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Effect of Hydrothermal Treatment on the Antioxidant Activity of Rice Hull Extracts

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Abstract Hydrothermal treatment of rice hull was hydrothermal carried out at 105, 110, 121°C for 15, 30, 60 min, respectively, using a conventional autoclave. Antioxidant activity of the hydrothermal treated rice hull extract was evaluated by determining total phenol contents (TPC), 1,1-diphenyl-2-picylhydrazyl (DPPH) radical scavenging activity (RSA), reducing power, and ABTS RSA. TPC, DPPH RSA, reducing power, and 2,2-azinobis(3-ethylenebenzothiozoline-6-sulfonic acid) (ABTS) RSA of the extract were significantly increased with increasing treated temperature and time. For example, hydrothermal extracts at 121°C for 60 min increased the TPC, DPPH RSA, reducing power, and ABTS RSA to 0.840 mg/mL, 64.77%, 1.437, and 92.11%, respectively, while those of the extracts treated at 105°C for 60 min were 0.508 mg/mL, 51.23%, 0.819, and 45.22%, respectively. The results indicated that hydrothermal treatment of rice hull was very effective to increase phenolic compounds and antioxidant activity of rice hull extract.

Keywords: rice hull, hydrothermal treatment, total phenolic content, antioxidant activity

Introduction

During the past decade, it has been reported that hundreds of synthetic and natural antioxidants have been developed for food preservation. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinine have been widely used in food preventing oxidation (1). Antioxidants prevent rancidity, improve sensory scores, and provide improved consumer acceptance of food products (2). However, the use of these synthetic antioxidants in food is discouraged because of their toxicity (3) and carcinogenicity (4).

Agricultural and industrial residues are attractive sources of natural antioxidants (5). Rice hull, which represent 20% dry weight of the harvested rice, can serve as low cost feedstock for production of fuel alcohol in the USA and some other rice-producing countries (6). Rice hull contains an antioxidant defense system to protect rice seed from oxidative stress (7). Ramarathnam *et al.* (8) identified isovitexin as a natural component in white rice hull, which showed a strong antioxidant effect. To obtain natural antioxidants from plants, it is necessary to find an effective processing method to liberate them (9,10). Several methods such as high temperature and pressure (11), heat treatment (12), far-infrared radiation (13), fermentation (14), hydrothermal (hot water with high pressure) treatment (15), and enzyme treatment (16) have been studied to liberate and activate low molecular weight natural antioxidants. In our previous study (17), antioxidative polyphenolic compounds of rice hull were effectively cleaved by far-infrared irradiation while simple heat treatment could nor. In the present study, it was reported that hydrothermal treatment of rice hull with simple conventional was very effective to extract polyphenolic compounds and to increase antioxidant activity of rice hull extracts.

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Materials and Methods

Materials Rice hull from rice cultivar (*Oriza sativa* L.), one of Japonica type rice, was kindly supplied from Koseong Rice Processing Center (Koseong, South Korea). L-Ascorbic acid, gallic acid, 1,1-diphenyl-2-picylhydrazyl (DPPH) and 2,2-azinobis(3-ethylenebenzothiozoline-6-sulfonic acid) (ABTS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Folin-Ciocalteu reagent was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Hydrothermal treatment and preparation of rice hull extracts Each 10 g of rice hull was mixed with 100 mL of distilled water, and followed by hydrothermal treatment at 105, 110, and 121°C in an autoclave (model MLS-3020; Sanyo Electric Co., Ltd., Osaka, Japan) for 15, 30, 60 min, respectively. Then the mixture was filtered through a Whatman No. 1 filter paper, and filtrate was used to determine antioxidant activity.

Total phenolic content (TPC) TPC of the rice hull extract was determined using the method of Gutfinger (18). Rice hull extract was mixed with 1 mL of 2% Na_2CO_3 and 50% Folin-Ciocalteau reagent 0.2 mL and centrifuged at 13,400×g for 5 min. After 30 min incubation at room temperature, the absorbance was measured with a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) at 750 nm. TPC were expressed as gallic acid equivalents.

