Remarkable impact of amino acids on ginsenoside transformation from fresh ginseng to red ginseng

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A R T I C L E   I N F O

Article history:
Received 27 March 2018
Received in Revised form 9 March 2019
Accepted 5 April 2019
Available online 10 April 2019

Keywords:
amino acids
ginsenoside transformation
red ginseng
steaming treatment

A B S T R A C T

Background: Amino acids are one of the major constituents in Panax ginseng, including neutral amino acid, acidic amino acid, and basic amino acid. However, whether these amino acids play a role in ginsenoside conversion during the steaming process has not yet been elucidated.

Methods: In the present study, to elucidate the role of amino acids in ginsenoside transformation from fresh ginseng to red ginseng, an amino acids impregnation pretreatment was applied during the steaming process at 120°C. Acidic glutamic acid and basic arginine were used for the acid impregnation treatment during the root steaming. The ginsenosides contents, pH, browning intensity, and free amino acids contents in untreated and amino acid−treated P. ginseng samples were determined.

Results: After 2 h of steaming, the concentration of less polar ginsenosides in glutamic acid−treated P. ginseng was significantly higher than that in untreated P. ginseng during the steaming process. However, the less polar ginsenosides in arginine-treated P. ginseng increased slightly. Meanwhile, free amino acids contents in fresh P. ginseng, glutamic acid−treated P. ginseng, and arginine-treated P. ginseng significantly decreased during steaming from 0 to 2 h. The pH also decreased in P. ginseng samples at high temperatures. The pH decrease in red ginseng was closely related to the decrease in basic amino acids levels during the steaming process.

Conclusion: Amino acids can remarkably affect the acidity of P. ginseng sample by altering the pH value. They were the main influential factors for the ginsenoside transformation. These results are useful in elucidating why and how steaming induces the structural change of ginsenoside in P. ginseng and also provides an effective and green approach to regulate the ginsenoside conversion using amino acids during the steaming process.

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1. Introduction

Chinese herbal medicine is generally processed by several traditional methods (such as steaming, boiling, stir-frying, carbonization, calcination, etc.) before they are used for clinical purposes. On the basis of long-term experiences, the purposes of Traditional Chinese Medicine (TCM) processing are thought to enhance the efficacy, reduce or eliminate the toxicity and side effects, and to modulate the actions or properties of treated herbal medicines [1]. Many modern studies have demonstrated that most changes of chemical components in Chinese herbal medicine occurred during processing and then induced different therapeutic benefits [2]. Because TCM processing has a close relationship with the efficacy, safety, and quality of TCM, exploring the changes in chemical constituents of TCM during processing, especially the mechanism involved, is critical for developing the reliable quality control methodology and processing technology for TCM.

Panax ginseng Meyer is one of the most popular TCM in the world and has been demonstrated to have a wide range of pharmacological properties, such as anti-inflammatory, antidiabetic, antifatigue, antioxidant, antiobesity, and antitumor activities [3–6]. The main active components of ginseng are commonly considered

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to be triterpene saponins, termed ginsenosides, including widely studied neutral ginsenosides (e.g., Rb1, Rc, Rb2, Rd, Rg1, and Re) and the less studied malonyl ginsenosides (e.g., M-Rb1, M-Rc, M-Rb2, and M-Rd) (Fig. 1). The malonyl ginsenosides are also called “acidic ginsenosides”.

Traditionally, sun drying and steaming are two commonly used processing methods for *P. ginseng*. White ginseng (WG) is prepared by simply drying the fresh ginseng in sunlight, whereas red ginseng (RG) is made by steaming fresh ginseng at high temperatures. Many studies have revealed that RG possesses better pharmacological effects than fresh ginseng and WG, and the enhanced biological activities are mainly attributed to the structural changes in ginsenosides that occur during steaming process [7,8]. The contents of less polar ginsenosides (20(S)-Rg3, 20(R)-Rg3, Rg5, Rg6, Rk3, Rk1, Rh3, etc.) in fresh ginseng and WG could not be detected. When fresh ginseng was steamed at 120 °C, the amount of neutral ginsenosides and malonyl ginsenosides decreased sharply and almost disappeared, while the amount of the less polar ginsenosides increased rapidly [9,10]. These less polar ginsenosides exhibit better bioactivity and bioavailability than their original ginsenosides [11–13]. However, most past studies usually focused on the ginsenoside transformation and biological activity changes during the processing of RG. Very little work has been carried out on the transformation mechanisms involved in elucidating why and how steaming induces the structural change of ginsenoside in *P. ginseng*.

