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Research Article

Compositional changes and physiological activities of fresh ginseng extracts prepared at various temperatures in subcritical water

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ABSTRACT

Background: Subcritical water (SW) is regarded as an effective conversion technology for lignocellulosic biomass. The effect of SW on ginseng are limited to evaluate the ginsenoside composition of red ginseng, and there is little information on the effects of SW on fresh ginseng.
Methods: The general characteristics of ginseng extracts (GE) prepared with SW were evaluated in terms of brix, reducing sugar and residual solid content, and compositions of GE was estimated using chromatography. For utilization of GE as a bioactive food, the ginsenoside composition, antioxidative activity, angiotensin-converting enzyme (ACE) inhibitory activity, prebiotic potential and taste attributes were measured.
Results: Increasing SW temperature decreased residual solid content of ginseng and the soluble compounds of GE were yielded by SW at 250 °C. Despite that ginsenoside content decreased with SW temperature, a steep increase in Rg5 was observed at 200 °C. The SW at 200–250 °C manifested the highest antioxidant activities and ACE inhibitory activity of GE. However, the GE prepared at greater than 250 °C completely lost prebiotic potentials. Based on electronic-tongue, umami taste was enhanced by SW at 200 °C, but sweetness and bitterness were dominated at 250–300 °C.
Conclusion: The results demonstrated that SW has a potential application to convert lignocellulosic wastes generated from ginseng roots into bioactive food resource, and SW at ~200 °C can be potentially used to enhance the physiological activities of GE.

1. Introduction

Ginseng (*Panax ginseng* Meyer) is one of the most widely used medicinal herbs in the world [1]. Ginseng roots have various biological functions as they are rich in physiologically active compounds such as ginsenosides, organic acids, sugars, amino acids and antioxidants [1,2]. Among them, ginsenosides are important components providing the value of ginseng. The ginsenosides are particularly abundant in the epidermal part than in the inside of the main ginseng root body, and the ratio of epidermis is noted to be high in fine root hairs [1]. Therefore, preserving fresh ginseng root without loss of root hairs is an important factor in maintaining its commodity value. However, fresh ginseng has high moisture content (>70 %), and the presence of fine hairs can act as a barrier in washing away its surface contaminants, which results in ginseng's limited shelf life (<1 week) even under chilled storage condition [3]. Drying and steaming are commonly used to improve the physiological activity and preservation stability of fresh ginseng. However, processed ginseng, such as red ginseng or white ginseng, possess a strong bitter taste, which acts as a barrier for consumer preference, hence, they are commonly used as raw materials to extract physiologically active compounds [1,4].

Recently, the application of subcritical water (SW) has been recognized as a green technology for upcycling agro-industrial byproducts. SW is defined as pressurized hot water with a temperature range of 100~300 °C under greater than saturated vapor pressure [5]. As high enthalpic contribution of SW can hydrolyze the organic compounds of biomasses, there have been numerous investigations to produce functional peptides and fermentable sugar from agro-byproducts such as

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soymeal, rice bran, spent coffee ground as well as cereal husks [6–8]. Moreover, increasing SW temperature causes a decrease in dielectric constant of water, which enables water to act like an organic solvent such as acetonitrile, methanol, and ethanol [5]. These unique characteristics of the SW provide the possibility of using water for extracting less or nonpolar substances instead of organic solvent.

For ginseng, it was reported that SW at 200 °C showed an effective extractability of antioxidants from red ginseng compared with water or ethanol extraction [9], and specific ginsenosides such as Rg3 and Rh2 were obtained [10]. In addition, anticancer activities were discovered from ginseng leaf and stem extracts by SW at 190 °C [11]. These reports indicated that SW could enhance or modify the physiological activities of ginseng roots, depending on the SW temperature. Nevertheless, these studies focused only on the hydrothermal conversion of ginsenosides in red ginseng, and little information regarding the effect of SW on the entire characteristics of fresh ginseng extracts (GE) is available. Therefore, this current study investigated the effects of SW at a broad range of temperature (100–300 °C) on the characteristics and physiological activities of GE, in order to extend the utilization of whole fresh ginseng roots.

2. Materials and methods

2.1. Materials

Fresh ginseng roots (5-year-old) were purchased from Dong-Jin Pharmaceutical Co. (Geumsan, Korea). The ginseng roots were decontaminated twice with running water, thereafter the ginseng roots were gently drained using a sieve. The washed ginseng roots were dried at 60 °C for 6 h using a hot-air food dryer (LD-918H5, L'equip, Hwaseong, Korea) and coarsely ground for 1.5 min using a food blender (BL4258KR, Tefal, Haute-Savoie, France).

2.2. SW extraction

Dried ginseng roots were extracted using a lab-assembled SW device at the Biopolymer Research Center for Advanced Materials (Seoul, Korea). In brief, the device was consisted of a reactor (working volume of 130 mL), a vertical shaker, a heater, and a water-circulating water bath cooler as described previously [6]. For each treatment, 120 mL of 5 % (w/v) ginseng suspension was prepared by suspending dried ginseng roots into distilled water. The suspension was poured into the reactor and sealed tightly. The reactor was heated from ambient (~20 °C) to the target temperature at a heating rate of 4 °C/min. When the reactor reached the target temperature, it was cooled down to 40 °C by immersing the reactor into the 4 °C cooler (~25 min). The suspension was centrifuged at 3000×*g* for 15 min, and both precipitate and supernatant were separately collected. The supernatant was used as the GE, whereas the precipitate was used to estimate residual solid content. For the untreated control, the ginseng suspension was centrifuged under the same conditions without applying SW. For experimental replications, all these abovementioned procedures were repeated thrice using a new batch of the dried ginseng.

