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Research article

In situ analysis of chemical components induced by steaming between fresh ginseng, steamed ginseng, and red ginseng

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ABSTRACT

Background: The chemical constituents of *Panax ginseng* are changed by processing methods such as steaming or sun drying. In the present study, the chemical change of *Panax ginseng* induced by steaming was monitored *in situ*.

Methods: Samples were separated from the same ginseng root by incision during the steaming process, for *in situ* monitoring. Sampling was sequentially performed in three stages; FG (fresh ginseng) → SG (steamed ginseng) → RG (red ginseng) and 60 samples were prepared and freeze dried. The samples were then analyzed to determine 43 constituents among three stages of *P. ginseng*.

Results: The results showed that six malonyl-ginsenoside (Rg1, Rb1, Rb3, Rc, Rd, Rb2) and 15 amino acids were decreased in concentration during the steaming process. In contrast, ginsenoside-Rh1, 20(*S*)-Rg2, 20(*S, R*)-Rg3 and Maillard reaction product such as AF (arginine-fructose), AFG (arginine-fructose-glucose), and maltol were newly generated or their concentrations were increased.

Conclusion: This study elucidates the dynamic changes in the chemical components of *P. ginseng* when the steaming process was induced. These results are thought to be helpful for quality control and standardization of herbal drugs using *P. ginseng* and they also provide a scientific basis for pharmacological research of processed ginseng (Red ginseng).

1. Introduction

Korean ginseng (*Panax ginseng* Meyer) has been considered as one of the most valuable medicinal herbs in oriental countries for over 2,000 years and is now widely used as an alternative medicine and health enhancing supplement [1]. Approximately 8,000 tons of ginseng is produced per year and it is consumed all around the world, especially in Asia, [2] because of its renowned pharmacological efficacies such as maintaining homeostasis, enhancing immune-system function, antidiabetic effects, and adjusting blood pressure [3].

In traditional oriental medicines, the processing methods of medicinal herbs play an important role in the application and usage. Generally, the main purpose of processing medicinal herbs is to transform the properties of the plants or their products to increase their pharmacological effects and reduce toxicity or side-effects. The processing methods of medicinal herbs involve special manipulations, such as toasting, steaming, cooking, and fermentation. Ginseng is mostly consumed after various types of processing. Fresh ginseng (nonprocessed ginseng) is rarely used, because it is easily decomposed due to high water content (i.e., 70–80%) and it may coexist with soil microorganisms. The most common types of processed ginseng used are white ginseng (WG) and red ginseng (RG). WG is produced by drying the fresh ginseng in sunlight, and RG is manufactured by steaming the fresh ginseng at 95–100°C for 2–3 h, then drying. Processing conditions have a great influence on the chemical constituents of ginseng, which is the reason for differences among the types of processed ginseng [4]. Therefore, many researchers have studied the chemical change of ginseng and especially constituents such as ginsenosides [5–11], phenolics [12,13], and amino acids [14]. The research groups are always interested in the biological activity of ginseng and ginsenosides which have been generated during processing [15–18]. Recently, chemometric tools, called “metabolomics” have been applied for metabolite profiling and to identify the complicated constituents of

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steaming-induced components and different types of ginseng [19,20].

Although several studies have reported the chemical change of fresh and processed ginseng, there is currently limited sample preparation. In this report, *in situ* monitoring of chemical changes induced by steaming was performed. For the *in situ* analysis of chemical components in ginseng, samples were obtained from the same ginseng root after processing steps. Forty three components (ginsenosides, amino acids, free sugars, and some Maillard reaction products) were determined using various chromatographic techniques, such as ultra performance liquid chromatography photo diode array detector (UPLC-PDA), high performance liquid chromatography (HPLC) fluorescence detector, and high pressure ion chromatography pulsed amperometric detector.

2. Materials and methods

All the reagents used in this experiment were of extra pure grade. HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). All distilled water used in this experiment was purified by the Milli-Q gradient system (Millipore, Bedford, MA, USA) and the resistance value was measured as 18 MΩ prior to use.

2.1. Ginseng sample preparation

Ginseng samples (6-year-old aged *P. ginseng*) used in this experiment were obtained from the red ginseng manufacturing factory of Korea Ginseng Corporation (Buyeo, Chung-nam, Korea) as follows. First, each ginseng root sample was given a serial number to distinguish one from another. Before the steaming process, the washed fresh ginseng (FG) was given a longitudinal incision and one-third of the portion was separated and frozen at −80°C. The remaining two-thirds were steamed in a closed chamber at 98°C for 3 h. Immediately after steaming, the steamed ginseng (SG) was sliced in half and one portion was separated and kept frozen at −80°C. Finally, the remaining one-third piece of ginseng was dried in a chamber (65°C, 3 h) under daylight (for 13 d) to make RG. Frozen FG and SG samples were freeze-dried (Bondiro, PVTF010A, Ilshin Lab, Seoul, Korea) and RG was further dried in a dry-oven (60°C, 2 h; WiseVan, VS1202-D3, Daihan scientific, Seoul, Korea) and all samples were grinded to fine powder and stored at −20°C until used for analysis.

