Qualitative and quantitative analysis of furosine in fresh and processed ginsengs

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ABSTRACT

Background: Furosine (l-N-2-furoylmethyl-L-lysine, FML) is an amino acid derivative, which is considered to be an important indicator of the extent of damage (deteriorating the quality of amino acid and proteins due to a blockage of lysine and a decrease in the digestibility of proteins) during the early stages of the Maillard reaction. In addition, FML has been proven to be harmful because it is closely related to a variety of diseases such as diabetes. The qualitative analysis of FML in fresh and processed ginsengs was confirmed using HPLC-MS.

Methods: An ion-pair reversed-phase LC method was used for the quantitative analysis of FML in various ginseng samples.

Results: The contents of FML in the ginseng samples were 3.35–42.28 g/kg protein. The lowest value was observed in the freshly collected ginseng samples, and the highest value was found in the black ginseng concentrate. Heat treatment and honey addition significantly increased the FML content from 3.35 g/kg protein to 42.28 g/kg protein.

Conclusion: These results indicate that FML is a promising indicator to estimate the heat treatment degree and honey addition level during the manufacture of ginseng products. The FML content is also an important parameter to identify the quality of ginseng products. In addition, the generation and regulation of potentially harmful Maillard reaction products—FML in ginseng processing was also investigated, providing a solid theoretical foundation and valuable reference for safe ginseng processing.

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1. Introduction

Ginseng has been consumed as a dietary supplement and herbal medicine for thousands of years in China, Korea, and Western countries [1,2]. The processing of ginseng is known to have an influence on its bioactive components and pharmacological activities; therefore, its processing is crucial for ginseng’s dietary and medical functions [3,4]. During the storage (time, humidity, and temperature) and processing (steaming, drying, and excipients addition) of ginseng, reactions between the amino and carbonyl groups often develop randomly. These reactions are called as the Maillard reactions (MRs), amino-carbonyl reactions, or nonenzymatic model glycation reactions [5,6]. Because abundant carbonyl and amino compounds (reducing sugars or ginsenosides with amino acids or proteins) are contained in ginseng, various MRs may occur [7]. MRs in ginseng processing not only produce a large number of functional components but also generate a small amount of harmful substances which cannot be ignored [8]. In 2012, planted ginseng was advocated to be “homology of medicine and food” in China within 5 yr, stimulating higher standards with respect to the quality and safety of ginsengs [9].

Furosine (l-N-2-furoylmethyl-L-lysine, FML) is an amino acid derivative, generally binding with proteins to generate Amadori products (N-substituted 1-amino-1-deoxy-2-ketose) such as fructose-lysine, lactulose-lysine, and maltulose-lysine [10]. FML is one of the MR products (MRPs) from MRs of lysine with glucose and other reducing sugars or ginsenosides. The scheme for the formation of FML from the Amadori product of glucose is shown in Fig. 1. In addition, FML is also considered to be an important indicator of the extent of damage (reducing the quality of amino acid and proteins).
proteins due to a blockage of lysine and a decrease in the digestibility of proteins) during the early stages of MR. Harris et al. [11] reported that FML could degrade slowly to form many different advanced glycation end products (AGEs). Partial AGEs have been proven to be closely related to a variety of diseases, such as diabetes, and a high amount of AGEs in human bodies is considered harmful [11]. AGEs in human bodies are mainly derived from two pathways, in vivo transformation and in vitro intake, but the in vitro intake from foods and medicines is the main source. A number of previous researches have reported on the FML for identifying the quality or processing and storage effects in foods, such as liquid nutritional products [12], milk-cereal-based baby foods [13], dietary products [8], meat products [14], honey [15], pasta products [16], milk [17], as well as cookies, crackers, and breakfast cereals [16]. However, the presence of FML in ginseng products and suggestion to consider FML as an evaluating indicator of quality for ginseng has not been reported.

In the present study, qualitative and quantitative analysis of FML in fresh and processed ginseng was confirmed using HPLC-MS, and it was found that the FML content in various ginsengs was variable. The presence of FML in the acid hydrolysates of five kinds of ginseng samples was variable by comparing its retention time and mass fractions with that of an FML standard using HPLC-MS. The FML content in various ginseng products was analyzed using an ion-pair reversed-phase LC method and comparing its retention time and peak area with the standard. At the same time, the generation and regulation of potentially harmful MRPs-FML in ginseng processing was also analyzed, providing a solid theoretical foundation and valuable reference for safe ginseng processing and also providing a basis for the development of recommended ginseng dosage.

2. Experimental

2.1. Materials and methods

Five kinds of different ginseng samples were purchased from local markets in Ji’an, China and Cheong Kwan Jang, South Korea. Three of the five were solid samples (fresh, dried raw, and red ginseng), and the other two were liquors (red ginseng liquor and black ginseng concentrate). FML standard was purchased from NeoMPS (Strasbourg, France). Trifluoroacetic acid (TFA) was purchased from Sigma (San Francisco, USA). HPLC-grade acetonitrile was purchased from Fisher-Scientific (USA). Hydrochloric acid and other chemicals were of reagent grade.