2.3. General characteristics of the ginseng extracts

The brix of GE was measured in duplicates using a digital refractometer (RX-5000CX, Atago Co., Ltd., Tokyo, Japan). Reducing sugar content of GE was estimated in triplicate determinations based on 3,5-dinitrosalicylic acid (DNS) assay as described previously [12] without modification. The precipitates were dried at 105 °C for 24 h and weighed. Residual solid content was calculated by weight percentage of precipitate over the initial raw ginseng in suspension. The compositions of simple sugars and their thermal derivatives were measured in duplicates by a capillary ion chromatography (ICS-5000, Dionex, Sunnyvale, CA, USA) and a HPLC system (Ultimate300, Dionex), respectively, without modification of the method of Lee et al. [12].

2.4. Compositions and contents of ginsenosides

The compositions and contents of ginsenosides in GE were determined using a LC/MS system (LCMS-8050, Shimadzu Co., Kyoto, Japan) equipped with a CORTECS UPLC T3 column (1.6 μm, 2.1 mm × 150 mm, Waters). The GE were injected by 1 μL and eluted at a flow rate of 0.45 mL/min with an electrospray ionization (ESI) negative detection mode at 35 °C. Two mobile phases, i.e., 0.1 % (v/v) formic acid in water (phase A) and methanol containing 0.1 % formic acid and 10 % acetonitrile (phase B), were applied with a concentration gradient of 35 %~95 % phase B in 20 min. In total, 13 standard references including Rb1, Rb2, Rb3, Rc, Rd, Rg3, Re, Rf, Rg1, Rg2, Rh1, Rg5 and Rk1 were used.

2.5. Antioxidative activities

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical inhibition activity, total phenolic content and reducing power were measured to estimate antioxidative activities of GE. All assays were analyzed in triplicated determinations based on a previous literature [12] without modification.

2.6. Angiotensin-converting enzyme (ACE) inhibitory activity

Antihypertensive activity of GE was determined in triplicated determinations by ACE inhibitory activity using the method by Nagappan et al. [13] with minor modification. Aliquots 50 μL of GE were mixed with the same volume of 25 mU/mL ACE (rabbit lung, Sigma–Aldrich), and the mixture was incubated at 37 °C for 10 min. Thereafter, 100 μL of 25 mM N-Hippuryl-histidyl-leucine substrate in 50 mM sodium borate buffer containing 0.5 M NaCl (pH 8.3) was added. The mixture was further incubated at 37 °C for 1 h, and the ACE reaction was completed by adding 250 μL of 1 M HCl. Into the sample, 500 μL of ethyl acetate was added in a shaker (MS-100, Hangzhou Allsheng Instruments Co., Ltd., Hangzhou, China) to extract hippuric acid. The mixture was centrifuged at 4000×*g* for 10 min, and 400 μL of the supernatant was dried at 80 °C for 1 h. The dried mixture was dissolved in 1 mL of distilled water, and absorbance at 228 nm was taken. ACE inhibition was calculated using the following equation:

$$\text{ACE inhibition } (\%) = \frac{A_R - A_S}{A_R - A_B} \times 100 \quad \text{(eq. 1)}$$

where A_R, A_S, and A_B are the absorbance of the reference (without sample), sample, and blank (distilled water), respectively.

2.7. Prebiotic potential and electronic tongue assay

Prebiotic potential of GE was estimated in duplicates as described previously [12] without modification using two type-cultured human probiotic strains of *Lactobacillus rhamnosus* GG (LGG) and *Enterococcus faecium*. To evaluate taste of GE, aliquot 25 mL of each treatment was filtered through a 0.45 μm PVDC syringe filter (cat No. 6779-1304, Whatman), and the filtrates were applied to an e-tongue system (Astree, Alpha MOS, Toulouse, France) with seven cross-selective sensors coded as AHS (sourness), PKS (complex taste), CTS (saltiness), NMS (umami), CPS (complex taste), ANS (sweetness) and SCS (bitterness). The taste response data were acquired by #6 sensor module with acquisition time of 120 s and washing time of 10 s, and an average value from five runs was accessed via a software provided by the manufacturer (Alpha Soft ver. 14.2, Alpha MOS). Taste attributes of each treatment were measured in duplicates at ambient temperature.

2.8. Statistical analysis

A completely randomized design was selected to estimate the main effect (SW temperatures). Means and standard deviations (SD) were calculated from the averages obtained from three entirely repeated experiments (n = 3) and analyzed by one-way analysis of variance (ANOVA) using SPSS software (ver. 25, IBM, Armonk, NY, USA). When the main effect was significant ($p < 0.05$), Duncan's multiple range test was performed as a post hoc procedure.

3. Results and discussion

3.1. General characteristics of GE

The residual solid content of GE prepared at 100 °C was 76.1 %, which was close to the 76.8 % of the control (Fig. 1A). However, the solid content of GE was noted to gradually decrease with SW temperature, reaching 22.9 % at 250 °C ($p < 0.05$). Thereafter, the solid content of GE was not affected by SW temperature. It has been determined that SW was effective in hydrolyzing insoluble lignocellulosic fibers [6], which indicated that ~78 % of insoluble components of ginseng roots were liquefied and incorporated into GE by SW at 250 °C. The results implied that SW at 250 °C was effective in minimizing the generation of residual ginseng byproducts.

