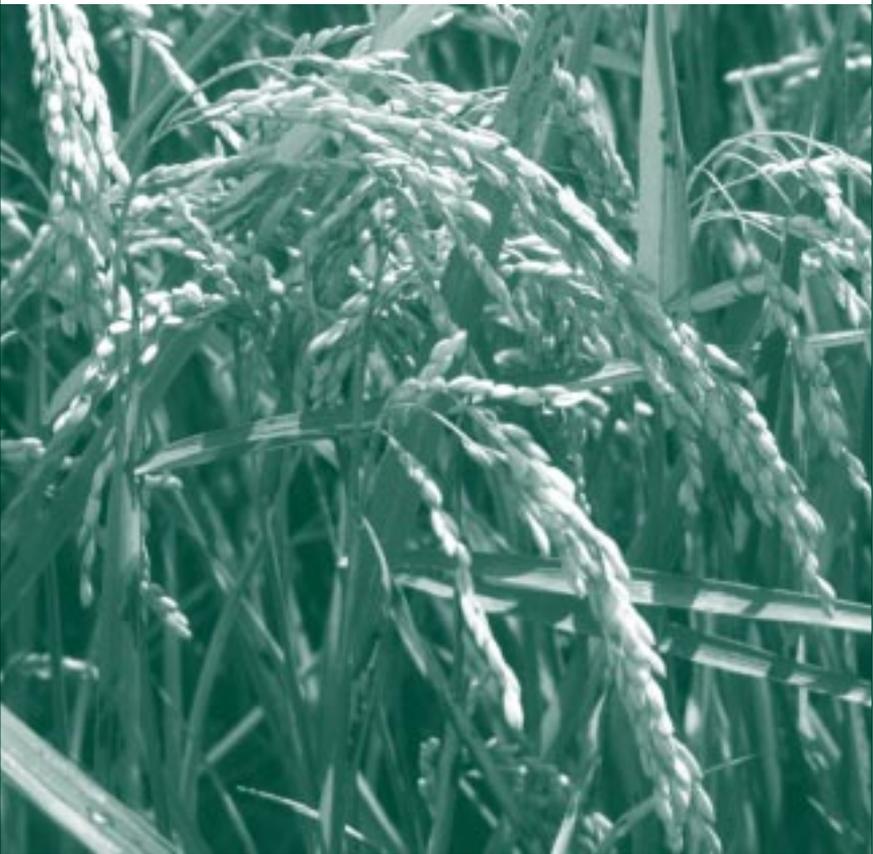


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# **Lipase and Lipoxygenase Activity, Functionality, and Nutrient Losses in Rice Bran During Storage**

**Fatemeh Malekian<sup>1</sup>, Ramu M. Rao<sup>2</sup>,  
Witoon Prinyawiwatkul<sup>2</sup>, Wayne E. Marshall<sup>3</sup>,  
Marlene Windhauser<sup>4</sup>, and Mohammed Ahmedna<sup>5</sup>**

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## **Introduction**

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Rice bran is a by-product obtained from the outer layer of the brown (husked) rice kernel during milling to produce white rice. It is rich in nutrients with 14%-16% protein, 12%-23% fat, and 8%-10% crude fiber. It is also a good source of B vitamins and contains minerals such as iron, potassium, calcium, chlorine, magnesium, and manganese (Saunders, 1985). Furthermore, recent United States Department of Agriculture (USDA) findings show that rice bran is as good as or even better than oat bran in reducing serum cholesterol and reducing the risk of heart disease. In addition, rice bran costs less and tastes better than oat bran (Urbanski, 1990).

Rice bran has great potential as a supplementary source of many nutrients. The use of rice bran as food and feed is limited, however, by its instability caused by hydrolytic and oxidative rancidity. Rice bran

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contains 12%-23% crude fat, depending on whether it is short-, medium-, or long-grain, locality, and variety of rice (Barber and Benedito de Barber, 1980). Immediately following the milling process, rapid deterioration of the crude fat in the bran by lipase and, to a lesser extent, oxidase occurs and makes the bran unfit for human consumption. The naturally occurring lipase enzyme in the rice bran hydrolyzes triglycerols (TG), which are primary lipids. The resulting fatty acids increase bran acidity and reduce pH; an off-flavor and soapy taste is produced, and functional properties change. Rice bran contains several types of lipase that are site specific and cleave the 1,3-site of triglycerols. Depending on the type of lipases present in the bran, storage conditions, and packaging methods, spoilage due to lipase continues (Takano, 1993).

