Antioxidant Activity of Organic Solvent Extracts from Far Infrared-Treated Rice Hulls

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Abstract Methanolic extracts of rice hulls with or without far infrared (FIR) irradiation were sequentially fractionated with solvents (hexane, chloroform, ethyl acetate, butanol, and water), and antioxidant activities of the fractions were analyzed for total phenol contents (TPC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging capability, reducing power, and antioxidant potency. Yield of chloroform fraction increased significantly from 6.74 to 20.78% after FIR irradiation, while those of ethyl acetate and butanol fractions slightly decreased. Antioxidant activity of ethyl acetate fraction increased significantly by FIR radiation as TPC and DPPH radical-scavenging activity increased from 0.07 to 0.19 mM and 30.09 to 80.19%, respectively. Lard induction time of ethyl acetate fraction increased from 1.15 to 1.49 hr by FIR radiation. GC-MS analysis indicated amounts of phenolic compounds (3-vinyl-1-oxy benzene and benzaldehyde) in ethyl acetate fraction of FIR-irradiated rice hull methanolic extract were greater than those of nonirradiated ones.

Keywords: rice hull extract, solvent fraction, far infrared, antioxidant

Introduction

Plants contain a diverse group of phenolic compounds including simple phenolics, phenolic acids, anthocyanins, hydrocinnamic acid derivatives, and flavonoids, among which phenolic acids and flavonoids are the most active antioxidant compounds (1). Phenolic compounds possess antioxidant properties by hydrogen donation through the hydroxyl group, and the subsequently formed radicals are stabilized by resonance delocalization throughout the phenolic ring structure (2). In addition, many phenolics contain acid or ring groups that may participate in metal chelation (3).

Rice hull contains phenolic compounds such as isovitexin, phytic acid, vanillic acid, syringic acid, and ferulic acid (4-7). Our pervious studies revealed that methanolic extracts of rice hull contain several phenolic compounds including cinnamic and benzoic acid derivatives and far infrared (FIR) irradiation between 2 and 14 µm significantly increased the antioxidant activities of rice hull extracts (8, 9), because FIR irradiation on rice hull liberated and activated the covalently bound phenolic compounds that have antioxidant activities.

For concentration and isolation of useful components from plant extracts, fractionation with several organic solvents has been widely applied. In the present study, the antioxidant activities of organic solvent fractions from rice hull extract with or without FIR irradiation were determined. These results will be important in developing an effective method for the production of food-grade natural antioxidant from rice hull extracts.

Materials and Methods

Materials Rice hulls from a Japonica-type rice cultivar (Oriza Sativa L.) were purchased from a milling plant in Kimcheon, Korea. They were ground in a mill and passed through a 48-mesh sieve. 2-Thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), tannic acid, fish oil, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and lard were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Folin-Ciocalteu reagent was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

FIR irradiation onto rice hulls Rice hulls (50 g) were put in a wooden box (50 cm×40 cm×40 cm) and irradiated at 2-14 µm for 30 min with an FIR heater (35×10 cm; output 300 W; Hakko Electric Machine Works Co., LTD., Nagano, Japan). The sample-holding tray in the middle of the treating box was placed to parallelly face the FIR heater, and the distance between sample and heater was 20 cm.

Preparation of solvent fractionation extracts Rice hull samples (300 g), with or without 30-min FIR irradiation, were extracted in 1.5 L methanol overnight at room temperature. The extract was filtered through a Whatman nylon membrane filter (0.2 µm), and the filtrate was evaporated to dryness under reduced pressure on a rotary evaporator at 40°C. The dried extract was dissolved in 300 mL of 10% methanol, and 300 mL of hexane was added. The mixture was then partitioned into hexane and aqueous layers. After separation of the hexane layer, 300 mL of chloroform was added to the aqueous layer and partitioned, and the chloroform layer was separated. Using the same procedure, ethyl acetate, n-butanol, and final aqueous layers were separated (10). The separated layers were evaporated to dryness under reduced pressure and weighed to determine the yields. Each solvent fraction was redissolved in methanol (1 g/100 mL) and centrifuged.