The SW temperature affected brix and reducing sugar content of GE (Fig. 1B). It was observed that brix and reducing sugar content of GE increased gradually with SW temperature and reached to maximum at 200 °C thereafter decreasing with temperature ($p < 0.05$). Again, the former increase in brix accounted for the hydrolysis of proteins and fibers or for the extraction of less polar compounds in ginseng roots. Since the majority of ginseng roots was carbohydrates, the former increase in reducing sugar content also supported the SW-mediated hydrolysis of ginseng root fiber. However, the latter decrease in brix and reducing sugar has probably resulted from hydrothermal degradation of the hydrolysates, which was identically reported previously [6–8]. In lignocellulosic biomass, monosaccharides can react to the phenolic compounds of lignin under higher SW temperature (>257 °C), and they were converted into an insoluble complex [14], which supported the decrease in brix of GE in this present study.

For monosaccharide compositions, six simple sugars, including arabinose, galactose, rhamnose, glucose, xylose and mannose, were detected in control extracts (Fig. 1C). Increasing SW temperature to 100–150 °C tended to increase the glucose content in GE, whereas there was no obvious change in monosaccharide compositions of GE prepared at 100–150 °C compared to the control. Alternately, glucose and mannose were predominant simple sugar of GE prepared at 200 °C while xylose disappeared. Sucrose, cellulose and dextran were key precursors of glucose in ginseng roots. However, it was reported that cellulose and starch were intensively hydrolyzed by SW at 250 °C [6,12], which shows that sucrose hydrolysis was intensively manifested at 200 °C. Nevertheless, fructose was not detected in GE prepared at 200 °C, as fructose was converted into thermal derivatives such as acids and 5-hydroxymethylfurfural (HMF), which were detected in GE prepared at 200 °C (Fig. 1D). The result was also supported by a previous report [15]. As SW temperature increased to greater than 250 °C, simple sugars were not detected in GE. These results would explain that monosaccharides would undergo thermal conversion into thermal derivatives. This thermochemical reaction resulted in greater yield of formic acid and acetic acid as well as maximum amount of HMF at 250 °C. Generation of furfural has also reflected the thermal decomposition of free glucose. The thermal derivatives of sugar including formic acid, HMF, and furfural were diminished in GE prepared at 300 °C. Instead, acetic acid was yielded at maximum by SW at 300 °C. Due to the complexity of the components consisting of ginseng roots, the detailed mechanisms involved in the hydrothermal conversion of ginseng carbohydrates into thermal derivatives were not clearly understood in this present study, nevertheless, this current study was able to demonstrate that SW could liquefy the components of insoluble ginseng fibers.

3.2. Compositions and contents of ginsenosides in GE

As all ginsenosides quantified in this study were not detected in GE applied with SW at greater than 250 °C, the ginsenosides in GE prepared only at 100–200 °C were compared to the untreated control (Fig. 2).

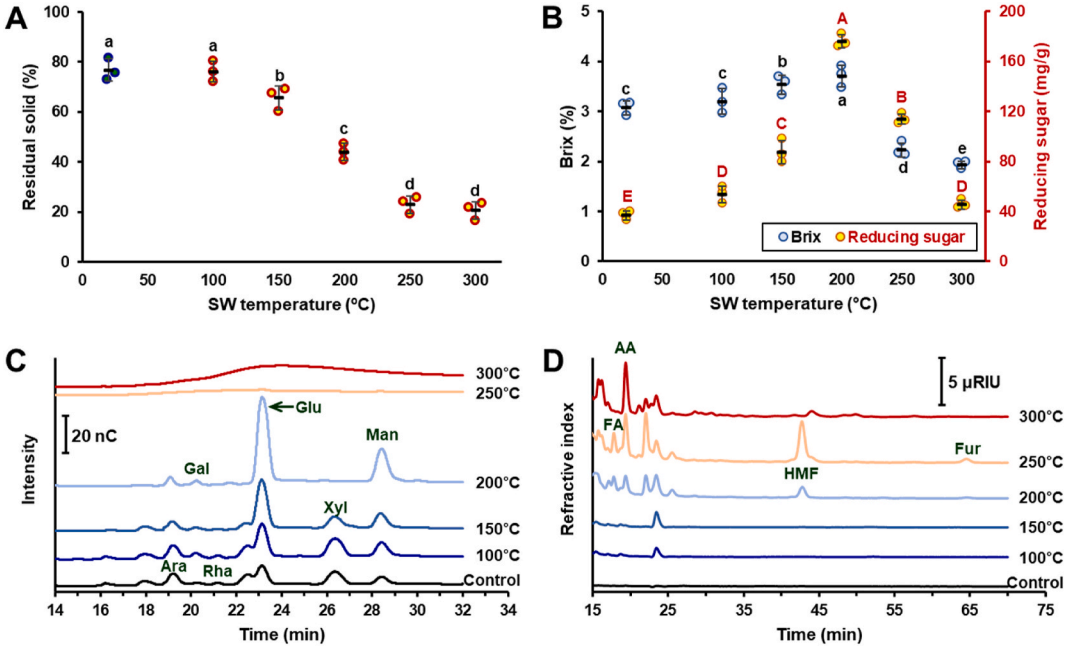

Fig. 1. Effects of subcritical water (SW) temperatures on residual solid content (A), brix and reducing sugar content (B), simple sugar compositions (C) and thermal derivatives (D) of ginseng extracts. Vertical bars indicate standard deviations (n = 3). Different letters (a-e, A-E) indicate significant differences ($p < 0.05$). Abbreviations of each compounds indicate arabinose (Ara), galactose (Gal), rhamnose (Rha), glucose (Glu), xylose (Xyl), mannose (Man), formic acid (FA), acetic acid (AA), 5-hydroxymethylfurfural (HMF) and furfural (Fur), respectively.

