Effects of Microwave Heat, Packaging, and Storage Temperature on Fatty Acid and Proximate Compositions in Rice Bran

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The effect of microwave heat, packaging methods, and storage temperatures on proximate and fatty acid compositions of rice bran during 16 weeks of storage was examined. Freshly milled raw rice bran was adjusted to 21% moisture content and microwave heated for 3 min. Raw and microwave-heated brans were packed in zipper-top bags and/or vacuum-sealed bags and stored at 4−5 and/or 25 °C for 16 weeks. The moisture content decreased significantly from an initial 8.4 to 6.4% in microwave-heated samples regardless of packaging methods and storage temperatures. Protein, fat, linoleic, and linolenic contents did not change significantly in all raw and microwave-heated samples during 16 weeks of storage. The microwave-heated rice bran packed in zipper-top bags can be stored at 4−5 °C for up to 16 weeks without adverse effect on proximate and fatty acid composition quality under the conditions employed in this study.

Keywords: *Rice bran; microwave heat; proximate composition; fatty acid composition; storage; packaging*

INTRODUCTION

Rice bran is rich in nutrients with a protein content of 14−16%. It is also high in lysine content. The reported protein efficiency ratio (PER) is 1.6−1.9, compared with the value of 2.5 for casein (Saunders, 1990). Major carbohydrates in rice bran are hemicellulose (8.7−11.4%), cellulose (9−12.8%), starch, and β-glucan (1%). Rice bran contains 15−23% oil.

Crude rice bran oil contains 3−4% waxes and ∼4% unsaponified lipids. Oryzanol and vitamin E, potent antioxidants, are present in rice bran (Saunders, 1985). Rice bran is rich in B-complex vitamins. The mineral composition of rice bran depends on the nutrient availability of the soil in which the crop is grown. Rice bran also contains small quantities of the minerals: iron, aluminum, calcium, chlorine, sodium, potassium, magnesium, manganese, phosphorus, silicon, and zinc. Bran contains 80% of rice kernel iron (Lu and Luh, 1991).

Three major fatty acids, palmitic acid (12−18%), oleic acid (40−50%), and linoleic acid (30−35%), make up 90% of the total fatty acids of rice bran oil. Fatty acids are important in a number of functions in the human body. Linoleic acid, with two double bonds, is one of the essential fatty acids and is found at high concentrations in vegetable oil and, to a smaller extent, in meats. It is the basic structural element of fats in the body, and, therefore, is one of the essential nutrients. Rice bran is a good source of linoleic acid. The amount of linoleic acid in a normal diet is ∼2% of the total calories. Two to three tablespoons of soybean oil will supply the needed amount (Labuza, 1977). With rice bran oil (30−35% linoleic acid), the required amount is ∼3−4 tablespoons per day. Monounsaturated fatty acids, especially in a cis-form such as oleic acid, appear to exert a neutral effect or to be mildly hypocholesterolemic (Kris-Etherton and Yu, 1997). Storage of rice bran, especially at room temperature, for extended periods leads to degradation of triglycerides in the oil and ultimately to the formation of off-flavors and odors. Lipid peroxidation by lipases and lipoxygenases is thought to be the primary cause for bran degradation (Ramarathnam et al., 1989). Several methods have been reported that combat this deterioration. Extrusion cooking (Lin and Carter, 1973; Sayer et al., 1982), microwave heat (Hafez et al., 1985a; Tao et al., 1993), and γ-radiation (Hafez et al., 1985b; Ramarathnam et al., 1989) have been employed with different degrees of success. Recently, Ramezanzadeh et al. (1999a,b) showed that rice bran deterioration could be effectively reduced by an initial microwave heat treatment followed by packaging of the bran in zipper-top bags and storing the bags at 4−5 °C. This method was found to be effective for at least 16 weeks of storage time. Control over the development of bran rancidity could potentially prevent unwanted changes in bran nutrients such as fatty acid composition, essential fatty acid (linoleic acid) content, and contents of protein, fat, moisture, minerals (ash), and carbohydrate. This study was a continuation of two previously published articles (Ramezanzadeh et al., 1999a,b). The objective was to determine the effect of microwave treatment, packaging