Spoilage caused by 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). The reactions also depend on fatty acid composition (Nawar, 1985). LOX is found in a variety of plants, particularly legumes, such as soybeans, mungbeans, navy beans, green beans, peas, and peanuts, and in cereal, such as rye, wheat, oat, barley, and corn (Tappel, 1963). Unlike lipase, and like most other enzymes, LOX activity is accelerated by adding 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 6-10 counting from the methyl terminus (Shastry and Rao, 1975). It also causes off-flavor and off-odor in food because of its reaction with unsaturated fatty acids. There is little published information on the role of LOX in rice bran, especially in regard to storage characteristics.

Bran, after proper stabilization, can serve as a good source of protein, essential unsaturated fatty acids, calories, and nutrients such as tocopherols and ferulic acid derivatives. To process bran into a food grade product of good keeping quality and high industrial value, all the components causing deterioration must be removed or their activity arrested. Important in this respect is that inactivation of lipase and LOX enzymes must be complete and irreversible. At the same time, the valuable nutrients must be preserved.

Several different thermal methods are used for rice bran stabilization (to inhibit lipase activity). Most of the processes involve dry or moist heat treatment. Use of chemicals and irradiation has been unsatisfactory or impractical. The drawbacks common in all heat treatment methods are: (1) severe processing conditions capable of damaging valuable components of bran, (2) substantial moisture removal, and (3) complete and irreversible inactivation of enzyme not achieved. It is suggested that moist heat treatment may be more effective than dry heat (Barber and Benedito de Barber, 1980), but few processes that use steam have achieved satisfactory results. To achieve proper stabilization, every discrete bran particle must have a proper moisture content, depending upon the time and temperature of the treatment. Furthermore, moist heat results in agglomeration of bran, resulting in lumpy bran. Extrusion cooking for bran stabilization has been shown to be effective but requires large capital investment. Operating and equipment maintenance costs make the process uneconomical.

In recent years, use of microwave energy as an inexpensive source of heat for thermal processing of foods has offered an alternative energy source for stabilization of rice bran. Microwave heat processing of foods offers savings in time and energy. The use of microwave heat for stabilization of rice bran was shown to be effective in controlling deterioration of bran (Wu, 1977; Rhee and Yoon, 1984). Compared with other heat treatments, microwave heating is efficient, economically superior, shorter in processing time, has little effect on the nutritional value of bran, and has little or no effect on the original color of bran (Tao, 1989).

Use of microwave heating to stabilize rice bran may affect the bran functionalities. Functional properties of foods are defined as those that affect the use of an end product (Han and Khan, 1990). It is important, for marketing a product, to be cognizant of the properties that determine acceptability of a food or food ingredient. Therefore, functionality can be defined as a set of properties that contributes to the desirable color, flavor, texture, and nutritive value of a product. Rice bran, if properly processed and used, can provide good volume, appealing color, and excellent texture in popular, finished baked goods (Farmer's Rice Cooperative, 1990).

The deterioration of rice bran by lipase and LOX is affected by storage temperature and packaging conditions. Oxidative rancidity by LOX should increase in the presence of oxygen and the rate of hydrolytic, and oxidative rancidity should increase with increased storage

temperature and packaging conditions. Therefore, bran stored in sealed bags should have a longer shelf life than bran exposed to the atmosphere. There seems to be confusion in published literature (Champagne et al., 1992), however, in that lipase and LOX are found to be more active for bran samples stored under vacuum. This was attributed to anaerobic microorganisms present in the bran.