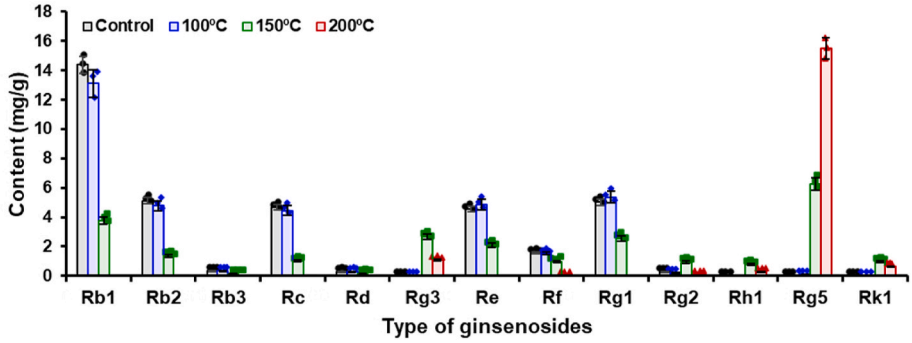

Fig. 2. Effects of subcritical water temperatures on ginsenoside content of ginseng extracts. Vertical bars indicate standard deviations (n = 3).

Major intact ginsenosides detected in control GE were Rb1, followed by Rb2, Rg1, Rc and Re, which were generally reported as the main ginsenosides of fresh ginseng root [16]. Based on literature, Rf is another intact ginsenosides abundant in fresh ginseng root, but the less polarity of Rf would account for poor extraction by water in the control [17]. The compositions and contents of ginsenosides in GE prepared at 100 °C did not differ from the untreated control, whereas substantial decrease in the major intact ginsenosides was found in GE prepared by SW at 150 °C ($p < 0.05$). Concomitantly, substantial increases in Rg3, Rg2, Rh1, Rg5 and Rk1 were observed at this SW condition ($p < 0.05$).

It is known that the intact ginsenosides undergo thermal denaturation, which affects the compositions and content of ginsenosides [18]. Although the mechanisms underlying the thermal conversion of ginsenosides were not completely elucidated due to their complexities, it was reported that protopanaxadiol (Rb1, Rc, Rb2) and protopanaxatriol (Rg1, Re) were converted into different types of ginsenosides [1]. The increases in Rh1 and Rg2 at 150 °C would be related with the thermal conversion of Rg1 and Re, respectively [19]. However, the increases in Rh1 and Rg2 were minor compared to the decrease in Rg1 and Re, reflecting that the converted ginsenosides could be transformed again into Rh4+Rk3 and F4+Rg6, respectively [19], which were not quantified in this study. Alternately, protopanaxadiol-type ginsenosides (Rb1, Rc, Rb2) were known to convert into Rg5, Rk1 and Rz1 [1,19], which would explain the steep generation of Rg5 in this study.

The contents of all the detected ginsenosides were noted to decrease by SW at 200 °C, excluding Rg5 as it yielded the maximum at 200 °C. Based on the result, it was suggested that SW had a potential technology to produce Rg5 from fresh ginseng root. However, all ginsenosides including Rg5 were not detected in GE prepared by SW at greater than 250 °C. Despite numerous investigations, mechanisms of thermal conversions of ginsenosides have been elucidated just under mild thermal treatment condition (steaming for red ginseng processing). Although, this study has revealed the similar pattern of ginsenoside conversions to those previously reported, it was not completely understood whether the

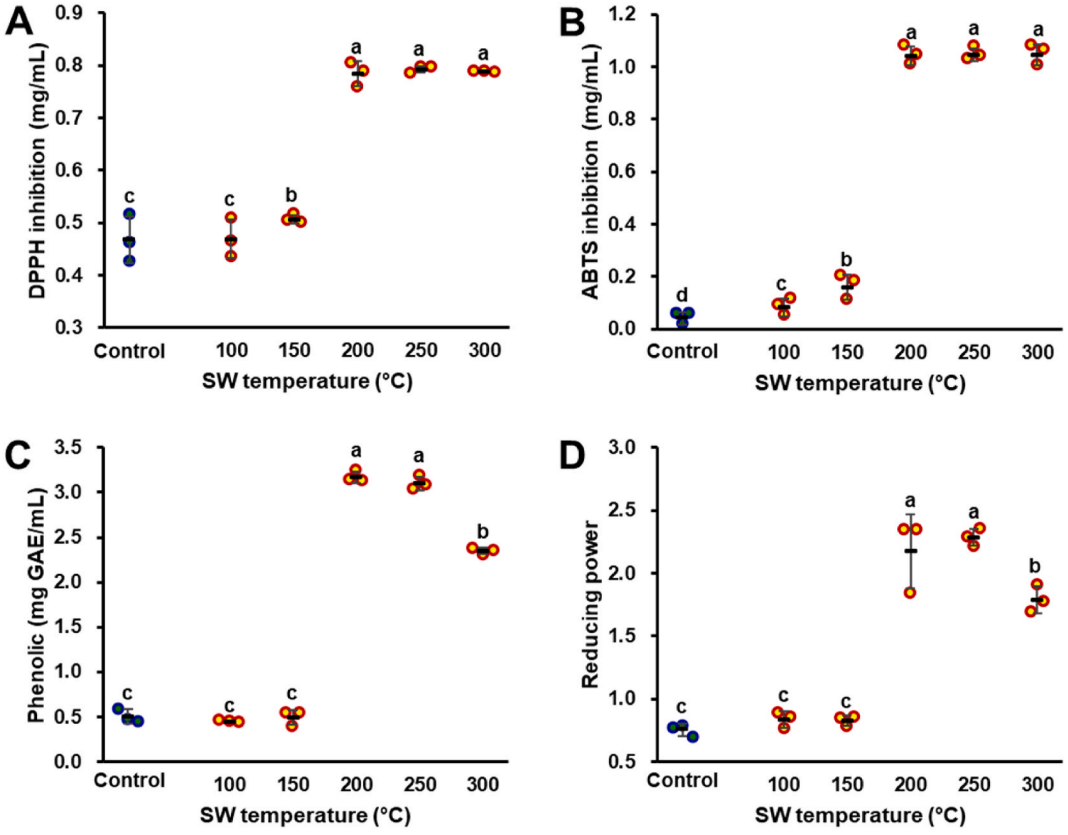

Fig. 3. Effects of subcritical water (SW) temperatures on DPPH radical scavenging activity (A), ABTS radical inhibition (B), total phenolic content (C) and reducing power (D) of ginseng extracts. Vertical bars indicate standard deviations (n = 3). Different letters (a–d) indicate significant differences ($p < 0.05$).

