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Research article

Remarkable impact of steam temperature on ginsenosides transformation from fresh ginseng to red ginseng

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A B S T R A C T

Background: Temperature is an essential condition in red ginseng processing. The pharmacological activities of red ginseng under different steam temperatures are significantly different.
Methods: In this study, an ultrahigh-performance liquid chromatography quadrupole time-of-flight tandem mass spectrometry was developed to distinguish the red ginseng products that were steamed at high and low temperatures. Multivariate statistical analyses such as principal component analysis and supervised orthogonal partial least squared discrimination analysis were used to determine the influential components of the different samples.
Results: The results showed that different steamed red ginseng samples can be identified, and the characteristic components were 20-gluco-ginsenoside Rf, ginsenoside Re, ginsenoside Rg1, and malonyl-ginsenoside Rb1 in red ginseng steamed at low temperature. Meanwhile, the characteristic components in red ginseng steamed at high temperature were 20*R*-ginsenoside Rs3 and ginsenoside Rs4. Polar ginsenosides were abundant in red ginseng steamed at low temperature, whereas higher levels of less polar ginsenosides were detected in red ginseng steamed at high temperature.
Conclusion: This study marks the first time that differences between red ginseng steamed under different temperatures and their ginsenosides transformation have been observed systematically at the chemistry level. The results suggested that the identified chemical markers can be used to illustrate the transformation of ginsenosides in red ginseng processing.
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1. Introduction

Q3 Ginseng (*Panax ginseng* Meyer) is a famous traditional Chinese herb used for its medicinal properties and as a functional food to maintain balance in the human body in China, Korea, and Japan for more than 1,000 yr [1,2]. Its bioactive ingredients include acidic polysaccharides, ginsenosides, proteins, and phenolic compounds [3–6].

In China, there are three kinds of ginseng according to their growth environment: cultivated ginseng, mountain-cultivated ginseng, and mountain wild ginseng [7]. Cultivated ginseng is often processed into white ginseng, red ginseng, sugared ginseng, and active ginseng. Among these ginseng products, white ginseng and red ginseng are the most widely used in clinical applications because of their considerable pharmacological activity. Red ginseng is often used for "boosting yang" and replenishing vital essence with the "warming effect" [8]. Moreover, red ginseng exhibits more potential anticancer activity than white ginseng likely because of the abundant amount of rare ginsenosides generated from processing, such as ginsenosides Rg3 and Rh2 [9,10]. Traditionally, red ginseng is steamed at 90–100°C for 2–3 h and then dried until moisture qualified. The process condition directly influences the pharmacological activity of red ginseng. Research has revealed that the steaming temperature in red ginseng processing may affect the composition of ginsenosides, and the high steaming temperature could enhance the biological activity of red ginseng [11,12]. Thus, it is necessary to identify the chemical components present in red ginseng processed under different steaming temperatures and the "chemical marker" components between different processed samples to provide the chemical basis for the pharmacological activities.

Typically, ginsenosides are considered the main bioactive components, and they exhibit antioxidant, antiinflammatory,

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anticancer, antiapoptotic, and immune-stimulant pharmacological activities [13–17]. According to different aglycones, ginsenosides can be classified into three types: protopanaxadiol type, such as ginsenoside Rb1, Rc, Rb2, and Rd; protopanaxatriol type, such as ginsenoside Rg1 and Re; and oleanolic acid type, which includes ginsenoside Ro and polyacetyleneginsenoside Ro [18]. Additionally, ginsenosides Rb1, Rb2, Rc, Rd, Re, and Rg1 are the most abundant ginsenosides in *P. ginseng*. The rest, which are the minority in *P. ginseng*, are called rare ginsenosides, and they also have significant pharmacological activities [19]. These ginsenosides can transform from one form to another during steam processing. Malonyl-ginsenosides, which are considered an important form of ginsenosides in fresh ginseng, can demalonylate and transform to their corresponding ginsenosides upon processing. Some ginsenosides can deglycosylate into another ginsenoside; for example, ginsenosides Re, Rg1, Rd can deglycosylate into ginsenosides Rg2, Rh1, Rg3. Some ginsenosides can dehydrate into another ginsenoside; for example, ginsenosides Rg5, Rg6 are the dehydration products from ginsenosides Rg3, Rg2. Other ginsenosides such as Rs1 and Rs2 are the decarboxylization products of malonyl-ginsenoside Rb2 and malonyl-ginsenoside Rc. Moreover, the type of "S" ginsenosides that is the natural form existing in ginseng can transform into the type of "R" ginsenosides during the steaming process.

