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Effect of Freeze-Drying and Hot Air-Convection Drying on the Antioxidative Activity of Butterbur (*Petasites japonicus*)

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ABSTRACT The principal objective of this study was to assess the antioxidative activities of *Petasites japonicus* against oxidative stress in bovine brain tissue. *Petasites japonicus* is found with a relatively widespread distribution, and is cultivated as a culinary vegetable in Korea. *Petasites japonicus* samples were dried either by freeze-drying or by hot air-convection drying (80°C), then evaluated for their antioxidative activity by measuring l-dipheny-l,2-picrylhydrazyl (DPPH) radical scavenging, and by measuring thiobarbituric acid-reactive substances (TBARS) in brain homogenates subjected to Fe^{2+}-mediated lipids with or without the addition of botanical extract. Hot air convection-drying resulted in a slight increase in the extraction yield as compared with freeze-drying. However, total phenol and flavonoid contents in freeze-dried *Petasites japonicas* were significantly higher than those of hot air convection-drying. Freeze-drying increased the free radical scavenging activity of *Petasites japonicas*, leaves, and stems by 52.6, 28.6, and 248.0%, as compared with hot air convection-drying. Additionally, the IC_{50} values measured by TBARS in hot air convection-dried *Petasites japonicas*, leaves, and stems were increased by 36.0, 31.6, and 15.9%, as compared to those of freeze-drying. Although antioxidative activity was reduced slightly by heat processing in *Petasites japonicas*, freeze-drying for each portion of *Petasites japonicus* was the most appropriate for use as a functional food and pharmaceutical material.

KEYWORDS: antioxidative activity, flavonoids, free radical scavenging activity, petasites japonicas, phenol content

INTRODUCTION

The consumption of natural products with potential health benefits has grown continuously at a rate of 5-10% per year, and more research has emphasized the discovery of novel bioactive compounds (Briskin 2000; Kris-Etherton et al 2002). Among a variety of sources, plants are regarded as crucial sources of bioactive compounds such as phenolics, with a variety of biological activities such as antioxidant, anticancer, antimicrobial, and anti-inflammatory effects--by neutralizing the negative effects of reactive oxygen species (ROS). The importance of phenolics, and flavonoids in particular, is attributable to their ability to function as efficient free radical scavengers (Havsteen 2002; Tsao and Deng 2004; Vinson et al 2001). In general, synthetic antioxidants including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone (TBHQ), have been widely utilized in food; however, some adverse effects have been reported (Russo 2007). Therefore, an increasing number of investigations of new natural sources of antioxidants are being conducted (Larson 1988; Wanasundara and Shahidi 1994).

Butterbur (*Petasites japonicas*), a perennial plant grown extensively in Korea and Japan, is utilized as an edible vegetable. Recently, several investigators reported that petasinophenol and flavonoid glycosides, isolated from *Petasites japonicas*, inhibited eukaryotic DNA polymerase rhamda and DNA polymerase alpha, respectively (Mizushina et al 2002; Mizushina et al 2003). In the author's previous study, it was demonstrated that in an extract of leaves of *Petasites japonicas*, the butanol-soluble fraction evidenced a remarkable antioxidative action in a DPPH radical scavenging assay (Min et al 2005). It is known that processing and cooking induces significant changes in chemical composition, influencing the concentration and bioavailability of bioactive compounds in edible vegetables. However, both positive and negative effects have been reported depending upon differences in process conditions and nutritional characteristics of vegetable species (Bernhardt and Schlich 2006; Gliszczyńska-Swigło et al 2006; Nicoli et al 1999). *Petasites japonicas* is a member of a group of vegetables

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that are generally heat-treated prior to eating; thus it is crucial to know which type of drying and processing method besides blanching or steaming is optimal for widely usage as a functional food materials and the preservation of health-promoting compounds present in different portions of this vegetable. The effects of processing on the antioxidative activities of some edible plants, such as red pepper seed (Sim and Han 2008), sweet potato (Lee et al 1999), dropwort (Choi et al 1992), and lettuce (Kim et al 2007) were reported, however, no investigation on the effects of heating treatment on the antioxidative activity of *Petasites japonicas* by different portions is available, although most vegetables are typically consumed after cooking. Therefore, the primary objective of this study was to evaluate the effects of heating treatment on the antioxidative activities of *Petasites japonicas*, leaves, and stems among edible plants possessing potent antioxidative activity in Korea.