thermally transformed ginsenosides underwent further conversion into unidentified types of ginsenosides or thermally degraded into metabolites at elaborated SW temperature (>250 °C), which warranted further explorations.

3.3. Antioxidative activities of GE

As depicted in Fig. 3, antioxidative activity of GE was steeply increased at 200 °C regardless of type of assay. For DPPH radical scavenging activity and ABTS radical inhibition activity, the high antioxidative activity of GE was maintained up to 300 °C, whereas total phenolic content and reducing sugar were decreased at 300 °C. It was reported that chlorogenic acid, gentisic acid, *p*-coumaric acid and rutin were the most abundant phenolic compounds in ginseng, and *p*-coumaric acid and ferulic acid were strongly correlated to the radical scavenging activity of ginseng [20]. In general, organic solvents such as methanol were applied to extract the phenolic compounds in ginseng root [21], and water extraction adopted in this study would account for the limited antioxidative activities of control and the GE prepared at relatively lower SW temperature (100–150 °C). However, the dielectric constant of water was determined to decrease with SW temperature, and the dielectric constants of SW at 150–200 °C were similar to those of organic solvents such as acetonitrile and methanol, which could explain the maximum phenolic content of GE [5]. In addition, 180–260 °C of SW was reported to effectively hydrolyze protein and polysaccharides, which could be responsible to the maximum antioxidative activity of GE at 200–250 °C in this study [12,22]. Furthermore, lignin, which accounted for ~35 % of the insoluble dietary fiber of ginseng, could undergo depolymerization into oligolignols and monomeric phenols by SW, which would also contribute to the oxidative activities of GE [23,24]. Alternately, it was reported that phenolic compounds took part in an interaction with Maillard products under high temperature condition, leading to decreases in phenolic compounds as well as 5-HMF [25]. These interactions were also confirmed by a decrease in the total phenolic compounds and reducing power of GE prepared at 300 °C in this study.

3.4. ACE inhibitory activities of GE

As an indicator of antihypertensive effect, ACE inhibitory activity of GE is depicted in Fig. 4. ACE inhibition of GE prepared by SW at 100–150 °C was ranged to 57.1 %~58.3 %, which did not differ from 58.8 % of the untreated control (Fig. 6). Conversely, GE prepared by SW at 200 °C exhibited 95.7 % of the highest ACE inhibition activity among all SW treatments ($p < 0.05$). With further increase in SW temperature, the ACE inhibitory activity was gradually decreased and reached to 78.3 % at 300 °C of SW ($p < 0.05$).

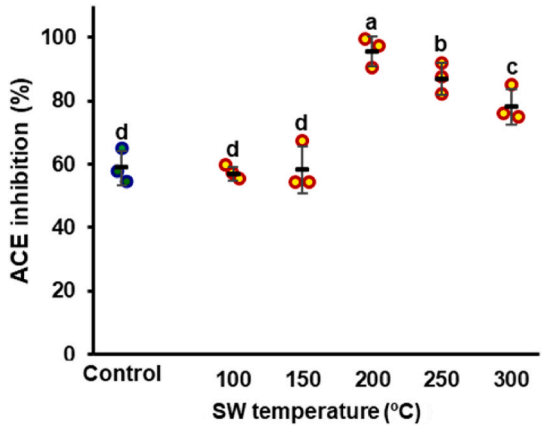

Fig. 4. Effects of subcritical water (SW) temperatures on angiotensin-I converting enzyme (ACE) inhibition of ginseng extracts. Vertical bars indicate standard deviations (n = 3). Different letters (a–d) indicate significant differences ($p < 0.05$).

In this study, the presence of ginsenosides can be considered an important factor affecting the ACE inhibitory effect of GE, as antihypertensive effect of ginsenosides has been widely introduced. Ali et al. [26] demonstrated that Rg5 exhibited better ACE inhibition than those of Rg1, Re, Rb1, Rc, and Rb2. Although Rh1 and Rh2 were reported as the best ginsenoside for ACE inhibition [26], these ginsenosides were intermediates in thermal conversion of intact ginsenosides and produced in a limited amount in red ginseng [1,27]. Liu et al. [28] postulated that Rg5 had an excellent ACE inhibitory activity and good activity in heart failure. Based on the literatures, SW at 200 °C would be the best extraction condition to maximize the physiological activity of fresh ginseng, and SW was a suitable process to produce high yield of Rg5 from fresh ginseng roots.

Alternately, peptides could be another factor affecting the antihypertensive activity [29]. From this perspective, ginseng root is believed to be a good candidate for antihypertensive peptides because it is rich in proteins. The SW was considered an effective technique to hydrolyze proteins [7,8,30], and the hydrolysis efficiency reported in these experiments was at maximum at the SW temperature range of 190~250 °C. However, thermal degradation of the protein hydrolysates was followed with a further increase in SW temperature [7,8,22], which could explain the latter decrease in ACE inhibition of GE prepared at 250–300 °C in this study.