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Table 1. Fatty Acid Composition of Microwave-Heat-Stabilized and Raw Rice Bran at 0 and 16 Weeks of Storage

			fatty acid %[a]				
			$C_{16:0}$ palmitic	$C_{18:0}$ stearic	$C_{18:1}$ oleic	$C_{18:2}$ linoleic	$C_{18:3}$ linolenic
raw (control)	0 weeks		14.5 ± 0.5^{a}	2.3 ± 0.2^{a}	47.9 ± 0.2^{a}	32.4 ± 0.5^{a}	1.1 ± 0.0^{a}
	16 weeks	ZRT[b]	19.9 ± 0.1^{d}	2.7 ± 0.0^{a}	42.0 ± 0.2^{c}	32.4 ± 0.1^{a}	1.2 ± 0.0^{a}
		ZRef	17.1 ± 3.0^{b}	2.6 ± 0.5^{a}	45.0 ± 4.0^{b}	30.5 ± 4.3^{a}	1.1 ± 0.2^{a}
		VRT	17.0 ± 2.0^{b}	2.7 ± 0.3^{a}	45.5 ± 2.0^{ab}	33.4 ± 0.7^{a}	1.1 ± 0.2^{a}
		VRef	18.5 ± 0.2^{c}	2.5 ± 0.0^{a}	45.9 ± 0.1^{ab}	30.2 ± 0.1^{a}	1.1 ± 0.0^{a}
microwave	0 weeks		14.3 ± 0.1^{a}	2.2 ± 0.1^{a}	48.0 ± 0.1^{a}	32.6 ± 0.2^{a}	1.1 ± 0.0^{a}
	16 weeks	ZRT	18.1 ± 0.5^{c}	2.5 ± 0.1^{a}	46.7 ± 0.5^{ab}	29.9 ± 0.3^{a}	1.1 ± 0.0^{a}
		ZRef	18.1 ± 0.4^{c}	2.5 ± 0.1^{a}	46.0 ± 0.3^{ab}	30.3 ± 0.2^{a}	1.2 ± 0.0^{a}
		VRT	14.7 ± 0.3^{a}	2.8 ± 0.0^{a}	44.2 ± 0.2^{ab}	34.9 ± 0.1^{a}	1.3 ± 0.0^{a}
		VRef	18.3 ± 0.0^{c}	2.4 ± 0.0^{a}	45.9 ± 0.0^{ab}	30.4 ± 0.0^{a}	1.2 ± 0.0^{a}

[a] Means within a column with different letters are different ($p < 0.05$). [b] Z = zipper-top bags; V = vacuum bags; RT = room temperature (25 °C); Ref = refrigerated temperature (4−5 °C).

methods, and storage temperatures on proximate and fatty acid compositions in rice bran during 16 weeks of storage.

MATERIALS AND METHODS

Rice Bran Collection. Long-grain rice (Lemont) cultivated at the Louisiana State University Rice Experiment Station, Crowley, LA, was used for this study. The rice samples were dehusked and milled using a Satake (friction type) milling system (Satake USA, Houston, TX). Rice bran was collected in a barrel lined with a black plastic bag. Dry ice was added continuously to the barrel during the milling process to prevent hydrolysis of fatty acids by lipase activity. The bag was tightly tied and stored (within 15 min after milling) in an ultralow freezer (−78 to −80 C°) until the day of sample preparation (within 10 days). The rice bran was sieved with a 20-mesh sieve to remove broken pieces of rice and husks. A thermometer was placed in the rice bran samples to monitor the temperature (i.e., 0−2 °C) during the sieving process.

Microwave Heat Stabilization. One hundred and fifty grams per batch of raw rice bran was heated in a microwave oven (model R3A96, Sharp Electronic Corp., Mahwah, NJ) at 850 W and 2450 MHz. The microwave chamber was preheated at 100% power for 3 min. The moisture content of the raw rice bran was adjusted from 7.5 to 21% by adding deionized water (Tao, 1989; Malekian, 1992). The sample was thoroughly mixed to evenly distribute the water. Each sample was placed in a plastic zipper-top bag (Hefty One Zip multipurpose storage bag, gallon size, Mobile Chemical Co., New York). The sample was spread out evenly to a thickness of 0.5 cm, and the bag was sealed. The sample was heated at 100% power for 3 min. The temperature of the sample after heating in the microwave was 107 ± 2 °C. The sample was allowed to cool to room temperature (∼25 °C) and stored in an ultralow freezer (−78 to −80 °C) until packaging (within 2 days).

