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Effect of Far-Infrared Radiation on the Antioxidant Activity of Rice Hulls

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After far-infrared (FIR) radiation onto rice hull, a methanolic extract was prepared for the determination of antioxidant ability. After 30 min of FIR treatment, the radical scavenging activity and total phenol contents of rice hull extracts increased from 47.74 to 79.63% and from 0.12 to 0.19 mM, respectively, compared to control. Inhibition of lipid peroxidation in extracts was also increased from 41.07 to 47.96%. According to the GC-MS analysis, more phenolic compounds (*p*-coumaric acid, 3-vinyl-1-oxybenzene, *p*-hydroxybenzaldehyde, vanillin, *p*-hydroxybenzoic acid, and 4,7-dihydroxyvanillic acid) were detected in FIR-irradiated rice hull extract. These results indicated that FIR radiation onto rice hull could liberate and activate covalently bound phenolic compounds that have antioxidant activities.

KEYWORDS: Rice hull extract; far-infrared; antioxidant; total phenolic contents

INTRODUCTION

Rice is the principal cereal in Asia, some countries in Africa, and Latin America. More than one million tons of rice hulls have been produced annually in South Korea after the processing of rice. However, rice hulls are wasted or destined to undervalued uses. Currently, agricultural and industrial residues are attractive sources of natural antioxidants (*1*). The extraction of antioxidant compounds from residue materials such as hulls, seed coats, peels, grape seeds, olive rape, and cocoa byproduct has been reported (*2*). In general, seed coat plays an important role in protecting seeds from oxidative damage because the seed coat possesses large quantities of endogenous antioxidants such as phenolic compounds (*1*, *3*).

Rice hull also contains an antioxidant defense system to protect rice seed from oxidative stress. Ramarathnam et al. (*4*) identified isovitexin as a natural component in white rice hull, which showed a strong antioxidant effect. Wu et al. (*5*) reported that wild rice kernels contain 2.1−2.4% of phytic acid, a strong metal chelating agent, and thus possess antioxidant activity. Another study (*6*) showed that rice hull contained several kinds of strong antioxidants such as anisole, vanillin, and syringaldehyde. Thus, rice hull is an attractive source of natural antioxidants.

Many natural plant antioxidants, however, exist either as bound forms to high molecular weight compounds or as part of the repeating subunits of high molecular weight polymers (*7*). Several methods including far-infrared (FIR) radiation are known to liberate and activate low molecular weight natural antioxidants (*8*). FIR rays are defined as electromagnetic waves having a wavelength of longer than 4 μm but shorter than microwaves ($\lambda > 0.1$ cm). FIR rays are biologically active (*9*) and transfer heat to the center of materials evenly, without degrading the constituent molecules of the surface (*10*). FIR, however, may have the capability to cleave covalent bonds and liberate antioxidants such as flavonoids, carotene, tannin, ascorbate, flavoprotein, or polyphenols from repeating polymers (*7*, *8*). The objective of this work was to determine the effect of FIR radiation on the antioxidant activity of rice hull extract.

MATERIALS AND METHODS

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

FIR Radiation onto Rice Hulls. Rice hulls (5.0 g) in a wooden box (50 cm × 40 cm × 40 cm) were irradiated with an FIR heater (35 × 10 cm, output 300 W, Hakko Electric Machine Works Co., Ltd., Nagano, Japan), which emitted radiation between 2 and 14 μm wavelength range. The sample holding tray in the middle of the treatment box was placed to face an FIR heater in a parallel position, and the distance between sample and heater was 20 cm. To determine the effect of heat generated by FIR radiation, another batch of rice hull samples was treated in an oven at 100 °C.

Preparation of Rice Hull Extracts. Each 3 g of rice hull samples treated with FIR for a given time was mixed with 100 mL of methanol

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for 1 h at room temperature and filtered through a Whatman no. 1 filter paper, and the filtrate was used to determine antioxidant activity.