MATERIALS AND METHODS

Materials

1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, α-tocopherol, thiobarbituric acid (TBA), trichloroacetic acid (TCA), Folin-Ciocalteu's phenol reagent, caffeic acid, tannic acid and 1,1,3,3-tetraethoxypropane were purchased from the Sigma Chemical Company (MO, USA). Trolox from Acros Organics (NJ, USA), ethanol, acetic acid, methanol and hydrochloric acid from Merck (Darmstadt, Germany), sodium hydroxide, and ferric sulfate [$Fe(III)_2(SO_4)_3$] from Aldrich Chemical Company (MO, USA) were used.

Petasites japonicas samples

Petasites japonicas were collected from agricultural fields in Kongju, Korea in May, 2004.

Petasites japonicas were separated into three portions -- whole (leaf and stem), leaf, and stem -- and then washed, drained, weighed, and dried. *Petasites japonicas* was processed by freeze-drying (−70°C) and hot air-convection drying (80°C). The dried samples were ground into a powder to pass through a 200 mesh sieve. The powdered samples (0.3 g) were immersed in absolute methanol (10 mL) and stored in darkness (15°C) for 3 days, after which the solvent fraction was collected. The extraction was repeated three times and the solvents were removed using a rotary evaporator. After rotary evaporation, the residue was dissolved in solvent, and the solvent fractions were assayed for antioxidative activity.

Determination of total phenolics

The total phenolic contents of *Petasites japonicas* were determined via the Folin-Ciocalteu colorimetric method (Singleton and Rossi 1965). One milliliter of *Petasites japonicas* extract was mixed with 0.5 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22°C for 5 min; 1.0 mL of sodium bicarbonate (60 g/L) solution was added to the mixture. After 30 min at 22°C, absorbance was measured at 760 nM. The results were expressed as milligrams of tannic acid equivalent per gram of the sample.

Flavonoid content

The total flavonoid contents of the *Petasites japonicas* extracts were determined via the David deformed method (KFN 2000). The *Petasites japonicas* extracts (1.0 mL) were mixed with 10 mL of diethylene glycol and 0.1 mL of 1 N NaOH. The mixtures were thoroughly shaken and permitted to stand for 1 hr at 37°C. The absorbance was then determined at 420 nm. The flavonoid contents were acquired using a standard curve obtained from various concentrations of rutin (RE).

Preparation of bovine brain homogenate

The bovine brain was obtained from a slaughterhouse in Daejeon. The brain was washed in cooled 0.15 M NaCl, kept on ice, and subsequently blotted on filter paper. The brain tissue was then homogenized for 2 min in a glass-teflon homogenizer with an equal volume of cold 10 mM phosphate buffer. The homogenates were used to analyze the contents of thiobarbituric acid- reacting substances (TBARS).

Antioxidative activities of *Petasites japonicas*

The antioxidative activities of *Petasites japonicas* were measured via a DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay, the TBA (thiobarbituric) method, and a Folin-Ciocalteu assay, according to the method described in our previous study (Zao et al 2004). In brief, samples (0~1 mg/mL) in 4 mL methanol were added to a solution of DPPH (10 mM, 1 mL) in methanol. The mixture was shaken and allowed to stand for 10 min at room temperature; the absorbance of the resultant solution was spectrophotometrically measured at 517 nm. The percentage of scavenging effect (%) was then calculated as follows:

Scavenging effect % (capacity to scavenge the DPPH radical) =[(1−absorbance of sample)/absorbance of control]×100

α-Tocopherol, ascorbic acid and trolox were used as positive controls. The IC_{50} values are reflective of the concentration of *Petasites japonicas* extract necessary to exert a 50% free radical scavenging effect.