Identification of ethyl acetate fraction of rice hull extract Dried ethyl acetate fraction of the rice hull methanol extract was dissolved in ethanol (200 mg/mL) and centrifuged.
Total phenolic contents (TPC)  TPC of each rice hull extract fraction was determined using the Folin-Ciocalteu reagent with tannic acid as a standard (11). One milliliter of each fraction was mixed with 1 mL of 50% Folin-Ciocalteu reagent with tannic acid as a standard (11). One milliliter of each sample (10 mg/mL) was added to the lard (2.5 g), and mixed vigorously with a vortex for 8 s immediately before Rancimat measurement.

Radical scavenging activity  Antioxidant activity was determined based on the radical scavenging activity of the sample (12). After mixing 1 mL of 0.041 mM DPPH in ethanol with 0.2 mL of rice hull extracts for 10 min, the optical density (OD) was measured at 517 nm. Results were expressed as a percentage DPPH-radical scavenging activity of the sample and were calculated according to the following equation:

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\% \text{DPPH-radical scavenging activity} = \frac{\text{control OD} - \text{sample OD}}{\text{control OD}} \times 100
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Reducing power  Reducing power of the solvent fractions of rice hull extract was determined using the method of Oyaizu (13). Extracts (1 mg/mL) in phosphate buffer (2.5 mL, 0.2 M, pH 6.6) were added to potassium ferricyanide (2.5 mL, 10 mg/mL), and the mixture was incubated at 50°C for 20 min. Trichloracetic acid (2.5 mL, 100 mg/mL H₂O) was added to the mixture and centrifuged at 13,400 × g for 5 min. The supernatant (1 mL) was mixed with distilled water (1 mL) and ferric chloride (0.1 mL, 10 mg/mL H₂O), and the absorbance was measured at 700 nm.

Rancimat method  Induction periods of lard as affected by the addition of antioxidant were determined using a Metrohm 793 Rancimat (Herisan, Switzerland) (14). Oxidation was carried out at 100°C with an airflow rate of 20 L/hr. One milliliter of each sample (10 mg/mL) was added to the lard (2.5 g), and mixed vigorously with a vortex for 8 s immediately before Rancimat measurement.

Statistical analysis  All measurements except GC/MS analysis were done in triplicates, and Students t-test was used to determine the difference between mean values (p < 0.05) of FIR irradiated and nonirradiated samples (15).
scavenging activities as that of BHT, an indication that, in rice hull extracts, antioxidant activities are closely related to the total phenol contents.

Reducing power Reducing power of grape seed is associated with its antioxidant activity (19). Duh (20) reported that reducing properties are generally associated with the presence of reductones. Figure 4 shows the reducing powers of solvent fractions of FIR-irradiated and nonirradiated rice hull extracts using the potassium ferricyanide reduction method. The increased reducing powers of methanol, hexane, chloroform, and ethyl acetate fractions by FIR irradiation were 0.35, 0.50, 0.13, and 0.80 absorbance values, respectively, whereas those of butanol and water fractions were not significantly changed. The highest reducing power was observed in the ethyl acetate fraction of FRH, also showing that the reducing power of rice hull extracts is related to the total phenol content.

Rancimat analysis Rancimat method is commonly used to evaluate the antioxidant potency of various antioxidants (21). The longer induction period of lard with the addition of antioxidant compared to that of the control (pure lard) increased the antioxidant activity of the antioxidant compound. Table 1 shows the induction times of lard affected by the addition of each solvent fraction. Chloroform fraction showed the highest inhibition of lipid oxidation. However, the lipid oxidation-retarding time of ethyl acetate fraction of FIR-treated rice hull extract showed the greatest increase.
among the fractions, an increase from 1.15 to 1.49 hr.

**GC analysis for ethyl acetate fraction of rice hull extracts**

Phenolic compounds with antioxidant activity (benzoic, cinnamic, and indole acetic acids) were detected in the ethyl acetate fraction of nonirradiated rice hull methanolic extract (Fig. 5A). However, the ethyl acetate fraction of FIR-irradiated rice hull methanolic extract showed new phenolic compounds such as 3-vinyl-1-oxy benzene and benzaldehyde (Fig. 5B). Furthermore, the amount of cinnamic acid, a well-known antioxidant phenolic compound, was higher than that from nonirradiated rice hull. These results coincide with the increase of TPC in ethyl acetate fraction of rice hull extracts from 0.07 to 0.19 mM by FIR irradiation (Fig. 2).

In our previous study (8), FIR irradiation of rice hulls liberated phenolic compounds, and increased the contents of active compounds in the extracts. FIR irradiation activated phenolic compounds in rice hull, thus increasing the antioxidant activity of rice hull extract.

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