DPPH radical scavenging activity (RSA) Antioxidant activity of the rice hull extract was determined by DPPH RSA (19). After mixing 0.1 mL of rice hull extract with 0.9 mL of 0.041 mM DPPH radical in ethanol for 30 min, the absorbance of the sample was measured at 517 nm. RSA was expressed as percentage according to the following

formula:

% DPPH RSA=(1−sample O.D./control O.D.)×100

Reducing power Reducing power of the rice hull extract was determined according to the method of Oyaizu (20). The rice hull extract (1 mL, 1 mg/mL), phosphate buffer (1 mL, 0.2 M, pH 6.6), and potassium ferricyanide (1.0 mL, 10 mg/mL) were mixed and incubated at 50°C for 20 min. Trichloroacetic acid (1.0 mL, 100 mg/mL) was added to the mixture and centrifuged at 13,400×g for 5 min. The supernatant (1.0 mL) was mixed with distilled water (1.0 mL) and ferric chloride (0.1 mL, 1.0 mg/mL), and then the absorbance was measured at 700 nm.

ABTS RSA The ABTS RSA was evaluated with the method of Pellegrini *et al.* (21). Rice hull extract (0.1 mL), potassium phosphate buffer (0.1 mL, 0.1 M, pH 5.0), and H_2O_2 (20 μL, 10 mM) were mixed and incubated at 37°C for 5 min. After a preincubation, ABTS (30 μL, 1.25 mM, in 0.05 M phosphate-citrate buffer, pH 5.0) and peroxidase (30 μL, 1 Unit/mL) were added to the mixture and then it was incubated at 37°C for 10 min. The optical density (O.D.) level was obtained with a multiplate reader (Sunrise RC/TS/TS Color-TC/TW/BC/6Filter; Tecan Austria GmbH, Grödig, Austria) at 405 nm, and ABTS RSA was calculated with the following formular:

ABTS RSA (%)=[1−(sample O.D./control O.D.)]×100

Statistical analyses All measurements were performed in triplicate, and analyses of variance were conducted by the general linear model procedure using (22). Student-Newman-Keul's multiple range tests were used to determine the significant differences between the mean values for the treatments ($p<0.05$).

Results and Discussion

Total phenolic content (TPC) Phenolic substances have been reported to be widely distributed in plants (23,24). The function of phenolic in plants seems to vary extensively, though mainly as attractants of agents for pollination. Rice hull contains many phenolic compounds such as isovitexin, phytic acid, vanillic acid, syringic acid, and ferulic acid (25,26), which showed antioxidant activity. Effect of hydrothermal treatment on the TPC of rice hull extract was determined by the Folin-Ciocalteau assay, and the results were shown in Table 1. The TPC of rice hull extracts significantly increased with hydrothermal treatment. The TPC of rice hull extracts increased from 0.508 mg/mL GAE in hydrothermal treatment at 105°C for 15 min to 0.840 mg/mL GAE at 121°C for 60 min, while that of non-heated rice hull extracts at 25°C for 12 hr was 0.364 mg/mL GAE (data not shown). These results indicate that phenolic compound in rice hull can be liberated by hydrothermal treatment. In our previous studies, simple heat treatment at 100°C could not cleave covalently bound phenolic compounds from rice hull (17), however, hydrothermal treatment in this study was efficient to extract them. Water at the range of 105 to 121°C in this experiment is subcritical water state. Subcritical water has unique characteristics such as high density, high reactivity, and good solubility for a series of organic compounds having relatively low molecular weights. Based on these features, subcritical water has been used as an extractant instead of organic solvent for its environmentally friendly characteristics and high catalytic activity. For example, subcritical water was used to extract nutraceuticals from licorice roots (27), flavor compounds from rosemary (28), and protein and amino acids from rice bran (29). In this study, we found that subcritical water (hydrothermal) treatment was also effective and practical technique to extract phenolic compounds from rice hulls.

Table 1. Effect of hydrothermal treatment on total phenolic content of rice hull extracts (mg/mL)

Temperature (°C)	Time (min)			
	15	30	60	SEM[1)]
105	0.508^{by}[2)]	0.515^{by}	0.576^{ay}	0.008
110	0.510^{ay}	0.435^{cz}	0.467^{bz}	0.004
121	0.580^{bx}	0.720^{bx}	0.840^{ax}	0.001
SEM[1)]	0.003	0.011	0.007	

[1)]Standard errors of the mean (n=9).
[2)]Different letters within a row $^{a-c}$ and within each extract $^{x-z}$ are significantly different at $p<0.05$ (n= 3).

On the other hand, TPC of ethyl acetate fractions of rice hulls increased significantly by far-infrared treatment from 0.07 to 0.19 mM (30). Ethyl acetate is easily miscible with hydrophobic organic solvents such as ether, chloroform, and acetone, however, only 1 mL of ethyl acetate dissolves in 10 mL of water (31). Water in the hydrothermal condition in this study was subcritical state with increased solubility for organic compounds, therefore hydrothermal treatment enhanced significantly TPC of rice hull extracts.

