

# Prevention of Hydrolytic Rancidity in Rice Bran during Storage<sup>†</sup>

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The effect of microwave heating, packaging, and storage temperature on the production of free fatty acids (FFA) in rice bran was examined. Freshly milled raw rice bran was adjusted to 21% moisture content and heated in a microwave oven at 850 W for 3 min. Raw and microwave-heated rice bran were packed in zipper-top bags or vacuum-sealed bags and stored at 4–5 or 25 °C for 16 weeks. FFA content of bran was measured at 4-week intervals. Total FFA increased rapidly over the 16-week period from the initial value of 2.5% in raw bran stored at 25 °C to 54.9% in vacuum bags and 48.1% in zipper-top bags. However, total FFA of raw bran stored at 4–5 °C increased at a slower rate from an initial value of 2.5 to 25.4% in vacuum bags and 19.5% in zipper-top bags. After 16 weeks of storage, total FFA of microwave-heated bran stored at 25 °C increased from 2.8 to 6.9 and 5.2%, respectively, for samples stored in vacuum bags and zipper-top bags. Total FFA of microwave-heated samples stored at 4–5 °C did not change significantly with storage time. Results showed that hydrolytic rancidity of rice bran can be prevented by microwave heating and that the recommended storage condition for microwaved rice bran is 4–5 °C in zipper-top bags.

**Keywords:** *Rice bran; free fatty acid; hydrolytic rancidity; microwave heat; storage*

## INTRODUCTION

Rice bran is a byproduct of the milling process. It constitutes nearly 7–8% of the total rice grain (Henderson and Perry, 1976). When bran layers are removed from the endosperm during the milling process, the individual cells are disrupted and the rice bran lipids come into contact with highly reactive lipases. These enzymes are both endogenous to the bran and of microbial origin and initiate hydrolytic deterioration of kernel oil (Champagne et al., 1992). Most enzymes are effective in aqueous systems in which both the enzyme and substrate are soluble. In the case of lipase, the substrate is insoluble in water and the enzyme is active at the oil–water interface (Laning, 1991). Freshly milled rice bran has a short shelf life because of the decomposition of lipids (triacylglycerols) into free fatty acids (FFA) by lipases, making it unsuitable for human consumption or the economical extraction of edible oil (Barnes and Galliard, 1991). However, if the bran is subjected to a short-time high-temperature heat treatment immediately after milling, the lipase activity is deactivated and stable bran is produced. This study was undertaken to determine the effect of microwave heating, packaging, and storage temperature on lipase activity in rice bran.

## 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 experiment. The rice samples were dehusked and milled (friction type) using a Satake milling system (Satake USA, Houston, TX). Rice bran was collected in a barrel lined with a black plastic bag. Dry rice 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 7.0 to 21% by adding 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 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  $107 \pm 2$  °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). 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 polyethylene 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

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stored at 25 °C. The storage temperatures were monitored and recorded daily.

**FFA Determination.** FFA were determined in duplicate using the method of Hoffpauir et al. (1947) modified to use *m*-cresol purple indicator instead of phenolphthalein. Alcoholic sodium hydroxide was prepared by adding one pellet (0.0894–0.1035 g) of NaOH to 500 mL of absolute ethanol. The solution was mixed for 30–60 min until the sodium hydroxide pellet was completely dissolved. A stock solution of *m*-cresol purple was prepared by dissolving 0.1 g of *m*-cresol purple (C-1393, Sigma Chemical Co., St. Louis, MO) in 24 mL of 0.01 N NaOH solution and was diluted to 250 mL. The working solution of *m*-cresol was prepared by mixing 1 mL of stock solution with 100 mL of absolute ethanol. Approximately 1 mL of alcoholic sodium hydroxide was added by titration to the *m*-cresol purple working solution to reach a grayish purple endpoint. To standardize alcoholic NaOH, 1 mL of 0.1 N hydrochloric acid was added to 25 mL of *m*-cresol purple working solution and 10 mL of petroleum ether in a 250-mL Erlenmeyer flask. This solution was titrated with the alcoholic sodium hydroxide to a purplish gray endpoint. The concentration of alcoholic sodium hydroxide was calculated by dividing 0.1 by the volume used for the titration. A titration blank was determined by titrating 25 mL of *m*-cresol purple working solution plus 10 mL of petroleum ether with the alcoholic sodium hydroxide.

