

Effect of Far-Infrared Irradiation on the Antioxidant Activity of Defatted Sesame Meal Extracts

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To determine the effect of far-infrared (FIR) irradiation on the antioxidant activities of sesame meal, half of sesame seeds were FIR-irradiated and then oil was extracted from the seeds. The resulting defatted sesame meal (DSM) was extracted with methanol, and the antioxidant activities of methanolic extract were determined. FIR irradiation of sesame seeds for 30 min increased the total phenol content from 34.0 to 59.0 μM and radical scavenging activity of DSM extracts from 26.40 to 68.76%. The induction time of lipid oxidation of oil added to extracts was also retarded from 0.82 to 0.96 h. According to the gas chromatography–mass spectrometry analysis, several low molecular weight phenolic compounds, such as *p*-hydroxy benzoic acid, vanillic acid, *p*-coumaric acid, isoferulic acid, and *o*-coumaric acid, were frequently detected in FIR-irradiated DSM extracts as compared to unirradiated ones. These results indicated that FIR irradiation of sesame seeds increased the antioxidant activity of methanolic extracts of DSM.

KEYWORDS: Defatted sesame meal extract; far-infrared irradiation; antioxidant activity; phenolic compounds

INTRODUCTION

Sesame (*Sesamum indicum* L.) seed is one of the most important oil seed crops in the world. Sesame seed is composed of 45–50% lipid, 5–6% moisture, 10–15% carbohydrate, 5–6% ash, 4–5% fiber, and 15–20% protein. Sesame seed is not only a good source for edible oil but is also widely used in baked foods and confectionery products (1). Sesame oil is highly resistant to oxidative changes during storage due to high concentrations of various antioxidants in it. Sesame oil significantly decreased lipid peroxidation and serum nitrite levels and attenuated cecal ligation and puncture-induced hepatic injury in rats (2). In addition, compounds in sesame oil showed anticarcinogenic (3), blood pressure lowering (4), and serum lipid lowering effects (5, 6) in experimental animals and humans.

Defatted sesame meal (DSM) obtained from oil extraction is either used as a feed ingredient for domestic animals or composted. It has been reported that sesame oil extracted from seeds with hulls is more stable than that from dehulled seeds (7), indicating that antioxidant components may exist in the sesame hull. Chang and others (8) reported that the sesame coat has a significant antioxidant activity in various in vitro systems. Feeding defatted sesame oil meal showed a hypocholesterolemic effect in rabbits (9) and promoted the detoxifying capability of ethanol-medicated rats (10).

Antioxidants such as flavonoids, tannins, coumatins, curcuminoids, xanthon, phenolics, and terpenoids are found in various plant products (e.g., fruits, leaves, seeds, and oils) (11). For this reason, there is a growing interest in separating these plant antioxidants and using them as natural antioxidants. Some components from fruit and vegetable extracts showed as strong antioxidant effects as synthetic antioxidants in model systems (12). In general, the seed coat plays an important role in protecting seeds from oxidative damage because the seed coat possesses a large quantity of endogenous antioxidants such as phenolic compounds (13, 14).

Many natural plant antioxidants, however, exist either as a bound form to high molecular weight compounds or as a part of repeating subunits of high molecular weight polymers. Several methods including far-infrared (FIR) irradiation are known to liberate and activate low molecular weight natural antioxidants in plants (15). FIR rays are defined as electromagnetic waves, which have wavelengths longer than 4 μm but shorter than microwaves ($\lambda > 0.1$ cm). FIR rays are biologically active (16) and transfer heat to the center of materials evenly without degrading the constituent molecules on the surface. FIR, however, may have the capability to cleave covalent bonds and liberate antioxidant compounds such as flavonoids, carotene, tannin, ascorbate, flavoprotein, or polyphenols from repeating polymers (15, 17). Therefore, the objective of this research was to elucidate the effect of FIR irradiation on the antioxidant activities of extracts from DSM.