The ongoing development in ultrahigh-performance liquid chromatography (UPLC) coupled with MS-based metabolomics has the advantages of rapid analysis time, high resolution, selectivity, and sensitive analysis of components in complex medicinal herb mixtures. Recently, UPLC coupled with multivariate statistical analysis (MVA) was used to identify ginseng products, such as the commercial white and red ginseng [8], *P. ginseng* at different ages [20], white ginseng of different origins [21], and white ginseng, commercial red ginseng, and self-manufactured red ginseng [22]. These studies did not discuss the chemical changes of red ginseng processed at different steaming temperatures. However, the different marker components of red ginseng processed under different steaming temperatures have not been discovered. In our study, we developed a sample profiling strategy combining UPLC–quadrupole time-of-flight tandem mass spectrometry (UPLC–QTOF-MS/MS) and MVA as the analytical tools to compare the chemical contents of red ginseng in different steaming temperatures. This method allows us to understand the subtle differences between such ginseng products. At the same time, it can illuminate the transformation of ginsenosides into red ginseng at different steaming temperatures from the chemical components.

2. Materials and methods

2.1. Reagents

Fisher Optima grade acetonitrile, methanol, and isopropanol were purchased from Thermo Fisher Co. (Waltham, MA, USA). Formic acid and leucine enkephaline were purchased from Sigma Aldrich (St. Louis, MO, USA). Deionized water was obtained from our laboratory via a Milli-Q water purification system (Millipore Corporation, Bedford, MA, USA). Ginsenoside Rg1, Re, Rb1, Rf, Rb2, and Rb3 standards were purchased from the National Institute for the Pharmaceutical and Biological Products (Beijing, China). Ginsenoside Rc, Rg2 standards were obtained from Beijing Xiantong era Pharmaceutical Co. Ltd. (Beijing, China). The standards were dissolved in methanol and stored at 4°C.

2.2. Ginseng samples and sample processing

Twenty ginseng samples, which came from 6-yr cultivated ginseng collected from Jingyu county, Jilin province, China, were used. All of these samples were fresh ginseng and then steamed at different temperatures. Red ginseng (HL) was made by steaming fresh ginseng at 100°C for 3 h, drying at 70°C for 12 h, and then drying until the water qualified at 50°C. Red ginseng (HH) was made by steaming fresh ginseng at 120°C for 3 h, drying at 70°C for 12 h, and then drying until the water qualified at 50°C.

All of these processed samples were identified by Professor Xiangri Li (School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing, China) and deposited in the specimen cabinet of traditional Chinese medicine of Beijing University of Chinese Medicine.

2.3. Sample preparation

Fine roots of red ginseng samples processed at different steaming temperatures were pulverized to fine powder and sieved with 65 meshes. Then, accurately weighed powder (0.4 g) from each red ginseng powder was dissolved in 50 mL methanol, filled with plug, weighed, and ultrasonic extracted for 30 min. After cooling to room temperature, the weight loss was replenished with methanol and then filtrated. Then, with precision we drew subsequent filtrate (25 mL) and concentrated it into residue, which was then dissolved in methanol in a 10-mL volumetric flask. The extraction solution was injected into the UPLC system after being filtered through a 0.22-μm filter membrane.

2.4. Steaming model experiment

We performed the red ginseng steaming model experiment using ginsenoside Rb1. In the steaming model experiment, a certain amount of ginsenoside Rb1 was steamed at 100°C and 120°C for 3 h, respectively. After drying at 70°C for 12 h and then drying at 50°C, the residue was then dissolved in methanol in a 5-mL volumetric flask. Then after being filtered through a 0.45-μm filter membrane, this solution was injected into the high-performance liquid chromatography (HPLC) system.

2.5. HPLC and UPLC–QTOF conditions

2.5.1. HPLC conditions

Changes in constituents via the steaming process were carried out using a Waters 2695 high performance liquid chromatograph coupled to a Waters 2489 detector with a C18 reversed phase column (250 mm × 4.6 mm, 5 μm) using the solvent gradient system. The mobile phase consisted of water (Solvent A) and acetonitrile (Solvent B), and the flow rate was 1 mL/min. The column oven temperature was set at 30°C. The gradient elution was used as follows: 0 min, 15% B; 10 min, 40% B; 25 min, 50% B; 38 min, 76% B. The total run time was 38 min, and the sample injection volume was 20 μL.

2.5.2. UPLC conditions

UPLC separation was performed on an ACQUITY UPLC system (Waters Corporation, Milford, MA, USA) with an ACQUITY UPLC BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm). The column oven temperature was set at 40°C, and the flow rate was maintained at 400 μL/min. The mobile phase solvents A and B consisted of water with 0.1% formic acid and acetonitrile, respectively. The UPLC elution conditions were programmed as follows: 0–15 min (95–35% A), 15–18 min (35–0% A), 18–20 min (0–0% A), 20–22 min (0–95% A), 22–25 min (95–95% A). The total run time was 25 min, and the sample injection volume was 2 μL.

2.5.3. MS conditions

MS detection was performed on a quadrupole orthogonal acceleration time-of-flight tandem mass spectrometer (Waters

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