3.5. Prebiotic potentials of GE

Ginseng roots are known to be rich in soluble edible fibers, and the SW was deemed favorable for hydrolyzing root fibers [31,32]. As Lee et al. [6] indicated that there was no cytotoxic evidence of lignocellulosic biomass treated by SW at 100–350 °C, it was expected that GE would be available as potential prebiotics. In this study, control GE acted as a good medium for the growth of selected human gut microbiota, and both strains reached 7.67–8.27 log CFU/mL after 24 h of incubation (Fig. 5). The GE prepared at 100 °C tended to increase the prebiotic potential compared with the control, however, the bacterial counts of both strains were not substantial to those of the control. Regardless of strains, the bacterial counts decreased in GE prepared at temperatures higher than 150 °C ($p < 0.05$), moreover, *E. faecium* showed greater depression compared to the LGG strain ($p < 0.05$). In addition, both strains were not noted in GE prepared at greater than 250 °C.

Similar results on prebiotic potentials were obtained using rice husk hydrolysates prepared by SW at 150–300 °C [6]. It has been reported that pH and thermal derivatives of sugars are major barriers to inhibit the microbial growth. It was known that optimal pH for growth of both strains was pH 6.0–7.0 [12]. Because sugars were hydrothermally converted into acids under SW process, hydrolysates prepared by SW exhibited normally low pH than untreated control [6]. This pattern of pH changes was also observed in this study. Control and GE prepared at 100 °C showed a pH of 5.12–5.17, and the increasing SW temperature gradually decreased the pH of GE to 3.66 at 250 °C, thereafter, pH was not affected by SW temperature (data were not shown). When the pH of all GE was adjusted to 7.0 to eliminate the impact of pH, GE prepared at 150–200 °C exhibited greater bacterial counts ($p < 0.05$), particularly, the growth of *E. faecium* was highly recovered in GE prepared at 150 °C. Nevertheless, the prebiotic potentials of GE were generally lower than the control, still no bacterial counts in GE prepared at greater than 250 °C were found, indicating that pH had minor impact on prebiotic potential of GE. Alternatively, 5-HMF is known to absorb on the microbial surface and inhibits the microbial growth [33], which could explain the loss of prebiotic potential of GE prepared at greater than 250 °C. Therefore, the results indicated that extremely high SW temperature (>250 °C) was not suitable for thermochemical conversion of ginseng roots from the prebiotic application point of view.

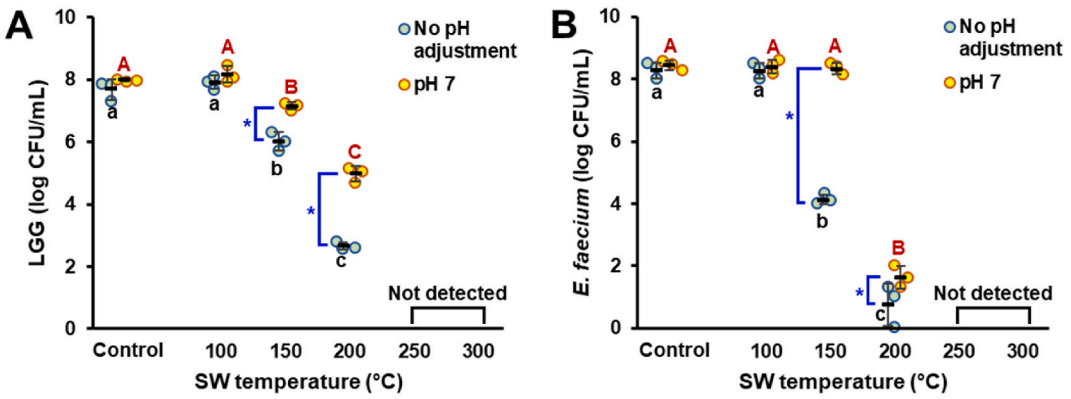

Fig. 5. Effects of subcritical water (SW) temperatures on growth of *Lactobacillus rhamnosus* GG (A) and *Enterococcus faecium* (B) in ginseng extracts with and without pH adjustment. Vertical bars indicate standard deviations (n = 3). Different letters (a-c, A-C) and asterisk (*) indicate significant differences ($p < 0.05$).

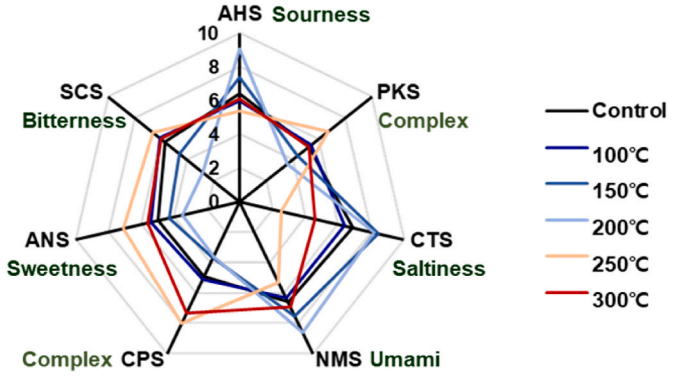

Fig. 6. Effects of subcritical water temperature on taste attributes of ginseng extracts based on the responses of electronic tongue.