2.2. Sample preparation

The preparation of the samples follows the traditionally-adopted procedures [18,19]. Briefly, five kinds of ginseng samples (3.0 g each) were hydrolyzed with 6M HCl at 110°C for about 22 h in a screw-capped Pyrex vial with PTFE-faced septa. The hydrolysates were filtered with a medium-grade filter paper, and then a 2 mL portion of the filtrate was applied to a Millipore Sep-Pak C18 cartridge (Massachusetts, USA) pre-wetted with 5 mL methanol and subsequently with 10 mL water. The FML portion was eluted with 3 mL of 3M HCl, and the resulting solutions were collected for HPLC-MS or HPLC analysis.

2.3. Protein content analysis

The protein content in fresh and processed ginseng was measured on a Dumas Nitrogen Analyzer (Velp NDA 701-Monza, Brianza–Italy), according to a previous method with minor modification [20]. The total nitrogen level was converted to protein content using a conversion factor of 6.25. The working conditions of NDA were as follows: O2 gas at 400 mL/min, He gas at 195 mL/min, combustion reactor at 1030°C, reduction reactor at 650°C, and pressure at 881.0 mbar.

2.4. Qualitative analysis of FML

To identify the presence of FML in fresh and processed ginseng samples, HPLC-MS analysis was performed. Qualitative analysis of FML was performed at 25°C using HPLC (Agilent1200, USA) coupled
with 6310 electric spray-ion trap mass spectrometer (Agilent, USA), which consisted of a column oven (G1316A), a pump (G1311A), a degasser (G1322A), and an automatic sampler (G1329A). The chromatographic separation was performed on a YMC hydrosphere C18 column (4.6 × 250 mm, 5 μm, Tokyo, Japan) and the liquid chromatograph working in electrospray ionization mode under atmospheric pressure and positive polarity (API-ES positive). Other HPLC-MS conditions were as follows [7], water/formic acid (99.6:0.4, v/v) at a flow rate of 0.5 mL/min. The ion mode set at selective monitoring was m/z 255, corresponding to FML “[M+H]⁺”.

2.5. Quantitative analysis of FML

HPLC analysis of FML was performed at 25°C on an HPLC instrument (Agilent1200, USA) with a UV detector (G1315D). The other accessories were as described in section 2.4. An ion-pair reversed-phase (by adding TFA into the mobile phase)-LC method was developed for the determination of FML in different ginseng samples. The sample acid hydrolysates were subjected to quantitative analysis, which was performed by the external standard method using a commercial standard of pure FML. Results were expressed as g/kg protein, and all the analyses were performed in triplicate.

3. Results and discussion

3.1. Quantitative analysis of FML

3.1.1. Identification of FML by HPLC-MS

The comparison of the retention times and mass fragmentation patterns for the acid hydrolysates of the ginseng samples with those of the reference FML established the presence of FML in the ginseng samples. Fig. 2 shows the HPLC-MS chromatographs of the reference FML and other samples (standard, fresh ginseng, dried raw ginseng, red ginseng, red ginseng liquor, and black ginseng concentrate; Figs. 2A–2F). It reached a good resolution for the

Fig. 2. Selective ion monitoring of furosine by HPLC-MS in a solution of furosine. (A) Furosine standard. (B) Fresh ginseng. (C) Dried raw ginseng. (D) Red ginseng. (E) Red ginseng liquor. (F) Black ginseng concentrate.

Fig. 3. (A) Selective ions monitoring of furosine standard by HPLC-MS. (B) Mass spectrum of the observed furosine fragmentation pattern of furosine standard. (C) Mass spectrum of the observed furosine fragmentation pattern of red ginseng. (D) The assignment (or interpretation) of the fragmentation.
separation of FML under the HPLC condition. The selective ion monitoring fraction of mass spectral analysis for the FML standard (Fig. 3A) and the ginseng samples showed the same fragmentation patterns of 237, 192, 130, and 84 (Figs. 3B and 3C), and the assignment of the fragmentation pattern is shown in Fig. 3D, demonstrating the presence of FML in the ginseng samples. These results were in good agreement with those of the previous report in food products (pasta, milk, and tigelle bread) [21].

3.1.2. Confirmation of FML by UV scanning

From the retention time of FML at about 10.07 min in ultraviolet spectrum, the red ginseng had the same UV absorption spectrum with the FML standard (Figs. 4A and 4B), and the other four ginseng samples had the same absorption, further confirming that the chromatographic peak at about 10.07 min corresponds to FML. The results further support the presence of FML in the ginseng samples.