Fe^{2+}-mediated lipid peroxidation in the bovine brain was induced with 0.2 mM Fe^{2+} and 25 μM ascorbic acid *in vitro*, as previously described by Lee (1999). The extent of lipid peroxidation was assayed in terms of TBARS contents, according to the method described by Bidlack and Tapple (1973). Brain homogenates containing Fe^{2+} and ascorbic acid, with or without plant extract, were placed in a shaking water bath for 30 min at 37°C; equal volumes of 15%

trichloroacetic acid (TCA) and 0.75% thiobarbituric acid (TBA) were then added. The reaction mixtures were heated for 15 min in boiling water, kept on ice for 5 min, and then centrifuged for 10 min at 3,000 rpm to separate corpuscolate particles. The absorbance of the supernatant was read at 533 nm with a spectrometer (Model 80-2088-64, Pharmacia Biotech. Co., Cambridge, England). Calibration was conducted using a malondialdehyde standard prepared by the hydrolysis of 1,1,3,3,-tetraethoxypropane. The antioxidative activity was expressed as the percentage reduction of TBARS relative to the control using the following equation.

$$\text{Antioxidative activity (\%)}=[1-(A_4-A_2)/(A_3-A_1)]\times 100$$

in which the sample was comprised of *Petasites japonicas* extracts, A_1 is the absorbance from the incubation containing brain homogenate only, A_2 is the absorbance from the incubation containing the brain homogenate and sample, A_3 is the absorbance from the incubation containing brain homogenate, ascorbate, and Fe^{2+}, A_4 is the absorbance from the incubation containing brain homogenate, ascorbate, Fe^{2+}, and sample.

Statistical analysis

The results of the treatments were expressed as the means ±standard deviations (SD). The experiments for extraction yield, total phenol and flavonoid contents were run in triplicate. The data were analyzed with SPSS (Statistical Analysis Program, version 12.0) in order to determine the effects of the *Petasites japonicas* extracts on the antioxidative activity. The differences between means were evaluated by the Student's *t*-test, and the statistical significance was defined at $p<0.001$.

RESULTS AND DISCUSSION

Extraction yield of *Petasites japonicas* by different drying condition

Table 1 shows the effects of heating on the extraction yield of *Petasites japonicas* by different portions, such as leaves and stems. Freeze-drying reduced the extraction yield of whole *Petasites japonicus* by 30.8%, as compared with hot air convection-drying. Additionally, the extraction yield in freeze-dried *Petasites japonicus* leaves and stems were decreased significantly, by 51.5% and 33.0% as compared with the hot air convection-dried samples. Also, these extraction yields were higher than those of previous study that is, the total yield of water extracts of 28 functional herb was in the range of 5.33~36.71% (Lee et al 2007).

Total phenol contents of *Petasites japonicus* processed with different drying condition

Phenolic compounds are secondary plant metabolites found in several plants. Antioxidative phenolic compounds show promise as health-promoting phytochemicals, as they have been demonstrated to evidence beneficial antimutagenic, anticarcinogenic, cholesterol-lowering, and antimicrobial properties (Im et al 2008; Mattila and Hellström 2007). Table 2 presents the total phenol contents of the different drying-pretreated whole *Petasites japonicus*, leaves, and stems. The total phenol content in freeze-dried *Petasites japonicus* for the whole sample, *Petasites japonicus* leaves, and *Petasites japonicus* stems were increased markedly, by 1.7 times, 1.6 times, and 7.7 times as compared to hot air convection-drying. These significant losses in hot air convection-drying could be attributed to the breakdown of phenolics during thermal processing. Consistent with our results, it has been reported that thermal treatment reduced the total phenolic contents, antioxidative, and free radical scavenging activities of certain leafy vegetables. In particular, the leaves of *C. sativum* were found to contain sizeable quantities of caffeic acid, ferulic acid, gallic acid, and chlorogenic acid--all of which evidence potent antioxidative activity (Bajpai et al 2005). Im et al (2008) previously reported that the total phenolic acid contents of potato plant flowers were 21 and 59 times as high as those seen in the leaves and stems. In a previous study, it was reported that heat processing reduced the total phenolic contents in certain vegetables, such as kale, spinach, cabbage, swamp

Table 1. Extraction yield (%) of *Petasites japonicus* by different drying condition

Treatment	Whole	Leaf	Stem
Hot air convection-drying	19.67±0.40	16.92±0.30	35.14±0.50
Freeze-drying	13.62±0.30***	8.21±0.20***	23.53±0.40***

The values are mean±SD. ***: Values within the same column are significantly different by Student's *t*-test ($p<0.001$).

