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 malonylginsenoside Rb1 in red ginseng steamed at low temperature. Meanwhile, the characteristic components in red ginseng steamed at high temperature were 20R-ginsenoside R3 and ginsenoside Rg4. 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

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 Rb2 [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, anti-inflammatory, and other health benefits [13–16].
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 Pharmaceutical and Biological Products (Beijing, China). Ginsenoside Rg1, Re, Rb1, Rf, Rb2, Rd, and Rg2 were purchased from Aldrich (St. Louis, MO, USA). Deionized water was obtained from a Milli-Q water purification system (Millipore Corporation, Bedford, 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 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 content was 67%. 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 content was 67%. All of these samples were identified by Professor Xiaori Li (School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing, China) and deposited in the specimen cabinet of the Chinese Academy of Medical Sciences.

2.3 Sample preparation

Fine roots of red ginseng samples processed at different steaming temperatures were pulverized to fine powder and sieved with 50–65 meshes. Each sample was accurately weighed (0.4 g). The sample powder was mixed with plug, weighed, and ultrasonicated for 30 min. After cooling to room temperature, the weight loss was replenished with ethyl alcohol and then filtrated. Then, with precision we drew subsequence 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 steaming 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 chromatography 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 1.7 μm 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 0.5 mL/min. The mobile phase consisted of solvent A (water) and solvent B (acetonitrile). The mass range was 200–2000 m/z. The ESI source was set at a positive ion mode. The electrospray ionization source temperature was 350°C. The cone voltage was 35 V.

2.5.3 MS conditions

MS detection was performed on a quadrupole orthogonal acceleration time-of-flight tandem mass spectrometer (Waters QTOF, Waters Corporation, Milford, MA, USA) operating under the following conditions: capillary voltage, 3500 V; fragmentor, 350 V; source temperature, 120°C; desolvation temperature, 500°C; desolvation gas flow rate, 800 L/h; cone gas flow, 50 L/h; and cone voltage, 35 V. The mass range was 200–2000 m/z. The ESI source was set at a positive ion mode. The electrospray ionization source temperature was 350°C. The cone voltage was 35 V.