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Effect of processing methods on the concentrations of bioactive components of ginseng (*Panax ginseng* C.A. Meyer) adventitious roots

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Abstract

Adventitious roots of Korean mountain ginseng (*Panax ginseng* C.A. Meyer) were processed by using forced air drying methodology at 30, 50 and 70 °C for 1, 3, 5, 10 and 20 h with objective of developing suitable processing/drying technique. Drying of adventitious roots at 50 °C for 10 h was found suitable as desirable moisture content (3.13 g water/dry matter i.e. 10%) could be reached with dried roots. Roots which were dried by such treatment were also possessing higher amount of ginsenosides (1.5 mg g^{-1} DW triols, 15.9 mg g^{-1} DW diols and 17.4 mg g^{-1} DW of total ginsenosides) and phenolics. Adventitious roots were also processed by using far infrared and freeze drying methods and results revealed that forced air drying method is superior to far infrared and freeze drying methods.

Keywords: Adventitious roots; Forced air drying; Far infrared drying; Freeze drying; Ginseng; Ginsenosides

1. Introduction

Ginseng (*Panax ginseng* C.A. Meyer) is one of the important nutraceutical and medicinal plants, which is used worldwide. The ginseng products are increasingly popular as health food around the world (Li, 1995). The principal active components of ginseng are triterpenoid saponins known as ginsenosides. Ginsenosides are attributed with cardio-protective, immunostimulatory, anti-fatigue and hepato-protective physiological and pharmacological effects (Wu & Zhong, 1999). Ginsenosides are divided into three groups based on their structure, i.e., the Rb group (protopanaxadiol including Rb1, Rb2, Rc, Rd and others), and Rg group (protopanaxatriols including Rg1, Rg2, Re, Rf and others) and the Ro group (oleanolic acid). The other most important bioactive components of ginseng are biophenols and polysaccharides. Plant organ cultures have become alternatives to whole plant for the production of valuable bioactive compounds. Adventitious roots of Korean mountain ginseng were cultivated in large-scale bioreactor for the production of biomass which can meet out the demand of pharmaceutical, nutraceutical and herbal industry (Choi et al., 2000).

Processing (drying) is one of most important step in the production and commercialization of adventitious roots. Adventitious roots (with approximately 90% moisture content, wet basis) have to be dried to low moisture content (5–10%) so that raw material can be stored and used in various health and pharmaceutical products. Currently, drying methodology is available for natural roots and commercial drying practice is to use forced air at low temperature to achieve the quality product (Davidson, Li, & Brown, 2004a, 2004b). Several researchers have studied the drying conditions of field cultivated roots of American ginseng and reported the degradation of quality attributes, such as color, ginsenosides levels and texture (Li & Morey, 1987; Sokhansanj, Bailey, & van Dalfsen, 1999) when roots are dried above 40 °C, whereas researchers in China and Korea have used relatively high air temperatures (up to

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55 °C) with Asian ginseng to reduce drying time and indicated that the dried quality is satisfactory. Researchers have also investigated the efficient extraction methods of active ingredients from natural roots as well as cultured cells of ginseng (Du, Wills, & Stuart, 2004; Vongsangnak, Gua, Chauvatcharin, & Zhong, 2004). No such work has been done with the adventitious roots of ginseng. Therefore, the major objective of the present study was to develop suitable technique/s for processing/drying of adventitious roots of Korean mountain ginseng without the deterioration of bioactive components of roots. We have used forced air drying method for drying of adventitious roots of ginseng at different temperature levels. We also evaluated the far infrared radiation drying and freeze drying methods for drying the adventitious roots of ginseng.