Table 2. Total phenol contents (mg/mL) of *Petasites japonicus* by different drying condition

Treatment	Whole	Leaf	Stem
Hot air convection-drying	0.63±0.02	1.00±0.01	0.06±0.01
Freeze-drying	1.07±0.03**	1.57±0.06*	0.46±0.01***

The values are mean±SD (n=3).
*, **, ***: Values within the same column are significantly different by Student's *t*-test ($p<0.05$, $p<0.01$ and $p<0.001$, respectively).

Table 3. The flavonoid contents (mM) of *Petasites japonicus* by different drying condition

Treatment	Whole	Leaf	Stem
Hot air convection-drying	3.02±0.07	4.03±0.21	0.22±0.01
Freeze-drying	3.77±0.04**	3.83±0.09	0.71±0.01**

The values are mean±SD (n=3).
**: Values within the same column are significantly different by Student's *t*-test ($p<0.01$).

cabbage, and shallots (Ismail et al 2004). On the other hand, Miglio et al (2008) reported that heating exerted the most detrimental effects on carrot polyphenols, as compared to steaming and frying.

Flavonoid contents of *Petasites japonicus* processed with different drying condition

Flavonoids -- a class of compounds that includes flavones, isoflavones, flavonones, anthocyanins, and catechins -- are one of the most diverse and widespread groups of natural compounds. These compounds evidence several biological activities, including radical scavenging and strong antioxidative activities characterized by a chain-breaking mechanism (Guo and Wang 2007; Iqbal et al 2007). Table 3 shows the total flavonoid contents of the different drying-pretreated *Petasites japoniuas*. Consistent with the results of the total phenolic contents, the flavonoid contents (mg/mL) in the freeze-dried whole *Petasites japonicus* (3.02±0.07 vs. 3.77 ±0.04 mM), leaves (4.03±0.21 vs. 3.83±0.09 mM), and stems (0.22±0.01 vs. 0.71±0.01 mM) were significantly higher than those measured in the hot air convection-dried sample. In general, it has been shown that several vegetables, which are harvested when completely ripe, evidence losses in total carotenoids after drying. In particular, drying in industrial hot air-dryers tends to reduce total carotenoid concentrations (Mínguez-Mosquera et al 1993). Lee et al (2008) reported that commercial, dehydrated onion products harbored low amounts of flavonoids, or no flavonoids. In a previous study, it was reported that a markedly greater reduction in ascorbic acid and beta-carotene was observed in dried, blanched, and cooked spinach and amaranth leaves (Yadav and Sehgal 1995). Agostini et al (2004) reported that the antioxidative capacities of the flavonoids of red apples with and without skin, strawberries, tomatoes and onions were diminished to a relatively higher degree following dry-heat thermal treatment. On the other hand, Veda et al (2008) reported that the thermal processing of some lime juice and the antioxidant species turmeric and onion enhanced their antioxidative activities. However, it is generally believed that many food antioxidative components can be significantly lost as a consequence of industrial sterilization, pasteurization, and dehydration, as well as by home-cooking (Jonsson 1991).

DPPH free radical scavenging activities of dried *Petasites japonicus*

The DPPH free radical scavenging activities of dried

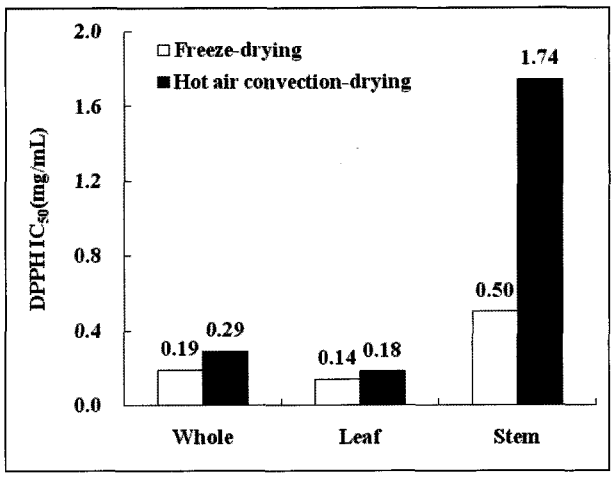

Fig. 1. DPPH free radical scavenging activity of *Petasites japonicus* by different drying condition.