The primary goals of this investigation were (1) to explore the feasibility of using microwave heat to inactivate lipase and LOX and thereby to extend the shelf life of rice bran, (2) to determine the optimum storage and packaging conditions with the fewest adverse effects on functionality, and (3) to determine changes in functionality of rice bran as a result of heat treatment. This was accomplished under two phases, each phase with specific objectives:

**Phase I:** “Functional, nutritional, and storage characteristics of rice bran as affected by microwave heating and extrusion stabilization methods.” The specific objectives of the first phase were:

1. To compare microwave heating and extrusion as methods to stabilize rice bran;
2. To determine the effect of microwave heating and extrusion on functional properties of rice bran;
3. To study the effect of packaging methods (vacuum pack vs. zipper-top bags) and storage temperature (4-5 degrees C) during 8 weeks of storage on lipase activity and functional properties of rice bran.

**Phase II:** “Prevention of hydrolytic and oxidative rancidity and nutrient loss in rice bran during storage.” The specific objectives of the second phase were:

1. To determine lipase activity in microwave-heat stabilized rice bran during 16 weeks of storage in two different types of packaging (zipper-top bags vs. vacuum pack) and two different storage temperatures (4-5 degrees C vs. 25 degrees C);
2. To determine the effect of microwave heating on lipoxigenase (LOX) activity;
3. To determine the effect of microwave heating, packaging methods, and storage time and temperature on fatty acid content and proximate composition of rice bran.



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## Review of Literature

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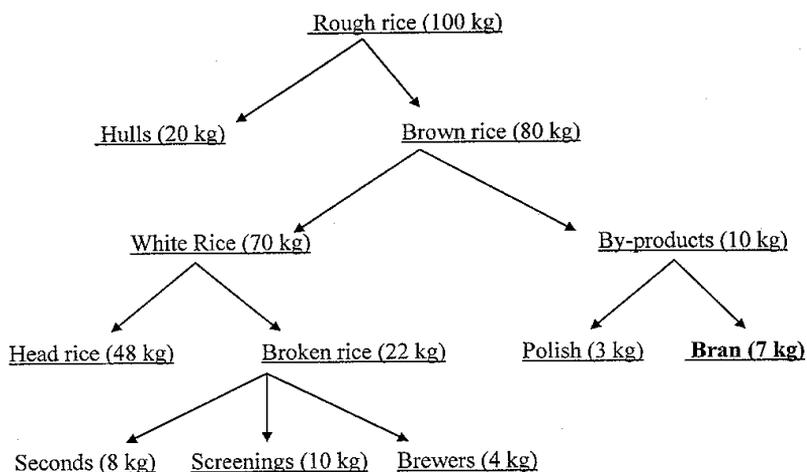
### **Rice Bran Production**

Rice is unique among the world's major crops because of its many uses and its capability to adapt to climatic, agricultural, and cultural conditions. Its ability to grow and produce high caloric food per unit area on all types of land makes rice the world's most important cereal crop (Mikkelsen and de Datta, 1991). The importance of rice as the number one staple in the developing countries will grow as the human population increases at a higher rate than the developed world. By the year 2000, rice and rice products will be the chief source of energy for 40% of the world's people, thereby surpassing wheat (Chang and Luh, 1991).

Rice bran is a by-product produced during the process of milling. The bran constitutes nearly 7%-8.5% of the total grain. The product fractions from standard milling of rice are shown in Figure 1 (Henderson and Perry, 1976). The bran consists of the pericarp, tegmen (the layer covering the endosperm), aleurone, and sub-aleurone (Houston, 1972).

### **Rice Bran Composition**

When bran layers are removed from brown rice during milling, rice bran is produced. Rice bran is rich in nutrients with a protein content of 14%-16%. The nutritional value of rice bran protein is relatively high because of the high lysine content, one of the essential amino acids. The reported protein efficiency ratio (PER) is 1.6-1.9, compared with the value for casein of 2.5 (Saunders, 1990). Major carbohydrates in rice bran are hemicellulose (8.7%-11.4%), cellulose (9%-12.8%), starch (5%-15%), and  $\beta$ -glucan (1%). Rice bran contains 15%-23% oil. Three major fatty acids, palmitic (12%-18%), oleic (40%-50%), and linoleic (30%-35%), make up 90% of total fatty acids.