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Table 1. Effect of FIR Irradiation on TPCs, DPPH RSA, and Induction Time (IT) of Lipid Peroxidation of Methanol Extracts from DSMs

	FIR irradiation time (min)									SEM
	0	10	20	30	40	50	60	90	120	
TPC (μM)	33.98 ^a	52.52 ^a	47.07 ^f	58.76 ^d	68.05 ^c	69.40 ^c	72.85 ^b	73.25 ^b	79.74 ^a	0.717
RSA (%)	26.40 ^b	63.26 ^f	60.29 ^g	68.76 ^e	74.04 ^d	75.25 ^c	76.35 ^b	76.24 ^b	77.78 ^a	0.304
IT (h)	0.643 ^b	0.570 ^b	0.573 ^b	0.623 ^b	0.720 ^a	0.757 ^a	0.777 ^a	0.791 ^a	0.793 ^a	0.019

^{a–h}Different letters within a row are significantly different ($P < 0.05$), $n = 3$.

MATERIALS AND METHODS

Materials. White sesame seeds (*S. indicum* L.) were purchased from a local market in Masan, South Korea. 2-Thiobarbituric acid, tannic acid, lard oil, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO). Folin–Ciocalteu reagent was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

FIR Irradiation onto Sesame Seed. Whole sesame seeds (20 g) in a wooden box (50 cm \times 40 cm \times 40 cm) were irradiated using a FIR heater (35 cm \times 10 cm, output 300 W, Hakko Electric Machine Works Co., Ltd., Nagano, Japan), which emitted radiation at the wavelength range of 2 and 14 μm at 200 $^{\circ}\text{C}$ for 10, 20, 30, 40, 50, 60, 90, or 120 min. The sample holding tray in the middle of treating box was placed to face a FIR heater in a parallel position, and the distance between the sample and the heater was 5 cm. After irradiation, the seeds were allowed to cool to ambient temperature before oil extraction.

Preparation of DSM and Its Methanolic Extract. The irradiated sesame seeds (20 g) were crushed, and oil was extracted using an electric oil extractor (model Do-9001; Donga Oscar Co., Gimhae, Korea). The remaining oil in residue was extracted with 100 mL of *n*-hexane by vigorous shaking in a three-cycle shaker, filtered through Whatman no. 1 filter paper. The residue (DSM) was collected and dried at room temperature.

The DSM (10 g) was extracted with 100 mL of methanol in a shaking incubator overnight at room temperature and filtered through Whatman no.1 filter paper. The residue was re-extracted under the same conditions. The first and second extracts were pooled and filtered through a Whatman nylon membrane filter (0.2 μm , Millipore filtration kit MA 01730, Millipore Co., Bedford, England). The methanol in the filtrate was evaporated under vacuum at 40 $^{\circ}\text{C}$ using a rotary evaporator (model Eyela N-1000; Tokyo Rikakikai Co., Tokyo, Japan). The dried methanolic extract of DSM was redissolved in methanol (1 mg/mL) and used for further analyses.

Total Phenolic Content (TPC). The TPC of DSM extracts was determined using a method described by Gutfinger (18). One milliliter of DSM extract (1 mg/mL) was mixed with 1 mL of 50% Folin–Ciocalteu reagent and 1 mL of 2% Na_2CO_3 and centrifuged at 13400g for 5 min. The absorbance at 750 nm was measured with a spectrophotometer (model UV-1601; Shimadzu, Tokyo, Japan) after 30 min of incubation at room temperature. TPC was expressed as a tannic acid equivalent.

Radical Scavenging Activity (RSA). The effect of extracts on DPPH RSA was estimated using a method described by Blois (19). After mixing 1 mL of 0.041 mM DPPH in ethanol with 0.2 mL of DSM extract (1 mg/mL) for 10 min, the absorbance was measured at 517 nm. RSA was expressed as a percentage inhibition and was calculated using the following formula:

$$\% \text{ DPPH RSA} = (1 - \text{sample OD/control OD}) \times 100$$

Rancimat Method. The peroxidation induction period of lard was affected by the addition of antioxidant was determined according to the method of Chen and Ho (20). A Metrohm 793 Rancimat instrument (Herisan, Switzerland) was used to determine the oxidation of lard with or without the addition of sesame meal extract. Oxidation was induced at 120 $^{\circ}\text{C}$ with air at a flow rate of 20 L/h. One milliliter of each sample (10 mg/mL) was added to lard (2.5 g) and then mixed vigorously with vortex for 8 s immediately before starting the Rancimat measurement.