Petasites japonicus were measured and are shown in Fig. 1. DPPH is a stable free radical which accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Oh et al 2002). The IC_{50} value, a measure of the extract concentration required for a 50% inhibition of the free radical DPPH, was determined (IC_{50}). In the current study, the IC_{50} value in hot air convection-dried whole *Petasites japonicus* and *Petasites japonicus* leaves were increased by 52.6% and 28.6%, as compared to the freeze-dried samples. In particular, the antioxidative activity observed after the DPPH method in freeze-dried *Petasites japonicus* stems was increased markedly by 248.0%, as compared to the hot air convection-dried samples, thereby indicating that freeze-drying is more effective for the scavenging of free radicals than is hot air convection-drying. Data regarding antioxidative activity in dried *Petasites japonicus* samples is currently quite limited. In a previous study, the effects of *in vitro* growth and the antioxidant effects determined by DPPH of a methanol extract for *Petasites japonicus* were reported (Mun and Ryu 1994). Gliszczyńska-Swigło et al (2006) previously noted that the antioxidative activities of water-cooked broccoli evidenced a 29% reduction as compared to what was observed in polyphenol extracts. In a previous study, it has been reported that, as compared to raw soybeans, thermal processing methods induced significant reductions in total phenolic contents, total flavonoid contents, DPPH free radical scavenging activity, ferric-

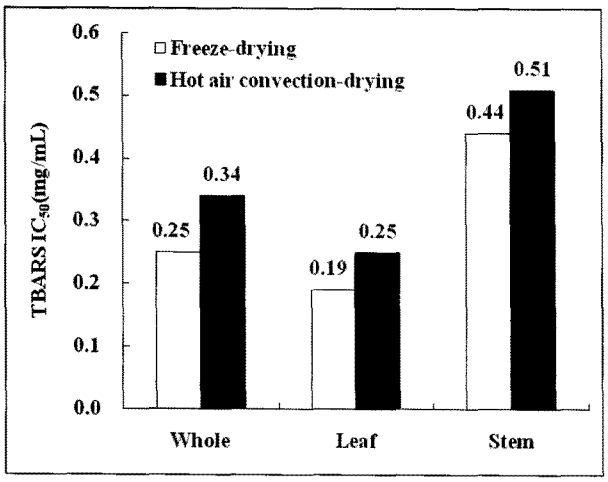

Fig. 2. Antioxidative activities by TBARS of *Petasites japonicus* by different drying condition.

reducing antioxidative power, and oxygen radical-absorbing capacity in black soybeans; these results are similar to our own (Xu and Chang 2008). Therefore, such dietary antioxidants from fresh or freeze-dried *Petasites japonicus* may be crucial to the prevention of some diseases induced by oxidative stress, via a protective effect against free radical damage to cellular DNA, lipids, and proteins *in vivo*.

Antioxidative activities by TBARS of *Petasites japonicus*

The antioxidative activity of the TBARS of *Petasites japonicus* under different drying conditions is shown in Fig. 2. In the current study, when whole *Petasites japonicus*, leaves, and stems were dried via a freeze-drying method, very strong antioxidative activities against lipid peroxidation were observed. The IC_{50} value in hot air convection-dried whole *Petasites japonicus* was increased by 36.0% as compared to those noted in the freeze-drying group. Additionally, the IC_{50} values in the hot air convection-dried *Petasites japonicus* leaves and stems were increased by 31.6% and 15.9% as compared to freeze-drying. These results showed that *Petasites japonicus* evidenced more profound antioxidative activities when freeze-dried than when dried via hot air convection. Consistent with the above results, Jang et al (2008) reported that the methanol fractions of onion dried with either a freeze-dryer or a low temperature vacuum dryer evidenced a relatively stronger inhibitory effect on intracellular reactive oxygen species. Oh et al (2002) previously reported that the antioxidative activities of vegetables were reduced by 10~81% in brain tissue as the result of heat treatment, whereas less than a 20% reduction was observed in *Petasites japonicus*, as compared with freeze-drying. Additionally, it has been reported that the pro-oxidant activity of *Cichorium* vegetables was unstable with heating, whereas their activity increased after freeze-drying and freezing (Papetti et al 2002). Our results indicated that for each portion of *Petasites japonicus*, a preferential dry processing method might be selected to preserve or minimize the loss of its nutritional and antioxidative activities. This selection may help consumers in choosing cooking practices to improve the nutritional quality of *Petasites japonicus*, and may also facilitate its use as a functional food and pharmaceutical material.

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