Prevention of Oxidative Rancidity in Rice Bran during Storage†

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The effect of microwave heat on lipoxygenase (LOX) activity in rice bran under various storage conditions was examined. Raw rice bran from the long-grain variety Lemont was adjusted to 21% moisture content and heated in a microwave oven at 850 W for 3 min. Raw and microwave-heated rice bran samples were packed in zipper-top bags or vacuum packs and stored at room temperature (25 °C) or in the refrigerator (4−5 °C) for 16 weeks. Samples were analyzed for LOX activity at 4-week intervals. LOX activity did not significantly change from its initial value at week 0 for zipper-top and vacuum-packed samples while stored at 4−5 °C for 12 weeks, but decreased at week 16. Vacuum packing did not show a significant impact on LOX activity during 16 weeks of storage. Microwave-heated samples stored in the refrigerator did not show significant change in LOX activity for up to 12 weeks but showed a significant ($p < 0.05$) decrease at 16 weeks. Results showed that oxidative rancidity of rice bran could be prevented by microwave heating the samples, packing in zipper-top bags, and storing at 4−5 °C for up to 16 weeks.

Keywords: *Rice bran; oxidative rancidity; lipoxygenase activity; storage; microwave heat*

INTRODUCTION

Microwave processing of rice bran results in the inactivation of the lipase responsible for hydrolytic rancidity (Tao, 1989; Malekian, 1992), but no information is available in the published literature as to how the deleterious effect of oxidative rancidity on bran can be controlled during storage. Oxidative rancidity involves a reaction between the lipid and molecular oxygen. The reaction takes place at the double bonds of unsaturated fatty acids and can be accelerated by singlet oxygen, free radicals, metal ions (iron, copper, and cobalt), light, radiation, and enzymes containing a transition metal prosthetic group such as lipoxygenase (LOX) (Barnes and Galliard, 1991). Also, the reactions involved are dependent on fatty acid composition (Nawar, 1985). Unlike lipase, and like most other enzymes, LOX activity is accelerated by the addition of water to cereal products (Barnes and Galliard, 1991).

LOX specifically oxygenates polyunsaturated fatty acids and/or their esters and acylglycerols containing the *cis,cis*-1,4-pentadiene double-bond system located between carbons 6 and 10 counting from the methyl terminus (Shastry and Raghavendra Rao, 1975). Under appropriate conditions LOX leads to deterioration of fat-soluble vitamins and essential fatty acids (linoleic and linolenic acid) of oils and fats. It also causes off-flavor and off-odor in food because of its reaction with unsaturated fatty acids.

Little research has been done on the kinetics of LOX-catalyzed reactions because of the complicated nature of the system. This study was undertaken to examine the effect of microwave heat on LOX activity in rice bran samples in zipper-top bags and vacuum packs and stored at 25 and 4−5 °C for 16 weeks.

MATERIALS AND METHODS

Rice Bran Collection. Rice variety Lemont (long grain), cultivated at the Louisiana Rice Experiment Station, Crowley, LA, was used for this experiment. The rice samples were dehusked and milled (friction type) by a Satake milling system (Satake USA, Houston, TX) at the Biological and Agricultural Engineering Department of Louisiana State University. 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 the hydrolysis of fatty acids by lipase activity. The bag was tied tightly and was delivered (within 15 min) to the Pennington Biomedical Research Center (PBRC) laboratory. The bags were placed in the ultralow freezer (−78 to −80 °C) until the day of sample preparation (within 10 days). On the day of sample preparation, the samples were 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 (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 power and 2450 MHz. The microwave chamber was preheated at 100% power for 3 min. The moisture content of raw rice bran samples was adjusted from an initial value of 7.4 to 21% by adding water (Tao, 1989; Malekian, 1992). The sample was mixed thoroughly to evenly distribute the water. Each sample was placed in a zipper-top 1 gal storage bag and 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 microwave heating was 107

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± 2 °C. The sample was allowed to cool to room temperature. The samples were stored in an ultralow freezer (−78 to −80 °C) until the day of packaging (within 2 days). Approximately 2450 g of rice bran was used for this experiment.