Total Phenolic Contents (TPC). The TPC of rice hull extracts was determined using the method of Gutfinger (*11*). Rice hull extract was mixed with 1 mL of the 50% Folin−Ciocalteu reagent and 1 mL of 2% Na_2CO_3 and centrifuged at 13400*g* for 5 min. The absorbance of the mixture was measured with a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) at 750 nm after 30 min of incubation at room temperature. TPC were expressed as millimolar tannic acid equivalents.

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

$$\%\ \text{DPPH radical scavenging activity} = [(\text{control OD} - \text{sample OD})/\text{control OD}] \times 100$$

TBARS. Lipid peroxidation was evaluated using a thiobarbituric acid reactive substance (TBARS) method (*13*). A fish oil emulsion was prepared by homogenizing 0.5 g of fish oil, 0.5 g of Tween 20 in maleic acid buffer (8 mL, 0.1 M, pH 6.0), and 90 mL of distilled water. A reaction mixture containing rice hull extract (0.1 mL), fish oil emulsion (0.5 mL), distilled water (0.4 mL), and $FeCl_2$ (0.1 mL, 50 ppm) was mixed with a vortex. The reaction mixture was incubated at 37 °C for 1 h. After the addition of 0.05 mL of BHT solution (7.2% in ethanol) and 2 mL of TCA/TBA solution (7.5% TCA and 0.19% TBA, w/v), color was developed in boiling water for 15 min followed by cooling in ice water. The mixture was centrifuged at 13400*g* for 10 min, and the OD of the supernatant was measured at 532 nm. Results were calculated using the following equation:

$$\%\ \text{inhibition of lipid peroxidation} = [(\text{control OD} - \text{sample OD})/\text{control OD}] \times 100$$

Statistical Analysis. All measurements were done in triplicate, and Student's *t* test was used to determine the difference between mean values ($p < 0.05$) (*14*).

Identification of Rice Hull Extracts. Intact rice hull extract (IRH) or FIR-irradiated rice hull extract (FRH) was dissolved in ethanol (200 mg/mL) and centrifuged at 13400*g* for 5 min to precipitate undissolved materials. The supernatant was mixed with 4 volumes of BSA [*N*,*O*-bis(trimethylsilyl)acetamide] and derivatized in a water bath (70 °C) for 15 min (*15*). The compounds in IRH or FRH were identified using a gas chromatograph−mass spectrometer (GC6890/MS5973, Hewlett-Packard Co., Wilmington, DE). A split inlet (100:1) was used to inject samples (5 μL) into a combined column, an HP-5 column (30 m, 0.32 mm i.d., 0.25 μm film; Hewlett-Packard Co.) connected with an HP-35 column (7.5 m, 0.32 mm i.d., 0.25 μm film; Hewlett-Packard Co.) using a zero-volume connector. A ramped oven temperature was used (100 °C for 2 min, increased to 270 °C at 10 °C/min, and held for 6 min). The inlet temperature was 250 °C, and the carrier gas was He at constant flow of 1.5 mL/min. The ionization potential of the mass selective detector was 70 eV, and the scan range was *m*/*z* 19.1−400. Identification of compounds detected was achieved by comparing mass spectral data of samples with those of the Wiley library (Hewlett-Packard Co.).

RESULTS AND DISCUSSION

Effect of FIR Radiation on the Antioxidant Ability of Rice Hull Extracts. FIR radiation significantly increased TPC in rice hull (**Figure 1A**). The amount of TPC in rice hull increased from 0.12 to 0.20 mM after radiation of FIR for 50 min. The amount of TPC increased with radiation time and then reached a plateau after ∼30 min of radiation.

Phenolic compounds are the most active antioxidant derivatives in plants (*16*, *17*). Generally, the outer layers of plant such as peels, shells, and hulls contain large amounts of polyphenolic compounds to protect the inner materials. Rice hull also contains many phenolic compounds such as isovitexin, phytic acid, vanillic acid, syringic acid, and ferulic acid (*4*−*6*, *18*).