*P. ginseng* contains a complex mixture of chemical components, including ginsenosides, low-molecular-weight organic acids, amino acids, fatty acids, polysaccharides, phenolic compounds, and volatile oils [14]. In our previous work, we found that neutral ginsenosides were degraded into less polar ginsenosides during the *P. ginseng* root steaming, but not during the pure reference ginsenoside steaming. Further research revealed that a low concentration of low molecular weight organic acid was the determinant effecting factor for the ginsenoside transformation during processing of RG [15].

Amino acids are important organic compounds containing carboxyl (-COOH) and amine (-NH2) functional groups. On the basis of the number of acidic carboxylic groups and basic amino groups in the molecule, the amino acids are classified into three main groups: neutral amino acid (e.g., threonine, leucine, valine, tyrosine, proline, alanine, glycine, and serine), acidic amino acid (e.g., glutamic acid and aspartic acid), and basic amino acid (e.g., arginine, lysine, and histidine). Previous researches have shown that the concentrations of amino acids are abundant in *P. ginseng* [16,17]. They were used as an important index for the quality control of ginseng product [18]. However, whether amino acids play a role in ginsenoside conversion during the steaming process has not yet been elucidated. In addition, the degradation of ginsenosides in RG by traditional processing technology exhibits low yield and long processing time. As the building blocks of life, amino acids play a very useful role in human health and nutrition. They can be safely used in the food and pharmaceutical applications. However, there are no studies that have tried to develop an effective approach to promote the ginsenoside conversion using amino acids treatment during RG processing.

In this article, to elucidate the role of amino acids in ginsenoside conversion, an amino acids impregnation pretreatment was applied during RG processing. Acidic glutamic acid and basic arginine were used for the acid impregnation treatment during the root steaming. The ginsenosides contents, pH, browning intensity, and free amino acids contents in untreated and amino acid−treated *P. ginseng* samples were determined. Our results showed that amino acids can remarkably affect the acidity of *P. ginseng* sample by altering the pH value. They were the main influential factor for the ginsenoside transformation.

### 2. Materials and methods

#### 2.1. Chemicals

Ginsenoside Re, Rg1, Rb1, Rb2, Rc, Rd, 20(S)-Rg3, 20(R)-Rg3, 20(S) Rg2, 20(R)-Rg2, 20(S)-Rh1, and 20(R)-Rh1 were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Malonyl ginsenoside Rb1, Rb2, Rc, Rd, zingibroside R1, ginsenoside Ro, Rk1, Rk3, Rg5, Rg6, and F4 were isolated by our laboratory [19,20]. Arginine, glutamic acid, threonine, asparagine, lysine, phenylalanine, leucine, isoleucine, cysteine, methionine, valine, tyrosine, proline, alanine, histidine, glycine, and serine were purchased from Sigma-Aldrich (Shanghai, China). Methanol and acetonitrile were HPLC grade (Fisher Scientific, Pittsburgh, PA). Phenylisothiocyanate and triethylamine were obtained from Agela of the USA (Agela Technologies Inc., Wilmington, DE).