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 quart size zipper-top bags or in nonpermeable vacuum bags, which were subsequently sealed using the TurboVAC vacuum machine (Model SB 600, Howden Food Equipment, Oldenzaal, The Netherlands). The bags were marked for storage times of 0, 4, 8, 12, or 16 weeks. All bags were also marked for free fatty acids (FFA), LOX activity, and moisture content (at 0 and 16 weeks of storage). Half of the bags were stored at 4−5 °C and the other half at 25 °C. The storage temperature was monitored and recorded daily.

Standard Enzyme Preparation. Soybean lipoxygenase (L 7395, lot 118 F03422) was purchased from Sigma Chemical Co., St. Louis, MO. This standard enzyme contained 110600 units/mg of solid. An enzyme solution was made by adding 11.6 mL of 0.01 M borate buffer, pH 8.5 (Corning pH meter 340, Corning Inc., Corning, NY) to 1 mg of dried standard enzyme to obtain 10000 units of enzyme/mL of buffer. The enzyme standard solution was used for each analysis as a control.

Enzyme Extraction from Rice Bran Samples. In a beaker, 10 g of rice bran was mixed with 40 mL of 50 mM phosphate buffer, pH 7.0, for 30 min at room temperature. The sample was filtered using two layers of cheesecloth. The filtrate was collected and centrifuged (Model J2- HC, Beckman Instrument Co., Houston, TX) at 9000*g* for 15 min at 5 °C. The supernatant was collected and the volume recorded. Solid ammonium sulfate was added to each sample to obtain 50% saturation (Cooper, 1942). The sample was mixed gently and centrifuged at 9000*g* for 10 min at 5 °C. The volume was recorded. The supernatant was discarded, the precipitate was dissolved in 0.01 M borate buffer, pH 8.5, and the volume was adjusted until the previously recorded volume was obtained for each sample. The solution was collected in a 10 mL disposable syringe and filtered through a 0.2 μm pore size filter. The filtrate was used as the source of enzyme. For each assay, the enzyme extract was diluted (1:1 ratio) with 0.01 M borate buffer, pH 8.5.

LOX Activity Determination. LOX activity was determined in duplicate using the methods as described by Shastry and Raghavendra Rao (1975), Aurand et al. (1987), and Dixon and Webb (1961) with modifications.

LOX Activity. Enzyme activity was measured with a thermostated spectrophotometer (Model DU 640, Beckman Instruments). The instrument was set to record for 5 min, and the temperature was set at 25 °C. The cuvette with 2.9 mL of substrate solution was placed in the spectrophotometer. The enzyme solution (0.1 mL) was rapidly added and mixed well, and the increase in absorbance (*A*) at 234 nm versus the blank was recorded. One unit of LOX activity was defined as the change in absorbance (ΔA) of 0.001/min in a 3 mL volume (1 cm light path) when linoleic acid was used as a substrate (Shastry and Raghavendra Rao, 1975). The rate of increase is usually highest between 0.1 and 3 min, after which time it decreases.

FFA Determination. FFA were determined in duplicate using the method of Hoffpauir et al. (1947) modified to use *m*-cresol purple instead of phenolphthalein as an indicator.

Moisture Content. Moisture content was determined in duplicate using AOAC Method 985.14 (AOAC, 1991).

Statistical Analysis. A completely randomized design was used. To study the main effect for each factor, three-factor (heat treatment, packaging methods, and storage temperature) factorial (2 × 2 × 2) and four-factor (heat treatment, packaging methods, storage temperature, and storage time) factorial (2 × 2 × 2 × 4) arrangements were used for each variable. To compare the mean of the results, the Student−Newman−Keuls (SNK) test was performed at $\alpha = 0.05$. A statistical analysis of variance (ANOVA) was performed on all values using the SAS program version 6.12 (SAS, 1997).