Packaging and Storage of Rice Bran. Microwave-heated and raw rice bran samples were divided in half. Representative samples, weighing 70−75 g each, were packed in plastic zipper-top bags or nonpermeable vacuum bags, which were sealed using the TurboVAC vacuum machine (model SB 600, Howden Food Equipment, Oldenzaal, Netherlands). The bags were marked for storage times of 0, 4, 8, 12, and 16 weeks. Half of the bags were stored at 4−5 °C and the remainder stored at 25 °C. The storage temperatures were monitored daily.

Fatty Acid Composition. The fatty acid composition was determined in duplicate by gas chromatography (GC) according to American Oil Chemists' Society Method Ce 1b-89 (AOCS, 1991) with modifications. After the fat had been extracted and dissolved in 10 mL of petroleum ether, 100 μL was saved in a glass tube in the freezer (−25 °C) until the day of analysis.

Internal standard (IS) used was C:23 (N-23M, NuChek Prep Inc., Elysian, MN) and was prepared by weighing 25 mg of C:23 into a 25 mL volumetric flask, which was brought to volume with iso-octane. The calibration standard (GLC-85, NuChek Prep Inc.) of fatty acid methyl esters was prepared by emptying the contents of an ampule received (100 mg) in a 10 mL volumetric flask and bringing it to volume with hexane. Further dilutions were made to a concentration of 1 mg/mL.

To each sample was added 100 μL of IS. The solvent was evaporated between 40 and 45 °C under nitrogen gas. Then 1.5 mL of 0.5 N NaOH was added to each sample, blanketed with nitrogen, capped tightly, mixed, and heated with a vortex/heater at 100 °C for 5 min. The samples were cooled, and 2 mL of BF_3/methanol reagent (boron trifluoride/methanol 14% solution, B-1252, Sigma Chemical Co., St. Louis, MO) was added to each sample, blanketed with nitrogen, capped tightly, mixed, and heated at 100 °C for 30 min. The samples were cooled to 30−40 °C, and 1 mL of iso-octane was added to each sample. The samples were blanketed with nitrogen, capped tightly, and mixed for 30 s. Immediately, 5 mL of saturated NaCl was added, blanketed with nitrogen, capped tightly, and agitated. The samples were cooled to room temperature until the iso-octane layer was separated from the aqueous layer. The iso-octane layer was transferred to another vial, blanketed with nitrogen, and capped. The methanol/water phase was extracted again with an additional 1 mL of iso-octane, and the two extracts were combined and evaporated to 1 mL. The solubilized extract was transferred to a vial, capped, and then injected to the GC immediately.

The GC used was a Hewlett-Packard 5890 with an autosampler and equipped with a flame ionization detector (FID). The column used was a fused silica capillary column, 30 m in length, 0.25 mm i.d., and 0.20 μm film thickness (Supelco SP-2380, Supelco, Inc., Bellefonte, PA). The initial column temperature was programmed at 50 °C, held for 1 min, increased at 10 °C/min to 150 °C, held for 10 min, increased at 2 °C/min to 175 °C, held for 10 min, increased at 5 °C/min to 225 °C, and held for 7 min. The injector and detector port temperatures were maintained at 170 and 270 °C, respectively, and helium was used as a carrier gas. Fatty acids were identified by comparing their retention times with that of the calibration standard.

Proximate Analysis. Proximate composition of rice bran at 0 and 16 weeks of storage was determined in duplicate using AOAC methods (AOAC, 1991) for protein (Method 992.5), fat (Method 945.16A), moisture (Method 985.14), and ash (Method 920.153). Carbohydrate (percent) was determined by difference: [100 − (% protein + % fat + % moisture + % ash)].

Statistical Analysis. The analysis of variance (ANOVA) was performed on all values using the Statistical Analysis System (SAS) program version 6.12 (SAS, 1997). To compare the mean of the results, the Student−Newman−Keuls (SNK) test was done at $\alpha = 0.05$.

RESULTS AND DISCUSION

Fatty Acid Content. Oleic acid, linoleic acid, and palmitic acid are the dominant fatty acids in raw and microwave-heated rice bran (Table 1). Distribution of fatty acids (Table 1) did not show any significant difference between raw and microwave-heated rice bran during 16 weeks of storage except for the oleic and

Table 2. Effect of Microwave Heat Stabilization on Percent Linoleic Acid of Rice Bran Packed in Zipper-Top Bags (Z) and Vacuum Packed (V) Stored at Room Temperature (RT) and in the Refrigerator (Ref) during 16 Weeks of Storage