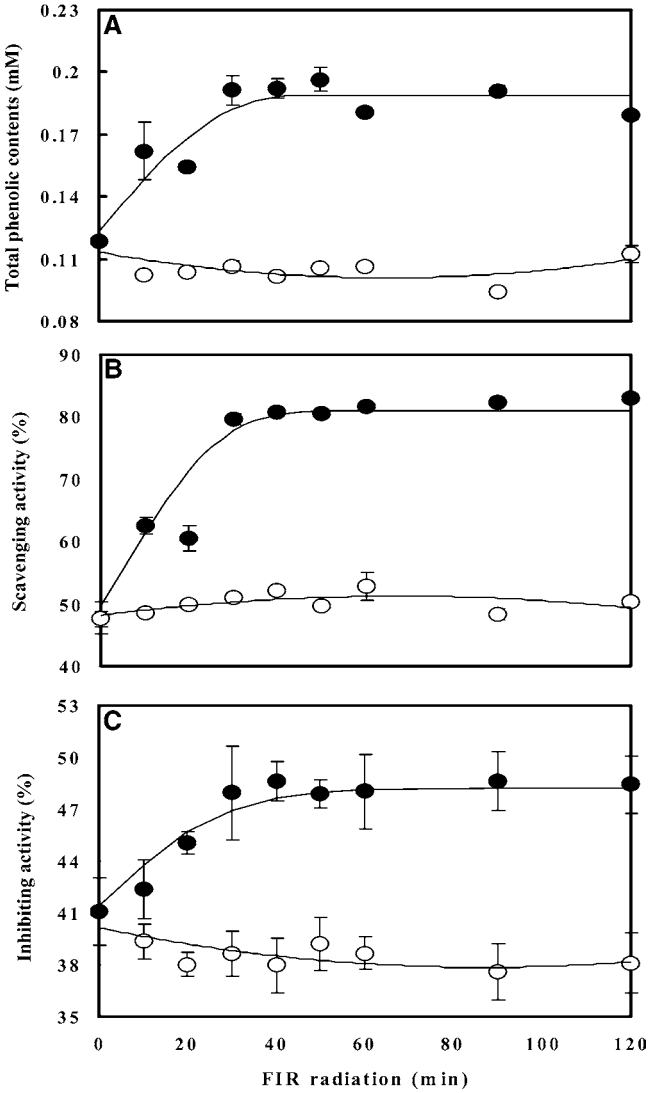

Figure 1. Effect of FIR treatment on (A) total phenolic contents, (B) DPPH radical scavenging activity, and (C) lipid peroxidation of rice hull extracts: (●) irradiated with FIR; (○) dried at 100 °C instead of FIR radiation. Error bars represent standard deviation of mean values. All values were compared by Student's *t* test ($p < 0.05$).

FIR radiation is accompanied by heat, which can accelerate reaction. However, simple heating of sample in a drying oven did not increase TPC in rice hull (**Figure 1A**). Although the drying process of oats resulted in a large loss of antioxidant compounds (*19*), such was not the case for rice hulls. NMR studies showed that water clusters become much smaller after exposure to FIR rays (*20*), and the peak width in ^{17}O NMR spectra of water kept around FIR radiator disks was reduced (*9*). These facts indicate that the motility of water molecules is highly activated by FIR radiation. This property of FIR is applied to many fields of life science (*9*). Niwa et al. (*8*) reported that FIR radiation heating significantly increased the content of natural medicinal products from an oriental pottery vessel. Our results indicated that FIR radiation cleaved and liberated polyphenols, which were either covalently bound to high molecular weight compounds or part of repeating subunits of high molecular weight polymers.