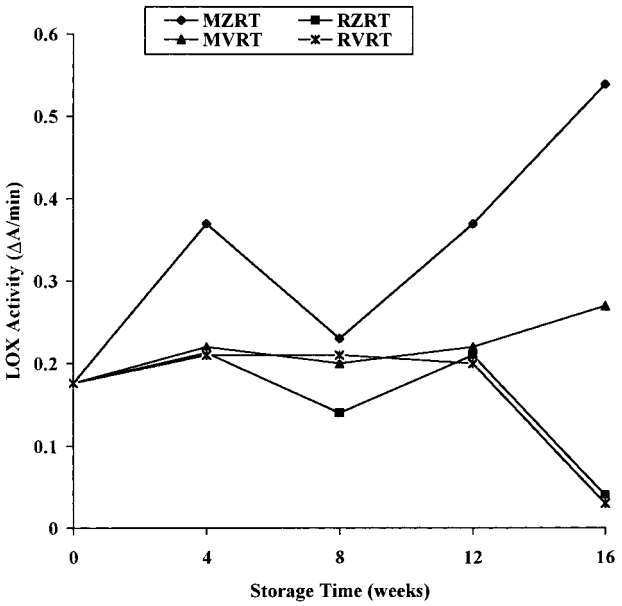

Figure 1. LOX activity in raw (control) and microwave heat stabilized rice bran packed in zipper-top bags or vacuum packed, stored at room temperature (25 °C). M, microwave heat stabilized; R, raw; Z, zipper-top bags; V, vacuum-packed; RT, room temperature.

RESULTS AND DISCUSSION

Effect of Microwave Heat on LOX Activity. Storage of both raw and microwave-heated bran had a significant ($p < 0.05$) effect on LOX activity for most of the samples examined at 25 °C (Figure 1). Significant fluctuations were observed in LOX activity for all samples except microwave-heated bran stored in vacuum packs, which showed only a small increase in activity between 12 and 16 weeks of storage. LOX activity in raw bran, regardless of packaging methods, did not deviate significantly from week 0 during the first 12 weeks of storage. However, these samples underwent a significant decrease in LOX activity between 12 and 16 weeks of storage. Major fluctuations in LOX activity were observed for microwave-heated rice bran packed in zipper-top bags. This sample exhibited significant changes in LOX activity at every storage time. Although a large decrease in LOX activity was seen between 4 and 8 weeks of storage, the trend was generally toward higher activity. At 16 weeks of storage, the LOX activity was over twice as high as in the microwave-heated sample that had been vacuum-packed.

In striking contrast to the samples stored at 25 °C, raw and microwave-heated bran showed very little fluctuation in LOX activity when stored at 4−5 °C regardless of packaging methods (Figure 2). There appeared to be a slight decrease in activity for all samples with increased storage time. In general, LOX activity differed significantly between samples stored at room temperature and those stored in the refrigerator.

Interaction between storage temperature and heat treatment showed a difference in LOX activity levels between microwave-heated samples packed in the zipper-top bags. LOX activity increased for the sample stored at room temperature (Figure 1), whereas there was not much change in the sample stored in the refrigerator (Figure 2). Microwave-heated samples showed a fluctuation with an increase between 0 and 4 weeks, then a decrease between 4 and 8 weeks, and a

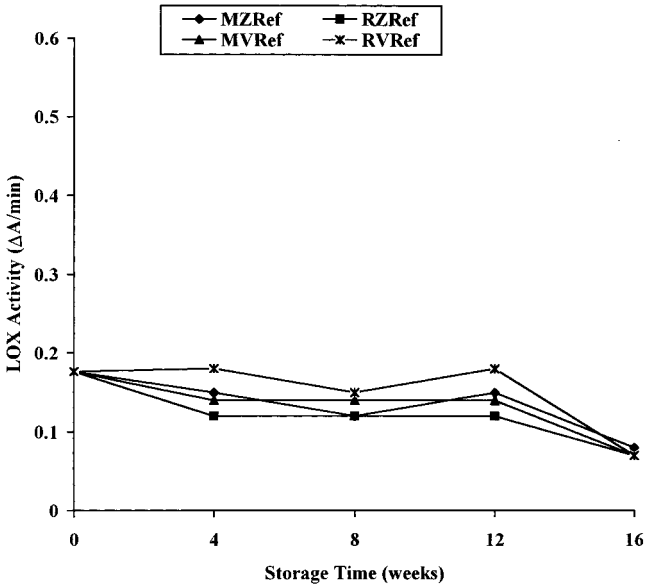

Figure 2. LOX activity in raw (control) and microwave heat stabilized rice bran packed in zipper-top bags or vacuum-packed, stored in the refrigerator (4−5 °C). M, microwave heat stabilized; R, raw; Z, zipper-top bags; V, vacuum-packed; Ref, refrigerated temperature.