**Fig. 1.** Structures of acidic and neutral ginsenosides from Panax ginseng. Ma, malonyl; glc, glucosyl; ara, arabinosyl; rha, rhamnose.
Fig. 2. Chromatograms of neutral ginsenosides transformation by amino acids steamed at 120°C for 1h. (A) Degradation of protopanaxadiol-type ginsenosides by glutamic acid. (B) Degradation of protopanaxadiol-type ginsenosides by threonine. (C) Degradation of protopanaxadiol-type ginsenosides by arginine. (D) Degradation of ginsenoside Re by glutamic acid. (E) Degradation of ginsenoside Re by threonine. (F) Degradation of ginsenoside Re by arginine.
2.2. Plant materials

Five-year-old fresh *P. ginseng* roots were collected from Fusong county, Jilin province, China. The voucher samples were identified by Dr. Guang-Zhi Sun and deposited in the College of Chinese Medicinal Materials at Jilin Agricultural University.

2.3. Steaming of neutral ginsenosides with amino acids

To imitate the RG steaming process, the same (5 mg) amounts of protopanaxadiol-type ginsenosides and amino acids (arginine, glutamic acid, threonine, asparagine, lysine, phenylalanine, leucine, isoleucine, cysteine, methionine, valine, tyrosine, proline, alanine, histidine, glycine, or serine) were dissolved in distilled water (1 mL) and were steamed at 120 °C for 1 h, respectively, using an autoclave. Re and amino acids were also steamed together in the same method.

2.4. Steaming of acidic ginsenosides with amino acids

The same amount (5 mg) of acidic malonyl ginsenoside Rb1 and arginine or glutamic acid were steamed together at 120 °C for 1 h, separately, using the same method that was used to steam the neutral ginsenosides.

2.5. Steaming of amino acid-treated *P. ginseng*

To infiltrate amino acid into *P. ginseng* roots, the treatment process was performed as follows. The roots of fresh ginseng were dried at 35 °C for 2–3 days with a hot air drier. When the drying

![Fig. 3. HPLC–principal component analysis (PCA) of neutral ginsenosides transformation by 17 amino acids after steaming treatment. (A) The score plot of PCA of protopanaxadiol-type ginsenosides degradation metabolites, using contents of ginsenosides Rb1, Rc, Rb2, Rd, 20S-Rg3, 20R-Rg3, Rk1, and Rg5 as input data. (B) The score plot of PCA of ginsenoside Re degradation metabolites, using contents of ginsenosides Re, 20S-Rg2, 20R-Rg2, 20S-Rh1, 20R-Rh1, Rg6, F4, RK3, and Rb4 as input data.](image)
process was completed, the dried ginseng samples were pulverized with an electric mill and sieved through a 40 mesh. Then a 10 g sample of *P. ginseng* root was accurately weighed and placed into a 100 mL round bottom flask. Ten milliliters of 1.5% (w/v) glutamic acid or 2.5% (w/v) arginine water solution was added into it. The impregnation processing was performed at room temperature for 2 h. Then the amino acid–treated *P. ginseng* samples were steamed from 0 to 2 h at 120 °C, respectively. After steaming process, the samples were dried at 35 °C for 2–3 days.

2.6. Measurement of browning intensity and pH

The extent of browning of untreated and amino acid–treated *P. ginseng* sample was determined as previous reported with minor modification [21]. One gram of each sample was extracted by ultrasonication with 70% EtOH for 30 min, and the extraction was repeated in three times. Then the solvent was evaporated under vacuum. The ginseng extracts were dissolved in 25 mL of distilled water-acetonitrile (1:1, v/v) and measured absorbance at a wavelength of 420 nm.

The pH of ginseng samples was measured according to the method of Yuan et al. [22] with some modification. The untreated or amino acid–treated *P. ginseng* sample (5 g) was extracted by ultrasonication with 20 mL of deionized water for 30 min. Then, the samples were centrifuged for 15 min at 2000g using an Eppendorf 5430R centrifuge (Hamburg, Germany). The solid residue was reextracted with deionized water (20 mL), and above steps are repeated. The supernatants were combined, filtered, and transferred into a 50 mL volumetric flask. The pH values were measured.