**Figure 1. Product fractions from standard milling of rice (Henderson and Perry, 1976)**

Crude rice bran oil contains 3%-4% waxes and about 4% unsaponified lipids. Oryzanol and vitamin E, potent antioxidants, are present in rice bran (Saunders, 1985). Rice bran is also rich in B-complex vitamins. The mineral composition of rice bran depends on nutrient availability of the soil in which the crop is grown. Rice bran contains iron (130-530 g/g), aluminum (54-369 g/g), calcium (250-1,310 g/g), chlorine (510-970 g/g), sodium (180-290 g/g), potassium (13,200-22,700 g/g), magnesium (8,600-12,300 g/g), manganese (110-880 g/g), phosphorus (14,800-28,700 g/g), silicon (1,700-7,600 g/g), and zinc (50-160 g/g). Bran contains 80% of rice kernel iron (Lu and Luh, 1991).

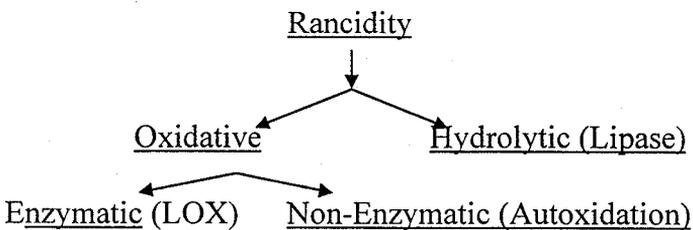
### **Health Benefits of Rice Bran**

Nutritional studies in animals and humans have shown a cholesterol-lowering potential for rice bran and rice bran fractions (Seetharamaiah and Chandrasekhara, 1989; Kahlon et al., 1990; Kahlon et al., 1991; Nicolosi et al., 1991; Rukmini and Raghuram, 1991; Newman et al., 1992; Hegsted et al., 1993). Among compounds whose hypocholesterolemic activity has been demonstrated in animal and/or human subjects are rice waxes, oryzanols (ferulic acid esters of triterpene alcohols), hemicelluloses, neutral-detergent fiber fractions, proteins, and oil components (Saunders, 1990). Rice bran can be used as a stool bulking agent (Tomlin and Read, 1988). Diets high in unsaturated fatty

acids such as oleic, linoleic, and linolenic acid, which are present in rice bran oil, lowered LDL-cholesterol when replacing saturated fat (Mattson and Grundy, 1985; McDonald et al., 1989).

### Use of Rice Bran

After the bran layer is removed from the endosperm during milling, the individual cells are disrupted, and the rice bran lipids come into contact with a highly reactive lipase enzyme. Freshly milled rice bran has a short shelf life because of decomposition of lipids into free fatty acids (FFA) (hydrolytic rancidity), making it unsuitable for human consumption and the economical extraction of edible rice oil. In rice bran, the hydrolysis is catalyzed by endogenous enzyme activity (lipases) and, to some extent, by microbial enzymes if the material is of poor quality (Barnes and Galliard, 1991). The hydrolysis of lipids in rice bran becomes apparent in several ways: off-flavor such as a soapy taste, increased acidity, reduced pH, changes in functional properties, and increased susceptibility of fatty acids to oxidation. The FFA undergo further decomposition (oxidative rancidity) and result not only in free radicals but also bad taste as well as loss of nutritional values. Types of rancidity are shown below (Barnes and Galliard, 1991):



### Functional Properties of Rice Bran

Rice bran is light in color, sweet in taste, moderately oily, and has a slightly toasted nutty flavor (Tao, 1989). Texture varies from a fine, powder-like consistency to a flake, depending on the stabilization process (Barber and Benedito de Barber, 1980). In addition to flavor, color, and nutritional properties (protein extractability and solubility), other properties such as water and fat absorption, emulsifying, and foaming capacity, are important factors in the potential use of rice bran in foods. Stabilized rice bran is known as a good source of both soluble and insoluble dietary fiber (25%-35%), which is almost twice as much as that of oat bran. Insoluble fiber functions as a bulking agent, while soluble fiber lowers cholesterol (Wise, 1989).