Lipid was extracted from rice bran with a Soxhlet apparatus (Tecator). The extracted lipid was dissolved in 10 mL of petroleum ether in a flask to which 25 mL of *m*-cresol purple working solution was added. The contents of the flask were then titrated with alcoholic sodium hydroxide. The percent FFA was determined using the calculation

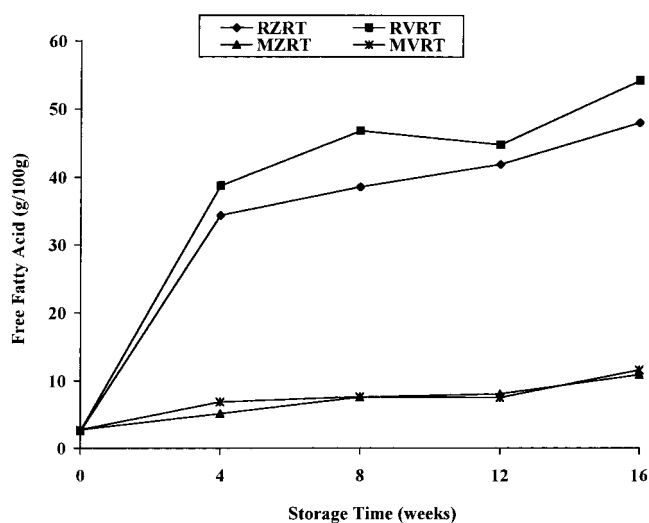
$$\text{FFA}\% = \frac{(\text{mL titrated} - \text{mL blank}) \times 28.2 \times \text{conc alcoholic-NaOH}}{\text{lipid weight (g)}}$$

where mL titrated = the mL of alcoholic sodium hydroxide that was used to change the color of the above solution (sample solution) to a grayish purple at the endpoint, mL blank = the mL of alcoholic sodium hydroxide that was used to change the color of the blank solution to a grayish purple at the endpoint, 28.2 = the multiplication factor obtained by multiplying the molecular weight of oleic acid (282) times the percentage (100) and divided by 1000 (milliliters in 1 L of solution), and conc alcoholic-NaOH = 0.1/mL of NaOH used to standardize.

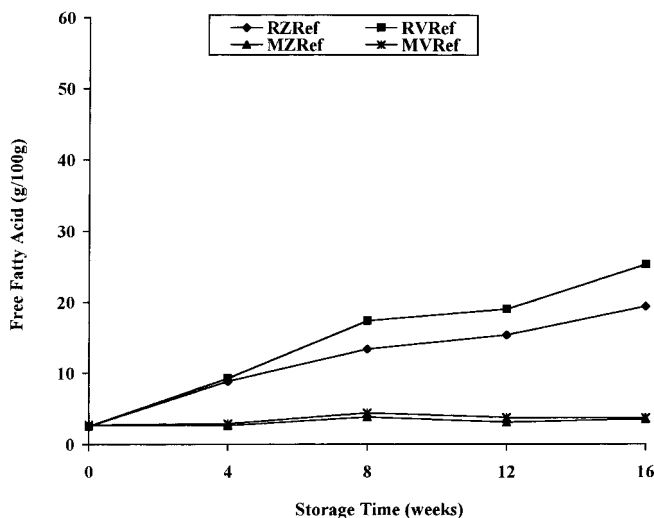
**Statistical Analysis.** A completely randomized design was used. To study the main effect for each factor, the four-factor factorial ( $2 \times 2 \times 2 \times 4$ ) arrangement was used. To compare the mean of the results, the Student–Newman–Keuls (SNK) test was performed at  $\alpha = 0.05$ . A statistical analysis of variance (ANOVA) was performed on FFA values using the SAS program, version 6.12 (SAS, 1997).

## RESULTS AND DISCUSSION

The effects of microwave heat on bran stability as measured by the change in total FFA are shown in Figures 1 and 2. The FFA level in raw rice bran increased rapidly from an initial value of 2.5 to 34.4 and 38.8% during 4 weeks of storage in zipper-top and vacuum packs, respectively, when stored at 25 °C. In contrast, the FFA level reached 8.9 and 9.3% in zipper-top bags and vacuum packs, respectively, when stored at 4–5 °C. There was no significant difference between these two levels (Figure 2). There was a slow but steady increase in FFA in the raw bran for both types of packaging at 25 °C between 4 and 16 weeks of storage. However, FFA content in the vacuum packs was consistently higher than in the zipper-top bags throughout the 4–16-week storage period at both 4–5 and 25 °C (Figures 1 and 2). At the end of the 16-week storage period, the FFA level reached 48.0 and 54.3% for rice bran in zipper-top bags and vacuum packs, respectively, stored at 25 °C, and 19.5 and 25.4% for raw bran in



**Figure 1.** Changes in free fatty acid content in raw (control) and microwave-heated rice bran packed in zipper-top bags and vacuum packs and stored at room temperature (25 °C). M, microwave heated; R, raw; Z, zipper-top bags; V, vacuum packs; RT, room temperature.