		% linoleic acid[a]				
		0 weeks	4 weeks	8 weeks	12 weeks	16 weeks
raw (control)		32.4 ± 0.1[a]	32.4 ± 0.1[a]	32.4 ± 0.1[a]	32.4 ± 0.1[a]	32.4 ± 0.1[a]
	ZRT		34.4 ± 1.0[a]	33.1 ± 0.4[a]	31.0 ± 0.6[a]	32.4 ± 0.1[a]
	ZRef		32.1 ± 1.9[a]	32.8 ± 0.3[a]	31.5 ± 0.1[a]	30.5 ± 4.2[a]
	VRT		33.9 ± 0.0[a]	32.3 ± 0.5[a]	31.4 ± 0.2[a]	33.4 ± 0.6[a]
	VRef		33.3 ± 0.2[a]	33.0 ± 0.7[a]	31.7 ± 0.1[a]	30.2 ± 0.1[a]
microwave		32.6 ± 0.1[a]	32.6 ± 0.1[a]	32.6 ± 0.1[a]	32.6 ± 0.1[a]	32.6 ± 0.1[a]
	ZRT		34.0 ± 0.1[a]	33.4 ± 0.5[a]	32.2 ± 1.1[a]	29.9 ± 0.3[a]
	ZRef		33.5 ± 0.0[a]	33.4 ± 0.7[a]	31.0 ± 1.2[a]	30.3 ± 0.2[a]
	VRT		33.7 ± 0.2[a]	33.1 ± 0.5[a]	31.6 ± 0.1[a]	34.9 ± 0.1[a]
	VRef		33.7 ± 0.1[a]	34.1 ± 0.0[a]	31.8 ± 0.1[a]	30.4 ± 0.0[a]

[a] For each row, means with different letters are different ($p < 0.05$).

Table 3. Proximate Composition (Percent) of Rice Bran Packed in Zipper-Top Bags (Z) and Vacuum Packed (V) Stored at Room Temperature (RT) and in the Refrigerator (Ref) during 16 Weeks of Storage

			protein[a]	fat[a]	moisture[a]	ash[a]	carbohydrate[a]
raw (control)	0 weeks		17.1 ± 0.6[a]	16.4 ± 0.0[a]	7.5 ± 0.1[b]	7.4 ± 0.1[bc]	51.7 ± 0.7[a]
	16 weeks	ZRT	17.4 ± 0.1[a]	16.9 ± 0.0[a]	7.0 ± 0.0[bc]	8.3 ± 0.2[a]	50.4 ± 0.0[b]
		ZRef	17.0 ± 0.2[a]	17.6 ± 1.1[a]	9.3 ± 0.1[a]	7.9 ± 0.1[ab]	48.2 ± 0.6[c]
		VRT	17.5 ± 0.2[a]	16.9 ± 0.2[a]	6.5 ± 0.2[c]	8.4 ± 0.2[a]	50.8 ± 0.4[b]
		VRef	17.0 ± 0.1[a]	16.9 ± 0.2[a]	8.5 ± 0.2[a]	7.8 ± 0.3[bc]	49.8 ± 0.7[bc]
microwave	0 weeks		17.5 ± 0.4[a]	17.5 ± 0.4[a]	8.4 ± 0.4[a]	7.6 ± 0.1[bc]	48.9 ± 0.3[bc]
	16 weeks	ZRT	17.7 ± 0.4[a]	17.9 ± 0.3[a]	6.4 ± 0.2[c]	8.4 ± 0.1[a]	49.7 ± 0.5[bc]
		ZRef	17.9 ± 0.3[a]	17.3 ± 0.0[a]	6.3 ± 0.1[c]	8.2 ± 0.1[a]	50.3 ± 0.3[b]
		VRT	18.0 ± 0.1[a]	17.0 ± 0.1[a]	6.3 ± 0.6[c]	8.4 ± 0.1[a]	50.4 ± 0.6[bc]
		VRef	17.5 ± 0.3[a]	17.1 ± 0.1[a]	7.6 ± 0.7[b]	8.3 ± 0.0[a]	49.5 ± 0.9[bc]

[a] Means within a column with different letters are different ($p < 0.05$).

palmitic acid contents. Distribution of fatty acids (Table 1) for raw rice bran was similar to data reported by Saunders (1990).