DPPH RSA DPPH method is one of simplest manner to measure the ability of from antioxidants to intercept free radicals. The effect of antioxidant on DPPH radical scavenging is thought to be due to their hydrogen donating ability. Hydrothermal treatment of rice hull significantly affected the DPPH RSA (Table 2). The highest DPPH RSA values (64.77%) of rice hull extract was found after hydrothermal treatment at 121°C for 60 min. Vitamin C, positive control, showed 22.49 and 91.58% of DPPH RSA at concentration of 10 and 100 μg/mL. The IC_{50} value (in mg/mL) of DPPH radical scavenging of far-infrared irradiated rice hull extract was 0.067, while those of BHT and α-tocopherol were 0.362 and 0.012, respectively (32). Although it is difficult to compare antioxidant activity of rice hull extracts with pure antioxidants, the significant antioxidant effects of hydrothermally treated rice hull extracts could be coming from the synergistic effects of various compounds in rice hull extract.

Reducing power The antioxidant activity has been reported to be concomitant with reducing power (20). The reducing powers of several rice hull extracts using the potassium ferricyanide reduction method are shown in Table 3. The reducing power of rice hull extract also increased from 0.655 (25°C for 12 hr, data not shown) to 1.437 by hydrothermal treatment at 121°C for 60 min. Vitamin C (100 μg/mL) exhibited 0.286 value of reducing power.

Table 2. Effect of hydrothermal treatment on DPPH radical scavenging activity of rice hull extracts (%)

Temperature (°C)	Time (min)				Positive control	
	15	30	60	SEM[1)]	Vit C (10 μg/mL)	Vit C (100 μg/mL)
105	51.23$^{bz2)}$	55.87^{bz}	58.79^{az}	0.002	22.49	91.58
110	54.71^{cy}	54.86^{by}	59.38^{ay}	0.001		
121	51.08^{cx}	62.00^{bx}	64.77^{ax}	0.001		
SEM[1)]	0.001	0.001	0.003			

[1)]Standard errors of the mean (n=9).
[2)]Different letters within a row $^{a-c}$ and within each extract $^{x-z}$ are significantly different at p<0.05 (n=3).

Table 3. Effect of hydrothermal treatment on reducing power of rice hull extracts (O.D.)

Temperature (°C)	Time (min)				Positive control	
	15	30	60	SEM[1)]	Vit C (10 μg/mL)	Vit C (100 μg/mL)
105	0.819$^{cz2)}$	0.860^{by}	0.973^{ay}	0.003	0.213	0.286
110	1.055^{ax}	0.840^{cy}	0.965^{by}	0.017		
121	1.339^{by}	1.400^{bx}	1.437^{ax}	0.028		
SEM[1)]	0.002	0.014	0.029			

[1)] Standard errors of the mean (n=9).
[2)]Different letters within a row $^{a-c}$ and within each extract $^{x-z}$ are significantly different at p<0.05 (n=3).

Table 4. Effect of hydrothermal treatment on ABTS radical scavenging activity of rice hull extracts (%)

Temperature (°C)	Time (min)				Positive control	
	15	30	60	SEM[1)]	Vit C (10 μg/mL)	Vit C (100 μg/mL)
105	45.22$^{bz2)}$	53.82^{az}	55.21^{az}	0.03	61.94	78.56
110	73.03^{ay}	62.31^{by}	65.62^{by}	0.03		
121	89.71^{ax}	90.54^{ax}	92.11^{ax}	0.03		
SEM[1)]	0.04	0.01	0.04			

[1)]Standard errors of the mean (n=9).
[2)]Different letters within a row $^{a-c}$ and within each extract $^{x-z}$ are significantly different at p<0.05 (n=3).

Reducing power was reported to be associated with the presence of reductones (33), thus hydrothermal treatment of rice hull might increase reductones of rice hull extract.

ABTS RSA ABTS, another stable free radical cation, was used to evaluate antioxidant activity of the rice hull extract. The ABTS systems have been commonly used to measure the total antioxidative status of various biological specimens with measuring radical scavenging by electron donation (34). As shown in Table 4, ABTS RSA increased with increasing hydrothermal temperature and time. The highest ABTS RSA (92.11%) was also detected in the extract at 121°C for 60 min, which was stronger than that of 100 μg/mL of vitamin C.

In our previous studies (17,30,32), far-infrared irradiation of rice hull could increase several antioxidative phenolic compounds in its methanolic extract. For simplicity and wide application, hydrothermal treatment of rice hull with conventional autoclave was carried out in this study, and appropriate temperature and time was found to be very effective to increase phenolic compounds and antioxidant activity of rice hull extract.

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