sharp increase throughout the rest of the storage period (Figure 1). This fluctuation has been demonstrated by Sharp and Timme (1986). They used long-grain (Starbonnet) brown rice that was packed in sealed polyethylene bags, sealed bags in metal cans, and punctured sealed bags sealed in a metal can under vacuum. They showed that LOX activity increased between months 1 and 2 and decreased between months 2 and 3, regardless of the storage temperature.

The LOX activity in rice bran that was microwave heat stabilized, vacuum-packed, and stored at room temperature did not increase as sharply as that of the samples packed in zipper-top bags (Figure 1). This could be due to lack of oxygen, the cosubstrate (Berry et al., 1997), which was considerably reduced during vacuum packaging. The native enzyme, a relatively inactive form, is high-spin ferrous, which is converted to the active ferric form by oxidation, possibly with atmospheric oxygen (Gardner, 1988).

The results from this study showed that the samples stored at room temperature had much higher LOX activity in most samples than samples stored in the refrigerator. This was likely due to the effects of light and storage temperature. The striking effect of light has been shown (Sowbhagya and Bhattacharya, 1976) in lightly milled, cured, and parboiled rice lipid during storage at room temperature. Sowbhagya and Bhattacharya (1976) concluded that a relatively low temperature, high moisture content, and storage in the dark were the important protectants of rice lipids against rancidification.

Microwave heat stabilized samples packed in zipper-top bags and stored at room temperature had much higher LOX activity than the raw rice bran kept under the same conditions. This could be due to the lack or loss of antioxidants present in rice bran samples during microwave heating. As early as 1943, Gyorgy and Tomarelli noticed that brown rice had beneficial antioxidant activity, which was reduced either upon milling or upon autoclaving (120 °C, 30 min). Rice bran and rice bran oil contain a good amount of potent antioxidants

Table 1. LOX Activity, FFA, and Moisture Content of Rice Bran Packed in Zipper-Top Bags (Z) or Vacuum-Packed (V), Stored at Room Temperature (RT) and in the Refrigerator (Ref) during 16 Weeks of Storage[a]

	storage (weeks)		LOX activity (ΔA_{234}/min)	FFA (%)	moisture (%)
raw (control)	0		0.18 ± 0.00^{b}	2.53 ± 0.2^{f}	7.5 ± 0.1^{b}
	16	ZRT	0.04 ± 0.01^{c}	48.01 ± 1.0^{b}	7.0 ± 0.0^{bc}
	16	Zref	0.07 ± 0.00^{c}	19.45 ± 1.8^{d}	9.3 ± 0.1^{a}
	16	VRT	0.03 ± 0.00^{c}	54.29 ± 0.6^{a}	6.5 ± 0.2^{c}
	16	Vref	0.07 ± 0.02^{c}	25.37 ± 0.3^{c}	8.5 ± 0.2^{a}
microwave	0		0.18 ± 0.00^{b}	2.75 ± 0.1^{f}	8.4 ± 0.4^{a}
	16	ZRT	0.54 ± 0.11^{a}	10.93 ± 0.6^{e}	6.4 ± 0.2^{c}
	16	Zref	0.08 ± 0.01^{c}	3.58 ± 0.1^{f}	6.3 ± 0.1^{c}
	16	VRT	0.26 ± 0.10^{b}	11.62 ± 0.0^{e}	6.3 ± 0.6^{c}
	16	Vref	0.07 ± 0.03^{c}	3.74 ± 0.2^{f}	7.6 ± 0.7^{b}

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

such as oryzanol, ferulic acid, and esters of unsaturated triterpenoid alcohols (Sowbhagya and Bhattacharya, 1976). These compounds can be lost at the time of milling (Sowbhagya and Bhattacharya, 1976) and/or lose their activity or be destroyed during microwave heating (Yoshida et al., 1991). Also, the increase in LOX activity in microwave-heated rice bran stored at room temperature observed in this study could be due to an increase in the concentration of transition metals such as copper, cobalt, chromium, and, especially, iron. This was demonstrated by Rao and Artz (1989), who extruded a cornstarch/soybean oil mixture. They reported that most of the metals were present at concentrations that were highly catalytic with respect to oxidation (LOX activity). Shastry and Raghavendra Rao (1975) reported that partially purified LOX from unfractionated rice bran of an Indica variety was activated by Fe^{2+}. Hiroyuki et al. (1986) indicated that rice LOX-3 was inactivated gradually during storage because of participation of metal ions and linoleic hydroperoxide.