Palmitic acid of raw and microwave-heated bran increased from ~14% at week 0 to an average of 17% at week 16, except for the microwave-heated rice bran in the vacuum bags stored at room temperature (MVRT) samples (Table 1). Stearic acid and linolenic acid did not change significantly. Oleic acid content decreased after 16 weeks of storage in both raw and microwave-heated samples; significant decreases were observed for raw rice bran stored in the zipper-top bags, regardless of storage temperature. Linoleic acid, the preferred substrate for lipoxygenase (LOX), decreased from an initial value of 32.6 to 29.9% in microwave-heated samples (ZRT) after 16 weeks of storage. Table 2 shows there was no significant ($p < 0.05$) change in linoleic acid at each storage interval. Hafez et al. (1985a) noticed no quantitative differences in fatty acid composition of raw and microwave-heated soybeans after heating the samples for 15 min, but protein digestibility decreased. Yoshida et al. (1991) reported that microwave heat acts differently on fatty acids from tocopherols: the higher the degree of unsaturation, the greater the chemical changes in ethyl esters. Ethyl linoleate showed the highest chemical changes. In our study, microwave-heated samples showed more change in unsaturated (oleic and, to a lesser extent, linoleic acid) than other fatty acids. Hafez et al. (1985b) noticed no significant ($p < 0.05$) changes in fatty acids (C16: 0, C18: 0, C18: 1, and C18: 2) at different radiation doses of γ-irradiation of soybean. They reported that high radiation doses caused a decrease in linoleic acid. Increases in moisture content and radiation dose did not affect fatty acids except for a reduction in linoleic acid content. γ-Irradiation of rice seeds with intact hull minimized the increase in the amount of unsaturated fatty acids in the free fatty acid, which are prone to oxidation in the presence of oxygen radicals generated by γ-irradiation.

Proximate Composition. Proximate composition of raw and microwave-heated rice bran is shown in Table 3. There were no significant ($p > 0.05$) changes in protein and fat of either raw or heated rice bran during storage. The data for raw bran agree with earlier findings of Wadsworth and Koltun (1986) and Yeo and Shibamoto (1991).

Moisture content was significantly ($p < 0.05$) higher in microwave-heat-stabilized samples than raw samples at 0 weeks. Raw samples with moisture content of 7.0% were adjusted to 21% before microwave heating. After microwave heating, the moisture content was 8.4%. The color of the raw bran was light tan, and after microwave heating, the color was darker with a toasted aroma. These changes could be due to a slight browning reaction. According to Yeo and Shibamoto (1991), browning intensities of an L-cysteine/D-glucose model system with microwave irradiation for 2.5 min at 22% moisture content were significant compared to samples with 14% moisture content. They reported that the reduction in the moisture content before and after heating and the production of darker color are because, at the initial stage of irradiation, the source of energy is solely or predominantly due to microwave irradiation, as there are more polarized dipoles (such as water) to undergo rotation and absorb microwave energy. However, as the irradiation proceeds, the source of energy is probably due to microwave and thermal effects. In addition, as the irradiation proceeds, water is removed from the system. This dehydration process could favor the formation of brown color and loss of moisture in our samples.

In raw samples the moisture content increased significantly ($p < 0.05$) in the samples packed in zipper-top bags and vacuum bags and stored in the refrigerator, whereas the samples stored at room temperature

had a slight decrease in moisture. However, in the microwave-stabilized samples, the moisture content decreased significantly ($p < 0.05$) for all samples, which could also be a contributing factor for an increase in LOX activity of samples stored at room temperature (Ramezanzadeh et al., 1999b).

Ash and carbohydrate showed significant fluctuations, probably due to changes in other nutrients. The amount of ash increased after 16 weeks of storage in both raw and microwave-heated samples. Carbohydrate showed significant ($p < 0.05$) decrease from an initial value of 51.8 to 48.2% in raw samples packed in zipper-top bags and stored in the refrigerator. However, the microwave-heat-stabilized sample started (0 weeks) at significantly lower carbohydrate content than the raw samples and did not change significantly during 16 weeks of storage. The results from this study agreed with previous findings (Tsen et al., 1977; Malekian, 1992). Microwave heat had little effect on the proximate composition of rice bran packed in the zipper-top bags and vacuum bags and stored in the refrigerator for 8 weeks (Malekian, 1992), as well as the breads made with soy and wheat flour (Tsen et al., 1977).

Conclusions. Fatty acid and proximate compositions did not change drastically in microwave-heated rice bran compared with raw samples kept under similar storage conditions. These results indicate that the stabilization of rice bran by microwave heating can be employed without concern as to deleterious changes to major nutrient concentrations in the bran.

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