2. Materials and methods

2.1. Adventitious root samples and drying experiments

Adventitious roots of Korean mountain ginseng (*P. ginseng* C.A. Meyer) were cultivated in suspension cultures (5 l capacity airlift bioreactors) by following the method of Yu, Gao, Hahn, and Paek (2002). Fresh roots were harvested and were dried in forced air drying unit (Model 36050-10, Cole-Parmer International, USA) at different temperatures including 30, 50 and 70 °C for 1, 3, 5, 10 and 20 h. Another set of samples was freeze dried by using freeze drying machine (FreeZone 12 Liter Console, Labconco Corporation, USA) at −70 °C for 30 min, 1, 3, 5, 10 and 20 h. One more set of samples was dried in far infrared radiation drying unit (Clean Vac8; Hanil, Korea) at 50 °C (inside the drying chamber) for 30 min, 1, 3, 5, 10 and 20 h and the outlet air velocity of 0.8 m s^{-1} was used for the experiment. Moisture contents were determined in samples removed after selected periods in all three sets of experiments. Moisture content was calculated on the dry basis as the ratio of moisture content at a particular time relative to the moisture content at the beginning of the drying process. The average initial moisture content was calculated as a weighted average that reflected the composition of the batch. The dried samples were used for the estimation of ginsenosides, total phenolics and the radical scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH).

2.2. Determination of total phenolic contents

The content of total phenolics were analyzed by the Folin–Ciocalteu colorimetric method (Folin & Ciocalteu, 1927) using gallic acid as a standard (Yu, Perret, Harris, Wilson, & Haley, 2003). Dried root samples were powdered (1 g), extracted with 100 ml ethanol (60 ml ethanol:40 ml water) for 30 min at 60 °C, and filtered through filter paper (Advantec, Toyo, Japan). Each reaction mixture contained 100 μl sample solutions, 6 ml distilled-deionzed water, 500 μl Folin–Ciocalteu reagent (Sigma and Aldrich, St. Louis, MO) and 1.5 ml of Na_2CO_3 (20 g/100 ml). The reagent blank was performed by replacing the gallic acid solution with 100 μl of acetone–water (1:1, v/v). After 2 h of reaction at ambient temperature, the absorbance of each reaction mixture was measured at 765 nm. The total phenolics were expressed as milligram gallic acid equivalents per gram of dry mass of adventitious roots.

2.3. Determination of ginsenoside content

Extraction and analysis of ginsenosides were carried out by the method of Yu et al. (2002). The ginsenoside fraction was analyzed using HPLC system (Shimadzu, Kyoto) consisting of 10AT pump, 10AXL autosampler, SPDM10A photodiode array detector, and CTO-10A column oven, 5 μM Licrhrosorb column ($250 \times 4.6\,mm^2$) (Altech, Deerfild, IL), and a C18 guard column, at 40 °C. The eluted peaks were detected at 203 nm and quantified against external standards of the ginsenosides Rf, Rb2, Rd (Karl Roth, Germany), Re, Rg1, Rg2, Rh1, Rh2, Rb1, Rb3, Rc and Rg3 (Wako, Osaka, Japan). The mobile phase was a gradient elution of water and acetonitrile (B), commencing with 20% B, rising to 22% B after 20 min then to 46% B after 45 min and 55% B after 50 min.

2.4. Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

The DPPH radical has been widely used to evaluate the free radical scavenging activity of natural anti-oxidants (Brand-Williams, Cuvelier, & Berset, 1995). Methanolic aliquots were mixed with 300 μl of 1 mM solution of DPPH* (Sigma and Aldrich, St. Louis, MO; prepared freshly each day and stored in the dark at 4 °C in a flask covered with aluminum foil) in 4 ml cuvettes and brought to a total volume of 3.0 ml with methanol. After incubation in the dark at room temperature for 15 min, the reaction mixture was assayed at 517 nm using a UV–vis spectrophotometer (UV-1650PC, Shimudzu, Japan). In order to eliminate interference with the DPPH* reaction by extracted pigments, blanks of the extracts were assayed using 300 μl of methanol instead of the DPPH* solution. A DPPH* blank sample (containing 2.7 ml of methanol and 300 ml of DPPH* solution) was prepared and assayed daily. All experiments were carried out in duplicate and repeated at least twice.

The percentage decrease in absorbance at 517 nm was recorded for each concentration and the percentage of quenching of the DPPH* radical was calculated on the basis of the observed decrease in absorbance of the radical. The inhibition percentage was calculated according to the formula:

$$\text{Inhibition percentage} = \left[(A_{DPPH} - A_{Extr})/A_{DPPH})\right] \times 100,$$

where A_{DPPH} is the absorbance value of the DPPH* blank sample and A_{Extr} was evaluated as the difference between