Increase in LOX activity during storage has been demonstrated by Dhaliwal et al. (1991). They concluded that drying the rice patty before storage did not affect LOX activity in milled rice, but activity increased significantly while samples were stored at room temperature for 12 months.

The effect of microwave heat on rice bran stability in terms of an increase in total FFA is shown in Table 1. The FFA level of microwave-heated rice bran packed in zipper-top bags and vacuum packs and stored at 25 °C during 16 weeks of storage was significantly higher than that of the samples at 0 week. However, there were no significant changes of FFA in rice bran samples packed in zipper-top bags and vacuum packs stored at 4−5 °C during 16 weeks of storage. The FFA level increased in raw bran for both types of packaging and storage temperatures during 16 weeks of storage. This is typical of the rapid development of hydrolytic rancidity in raw rice bran, which makes this product unsuitable for human consumption (Saunders, 1985; Tao, 1989; Malekian, 1992).

The moisture content of microwave-heated rice bran packed in zipper-top bags and stored at room temperature at the end of the 16-week storage period was significantly ($p < 0.05$) lower than that of the sample at 0 weeks (Table 1). The vacuum-packed microwave-heated sample (VRT) had the same amount of moisture as the samples packed in the zipper-top bags (ZRef). Although the moisture contents were nearly equal (6.3−

6.4%), the microwave-heated bran kept refrigerated (ZRef) had significantly lower LOX activity than those kept at room temperature, regardless of the packaging types (ZRT and VRT). These samples had been dehydrated, which may have led to lower water activity in the rice bran samples. A decrease in water activity accelerates the oxidation of lipids in food systems (Koch, 1961; Lee, 1975).

Moisture content in controlling LOX activity was important. Esaka et al. (1987) suggested that microwave heat was more effective in inactivating LOX in winged bean seeds of high moisture content. They concluded that microwave heating inactivates LOX of winged bean seeds in much less time than does conventional heating. The same conclusion was obtained for soybeans (Esaka et al., 1986). Wang and Toledo (1987) reported that LOX in microwave-heated high-moisture soybean samples was completely inactivated. The temperature of the soybeans was ~100 °C. This confirms that not only is the temperature of the sample important for LOX inactivation but also the moisture content of the sample plays an important role, because it results in higher energy absorption.

In our study, microwave heating did not inactivate LOX, and this could be mostly due to the moisture content of the samples. The moisture content in our rice bran samples was adjusted from an initial 7.4 to 21% before microwave heating. The temperature in rice bran samples reached 107 ± 2 °C during microwave heating. The amount of moisture in excess of 21% resulted in the bran becoming too lumpy, and a moisture level of <21% resulted in the bran being too dry with some charring (Tao, 1989; Malekian, 1992). In our study, the moisture content or time period was not enough to inactivate LOX.

CONCLUSIONS

LOX oxidizes polyunsaturated fatty acids rapidly, but its action is dependent on the earlier release of polyunsaturated fatty acids from triglyceride by the action of lipases. Thus, measurement of FFA provides a means of predicting the relative rates of deterioration of rice bran samples. The results of this study showed that even though microwave heating did not inactivate LOX enzyme, the FFA level was very low, and, therefore, there was a reduced amount of substrate present for LOX activity in microwave-heated samples stored in the refrigerator. Also, it can be concluded that, for prevention of lipid degradation in rice bran, samples can be treated with microwave heat to reduce FFA level, packed in zipper-top bags (the most commonly used packaging material), and stored in the refrigerator (4−5 °C). The condition above provides for less production of FFA and low LOX activity in rice bran samples.

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