically into two parts prior to treatment. HHP-processed red ginseng (Fig. 5B) showed a more glossy and reddish appearance and had significantly reduced 'inner white' compared with conventionally processed red ginseng (Fig. 5A). The lower availability of sugars and amino acids may lead to 'inner white', which imparts lower quality to red ginseng.[22] The improved microstructure of ginseng and increased gelatinisation may also result in reduced whiteness due to increased amounts of sugars, amino acids and MRPs.[23] In our study, there was improved and homogeneous colouration in HHP-processed ginseng, whereas white colour, similar to raw ginseng, was obvious in conventionally prepared ginseng, which indicates incomplete processing and may also result in lower consumer acceptance. Scanning electron micrographs showing the tissue structure of conventionally processed and HHP-processed red ginseng samples are presented in Fig. 6. Conventionally processed red ginseng showed visible intercellular spaces due to irregular gelatinisation during the steaming process (Figs 6A and 6B). A more magnified view of conventionally processed red ginseng clearly indicated the presence of intact cells that failed to release their contents to fill intercellular spaces (Fig. 6C). On the other hand, the tissue structure of HHP-processed ginseng seemed to be more 'complete' (Figs 6D and 6E) and, overall, swollen structures were produced in red ginseng (Fig. 6F). HHP-processed red ginseng showed visible gelatinisation of starch granules released from disrupted cells, and no prominent intercellular spaces within tissues were observed. HHP processing already triggered some gelatinisation of starch in fresh ginseng, and it led to more severe but homogeneous changes in tissue structure. Plant cell walls consist of pectin and crosslinking glycans; the latter increase the tensile strength of cellulose, whereas the coextensive network of pectin provides the cell walls with the ability to resist compression. Owing to the application of pressure, gelatinisation can be achieved at lower temperatures through mechanical disruption of molecular bonds.[24] Both the mechanical and thermal energy transferred to ginseng starch during HHP processing of red ginseng affect the breakdown of primary and secondary valence bonds as well as hydrogen bonds between neighbouring polymers in the starch structure.[25] HHP affects the structure of primary cell walls, which leak pectin, while secondary cell walls also become deformed, exuding internal water-soluble protein and secondary metabolites that then act as binding materials. During the steaming process, the pectin and protein exuded from cells facilitate gelatinisation, leading to more compact and stronger binding between structures. As mentioned previously, one of the important attributes of good-quality red ginseng is the absence of any tissue cavities.[3] HHP processing seems to greatly reduce the presence of these cavities in red ginseng by improving the microstructure of ginseng before heat treatment. HHP disrupted the structure of most cells in ginseng roots, resulting in the release of more sugars and amino acids before steaming, which subsequently enhanced the formation of MRPs, leading to more even colouration of red ginseng. Similarly, the release of cellular contents due to pressurisation and subsequently improved gelatinisation resulted in a more filled and smoother surface of red ginseng.

Effects of HHP process on browning intensity and colour of red ginseng

The effect of the HHP process on the formation of colour during the subsequent steaming and drying processes was also investigated by measuring the optical density at 420 nm ($OD_{420\ nm}$) of extracts prepared from conventionally processed and HHP-processed red ginseng. The results are presented in Table 2. The extracts prepared

Table 1. Amino acid contents of conventionally processed and HHP-processed red ginseng

Amino acid	0.1 MPa	200 MPa	400 MPa	600 MPa
Asparagine	0.043 ± 0.004	0.023 ± 0.001	0.085 ± 0.047	0.087 ± 0.034
Glutamic acid	0.142 ± 0.044	0.353 ± 0.035	0.641 ± 0.104	0.676 ± 0.106
Glutamine	0.025 ± 0.011	0.097 ± 0.043	0.086 ± 0.133	0.076 ± 0.054
Glycine	0.014 ± 0.007	0.016 ± 0.002	0.022 ± 0.002	0.028 ± 0.009
Histidine	0.064 ± 0.003	0.074 ± 0.004	0.084 ± 0.011	0.091 ± 0.020
Arginine	1.788 ± 0.849	4.968 ± 0.180	4.308 ± 0.776	4.449 ± 1.023
Threonine	0.042 ± 0.005	0.014 ± 0.004	0.038 ± 0.009	0.039 ± 0.012
Alanine	0.068 ± 0.021	0.134 ± 0.003	0.154 ± 0.020	0.169 ± 0.022
Proline	0.020 ± 0.006	0.023 ± 0.002	0.026 ± 0.005	0.027 ± 0.005
Tyrosine	0.033 ± 0.011	0.015 ± 0.001	0.022 ± 0.006	0.025 ± 0.006
Valine	0.094 ± 0.020	0.021 ± 0.004	0.040 ± 0.008	0.044 ± 0.012
Methionine	0.033 ± 0.006	0.017 ± 0.001	0.019 ± 0.004	0.017 ± 0.003
Isoleucine	0.036 ± 0.008	0.019 ± 0.001	0.022 ± 0.007	0.028 ± 0.005
Leucine	0.034 ± 0.016	0.019 ± 0.005	0.036 ± 0.008	0.044 ± 0.011
Phenylalanine	0.058 ± 0.006	0.040 ± 0.003	0.050 ± 0.008	0.054 ± 0.010
Tryptophan	0.218 ± 0.055	0.101 ± 0.006	1.191 ± 0.976	1.883 ± 1.143
Lysine	0.097 ± 0.021	0.013 ± 0.003	0.031 ± 0.010	0.032 ± 0.018
Cysteine	ND	ND	ND	ND
Asparagine	ND	ND	ND	ND
Serine	ND	ND	ND	ND