Composition of DSMs Extracts. Each DSM extract received either unirradiation or 30 min of FIR irradiation and were dissolved in ethanol (200 mg/mL) and centrifuged at 13400g 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 $^{\circ}\text{C}$) for 15 min (21). The compounds in the DSM extract were identified using gas chromatography/mass spectrometry (model GC6890/MS5973; Hewlett-Packard Co., Wilmington, DE). A split inlet (99:1) was used to inject samples (5 μL) into an HP-5 column (30 m, 0.32 mm i.d., 0.25 μm film; Hewlett-Packard Co.). A ramped oven temperature was used (100 $^{\circ}\text{C}$ for 2 min, increased to 270 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}/\text{min}$, and held for 6 min). The inlet temperature was 250 $^{\circ}\text{C}$, and the carrier gas was He at a constant flow of 1.5 mL/min. The ionization potential of the mass selective detector was 70 eV, and the scan range was 19.1–400 m/z . Identification of compounds was achieved by comparing mass spectral data of samples with those of the Wiley library (Hewlett-Packard Co.).

Statistical Analysis. All measurements were done in triplicate, and the analysis of variance was conducted by the General Linear Model procedure of SAS (SAS Institute) software (22). Student–Newman–Keul's multiple range tests and *t*-tests were used to compare the differences among mean values ($P < 0.05$).

RESULTS AND DISCUSSION

Effect of FIR Irradiation on the Antioxidant Activities of DSM Extracts. The effects of FIR irradiation on the antioxidant activities of DSM extracts were determined by TPC, RSA, and induction time for lipid peroxidation. Phenolic compounds are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because they form stable radical intermediates (23, 24). The TPC in methanolic extract of DSM increased significantly by FIR irradiation (Table 1). TPC in DSM increased from 34.0 μM in unirradiated control to 59.0 μM with 30 min of FIR irradiation. The TPC increased with the increase of FIR irradiation time but reached a plateau after about 60 min of irradiation. The DPPH RSA of the DSM extract also significantly increased with FIR irradiation (Table 1). After FIR irradiation for 30 min, the RSA of DSM extracts increased from 26.40 to 68.76%. RSA also increased with increasing FIR irradiation time and reached a plateau after about 60 min of irradiation in the TPC. The Rancimat method is commonly used to evaluate the potency of various antioxidants (25). The longer the induction period of lard, the stronger is the antioxidant activity of the compound. The induction time of lard increased from 0.82 to 0.96 h when sesame seeds were FIR-irradiated for 60 min (Table 1).

Roasting conditions of sesame seeds affect the antioxidant activity of sesame oil. Kim (26) reported that the storage stability of unroasted sesame oil was low, but roasting of sesame seed at 170 $^{\circ}\text{C}$ or higher significantly increased the stability of sesame oil. The highest sesamol content and storage stability of sesame oil was achieved when sesame seeds were roasted at 200–220 $^{\circ}\text{C}$. Yoshida and Takagi (27) reported that sesamol, a potent phenolic antioxidant, increased as the roasting temperature of sesame seeds increased to 180 $^{\circ}\text{C}$ or higher. We also found that roasting temperature and time of sesame seeds affected the antioxidant activity of DSM significantly (28). Simple heating increased the antioxidant activity of DSM (28) but not rice hull (17). FIR irradiation cleaved and liberated phenolic compounds