**Figure 2.** Changes in free fatty acid content in raw (control) and microwave-heated rice bran packed in zipper-top bags and vacuum packs and stored in the refrigerator (4–5 °C). M, microwave heat stabilized; R, raw; Z, zipper-top bags; V, vacuum pack; Ref, refrigerator.

zipper-top bags and vacuum packs, respectively, stored at 4–5 °C. These increases were significant ( $p < 0.05$ ) compared to baseline.

Figures 1 and 2 show the effects of storage time and temperature on FFA levels in microwave-heated rice bran packed in zipper-top bags and vacuum packs and stored at 25 and 4–5 °C for 16 weeks of storage. There was a small but steady increase in FFA level for both methods of packaging over the 16-week storage period when stored at 25 °C (Figure 1). There was essentially no change in FFA concentration in both packaging types when microwave-heated bran was stored at 4–5 °C for 16 weeks (Figure 2). The FFA changes between packaging types were not significant ( $p < 0.05$ ).

The increase in FFA content of raw rice bran in this study is similar to the results obtained by other researchers (Saunders, 1985; Tao, 1989; Martin et al., 1991; Champagne et al., 1992). This is typical of the rapid development of hydrolytic rancidity in raw rice bran, which makes this product unsuitable for human

consumption (Martin et al., 1993; Tao et al., 1993). A previous study (Tao, 1989) showed that FFA content of microwave-heated rice bran from a long-grain and a medium-grain variety increased only slightly during 4 weeks of storage at 25 °C. Malekian (1992) also showed that FFA content in microwave-heated rice bran stored in the refrigerator exhibited very little change for both types of packaging after 8 weeks of storage.

Interaction between storage temperature and packaging showed that the FFA content in raw bran samples from both packaging methods increased regardless of storage temperatures (4–5 and 25 °C). The FFA levels for both packaging methods were lower when the samples were stored at 4–5 °C. Although storage at 25 °C greatly increased the FFA level of the samples stored in zipper-top bags with increased storage time, the increase was greater in the vacuum-packed samples (Figure 1). Sharp and Timme (1986) noticed the same pattern in brown rice stored in bags versus vacuum-packed samples. This could be because the removal of air and oxygen by vacuum packing activates anaerobic microorganisms causing increased activity of the enzyme present in raw rice bran samples.

In microwave-heated rice bran, the FFA level increased slightly above 10% in samples stored at room temperature (25 °C) (Figure 1) and slightly above 3% for samples stored in the refrigerator (4–5 °C) (Figure 2) after 16 weeks of storage. Bran oils with an excess of 10% FFA and bran with >5% FFA are considered to be unsuitable for human consumption (Tao et al., 1993). The rate of FFA formation in bran or brown rice flour is high. Approximately 30% of the oil can be hydrolyzed to FFA within a week under conditions of high humidity and temperature (Champagne and Hron, 1992).

The ANOVA indicated that the packaging methods showed a significant ( $p < 0.05$ ) difference in FFA content between vacuum-packed and zipper-top bags in raw rice bran at the two storage temperatures. Untreated rice bran samples showed a significantly higher increase in FFA than microwave-heated rice bran regardless of packaging at both storage temperatures. The lowest FFA values were observed for microwave-heated samples stored in the refrigerator.

## CONCLUSIONS

The FFA values obtained in this study showed that microwave heat can be used as a method for inactivation of lipase to extend the shelf life of rice bran. Storage at 4–5 °C resulted in lower FFA values during 16 weeks of storage compared to FFA values at 25 °C. The vacuum packing did not show any significant benefit over zipper-top bags. Therefore, it can be concluded that, on the basis of the conditions employed in this study,

the recommended storage conditions for prevention of hydrolytic rancidity in microwave-heated rice bran are the use of zipper-top bags and a storage temperature of 4–5 °C, for up to at least 16 weeks of storage.

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