Values are mean ± standard deviation ($n = 3$). ND, not detected.

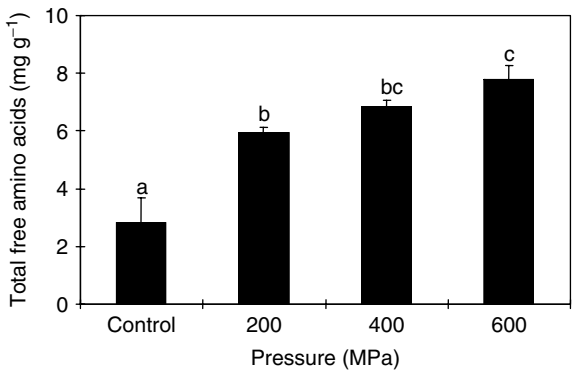

Figure 4. Total free amino acid content of non-pressurised and HHP-processed (200, 400 and 600 MPa for 1 min) ginseng. Bars represent standard error of mean ($n = 3$). Means with different letters are significantly ($P < 0.05$) different.

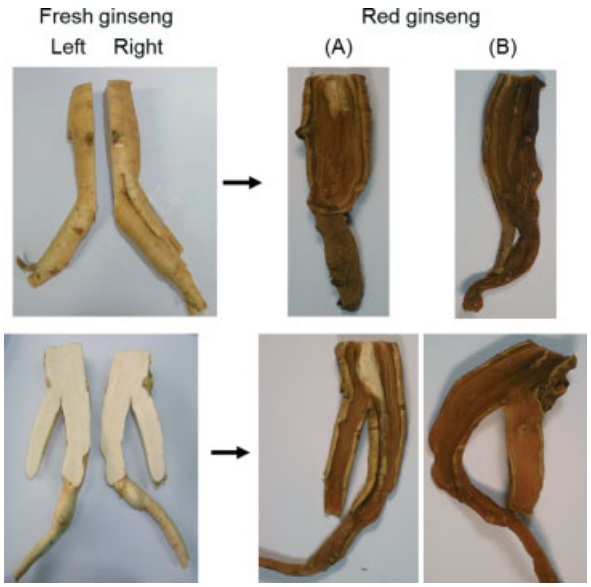

Figure 5. Appearance of (A) conventionally prepared and (B) HHP-processed red ginseng roots. Fresh ginseng roots were cut vertically into two parts prior to treatment.

from HHP-processed red ginseng showed significantly ($P < 0.05$) higher $OD_{420\ nm}$ values than the conventionally prepared extract. The Hunter's colour L, a and b values of extracts from different types of red ginseng are also shown in Table 2. There was a significant ($P < 0.05$) increase in a value or redness as a result of the HHP process, with the highest a value being observed for the extract from red ginseng processed at 600 MPa.