Soluble fiber can affect texture, jelling, thickening, and emulsifying properties (Olson et al., 1987). Since rice bran has more insoluble fiber, it has good water-binding capacity. According to James and Sloan (1984), the defatted extruded rice bran absorbs the most water and fat and has greater foaming capacity and stability compared to wheat bran in model systems. Water absorbed by rice bran in model tests is close to 200g water/100g bran, which compares favorably with commercially available 70% soy protein concentrate (Barber and Benedito de Barber, 1980). In baked products, the high water-binding capacity of rice bran helps maintain moisture and freshness.

High fat absorption capacity in extruded rice bran would be desirable in products such as meat extenders to help maintain juiciness and improve mouthfeel. The full fat extruded rice bran with less fat absorbency might be best for foods such as donuts and pancakes that are cooked in fat and for which absorption of fat is not desirable (James and Sloan, 1984). Barber and Benedito de Barber (1980) reported fat absorption capacity of rice bran in a model system at about 150g oil/100g bran, which is comparable to 70% soy protein concentrate of 110g oil/100g of bran.

The emulsification of rice bran protein concentrate is related to pH. A maximum value of 150 ml/g protein at pH 10.5 has been reported (Bera and Mukherjee, 1989). Rice bran protein concentrate has shown good emulsifying activity, stability, and capacity (Bera and Mukherjee, 1989). The emulsified layer, using raw bran in a model test, was 50% of the total volume of the emulsion, and emulsion stability after a 30-min heating was almost complete (Barber and Benedito de Barber, 1980). These properties suggest possible use of bran as fat emulsifiers in prepared foods (Bera and Mukherjee, 1989).

The foaming capacity aids in air incorporation, leavening, and texturization in baked products, meringues, and whipped toppings. Extruded defatted rice bran with 115.5% foaming value could be the best bran for achieving the above functions in food systems (James and Sloan, 1984). Extruded full-fat and raw rice bran have not shown any foaming properties (James and Sloan, 1984; Barber and Benedito de Barber, 1980).

A high level (20%) of rice bran in bakery products affects overall appearance, volume, taste, and structure. From 3%-8% sugar in stabilized rice bran contributes to an even browning reaction (Carol, 1990).

## **Hydrolytic Rancidity**

The oil in unmilled paddy rice and brown rice is relatively stable because the lipolytic enzymes within intact rice kernels are located primarily in the cross cells of the seed coat (tegmen), while most of the oil is stored in the aleurone layer and germ (Saunders, 1985). During the milling operation, this physical separation is disrupted, and lipase enzyme comes into contact with neutral fat, causing hydrolysis of fat to FFA and glycerol in the bran.

## **Types of Lipases**

Rice bran contains several types of lipases as well as phospholipases, glycolipases, and esterases (Takano, 1993). Rice bran lipase has an MW of 40 kDa, a pH optimum of 7.5-8.0, and an optimum temperature of 37 degrees C. The enzyme cleaves fatty acid ester bonds at the 1,3-site (Aizono et al., 1971). Phospholipases include phospholipase A1, phospholipase A2, phospholipase B, each acting on fatty acid ester parts, and phospholipase C and phospholipase D acting on the phosphate part (Takano, 1993). Triglycerols (TG), the main component of rice bran lipids, occur as spherosomes. Takano (1993) proposed the decomposition mechanism of rice bran lipids by lipases as follows: phosphatidylcholine, the major component of the spherosome membrane, is decomposed into phosphatidic acid by phospholipase D, and thus, spherosomes are disintegrated, then triglycerols (TG), which are protected by the membrane, come into contact with lipase and its decomposition process begins, causing an increase in free fatty acids.

