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Far infrared irradiation alters total polyphenol, total flavonoid, antioxidant property and quercetin production in tartary buckwheat sprout powder

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ABSTRACT

The effects of far infrared irradiation (FIR) on total polyphenol (TP), total flavonoid (TF) content, antioxidant properties and aglycone quercetin production in tartary buckwheat sprout (TBS) were investigated. The study showed that FIR treatment decreased the total antioxidant capacity and metal chelating property in TBS in a temperature dependent manner, however, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity increased with the temperature. Similarly, TP and TF content also increased with temperature. The HPLC result revealed that quercetin production was directly proportional to the temperature, and the maximum production (average 14.8 mg/g dw) of quercetin was at 120 °C which was 13.5 times higher than the untreated control sample of TBS. Overall, this research is expected to be helpful to improve the nutritional value of tartary buckwheat by optimizing the FIR conditions.

1. Introduction

Buckwheat (*Fagopyrum* spp.) is an annual crop belonging to the Polygonaceae family and cultivated in most Asian and European countries. Among the different varieties, common (*Fagopyrum esculentum*) and tartary (*Fagopyrum tartaricum*) buckwheat are the most popular varieties, which are recognized as healthy foods because of high rutin content (Mukoda et al., 2001). Nowadays, many people take interest in the utilization of buckwheat seed because of its high nutritional and pharmaceutical values. Especially, tartary buckwheat has been drawing attention for its high rutin content, which is beneficial to health. Similarly, quercetin is a flavonoid present in a glycoside or an aglycone form in most fruits and vegetables including buckwheat. It has been reported that the quercetin exhibits more potent biological activity than its corresponding glycosides (Kwon et al., 2004; Hou et al., 2004; Williamson et al., 1996). Therefore, due to the greater biological activity, safe and efficient production of quercetin, without using hazardous chemicals or expensive enzymes is crucial from a health and economic point of view.

Since a decade ago, the buckwheat sprout has been considered as a new vegetable. The sprout has mild flavor and attractive odor, so is used as fresh vegetable, salad, cooking ingredient, etc. (Kim and Kim, 2001). The sprouts are rich in flavonoid compounds (rutin and quercetin), phenolic acids, amino acids, minerals, vitamins and crude fibers (Kim et al., 2004). The powdered form of dried buckwheat sprout is used to make bread, snacks, and also added to make rice cake (Kim et al.; Kim and Kim, 2001).

Thermal processing changes the physical, chemical and biological properties of fruits and vegetables by altering carbohydrates, fats, vitamins, proteins, etc. to different extents (Hoyem and Kvale, 1977). Nowadays, several reports have shown that the far infrared (FIR) drying method is more advantageous over the conventional oven drying method (Eom et al., 2009; Niwa et al., 1988). The high penetration power (wave length 3–1000 nm) of FIR helps the exudation of chemical components without destroying the plant cells and thereby altering biological activity. Recently, some studies have been done on how buckwheat phenolics or antioxidant activity are affected by different processing methods (Sensoy et al., 2006; Sun and Ho, 2005; Zielinski et al., 2006). However, the

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use of FIR as a thermal treatment on buckwheat has not been studied yet.

The objective of this research was to study the alteration of total polyphenol, total flavonoid content and antioxidant properties of tartary buckwheat sprout (TBS), and also to monitor the maximum quercetin production in TBS resulting from FIR thermal treatment.

2. Materials and methods

2.1. Chemicals

Analytical grade organic solvents (methanol, acetonitrile, ethanol) used for the extraction of TBS and detection in HPLC were purchased from Merck KGaA Darmstadt, Germany. Gallic acid (GA), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, Mo). Folin-Ciocalteu's reagent was purchased from Wako Pure Chemicals, Japan. Rutin and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals and reagents used were of the highest commercially available purity.

2.2. Preparation of TBS extracts

Seven days old tartary buckwheat sprouts (TBS) were harvested, dried at oven temperature of 50 °C and powdered using a grinder. Two grams of powder were mixed with 4 ml H_20 in a glass petri dish (100 × 20 mm) and exposed to far infrared irradiation (FIR) at different temperatures (80, 100, 120, 140, 160 °C) for 1 h each. Further, the FIR treated powdered sprout samples were suspended in 200 ml of 80% ethanol and kept over-night in a shaker at room temperature. The extracts were filtered through Advantec 5B filter paper (Tokyo Roshi Kaisha ltd, Japan) and dried using a vacuum rotatory evaporator (EYLA N-1000, Tokyo, Japan) in a 40 °C water bath. Dried samples were weighed and kept at 4 °C for further analysis.

