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Drying Condition Affects Total Phenol Contents and Antioxidant Activities of *Hizikia fusiformis*

- Research Note -

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Abstract

This study was performed to evaluate the effect of drying methods on the polyphenol contents and antioxidant activities of *Hizikia fusiformis*. The seaweed was dried using four different conditions: vacuum drying (VD) at 20°C and 30°C and hot-air drying (HD) at 40°C and 60°C. After drying, the total phenol content, DPPH and nitrite scavenging activities of the water extracts were determined. The total phenol contents were significantly ($p<0.05$) higher in the vacuum dried samples than the hot-air dried samples. DPPH radical was scavenged 82.67% and 83.45% by VD-20 and VD-30, and 70.44% and 71.23% by HD-40 and HD-60, respectively. At pH 1.2, *Hizikia fusiformis* scavenged 87.43%, 88.45%, 72.14% and 73.45% of nitrite by the treatment VD-20, VD-30, HD-40 and HD-60, respectively. Interestingly, the nitrite scavenging activity was much more reduced at pH 3.0 than at pH 1.2. Through these experiments, we were able to show that the drying conditions affect the total phenol contents and antioxidant capacities of *Hizikia fusiformis*.

Key words: *Hizikia fusiformis*, drying conditions, polyphenol, antioxidant activity

INTRODUCTION

The brown alga *Hizikia fusiformis* is one of the most widely consumed seaweeds in Korea and is known to possess a number of therapeutic compounds, including a considerable amount of polyphenols (1). The phenol compounds protect living cells and tissues from the oxidative stress of free radicals, which causes aging and human degenerative diseases (2). The health benefits of this seaweed have drawn a considerable amount of attention and have encouraged people to consume seaweed on a daily basis (3,4).

Seaweeds are usually processed to dried products in order to reduce the moisture content and also to increase shelf life. The problem with drying, though, is that bioactive components could be lost, especially heat-labile antioxidants. Although seaweeds have received much attention as potential resources of antioxidants, the effect of drying conditions on the changes of antioxidant compounds has been rarely reported.

The experiments reported in this paper reveal that the drying conditions affect total phenol content and antioxidant activities of *Hizikia fusiformis*.

MATERIALS AND METHODS

Chemicals

All chemicals and solvents were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and all other reagents were of analytical grade.

Drying and extraction

Fresh *Hizikia fusiformis* was purchased from a local market (Busan, South Korea). The seaweed was thoroughly washed and randomly sliced and then stored at -20°C until further use. The samples were dried under four different conditions: vacuum drying at 20°C and 30 °C (VD-20 & VD-30) using Vacuum Dryer (HB-501V, Hanbaek Scientific Tech., Bucheon, Korea) and hot-air drying at 40°C and 60°C (HD-40 & HD-60) using Heat Dryer (KED-10, Korea Medi Co., Daegu, Korea).

Each dried sample (50 g, three replications) was ground and sieved through 40 mesh screen and then extracted with 500 mL of water at 80°C for 2 hr by continuous shaking (120 rpm). The water extracts were centrifuged at 8,000 rpm for 20 min. The supernatants were clarified by filtering through Whatman No.2 filter paper. The clarified water extracts were freeze-dried (Bondio, Ilsin, Daegu, Korea) and stored at -20°C prior to further use.

Measurement of total phenol content

Total phenol contents of aqueous extracts were determined using the Folin-Ciocalteu colorimetric method (5,6) with some modification. Briefly, each extract (2 mL) was mixed with 2 mL of 50% Foiln-Ciocalteu reagent and 2 mL of 10% Na_2CO_3. After 1 hr incubation

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at room temperature, the absorbance was measured at 700 nm using spectrophotometer (UV-1601, Shimadzu Co., Kyoto, Japan). The results were expressed as tannic acid equivalents (mg tannic acid/100 g extract), and the values were presented as means of triplicate analyses.

DPPH radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of each extract was measured according to the method of Mensor et al. (7) with slight modifications. A 5 mL aliquot of 0.2 mM DPPH solution in 95% ethanol was incubated with 0.5 mL of the extract. The reaction mixture was shaken and incubated for 10 min at room temperature. The DPPH radical scavenging capacity was monitored at an absorbance of 517 nm and determined by comparison with an ethanol control. The percentage (%) of DPPH radical scavenging activity was calculated by the following equation: Radical scavenging activity (%) $=[(A_c - A_s)/A_c] \times 100$, where A_c is the absorbance in the absence of sample, A_s is the absorbance in the presence of sample. All extracts were analyzed in triplicate.

Nitrite scavenging activity

Nitrite scavenging activity was determined under different pH conditions (pH 1.2 and 3.0) by measuring absorbance (8). An aliquot of 1 mL sample mixed with 1mL of 1 mM $NaNO_2$ was made up to 10 mL solution and adjusted to pH 1.2 and 3.0 using 0.1 N HCl. The reaction mixture was incubated at 37°C for 1 hr. Then 1 mL of the reaction mixture was mixed with 5 mL of 2% acetic acid and 0.4 mL of Griess reagent and placed at room temperature for 15 min. The absorbance was measured at 520 nm for residual amount of nitrite. The nitrite scavenging activity (%) was calculated using the following equation: Nitrite scavenging activity (%) $=\{1-(A_{abs} - C_{abs})/B_{abs}\}$, where A_{abs} is the absorbance at 520 nm after incubation at 60 min with 1 mM $NaNO_2$ solution sample, B_{abs} is the absorbance at 520 nm before incubation with $NaNO_2$ solution, and C_{abs} is the absorbance at 520 nm with the test sample.