2.2. Analysis of ginsenosides

Ginsenoside Rg1, Re, Rf, Rh1, Rb1, Rc, Rb2, Rb3, Rd, 20(*S*)-Rg3, and 20(*R*)-Rg3 standards were purchased from Chromadex (Irvine, CA, USA) and ginsenoside 20(*S*)-Rg2, 20(*R*)-Rg2 were obtained from Ambo Institute (Seoul, Korea).

The sample was prepared in a similar manner as in our previous studies [21]. A half gram of ginseng powder was weighed in a centrifugal tube (15 mL, polypropylene single use; BioLogix Group, Jinan, Shandong, China) and shaken vigorously after the addition of 10 mL of 70% MeOH. Extraction was performed in an ultrasonic cleaner (60 Hz, Wiseclean; Daihan Scientific, Seoul, Korea) for 30 min. After ultrasonic extraction, centrifugal separation (Legand Mach 1.6R; Thermo, Frankfurt, Germany) was performed for 10 min at 3,000 rpm. The resulting supernatant solution was filtered (0.2 μm, Acrodisk; Gelman Sciences, Ann Arbor, MI, USA) and injected into the UPLC-PDA system (Waters Co., Milford, MA, USA).

Malonyl (ma)-ginsenosides were analyzed by an indirect base-hydrolysis method as reported [22]. Acidic ginsenosides were hydrolyzed by adding 80 μL of 5% KOH to a portion (850 μL) of the ginseng extract. After 2 h, the solution was neutralized by adding 80 μL of 0.01 M KH_2PO_4 solution. The mixture was diluted with 850 μL of acetonitrile and injected into the UPLC-PDA system (Waters Co.).

The instrumental conditions of UPLC-PDA were as follows. The chromatographic separation was obtained by using an ACQUITY BEH C18 column (50 mm × 2.1 mm, 1.7 μm; Waters Co.) at 40°C. The binary gradient elution system consisted of: (A) 0.01 M KH_2PO_4 in water; and (B) acetonitrile. The separation was achieved using the following protocol: 0–0.5 min (15% B); 14.5 min (30% B); 15.5 min (32% B); 16.5 min (40% B); 17.0 min (55% B); 21.0 min (90% B); 25.0–27.0 min (15% B). The flow rate was set at 0.6 mL/min and the sample injection volume was 2.0 μL. The ginsenosides were determined at a UV wavelength of 203 nm using a photo diode array detector (Waters Co.).

2.3. Extraction of water soluble components

Sugar, amino-sugar, and maltol were extracted as follows. A 100 mg sample of ginseng powder was weighed in a centrifugal tube (15 mL, polypropylene-single use; BioLogix Group) and shaken vigorously after the addition of 10 mL of deionized water. Extraction was performed in an ultrasonic cleaner (60 Hz, Wiseclean) for 30 min. After ultrasonic extraction, centrifugal separation (Legand Mach 1.6R; Thermo) was performed for 10 min at 3000 rpm. The resulting supernatant solution was filtered (0.2 μm, Acrodisk; Gelman Sciences) and this filtrate was used as analytical solution for sugar, amino-sugar, amino acid, and maltol.

2.4. Analysis of sugar and amino-sugar

Glucose, fructose, maltose, and sucrose standard materials were purchased from Sigma-Aldrich (SUPELCO, Bellefonte, PA, USA). Arginyl-fructose (AF), arginyl-fructose-glucose (AFG) standard materials were obtained from Ambo Institute.

The sample solution was prepared by 10× dilution of water-soluble extraction filtrate. Chromatographic determinations were performed according to Joo et al [23] with some modifications. These components were determined using ICS-3000 high pressure ion chromatography and a pulsed amperometric detector with Au working electrode and Ag/AgCl reference electrode (Dionex, Sunnyvale, CA, USA). The chromatographic separation was obtained using a CarboPac PA-10 column (250 mm × 4 mm; Dionex, Sunnyvale, CA, USA) at 30°C. The gradient elution system consisted of: (A) 250 mM NaOH; and (B) water. The separation was achieved using the following protocol: 0–20 min (93% B); 30–35 min (50% B); 36–45 min (0% B); 46–60 min (93% B). The flow rate was set at 1.0 mL/min and the sample injection volume was 5.0 μL.

2.5. Analysis of amino acids

Simultaneous determination of 17 kinds of amino acids was performed using the AccQ·Fluor reagent kit (Waters Co.) and modified appropriately for the application of ginseng samples.

In this experiment the precolumn, derivatization method was used. Firstly 10 μL of 10× diluted filtrate was mixed with 70 μL AccQ·Fluor derivatization buffer and immediately mixed. Then 20 μL of AccQ·Fluor reagent was added to this solution and vortexed for 5 min. It was then allowed to stand for 2 min at room temperature, transferred to an auto-sampler vial and heated at 55°C for 10 min in a preheated heating block (HB-48, Wisetherm; Daihan Scientific).

The instrumental conditions of HPLC (model 2695; Waters Co.) were as follows. The chromatographic separation was obtained using a Discovery C18 column (250 mm × 4.6 mm, 5 μm,

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