DPPH radical scavenging activities of FRH also increased with FIR radiation (**Figure 1B**). After FIR radiation for 60 min,

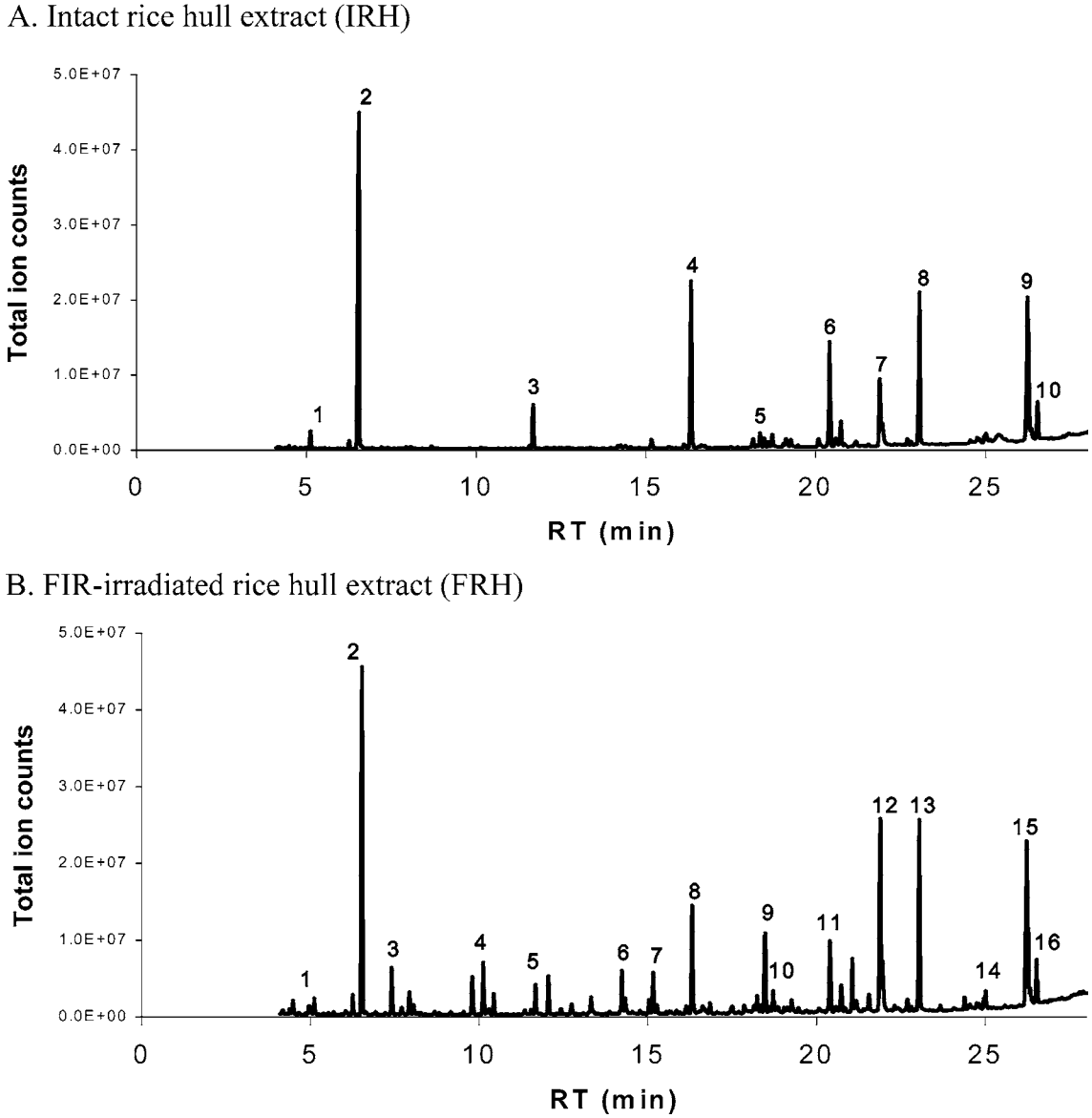

Figure 2. Typical gas chromatography of (A) intact rice hull extract (IRH) and (B) FIR-irradiated rice hull extract (FRH). Peaks: (A) 1, *o*-methoxycinnamic acid; 2, glycerol; 3, erythritol; 4, xylitol; 5, azelaic acid; 6, mannitol; 7, *p*-coumaric acid; 8, palmitic acid; 9, *N*-indolyl acetate; 10, stearic acid; (B) 1, *o*-methoxycinnamic acid; 2, glycerol; 3, 3-vinyl-1-oxybenzene; 4, *p*-hydroxybenzaldehyde; 5, erythritol; 6, vanillin; 7, *p*-hydroxybenzoic acid; 8, xylitol; 9, 4,7-dihydroxyvanillic acid; 10, azelaic acid; 11, mannitol; 12, *p*-coumaric acid (4-hydroxycinnamic acid); 13, palmitic acid; 14, isoferulic acid; 15, *N*-indolyl acetate; 16, stearic acid.