Statistical analysis

Statistical analyses were performed using SPSS version 12.0 for Windows (SPSS, Inc., Chicago, IL, USA). All results are expressed as mean ± standard deviation values. Statistical comparisons of the results among groups were performed by ANOVA followed by Duncan's multiple range tests. Values of $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Effect of drying conditions on phenol contents

Hizikia fusiformis was dried under four different conditions and extracted with hot water. The amounts of phenol compounds of water extracts from dried *Hizikia fusiformis* were shown in Table 1. Total phenol content was significantly ($p<0.05$) affected by the drying conditions: 857.79 mg/100 g at VD-20 and 742.56 mg/100 g at HD-60. Vacuum drying available in lower temperature could significantly ($p<0.05$) preserve a higher amount of phenol content than hot-air drying, which represents the importance of drying method for quality control of seaweeds.

Regarding the influence of the drying process on the phenol contents, the results of the previous studies varied. Ballistreri et al. (9) reported that sun drying process caused a substantial loss of phenolic compounds in pistachios. On the other hand, some reported that sun-drying and high temperature processing enhanced phenol contents. The total content of phenols increased significantly ($p<0.05$) after sun-drying of dates, whereas a significant amount of antioxidants and carotenoids was lost (10). Del Caro et al. (11) dried plums using high-temperature (70°C, 85°C) and low temperature (60°C) procedures and monitored phenol content during the storage. According to these investigators, the drying temperature significantly affected the polyphenol content, with different effects according to the class of polyphenols. Higher amounts of polyphenols were found in the prunes dried at the higher temperature. This effect can be explained by polyphenol oxidase still being active at the lower temperature, and capable of destroying the phenol compounds of the samples.

The previous studies (12-14) reported that heat treatment significantly enhanced the amount of polyphenol compounds in sweet corn and tomatoes. They discussed the fact that heat treatment liberated phenol compounds in plants, which could be more available to the body.

Table 1. Effect of different drying conditions on total polyphenol contents of water extracts from *Hizikia fusiformis*

VD-20[1)]	VD-30	HD-40	HD-60
857.79 ± 23.21[2)a]	850.21 ± 11.29[a3)]	732.12 ± 13.40[b]	742.56 ± 11.67[b]

[1)]VD-20 & VD-30 means vacuum drying at 20°C and 30°C, HD-40 & HD-60 means hot-air drying at 40°C and 60°C.
[2)]Values are represented in mg tannic equivalent/100 g extract as mean ± SD (n=3).
[3)]Different letters in a column are significantly different at $p<0.05$ by Duncan's test.

Table 2. Effect of different drying conditions on DPPH radical and nitrite scavenging activities of *Hizikia fusiformis*

Drying conditions[1)]	DPPH	Nitrite scavenging activity	
		pH 1.2	pH 3.0
VD-20	82.67±2.31[2)a]	87.43±1.05[a]	52.43±0.72[b]
VD-30	83.45±0.69[a3)]	88.45±0.73[a]	54.46±0.43[a]
HD-40	70.44±2.59[b]	72.14±0.41[b]	38.39±0.39[d]
HD-60	71.23±0.66[b]	73.45±0.82[b]	40.44±1.47[c]

[1)]VD-20 & VD-30 means vacuum drying at 20°C and 30°C, HD-40 & HD-60 means hot-air drying at 40°C and 60°C.
[2)]Values are represented in mg tannic equivalent/100 g extract as mean±SD (n=3).
[3)]Different letters in a column are significantly different at $p<0.05$ by Duncan's test.

Effect of drying conditions on DPPH and nitrite scavenging activity

The effects of drying conditions on DPPH and nitrite scavenging activity (%) of water extracts from *Hizikia fusiformis* are presented in Table 2.

DPPH radical of *Hizikia fusiformis* was scavenged at 82.67% and 83.45% by VD-20 and VD-30, respectively; 70.44% and 71.23% by VD-40 and VD-60, respectively. DPPH radical scavenging activities of hot-air dried products were significantly lower than those of vacuum dried products. As previously reported studies indicated (11, 15), DPPH radical scavenging activity seems to be highly correlated to the total phenol contents.

In particular, dietary phenol compounds are reported to reduce nitrite to NO in highly acidic environments, such as gastric conditions (16,17). Recently, this nitrite modulation to NO has emerged as an alternative health benefit of dietary polyphenols (18, 19). By promoting such a reaction, polyphenol might inhibit the formation of carcinogenic nitrosamines and yield a molecule (NO) that plays a beneficial role in vessel homeostasis and the immune system (20).

Hizikia fusiformis scavenged 87.43% and 88.45% of nitrite with the VD-20 and VD-30 treatment at pH 1.2. The nitrite scavenging activities of *Hizikia fusiformis* were significantly reduced to 72.14% and 73.45% with the HD-40 and HD-60 drying treatments, respectively. At pH 3.0, the VD-20 and VD-30 nitrite scavenging activities of *Hizikia fusiformis* were 52.43% and 54.46%, respectively, and 38.30% and 40.44% at HD-40 and HD-60, respectively. These results demonstrate that the nitrite scavenging activities of *Hizikia fusiformis* were influenced by the drying conditions and pH of the assay conditions. In summary, nitrite scavenging activities were significantly higher in the vacuum dried samples under greater acidity.

Polyphenols in *Hizikia fusiformis* acted as electron donors, so that the vacuum dried samples that contained more polyphenols showed higher scavenging activities of DPPH and nitrite. In conclusion, the drying conditions of the seaweed affected the total phenol content and the antioxidant. Therefore, the drying process could be critical for preserving the bioavailability of *Hizikia fusiformis*.

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