## **Lipase Activity**

In rice bran oil, as FFAs increase, the refining loss for edible oil production increases more rapidly because refining loss is 2-3 times the percentage of FFA. Refining of the crude oil with more than 10% FFA is considered uneconomical. Rice bran oil normally contains 1.5%-2% FFA right after milling. Less than 5% FFA is desirable in the crude oil for economic refining purposes (Enochian et al., 1980). The FFAs produced, especially polyunsaturated fatty acids such as linoleic acid (the best substrate for LOX), are subjected to oxidation by LOX. Because FFAs accumulate to unacceptable levels (more than 5%) within a few hours after milling, the lipase enzymes must be inactivated quickly. The value for FFA (% oleic acid) present is widely used as a quality indicator for fats and oils. The test is based on an alcohol extraction with sodium

hydroxide titration for endpoint neutralization using m-cresol purple as an indicator (Hoffpauir et al., 1947).

## **Rice Bran Stabilization**

Stabilization or inactivation of lipolytic enzymes in freshly milled rice bran has been of interest to researchers. Many procedures, such as those using pH (Prabhakar and Venkatesh, 1986), ethanol vapors (Champagne et al., 1992), and moisture and heat (Saunders, 1985), have been used to inactivate lipase to stabilize rice bran and extend its shelf life. According to Aizono et al. (1971; 1976), the rice bran lipases have pH optima of 7.5 - 8.0; if the pH either decreases or increases, the lipase activity decreases. Prabhakar and Venkatesh (1986) showed that lipase was active in its native state at pH 4.5. The pH of the bran had to be lowered to 4.0 to have a low-level enzyme activity. Even at pH 4.0, an increase of 3.0%-9.3% in free fatty acids occurred after 51 days of storage. Prabhakar and Venkatesh (1986) further concluded that chemical methods are not very efficient in rice bran stabilization. Less than 3% of the oil was removed from brown rice kernels extracted with ethanol at 24 degrees C, whereas extraction at 70 degrees C removed 15% of the oil. Brown rice kernels extracted with ethanol at 70 degrees C showed a slight increase in FFA and more susceptibility to oxidative rancidity during 6 months of storage (Champagne et al., 1992). The only practical method, which has commercial potential, is heat treatment of freshly milled rice bran (Desikachar, 1974). Depending on the type of heat treatment, the lipases may be either reversibly inhibited or permanently denatured. There are different types of heat stabilization procedures: retained moisture heating (Lin and Carter, 1973), added moisture heating (Saunders, 1985), dry heating in atmospheric pressure (Loeb et al., 1949), extrusion cooking (Sayre et al., 1982), and microwave heating (Tao, 1989; Malekian, 1992).

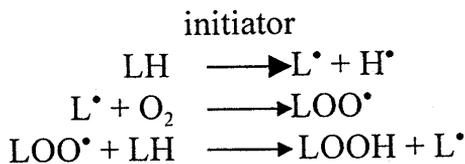
In extrusion cookers, added water, injected steam, or external heat may be required. Bran is held at 125-130 degrees C for a few seconds, then at 97-99 degrees C for 3 min prior to cooling to room temperature (Randall et al., 1985). In these methods, in addition to destruction of lipase activity, peroxidase activity is also destroyed. Long-term storage studies with this method indicate that stability against FFA development persisted for at least 4 months (Randall et al. 1985), in contrast to most processes using dry heat to stabilize with a shorter stabilized period. However, the major problems for these cookers are less flexibility and higher initial and operating costs.

## Microwave-heat Stabilization

Microwave heating is becoming increasingly popular and important in cooking and food processing. Microwave heating is considered to be one of the most energy-efficient types and a rapid method for heating food items (Yoshida et al., 1991). This method of cooking or processing saves time and energy. Microwave heating for bran stabilization has a significant advantage. It causes internal heating of particles within the microwave cavity, providing distribution of heat within the bran similar to conventional heating. The dipolar water molecules in the rice bran are excited by the electromagnetic waves, and the water molecules are made to spin. The resulting enhanced kinetic energy, along with the friction, produces the heat that results in the even distribution of heat (Roman, 1989). Since water molecules play an important role in this process, the initial moisture content is a critical factor in the microwave stabilization of rice bran. Rice bran stabilization with microwave heat has been practiced since 1979, although the method was not perfected (Liu et al., 1979). The microwave-heat treatment for extending the shelf life of soybean curds was successful (Wu, 1977). Tao (1989) and Malekian (1992) showed that exposure of fresh rice bran samples with 21% moisture content for 3 min inactivates lipase activity (increases in % oleic acid) for 8 weeks. Microwave heat had little effect on nutritional quality (proximate analysis) and the functional property (water and fat absorption capacity, emulsification, and foaming) of rice bran.