2.3. Estimation of total polyphenol and total flavonoid content

Total polyphenol (TP) content of FIR treated and untreated TBS samples were determined by the Folin-Ciocalteu assay. In brief, a sample aliquot of 1 ml of extract (1 mg/ml) was added to a test tube containing 0.2 ml of phenol reagent (1 M). The volume was increased by adding 1.8 ml of deionized water and the solution was vortexed and left for 3 min for reaction. Furthermore, 0.4 ml of Na_2CO_3 (10% in water, v/v) was added and the final volume (4 ml) was adjusted by adding 0.6 ml of deionized water. A reagent blank was prepared using deionized water. The absorbance was measured at 725 nm after incubation for 1 h at room temperature. The TP was calculated from a calibration curve ($R^2 = 0.999$) using gallic acid as a standard and expressed as mg of gallic acid equivalent (GAE) per g dry weight (dw).

Total flavonoid (TF) content was determined using the protocol of Eom et al. (2008). Briefly, an aliquot of 1 ml of the extract (1 mg/ml) was mixed with 0.1 ml of aluminum nitrate (10%) and 0.1 ml of potassium acetate (1 M). To the mixture, 2.8 ml of methanol (80%) was added to make a total volume of 4 ml. The mixture was vortexed and absorbance was measured after 40 min at 415 nm using a spectrophotometer (Hitachi U-2001, Japan). The TF was calculated from a calibration curve ($R^2 = 0.999$) using rutin and expressed as mg of rutin equivalent (RE) per g of dw.

2.4. DPPH radical scavenging activity

The antioxidant activity of the treated samples was determined on the basis of the scavenging activity of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical according to the method described by Braca et al. (2003) with slight modifications. Briefly, 1 ml of the extract at different concentrations (0.125 and 0.250 mg/ml) was added to 3 ml DPPH solution (0.15 mM). The mixtures were shaken vigorously and left to stand at room temperature in the dark for 30 min. Absorbance was measured at 517 nm using a spectrophotometer and the scavenging activity of extracts was calculated against a blank.

$$\text{Radical scavenging activity } (\%) = (A_0 - A_1)/A_0 \times 100$$

where, A_0 and A_1 were the absorbance of the control and the test sample, respectively.

2.5. Metal chelating assay and estimation of total antioxidant capacity

The TBS samples were analyzed for the metal chelating activity according to the procedure of Dinis et al. (1994) with slight modification. Briefly, 0.5 ml of sample extracts at different concentrations (1.0 and 0.5 mg/ml) was mixed with 0.1 ml of 1 mM $FeCl_2$ followed by the addition of 0.2 ml of 5 mM ferrozine. The mixtures were vortexed and kept at room temperature for 10 min. For a blank, the sample extracts were replaced with 80% ethanol. Finally, before measuring the absorbance with a spectrophotometer at 532 nm, the total volume was made up to 4 ml with the addition of 3.2 ml of 80% ethanol. The ability of extracts to chelate ferrous ions was calculated as follows:

$$\text{Chelating effect } (\%) = \left(1 - A_{\text{sample}}/A_{\text{control}}\right) \times 100$$

Total antioxidant activity was determined according to the protocol of Prieto et al. (1999) with minor modification. Briefly, extracts (1 mg/ml) were added to a tube containing 3 ml of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) making a total volume of 4 ml. The tubes were covered with aluminum foil, incubated at 95 °C for 90 min, cooled down, and the absorbance was measured at 695 nm against a blank. Higher absorbance of the reaction mixture indicated greater antioxidant capacity of the sample.

2.6. HPLC quantification of quercetin and rutin

To quantify quercetin and rutin content in the FIR treated TBS extracts, a high-performance liquid chromatography (HPLC) system (CBM-20A; Shimadzu Co, Ltd., Kyoto, Japan) with two gradient pump systems (LC-20AT; Shimadzu, Japan), an auto sample injector (SIL-20A; Shimadzu), a UV-detector (SPD-10A; Shimadzu) and a column oven (CTO-20A; Shimadzu) were used. The separation was performed on a C_{18} column (Synergi 4 μ MAX-RY, 150 × 4.6 mm, 4 micron Phenomenex. Inc., Torrance, CA, USA). HPLC conditions were as follows: Solvent A (water in 0.1% Trifluoroacetic acid) and solvent B (acetonitrile) was prepared. Gradient elution used was 0–10 min, 5–6% B; 10–15 min, 6–10% B; 15–45 min, 10–19% B; 45–65 min, 19–20% B. The flow rate of mobile phase solution was 1.0 ml/min, and detection was carried out at 355 nm. Ten microliter of each sample was injected. Quercetin and rutin were used as an external standard to monitor quercetin production.

2.7. Statistical analysis

All data were expressed as the mean value ± standard deviation (SD) of each experimental group ($n = 3$). Differences in the mean values among multiple groups were analyzed by one-way analysis