These results reveal that the HHP process resulted in accelerated browning reaction in red ginseng during the steaming and heat-drying processes due to the increased release of sugars and amino acids from the ginseng matrix. Subsequently, it led to increased formation of amino acid–sugar complexes due to Maillard reaction. Similarly, caramelisation of sugars can also occur, leading to overall browning of the product.[13] During Maillard reaction, α- or ε-NH_2 groups of amino acids or proteins covalently attach to sugars through glycosylation or glycation to form glycated proteins. The first glycation product or Schiff base rearranges to a more stable ketosamine or Amadori product. Amadori products can then form crosslinks between adjacent proteins or with other amino groups, resulting in polymeric aggregates called advanced glycation end-products.[26] The increased release of reducing sugars and free amino acids can enhance the formation of amino acid–sugar complexes and MRPs.[27] The increased formation of MRPs renders higher colour quality to red ginseng. MRPs are also reported to increase functional properties such as reducing power, DPPH radical-scavenging activity and antioxidant activity.[28] HHP

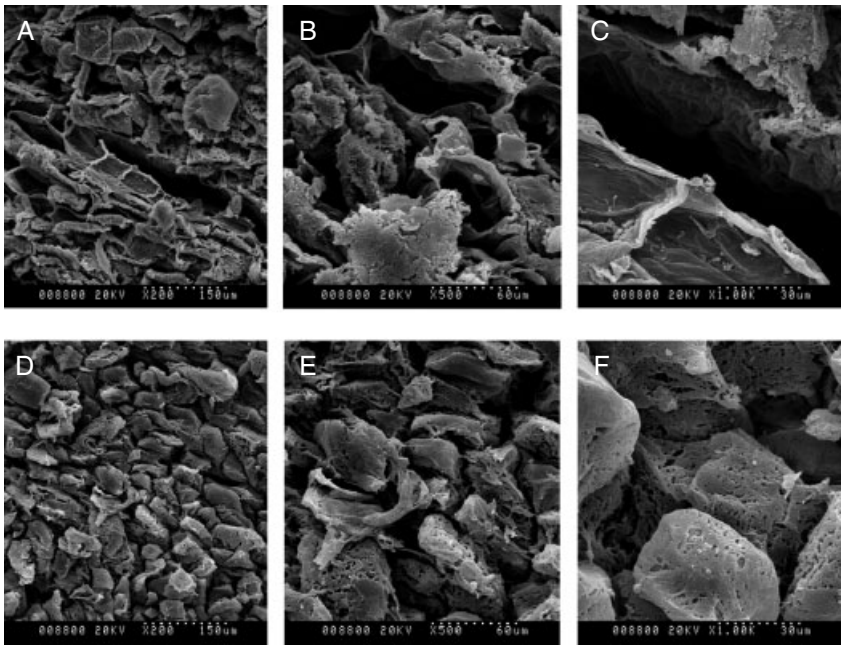

Figure 6. Scanning electron micrographs of (A–C) conventionally processed and (D–F) HHP-processed (600 MPa for 1 min) red ginseng at (A, D) 200×, (B, E) 500× and (C, F) 1000× magnifications.

Table 2. Optical density at 420 nm ($OD_{420\ nm}$) and Hunter's colour values *L*, *a* and *b* of extracts from conventionally processed and HHP-processed (600 MPa for 1 min) red ginseng

Pressure (MPa)	$OD_{420\ nm}$	*L* (lightness)	*a* (redness)	*b* (yellowness)
0.1	0.65 ± 0.01a	14.65 ± 0.99	−0.35 ± 0.06a	−0.42 ± 0.39
200	0.83 ± 0.07b	14.17 ± 0.72	−0.29 ± 0.09ab	0.06 ± 0.62
400	0.88 ± 0.06bc	14.02 ± 0.87	−0.23 ± 0.05ab	−0.12 ± 0.41
600	0.95 ± 0.07c	13.52 ± 0.76	−0.17 ± 0.08b	0.27 ± 0.64

Values are mean ± standard deviation ($n = 3$). Means with different letters in a column are significantly ($P < 0.05$) different.

affects the permeability of ginseng tissues and cells,[29] and these favourable structural attributes of HHP-processed ginseng aid in the development of brown colours and MRPs. HHP, which is commonly used for the inactivation of pathogens in foods and better retention of functional and nutritional ingredients in processed products,[30] can also be effectively used for processing higher-quality red ginseng.

CONCLUSIONS

The detailed histological observations showed that the structural changes induced by HHP at cellular level made HHP-processed red ginseng much superior to conventionally processed red ginseng. A more compact and filled structure was obtained in the case of HHP-processed red ginseng. The studies on reducing sugar and total free amino acid contents showed that the HHP process significantly enhanced the availability of these nutrients, which also need to be released for colour formation. Even colouration and visual attraction are important quality attributes of red ginseng, and they were improved by the HHP process through an increase in *a* value or redness and the formation of a more filled, even coloured and complete structure of red ginseng. The HHP process improved these quality attributes of red ginseng without any increment in steaming or drying temperatures; hence it is recommendable for manufacturing higher-quality red ginseng products.

ACKNOWLEDGMENTS

This study was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A050376).

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