![Fig. 4. Chromatograms of malonyl ginsenosides after steaming treatment. (A) Malonyl-ginsenoside Rb1. (B) Malonyl-ginsenoside Rb1 steamed at 120°C for 1 h. (C) Degradation of malonyl-ginsenoside Rb1 by glutamic acid steamed at 120°C for 1 h. (D) degradation of malonyl-ginsenoside Rb1 by arginine steamed at 120°C for 1 h.](image-url)
using a pH meter (Thermo Fisher Scientific, Waltham, MA) at room temperature. Measurement was performed three times.

2.7. Sample preparation for ginsenoside and amino acid analyses

For ginsenoside analysis, 1 g of untreated and amino acid-treated *P. ginseng* sample was extracted three times by ultrasonication with 70% MeOH at 25°C for 30 min. The extract was condensed under vacuum at 45°C. The solution was stored in a freezer at 4°C prior to analysis.

For amino acid analysis, 0.5 g of untreated and amino acid-treated *P. ginseng* was extracted by ultrasonication in 10 mL of 75% EtOH for 30 min, and the extraction was repeated in three times. The samples were centrifuged at 6000g for 20 min. The supernatants were pooled and evaporated under reduced pressure to dryness. The residue was placed in a 25 mL volumetric flask and diluted with 0.1 mol/L hydrochloric acid. The solution was stored at 4°C for HPLC analysis.

2.8. Precolumn derivatization

Precolumn derivatization was performed for amino acids before HPLC analysis. One milliliter of each *P. ginseng* extract and standards were derivatized with TEA acetonitrile solution and PITC acetonitrile solution and incubating at room temperature for 1 h as reported previously [23]. After the incubation, 400 μL n-hexane was added and mixed well for 2 min and stood for 10 min. The bottom layer was filtered through a 0.45 μm filter for HPLC analysis.

2.9. HPLC analysis

Ginsenoside analysis was performed using a Shimadzu LC-20A liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with an SPD-20A UV/vis detector and two LC-20AT pumps. The chromatographic separation was performed on a Cosmosil C18 analytical column (250 mm x 4.6 mm, 5 μm). The mobile phase consisted of acetonitrile (solvent A) and 0.05 mol L⁻¹ KH₂PO₄ (solvent B) using a gradient elution of 22% A at 0–20 min; 22%–29% A at 20–25 min; 29% A at 25–45 min; 29%–35% A at 45–55 min; 35%–50% A at 55–60 min; 50%–70% A at 60–75 min; and 70% A at 75–85 min. The flow rate was kept at 1 mL/min, and the column temperature was maintained at 25°C. The UV detection wavelength was at 203 nm, and the injection volume was 20 μL.

Amino acids were analyzed using an Ultimate® Amino Acid C18 column (5 μm, 250 mm x 4.6 mm). A gradient elution system was solvent A (0.1 mol L⁻¹ NaOAc: acetonitrile = 93:7, v/v, pH 6.5) and solvent B (acetonitrile: water = 80:20, v/v), the process was used as follows: 0–13 min, 0–7% B; 13–23 min, 7%–23% B; 23–29 min,
23°–25° B; 29–35 min, 35°–40° B; 35–40 min, 40°–100° B; and 40–45 min, 100° B. The flow rate was 1.0 mL/min, and the column temperature was maintained at 40°C. The UV detection wavelength was set at 254 nm, and the injection volume was 10 μL.

2.10. Statistical analysis

Statistical analysis was performed using a SPSS 20.0 system (SPSS Inc, Chicago, IL). Results were presented as mean ± SEM. A one-way ANOVA was used to determine whether the results had statistical significance. P value of less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. Degradation of neutral ginsenosides with amino acids