3.2. Quantitative analysis of FML

3.2.1. HPLC analysis of FML

A series of FML standard solutions (0.1–1 mmol/mL) were filtered through a nylon purification kit with a pore size of 0.45-μm cut-off (Massachusetts, USA) and then analyzed by the HPLC-pulsed amperometric detection system. TFA was added in the mobile phase, which could delay the retention time of highly polar FML on the reversed-phase column. A good linearity was obtained at concentration of FML in the range of 0.3–10 mg/L with an equation of $y = 325230x - 124444.4$ ($R^2 = 0.999$). The detection limit of FML for the method (three signal-to-noise ratio, $S/N = 3$) was 0.05 mg/mL, and the quantitation limit was 0.18 mg/mL.

3.2.2. Quantitative analysis of FML in ginseng samples

HPLC results indicated that FML was successfully separated in different ginseng samples. Quantitative analyses of FML in fresh and differently processed ginseng products (dried raw ginseng, red ginseng, red ginseng liquor, and black ginseng concentrate) under the given HPLC conditions were performed as shown in Fig. 5, and the data are shown in Table 1. FML was detected in all ginseng samples at concentrations ranging from 3.35 g/kg protein to 42.28 g/kg protein. These values of FML content indicated that ginseng protein was glycosylated to a considerable extent.

Fig. 4. UV spectrum of the compound corresponding to the peak at 10.07 min on HPLC fingerprint. (A) Standard. (B) Red ginseng.

Fig. 5. HPLC fingerprint for furosine analysis in ginseng samples. (A) Standard. (B) Fresh ginseng. (C) Dried raw ginseng. (D) Red ginseng. (E) Red ginseng liquor. (F) Black ginseng concentrate.
compared with other processed foods, such as ultra-high-temperature milk [13] (310–603 g/kg protein) or processed cheese [22] (3.5–366.6 g/kg protein). The FML content in fresh ginseng was low, but it was relatively high in black ginseng concentrate (Table 2). The FML content in processed ginseng products can be variable depending on many factors, such as processing methods, heating degree, and the excipients; however, fresh ginseng is not exposed to any of these factors. FML is one of the series of MRPs from carbonyl and amino compounds, and the processing conditions and the auxiliary materials supplemented to the black ginseng concentrate are more feasible to MR, thus a higher level of FML was obtained. The highest content of FML in black ginseng concentrate showed that even though honey addition to black ginseng concentrate during the production improved its organoleptic properties (flavor and taste), the presence of honey also favored the MR, and therefore decreased nutritional and medical values, basically through lysine losses. In addition, the content of FML in freshly-dried raw ginseng, 1-yr-stored dried raw ginseng, and 1.5-yr-stored-dried raw ginseng was 10.81 g/kg protein, 12.66 g/kg protein, and 14.78 g/kg protein, respectively, which with a relatively small change. Monitoring the content of FML during the processing of red ginseng indicates that the FML generation is slow during the steaming treatment, thereby giving values of 9.63 g/kg protein, 13.35 g/kg protein, and 15.69 g/kg protein after 60 min, 90 min, and 120 min, respectively, at 95°C. However, according to the above results, the main process for the formation of FML was air heating (drying). The FML amount increased markedly during the air heating process, reaching values of 24.16 g/kg protein, 28.16 g/kg protein, and 30.78 g/kg protein after 12 h, 24 h, and 36 h, respectively, at 70°C.

3.2.3. Precisions and recoveries

The FML concentrations of fresh ginseng, raw ginseng, red ginseng, red ginseng liquor, and black ginseng concentrate were obtained with the precision of the peak area calculation method, and relative standard deviation values of the FML content were 1.82%, 3.15%, 2.86%, 2.51%, and 2.66% by six parallel measurements, showing good precision.

To evaluate the accuracy of the method, the recovery of FML was studied by spiking a mixture of standard FML (1–2.5 times of the sample’s concentrate) into the red ginseng sample. According to the results shown in Table 2, the standard deviations for three replicates of each spiked sample of FML in red ginseng were less than 5% and most of them were less than 3%, thus confirming the accuracy of the detection and the absence of matrix effects.