## Oxidative Rancidity

The reaction of oxygen with unsaturated lipids (LH) involves free radical initiation, propagation, and termination processes (Frankel, 1984). Initiation takes place by loss of a hydrogen free radical ( $H^\bullet$ ). The resulting unstable lipid free radicals ( $L^\bullet$ ) react with oxygen to form peroxy radicals ( $LOO^\bullet$ ). In this propagation process,  $LOO^\bullet$  react with more LH to form lipid hydroperoxides (LOOH), the fundamental primary products of autoxidation (Frankel, 1984), as depicted in the following scheme:



Decomposition of lipid hydroperoxides is complex but has biological effects and causes flavor deterioration in fat-containing foods. This decomposition proceeds by homolytic cleavage of LO-OH to form alkoxy radicals LO $\cdot$ . These radicals undergo carbon-carbon cleavage to form breakdown products including aldehyde, ketones, alcohols, hydrocarbons, esters, furans, and lactones (Figure 2) (Frankel, 1982). LOX catalyzes the addition of oxygen to the chain reaction to form hydroperoxides. The complete lipid oxidation is composed of four parts (DeGroot et al., 1975): (a) the activation of enzyme, (b) the aerobic pathway, (c) the anaerobic pathway, and (d) the nonenzymatic pathway. The reactions are similar to those occurring during autoxidation, but LOX can act much more rapidly than autoxidation and is more specific in terms of end products.

### Mechanism of Lipoxygenase (LOX) Reaction

Lipoxygenase (linoleate: oxygen oxidoreductase E.C. 1.13.1.13) catalyzes the oxidation of methylene-interrupted unsaturated fatty acids and their esters such as linoleic and linolenic acids. LOX is very important to food scientists for a number of reasons. LOX can affect color, flavor (off-flavors in frozen vegetables, stored cereals, high-protein foods), and nutritive properties.

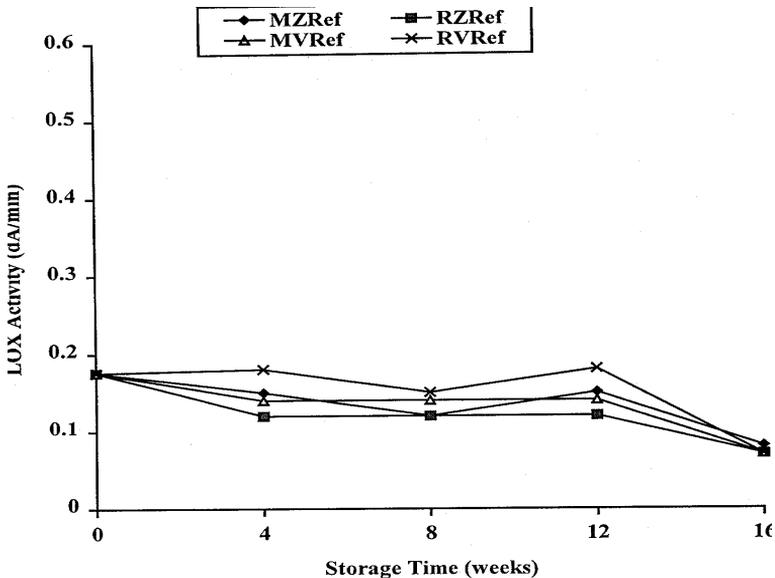
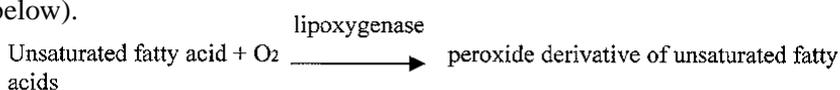


Figure 2. Products formed enzymatically from linoleic and linolenic acids in plants (Gardner, 1988).