In the present study, the effects of 17 amino acids on the degradation of neutral ginsenosides were investigated during the steaming at 120°C; among them, the glutamic acid had the most influence on the ginsenoside transformation. As shown in Fig. 2A and B, protopanaxadiol-type ginsenosides and Re were mostly converted into less polar ginsenosides by glutamic acid after 1 h of steaming at 120°C. Protopanaxadiol-type ginsenosides were degraded to 20(R)-Rg3, 20(S)-Rg3, Rg5, and Rk1; Re was degraded to 20(R)-Rg2, 20(S)-Rg2, 20(R)-Rh1, 20(S)-Rh1, Rk3, Rh4, F4, and Rg6. Compared with glutamic acid, the contents of less polar ginsenosides, which were produced by steaming treatment with neutral amino acids, was obviously low (Fig. 2B and E). In addition, ginsenoside Re was only converted into 20(R)-Rg2, 20(S)-Rg2, F4, and Rg6, while Re, F4, and Rg6 could not be further hydrolyzed by neutral amino acids at the terminal glycosyl moiety of C-6 to yield 20(R)-Rh1, 20(S)-Rh1, Rk3, and Rh4. However, protopanaxadiol-type ginsenosides and Re did not undergo any degradation by arginine during the steaming process (Figs. 2C, 2F).

Further, a principal component analysis assay was carried out on the data set obtained from the HPLC chromatogram. A 2-dimensional principal component analysis score plot was constructed using the contents of saponin degradation metabolites as input data. The plot showed that the samples could be obviously divided into three groups with steaming treatment by amino acids: an acidic amino acids-treated group (glutamic acid and aspartic acid), neutral amino acids-treated group (threonine, phenylalanine, leucine, isoleucine, cysteine, methionine, valine, tyrosine, proline, alanine, glycine, and serine), and basic amino acids-treated group (arginine, lysine, and histidine) (Fig. 3). These results indicate that acidic amino acids can promote the degradation of neutral ginsenosides during the steaming at 120°C, while basic amino acids could not hydrolyze neutral ginsenosides.

Several papers reported the structural changes of ginsenoside-neutral amino acid mixture by the Maillard reaction and its biological activities changes [24,25]. The results showed that neutral ginsenosides were dramatically converted into less polar ginsenosides by neutral amino acids during the heat processing. But, the results were not in agreement with our data obtained from steaming neutral ginsenosides. Previous reports focused on the ginsenoside transformation and Maillard reaction during heat processing. Maillard reaction model experiment was performed using a high concentration of ginsenosides and amino acids. In this work, to imitate the RG steaming process, a low concentration of amino acid was used for the transformation of ginsenosides.

3.2. Degradation of acidic ginsenosides with amino acids

The effect of amino acids on the degradation of acidic ginsenosides was also investigated. As shown in Fig. 4, after 1 h of steaming at 120°C, malonyl-ginsenoside Rb1 was transformed into Rb1, 20(S)-Rg3, 20(R)-Rg3, Rk1, and Rg5. When malonyl-ginsenoside Rb1 was heat processed with glutamic acid, the contents of the less polar ginsenosides 20(S)-Rg3, 20(R)-Rg3, Rk1, and Rg5 increased significantly, while neutral ginsenosides Rb1 decreased sharply and almost disappeared after 1 h of steaming, compared with those obtained after normal heat processing. When malonyl-ginsenoside Rb1 was heat processed with arginine, malonyl-ginsenoside Rb1 was only converted into Rb1, and less polar ginsenosides could not be generated.

Usually, ginsenosides could be hydrolyzed at the glycosyl moiety of C-3, C-6, and C-20 to yield less polar ginsenosides under a high acidic or alkaline condition [26,27]. It is difficult to degrade ginsenosides in a neutral environment. In our previous study, we found that neutral ginsenosides could not undergo any conversion during the individual ginsenoside steaming, while the malonylginsenosides were degraded into corresponding neutral ginsenosides, acetic acid, and malonic acid through decarboxylation, deacetylation, demalonylation reactions. Then, neutral ginsenosides were further hydrolyzed by these organic acids to yield less polar ginsenosides. These results indicate that a low concentration of low molecular weight organic acid was the determinant effecting factor for the ginsenoside transformation during processing of RG [15]. In the present study, the pH of untreated and amino acid-treated malonyl-ginsenoside Rb1 was measured. The pH of malonyl-ginsenoside Rb1 by normal heat processing was 6.02, whereas after being heat processed with glutamic acid and arginine, the pH values of the malonyl-ginsenoside Rb1 were 4.75 and 8.06, respectively. The pH of malonyl-ginsenoside Rb1 by heat
processing with arginine was higher than that by normal heat processing. The results indicate that arginine may neutralize organic acids, which were produced by steaming malonyl-ginsenoside Rb1, then, prevent the degradation of ginsenoside Rb1.