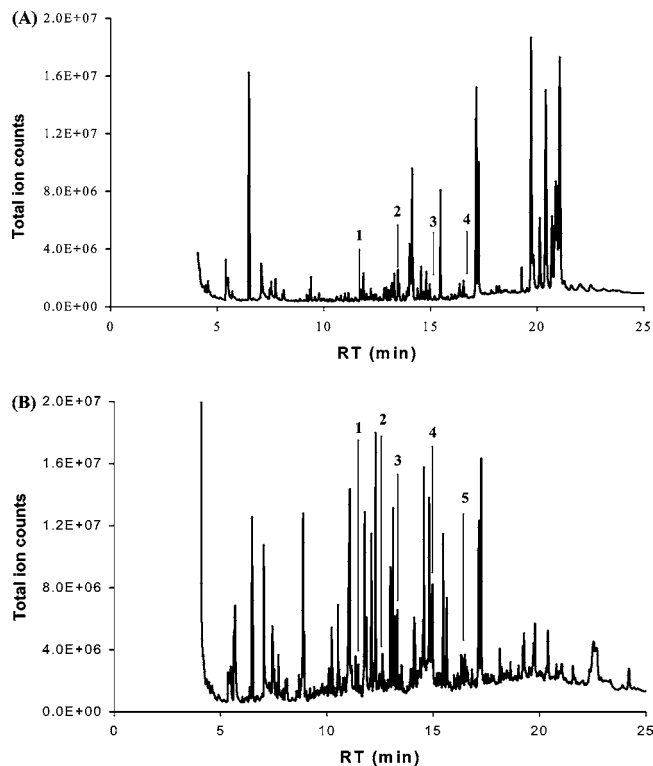


Figure 1. Gas chromatograms of DSM extracts, unirradiated (A) and FIR irradiated (B) at 200 °C for 30 min after BSA derivatization. The peaks in panel A are as follows: 1, *p*-hydroxybenzoic acid; 2, vanillic acid; 3, *p*-coumaric acid; and 4, isoferulic acid. The peaks in panel B are as follows: 1, *p*-hydroxybenzoic acid; 2, *o*-coumaric acid; 3, vanillic acid; 4, *p*-coumaric acid; and 5, isoferulic acid.

and increased the antioxidant activity of DSM and rice hull (17). These results indicate that phenolic antioxidant compounds present in several forms and effective processing steps for liberating antioxidant compounds from plants should be different depending upon plant species.

Identification of DSM Extracts. Phenolic compounds are the most active antioxidant derivatives in plants (29). Generally, the outer layers of a plant such as the peel, shell, and hull contain large amounts of polyphenolic compounds to protect inner materials. A number of phenolic acids are linked to various cell wall components such as arabinoxylans and proteins (30). The phenolic compounds in DMS were reported to contain 75.3% soluble esters and 24.7% of insoluble residue form (31).

Several low molecular weight phenolic compounds such as *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, isoferulic acid, and *o*-coumaric acid were detected in the methanolic extracts of DSM (Figure 1). In addition to sesamol and tocopherols, many other antioxidant compounds such as polyphenol compounds were present in sesame seeds and DSM (32). Fenton and others (33) reported that polyphenol compounds such as caffeic, *p*-coumaric, ferulic, *p*-hydroxybenzoic, sinapic, *trans*-cinnamic, and chlorogenic acids were present in the hydrolysate of rapeseed meal. There are slight differences in the kinds of phenolic compounds detected in unirradiated and FIR-irradiated DSM extracts. However, the amounts of *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, and isoferulic acid were higher in FIR-irradiated samples than the control, and *o*-coumaric acid was newly detected (Table 2).

It was reported that FIR irradiation and/or fermentation of plant increased antioxidant activity of its extract because those treatments liberated low molecular antioxidant compounds from

Table 2. Detected Phenolic Compounds in the Methanolic Extracts of DSM from FIR-Irradiated and Nonirradiated Sesame Seeds

compound	total ion counts $\times 10^4$	
	nonirradiated	FIR irradiated
<i>p</i> -hydroxy benzoic acid ^a	483	2842
<i>o</i> -coumaric acid ^a	0	1233
vanillic acid ^a	4563	8899
<i>p</i> -coumaric acid ^a	2371	25286
isoferulic acid	3027	3437
total ^a	10444	41697

^a The *t*-test shows the significant difference between two samples ($P < 0.05$), $n = 3$.

the repeating subunits of high molecular weighted polymers (14, 19, 20). This study also showed that FIR irradiation of sesame seeds helped liberate phenolic compounds from DSM and increased the antioxidant activity of DMS extract. FIR irradiation is simple and effective in releasing antioxidant compounds from agricultural byproducts and is easy to scale-up for industrial use.

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