the percent of DPPH radical scavenging activity increased from 47.74 to 81.60%. However, simple heating did not affect the radical scavenging property of rice hull extract. This indicated that the increase was not induced by heat but by the FIR ray. The DPPH radical scavenging activity was closely related to the amount of TPC in rice hull extracts.

Antioxidant activities of rice hull samples were determined by a TBARS method (**Figure 1C**). The antioxidant activity of rice hull extract increased after FIR radiation as in DPPH radical scavenging activity, whereas simple heating had no effect. Our previous studies showed that electromagnetic wave treatments such as microwave and γ-ray had no effect on the antioxidant activity of agricultural byproducts (*21*−*23*).

Antioxidant Compounds in Rice Hull Extracts. Only a few phenolic compounds (*o*-methoxycinnamic acid, *p*-coumaric acid, and *N*-indolyl acetate) were detected in IRH, and *p*-coumaric acid was the predominant compound of IRH (**Figure 2A**). FRH, on the other hand, contained many more phenolic compounds such as 3-vinyl-1-oxybenzene, *p*-hydroxybenzaldehyde, vanillin, *p*-hydroxybenzoic acid, 4,7-dihydroxyvanillic acid, and isoferulic acid, in addition to the phenolic compounds detected in IRH (**Figure 2B**). The TPC in rice hull extracts increased significantly by FIR radiation from 0.12 to 0.20 mM (**Figure 1A**). As in the chromatogram of IRH, *p*-coumaric acid (4-hydroxycinnamic acid) also was the predominant phenolic compound in FRH. Therefore, FIR radiation was effective in generating more antioxidant phenolic compounds from rice hull.

Various phenolic compounds with antioxidant activities have been reported in rice hull extracts: Ramarathnam et al. (*24*) reported that isovitexin, identified as a *C*-glycosyl flavonoid, was a main antioxidant compound in methanolic ($MeOH/H_2O$, 50:50) rice hull extract. Asamarai et al. (*6*) identified several phenol compounds, such as cinnamic acid and benzoic acid derivatives, from methanolic ($MeOH/H_2O$, 75:25) rice hull extract using GC-MS. A few alcoholic carbohydrates (erythritol, xylitol, and mannitol) and fatty acids (palmitic and stearic acid) also were shown in the chromatogram of FRH. Cinnamic acid derivatives, such as *o*-methylcinnamic acid and *p*-coumaric acid,

are well-known for their antioxidant activities (*25*). Hydroxycinnamic acids present as esters of quinic acid or glucose in plants. Ferulic and *p*-coumaric acids in plants may be linked to cell wall polysaccharides, ligin, suberin, and cutin, and diferulic acid serves as a cross-link between pentosan chains (*26*). Alkaline treatment of rice bran liberates ferulic acid by breaking the covalent bonds between the carboxyl group of ferulic acid and proteoglycan (*27*). Several phenolic carbohydrate esters, such as ferulic acid esters of arabinoxylan fragments and diferulic acid, were isolated from enzymatic digestion of rice endosperm cell wall (*28*).

Conclusions. FIR radiation of rice hull liberated phenolic compounds and thus increased the amounts of active compounds in extracts. FIR radiation released more phenolic compounds in rice hull and thus increased the antioxidant activity of rice hull extract. Although covalently bound phenolic compounds in oat hulls could be released by enzymatic treatments (*29*), FIR radiation with its simple process would be more effective in releasing antioxidant compounds from agricultural byproducts such as rice hull at an industrial scale.

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