3.3. Changes in ginsenosides of amino acid-treated P. ginseng samples during steaming process

As shown in Fig. 5 and Table 1, the content of malonyl ginsenosides in the untreated and amino acid-treated P. ginseng decreased sharply and almost disappeared after 0.5 h of steaming at 120°C; the concentration of less polar ginsenosides in glutamic acid–treated P. ginseng (20.49 mg/g) was approximately two-fold higher than that in untreated P. ginseng (9.2 mg/g) within the first 0.5 h. But, less polar ginsenosides, except for zingibroside R1, could not be generated when P. ginseng is processed with arginine.

After 2 h of steaming, neutral ginsenosides were mostly converted into less polar ginsenosides in glutamic acid–treated P. ginseng, while neutral ginsenosides in arginine-treated P. ginseng were degraded slightly. The proposed transformation pathways of ginsenosides in amino acid–treated P. ginseng during the steaming process are shown in Fig. 6. The ginsenoside transformation pathways were consistent with those of previous study [9,10]. These results indicated that amino acids remarkably affected ginsenoside transformation during the steaming process. Acidic amino acids can promote the degradation of neutral ginsenosides, while basic amino acids can prevent the ginsenoside conversion. Thus, our results provide an effective and green approach for regulating the ginsenoside conversion using amino acids during the steaming process.

3.4. Changes in amino acids, browning intensity, and pH of amino acid-treated P. ginseng during steaming process

Fig. 7 shows changes in the contents of amino acids in fresh P. ginseng, glutamic acid–treated P. ginseng, and arginine-treated P. ginseng during the steaming at 120°C. A total of 17 amino acids were quantified by HPLC. From 0 to 2 h of steaming, total contents
of free amino acids in fresh *P. ginseng*, glutamic acid–treated *P. ginseng*, and arginine-treated *P. ginseng* decreased significantly, ranging from 25.3 to 8.4 mg/g, 40.1 to 13.5 mg/g, and 50.9 to 14.2 mg/g, respectively. Meanwhile, the browning intensity of steamed *P. ginseng* samples significantly increased with increasing time, and the increased levels of browning products in glutamic acid–treated *P. ginseng* and arginine-treated *P. ginseng* were higher than those of untreated *P. ginseng* sample (Fig. 8A).

Similarly, a significant variation in the amino acids and browning intensity in RG during the steaming process has also been studied. Cho et al. [16] showed that the formation of Maillard reaction products (MRPs) depends on the processing conditions such as temperature and time, and the decrease of amino acid contents and increase of MRPs level in RG were considered to be related to the extent of the Maillard reaction. Moreover, many studies have shown that the MRPs possess the multiple biological activities, such as antihypertensive, antimicrobial, and antioxidant agents [25,28]. Therefore, our results suggested that amino acid–treated *P. ginseng* may possess better biological activities than untreated *P. ginseng* samples.

Among the 17 detected amino acids, arginine is the most abundant amino acid, it made up about 67.5% of the total free amino acids in fresh *P. ginseng*, 42.7% in glutamic acid–treated *P. ginseng*, and 82.7% in arginine-treated *P. ginseng*. Glutamic acid was only present in a high concentration in glutamic acid–treated *P. ginseng*, representing about 42.6% of total free amino acids. The glutamic acid content in glutamic acid–treated *P. ginseng* was approximately eight-fold higher than that in untreated *P. ginseng*. After 2 h of steaming, the content of arginine decreased significantly with the progression of thermal processing, reaching 5.1 mg/g in fresh *P. ginseng*, 6.6 mg/g in glutamic acid–treated *P. ginseng*, and 10.3 mg/g in arginine-treated *P. ginseng* (Fig. 7).