3.6. *E-tongue analysis of GE*

Based on e-tongue analysis, GE prepared at 100 °C exhibited similar tastes attributes to those of the control (Fig. 6). Increasing SW temperature up to 200 °C increased the responses of AHS, CTS and NMS sensors, which were related with sourness, saltiness and umami, respectively [34]. Moreover, the responses of PKS, CPS, ANS and SCS sensors decreased gradually with SW temperature. The former PKS and CPS were found to be linked to the complex taste, whereas the latter ANS and SCS were sensible to sweetness and bitterness tastes, respectively [34]. Despite the physiological advantages, strong bitterness acted as a major hurdle of ginseng products for consumer preference. It was known that ginsenosides, phenolic compounds, flavonoids as well as amino acids were responsible for the bitterness of ginseng [35]. As SW was an effective technique in hydrolyzing proteins and fibers, one could predict that sweetness and saltiness of GE could be enhanced with increasing SW temperature. These changes in taste characteristics of GE under SW would be responsible to the increase in umami and reduced bitterness of GE [36]. In addition, intact ginsenosides, particularly, Rb1 was reported to cause the strong bitterness of ginseng, as compared to other components [35]. At 200 °C, the majority of ginsenosides were Rg5 in this study, and the bitterness of Rg5 has not been clearly reported previously. Nevertheless, the thermal conversion of ginsenosides would explain the change of taste attributes of GE.

However, the tastes attributes of GE prepared at 250 °C exhibited different patterns of change compared with those prepared at lower than 200 °C. The tastes were characterized by the highest responses of PKS, CPS, ANS and SCS, reflecting that sweetness to bitterness was the major taste traits of GE prepared at 250 °C. Because of the low dielectric constant of SW, the SW at 250 °C acts as ethanol or acetone, and the extractions of polyacetylene and alkaloids, which are recognized as other factors related to the bitterness of ginseng, can be enhanced under this condition [5,36]. Although the factor affecting the taste attributes of GE was not completely understood in this study, the results indicated that GE prepared by SW at 200 °C can potentially improve consumer preferences and could be available as a taste enhancer or a beverage product.

4. Conclusions

Based on the results of this study, SW could improve the physiological activities of GE. The SW was able to not only maximize the content of Rg5 but also provide superior antioxidative and antihypertensive effects compared with the control. In addition, the hydrothermal hydrolysis of ginseng carbohydrates could improve the taste attributes of GE. Although SW at greater than 250 °C can possibly minimize the generation of residual byproducts of ginseng roots, reduction of the physiological activities of GE acted as its drawback. Eventually, this present study demonstrated that SW at ~200 °C was an optimal process condition for commercializing ginseng products such as beverages. Moreover, detailed hydrothermal conversion and isolation of specific ginsenosides in GE warrant further exploration.

CRediT authorship contribution statement

Jong Won Lee: Methodology, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Mi-Yeon Lee:** Funding acquisition, Formal analysis, Data curation, Software, Writing – original draft, Writing – review & editing. **SangYoon Lee:** Investigation, Writing – original draft, Writing – review & editing. **Geun-Pyo Hong:** Conceptualization, Funding acquisition, Methodology, Investigation, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no conflicts of interests.

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References

[1] Lee SM, Bae BS, Park HW, Ahn NG, Cho BG, Cho YL, Kwak YS. Characterization of Korean red ginseng (*Panax ginseng* Meyer): history, preparation method, and chemical composition. J Ginseng Res 2015;39:348–91.

[2] Yoon SH, Nam YM, Hong JT, Kim SJ, Ko SK. Modification of ginsenoside composition in red ginseng (*Panax ginseng*) by ultrasonication. J Ginseng Res 2016; 40:300–3.

[3] Kwon IS, Kim H, Hong GP. Utilization of pulsed infrared for the rapid semidrying of fresh ginseng with advanced qualities and extended shelf life. Food Control 2022; 138:109043.

[4] Xiao D, Shao H, Huo Y, Nugroho WA, Ogunniran BI, Fan W, Huo M. Reclamation of ginseng residues using two-stage fermentation and evaluation of their beneficial effects as dietary feed supplements for piglets. Waste Manage (Tucson, Ariz) 2022; 154:293–302.

[5] Carr AG, Mammucari R, Foster NR. A review of subcritical water as a solvent and its utilisation for the processing of hydrophobic organic compounds. Chem Eng J 2011;172:1–17.

[6] Lee JW, Nam J, Lim W, Lim TG, Hong GP. Subcritical water mediated hydrolysis of rice husk lignocellulose and upcycling applications of the hydrolysates. Food Bioprocess Technol 2024. in press.

[7] Ramachandraiah K, Koh BB, Davaatseren M, Hong GP. Characterization of soy protein hydrolysates produced by varying subcritical water processing temperature. Innov Food Sci Em 2017;43:201–6.

[8] Sunphorka S, Chavasiri W, Oshima Y, Ngamprasertsith S. Kinetic studies on rice bran protein hydrolysis in subcritical water. J Supercrit Fluids 2012;65:54–60.

[9] Cao R, Myint AA, Kim J. High-yield recovery of highly bioactive compounds from red ginseng marc using subcritical water extraction. J Ind Eng Chem 2022;109: 547–58.

[10] Lee JH, Ko MJ, Chung MS. Subcritical water extraction of bioactive components from red ginseng (*Panax ginseng C.A. Meyer*). J Supercrit. Fluid 2018;133:177–83.

[11] Lee KA, Kim KT, Chang PS, Paik HD. In vitro cytotoxic activity of ginseng leaf/stem extracts obtained by subcritical water extraction. J Ginseng Res 2014;38:289–92.

[12] Lee JW, Choi EJ, Ryu WB, Hong GP. Characterization of temperature-dependent subcritical water hydrolysis pattern of strong and floury rice cultivars and potential utilization of their hydrolysates. Food Chem 2024;445:138737.

[13] Nagappan H, Pee PP, Kee SHY, Ow JT, Yan SW, Chew LY, Kong KW. Malaysian brown seaweeds *sargassum siliquosum* and *sargassum polycystrum*: low density lipoprotein (LDL) oxidation, angiotensin converting enzyme (ACE), α-amylase, and α-glucosidase inhibition activities. Food Res Int 2017;99:950–8.