4. Conclusion

The present study investigated the qualitative and quantitative analysis of FML in fresh and processed ginseng, and the results showed that the lowest FML value was observed in the freshly collected ginseng sample, and the highest FML value was found in the black ginseng concentrate. In addition, it was found that the heat treatment and honey addition would increase the FML content. These results show that the content of FML can be a promising indicator to estimate the heat and honey addition level during ginseng processing. Lower temperature and lower level honey addition should be employed to avoid the generation of AGEs during the heating process of ginseng and other high polysaccharides-containing herbal medicines. This research provides useful information for the generation and regulation of potentially harmful MRPs in ginseng processing and also offers a solid theoretical foundation and valuable reference for safe ginseng processing. In addition, the presence of different values of FML in various ginseng products for this study can serve as a reference for the standard doses of the ginseng available doses per day.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

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References

[1] Wan JY, Fan Y, Yu QT, Ge YZ, Yan CP, Aloga RN, Li P, Ma ZH, Qi LW. Integrated evaluation of malonyl ginsenosides, amino acids and polysaccharides in fresh and processed ginseng. J Pharm Biomed Anal 2015;107:89–97.
[2] Paik DJ, Lee CH. Review of cases of patient risk associated with ginseng abuse and misuse. J Ginseng Res 2015;39:89–93.
[3] Jin Y, Kim VJ, Jeon JN, Wang C, Miu JW, Noh HY, Yang DC. Effect of White, Red and Black Ginseng on Physicochemical Properties and Ginsenosides. Plant Foods Hum Nutr 2015;70:141–5.
[4] Lee MH, Lee YC, Kim SS, Hong HD, Kim KT. Quality and antioxidant activity of ginseng seed processed by fermentation strains. J Ginseng Res 2015;39:178–82.
[5] Davidek T, Clety N, Devaud S, Robert F, Blank I. Simultaneous quantitative analysis of maillard reaction precursors and products by high-performance anion exchange chromatography. J Agric Food Chem 2003;51:7259–65.
[6] Somoza V. Five years of research on health risks and benefits of Maillard reaction products: an update. Mol Nutr Food Res 2005;49:663–72.
[7] Du QQ, Liu SY, Xu RF, Li M, Song FK, Liu ZQ. Studies on structures and activities of initial Maillard reaction products by electrospray ionisation mass spectrometry combined with liquid chromatography in processing of red ginseng. Food Chem 2012;135:832–8.
[8] Sell DR. Ageing promotes the increase of early glycation Amadori product as assessed by 1-N-(2-furoylmethyl)-L-lysine (furosine) levels in rodent skin
collagen The relationship to dietary restriction and glycoxidation. Mech Ageing Dev 1997;95:81.
[9] Chung HS, Lee VC, Kyung Rhee Y, Lee SY. Consumer acceptance of ginseng food products. J Food Sci 2011;76:5316–22.
[10] Gökmen V, Serpen A, Açar OC, Morales FJ. Significance of furosine as heat-induced marker in cookies. J Cereal Sci 2008;48:843–7.
[11] Harris CS, Beauleieu LP, Fraser MH, McIntyre KL, Owen PL, Martineau LC, Cuerrier A, Johns T, Haddad PS, Bennett SA. Inhibition of advanced glycation end product formation by medicinal plant extracts correlates with phenolic metabolites and antioxidant activity. Age 2011;77:196.
[12] McEwen JW, McKenna RJ, O’Kane KA, Phillips RR, Johns PW. Effect of carbohydrate DE on blocked lysine and furosine in a liquid nutritional product. Food Chem 2010;119:323–7.
[13] Bosch L, Alegria A, Farré R, Clemente G. Effect of storage conditions on furosine formation in milk–cereal based baby foods. Food Chem 2008;107:1681–6.
[14] Keiko Yamaguchi YN. Determination of furosine and fluorescence as markers of the maillard reaction for the evaluation of meat products during actual cooking conditions. Food Sci Technol Res 2012;18:67.
[15] Villamiel M, del Castillo MD, Corzo N, Olano A. Presence of furosine in honeys. J Sci Food Agri 2001;81:790–3.
[16] García Baños JL, Corzo N, Sanz ML, Olano A. Maltulose and furosine as indicators of quality of pasta products. Food Chem 2004;88:35–8.
[17] Van Renteghem R, De Block. J. Furosine in consumption milk and milk powders. Int Dairy J 1996;6:371–82.
[18] Pahn AA, Pedersen C, Stein HH. Application of the reactive lysine procedure to estimate lysine digestibility in distillers dried grains with solubles fed to growing pigs. J Agric Food Chem 2008;56:9441–6.
[19] Boucher S, Pedersen C, Stein H, Schwab C. Evaluation of the furosine and homoarginine methods for determining reactive lysine in rumen-undergraded protein. J Dairy Sci 2009;92:3951–8.
[20] Juhaimi FA, Ghafoor K, Özcan MM. Physical and chemical properties, antioxidant activity, total phenol and mineral profile of seeds of seven different date fruit (Phoenix dactylifera L.) varieties. Int J Food Sci Nutr 2012;63:84–9.
[21] Bignardi C, Gavazzi A, Corradini C. Determination of furosine in food products by capillary zone electrophoresis-tandem mass spectrometry. Electrophoresis 2012;33:2382–9.
[22] Villamiel M, Arias M, Corzo N, Olano A. Survey of the furosine content in cheeses marketed in Spain. J Food Protect 2000;63:974–5.