Similarly, glutamic acid also decreased significantly in *P. ginseng* samples after steaming process at 120°C. Previous studies have shown that the arginine derivatives such as arginyl-fructose and arginyl-fructosyl-glucose were rich in RG [29]. Arginyl-fructose and arginyl-fructosyl-glucose are formed through amadori rearrangement of arginine with maltose or glucose, respectively, in the early stages of the Maillard reaction [30]. These arginine derivatives have been shown to have potent antioxidant and anti-diabetic activities [31,32].

The initial pH of fresh *P. ginseng* was 6.50, whereas after amino acid treatment, the pH values of the arginine-treated *P. ginseng* and glutamic acid–treated *P. ginseng* were 8.24 and
Individual ginsenoside steaming and main in [15]. Therefore, these results indicate that amino acids can remarkably affect the acidity of P. ginseng sample by altering the pH value. They were the main influential factor for the ginsenoside conversion. Our results are useful in elucidating why and how steaming induces the structural change of ginsenoside in P. ginseng and also provides an effective and green approach to regulate the ginsenoside conversion using amino acids during the steaming process.

Conflicts of interest

The authors declare no competing financial interest.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (No. 31770378), Jilin Provincial Natural Science Foundation of China (No. 20180101183JC), and Tang Foundation for the Research of Traditional Chinese Medicines.

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Fig. 8. (A) Changes in the browning intensity of unsteamed and steamed P. ginseng samples by amino acid treatment. (B) pH of unsteamed and steamed P. ginseng samples by amino acid treatment. FG, fresh P. ginseng; Glu-FG, glutamate acid–treated fresh P. ginseng; Arg-FG, arginine-treated fresh P. ginseng. **p < 0.01, ***p < 0.05, compared with unsteamed P. ginseng sample.

5.69, respectively. The pH of arginine-treated P. ginseng was obviously higher than that of fresh P. ginseng and glutamic acid–treated P. ginseng. When untreated and amino acid–treated P. ginseng sample was steamed at 120°C for 2 h, the pH of fresh P. ginseng and arginine-treated P. ginseng decreased gradually with time, reaching 5.84 and 6.59, respectively. The pH values of the glutamate acid–treated P. ginseng decreased slightly (Fig. 8B). The results indicate that the pH decrease in RG was closely related to the decrease in arginine level during the steaming process. Similar results were also investigated in the black garlic manufacturing process. Choi et al. [33] showed that the pH of black garlic samples decreased sharply from 6.33 to 3.74 during steaming process. The pH decreases during the black garlic processing because of the formation of plenty of organic acids [34,35]. However, in our previous study, we found that the contents of total organic acids in RG did not significantly increase [15]. Therefore, these results indicate that amino acids can remarkably affect the acidity of P. ginseng sample. They were the main influential factor for the ginsenoside transformation

4. Conclusion

This work investigated the effects of amino acids on the degradation of neutral and acidic ginsenosides during the individual ginsenoside steaming and P. ginseng root steaming at 120°C. Our results showed that the concentration of less polar ginsenosides in glutamic acid–treated P. ginseng was significantly higher than that in untreated P. ginseng during the steaming process. However, less polar ginsenosides in arginine-treated P. ginseng increased slightly. Meanwhile, free amino acids contents, especially arginine and glutamic acid, in P. ginseng samples significantly decreased with increasing time. The pH also decreased in P. ginseng samples at high temperatures. The pH decrease in RG was closely related to the decrease in basic amino acids levels during the steaming process. Thus, amino acids can remarkably affect the acidity of P. ginseng sample by altering the pH value. They were the main influential factor for the ginsenoside conversion. Our results are useful in elucidating why and how steaming induces the structural change of ginsenoside in P. ginseng and also provides an effective and green approach to regulate the ginsenoside conversion using amino acids during the steaming process.

The authors declare no competing financial interest.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (No. 31770378), Jilin Provincial Natural Science Foundation of China (No. 20180101183JC), and Tang Foundation for the Research of Traditional Chinese Medicines.
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