[14] Yang F, Zhu P, Zheng H, Yang W, Wu S, Ye H, Che L. Interactions between cellulose and lignin during hydrolysis in subcritical water. J Supercrit Fluids 2023;199: 105943.

[15] Gao D, Kobayashi T, Adachi S. Kinetics of sucrose hydrolysis in a subcritical water-ethanol mixture. J Appl Glycosci 2014;61:9–13.

[16] Dai YL, Qiao MD, Yu P, Zheng F, Yue H, Liu SY. Comparing eight types of ginsenosides in ginseng of different plant ages and regions using RRLC-Q-TOF MS/MS. J Ginseng Res 2020;44:205–14.

[17] Yao F, Li X, Sun J, Cao X, Liu M, Li Y, Liu Y. Thermal transformation of polar into less-polar ginsenosides through demalonylation and deglycosylation in extracts from ginseng pulp. Sci Rep 2021;11:1513.

[18] Ryu J, Yoon J, Lee YW. Kinetic study of the thermal conversion of ginsenosides using lumped groups in steaming, hydrothermal reactions, and CO2-assisted hydrothermal reactions. J Supercrit Fluids 2021;167:105041.

[19] Lee SM. Thermal conversion pathways of ginsenosides in red ginseng processing. Nat Prod Sci 2014;20:119–25.

[20] Chung IM, Lim JJ, Ahn MS, Jeong HN, An TJ, Kim SH. Comparative phenolic compound profiles and antioxidative activity of the fruit, leaves, and roots of Korean ginseng (*Panax ginseng* Meyer) according to cultivation years. J Ginseng Res 2016;40:68–75.

[21] Alara OR, Abdurahman NH, Ukaegbu CI. Extraction of phenolic compounds: a review. Curr Res Food Sci 2021;4:200–14.

[22] Koh BB, Lee EJ, Ramachandraiah K, Hong GP. Characterization of bovine serum albumin hydrolysates prepared by subcritical water processing. Food Chem 2019; 278:203–7.

[23] Bogdan TV, Bobrova NA, Koklin AE, Mishanin II, Odintsova EG, Antipova ML, Petrenko VE, Bogdan VI. Structure of aqueous solutions of lignin treated by sub- and supercritical water: experiment and simulation. J Mol Liq 2023;383:122030.

[24] Jiang G, Bai X, Wu Z, Li S, Zhao C, Ramachandraiah K. Modification of ginseng insoluble dietary fiber through alkaline hydrogen peroxide treatment and its impact on structure, phytochemical and functional properties. LWT–Food Sci Technol 2021;150:111956.

[25] Han Z, Zhu M, Wan X, Zhai X, Ho CT, Zhang L. Food polyphenols and Maillard reaction: regulation effect and chemical mechanism. Crit Rev Food Sci Nutr 2022; 16:1–17.

[26] Ali MY, Zaib S, Jannat S, Khan I. Inhibition of angiotensin-I converting enzyme by ginsenosides: structure-activity relationships and inhibitory mechanism. J Agric Food Chem 2021;69:6073–86.

[27] Hu QR, Pan Y, Wu HC, Dai ZZ, Huang QX, Luo T, Li J, Deng ZY, Chen F. The ways for ginsenoside Rh2 to fight against cancer: the molecular evidences *in vitro* and *in vivo*. J Ginseng Res 2023;47:173–82.

[28] Liu J, Liu Y, Lin H, Zhou B, Yu H, Li L, Wang C, Li X, Li P, Liu J, Hao X. The effect of ginsenoside Rg5, isolated from black ginseng, on heart failure in zebrafish based on untargeted metabolomics. J Funct Foods 2021;76:104325.

[29] Chen R, Miao Y, Hao X, Gao B, Ma M, Zhang JZH, Wang R, Li S, He X, Zhang L. Investigation on the characteristics and mechanisms of ACE inhibitory peptides by a thorough analysis of all 8000 tripeptides via binding free energy calculation. Food Sci Nutr 2021;9:2943–53.

[30] Lee MY, Choi YC, Chun JY, Min SG, Hong GP. Effects of high pressure/high temperature processing on the recovery and characteristics of porcine placenta hydrolysates. Korean J Food Sci An 2013;33:474–80.

[31] Li S, Qi Y, Chen L, Qu D, Li Z, Gao K, Chen J, Sun Y. Effects of *Panax ginseng* polysaccharides on the gut microbiota in mice with antibiotic-associated diarrhea. Int J Biol Macromol 2019;124:931–7.

[32] Martins-Vieira JC, Torres-Mayanga PC, Lachos-Perez D. Hydrothermal processing of lignocellulosic biomass: an overview of subcritical and supercritical water hydrolysis. Bioenergy Res 2023;16:1296–317.

[33] Gubelt A, Blaschke L, Hahn T, Rupp S, Hirth T, Zibek S. Comparison of different Lactobacillus regarding substrate utilization and their tolerance towards lignocellulose degradation products. Curr Microbiol 2020;77:3136–46.

[34] Han D, Zhang CH, Fauconnier ML, Jia W, Wang JF, Hu FF, Xie DW. Characterization and comparison of flavor compounds in stewed pork with different processing methods. LWT–Food Sci Technol 2021;144:111229.

[35] Kim KT, Choi SY. Characterization of bitterness components of ginseng using electronic tongue. Korean J Food Preserv 2023;30:663–8.

[36] Wang W, Zhou X, Liu Y. Characterization and evaluation of umami taste: a review. Trends Anal Chem 2020;127:115876.