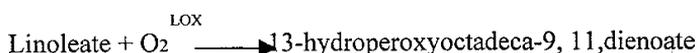
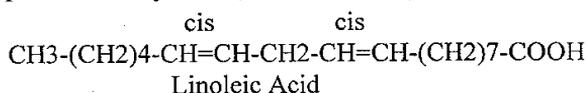
For example, there is destruction of vitamin A, loss of essential polyunsaturated fatty acids (linoleic acid), and interaction of enzymatic product with some essential amino acids that lower the quality of protein (Richardson and Hyslop, 1985).

Lipase and lipoxygenase enzymes, both of plant and animal origin, generally are activated when tissue is disrupted or injured. Sequential enzyme action on lipids starts with the release of fatty acids (lipolytic enzymes). Among the free fatty acids (FFA), the polyunsaturated are oxidized to fatty acid hydroperoxides by lipoxygenase (see equation below).



The most typical substrates are naturally occurring isomers of three essential fatty acids: linoleic, linolenic, and arachidonic acids. The next step leads to decomposition or enzyme conversion of hydroperoxides into a number of oxygenated fatty acids (Gardner, 1979). Hexanal is generally accepted as one of the major components responsible for off-flavor developing in long-term-stored rice kernels (Yamamoto et al., 1980).

LOX is an enzyme that imitates the autoxidation of polyunsaturated fatty acids, except that LOX is selective for the type of substrate it oxidizes and how the substrate is oxidized. In soybeans, several isoenzymes have been found. LOX-1 is the most thoroughly investigated species, and linoleic acid is the best substrate for this enzyme. Hamberg and Samuelsson (1967) concluded that a *cis*, *cis*-1,4-pentadiene moiety having a methylene group (a methylene between two double bonds) located at eight carbons from the terminal methyl end is necessary for oxidation of the substrate by soybean LOX. Such fatty acids are oxidized only at the sixth carbon, except linoleic acid (LH) is oxidized at both sixth and tenth carbons. With linoleic acid, the principal oxygenation products are optically active 9 and 13 hydroperoxide isomers (Theorell et al., 1947). Iron present in LOX appears to be involved in electron transfer during the incorporation of O<sub>2</sub> into unsaturated fatty acids containing *cis*,*cis*-1,4-pentadiene system (see as follows).



LOX must be in the oxidized ( $\text{Fe}^{3+}$ ) form for the oxidation reaction to proceed. Then the oxidized form of LOX can catalyze the stereospecific removal of hydrogen from the C-11 methylene group of linoleic acid (C18:2) or linolenic acid (C18:3) (O'Connor and O'Brien, 1991). A C-13 radical is formed, and LOX is reduced to the  $\text{Fe}^{2+}$  form. Under anaerobic or aerobic conditions, the reaction continues and the hydroperoxides may form other products (Figure 2) (Gardner, 1988). Hydroperoxide lyase produces aldehydes and aldehyde acids from hydroperoxide; isomerase produces epoxyhydroxyene fatty acids that are hydrolyzed to trihydroxyene fatty acids; hydroperoxide isomerase produces  $\alpha$ -ketol and  $\gamma$ -ketol fatty acids; and hydroperoxide cyclase produces 12-oxo-phytodienoic acid, a precursor of jasmonic acid. Jasmonic acid is a plant growth hormone that regulates plant responses to wounding and pathogens and, in addition, is an inducer of tuberization in the potato (Royo et al., 1996).

### **Inhibition and Inactivation of Lipoyxygenase**

LOX-produced off-flavors are a significant potential problem in products containing lipids. Many researchers have been working on optimizing conditions necessary for the inactivation of LOX in such products. Methods being investigated include addition of antioxidant, pH adjustment, and heat (O'Connor and O'Brien, 1991).

LOX activity in a model system is inhibited by various antioxidants (e.g., pyrocatechol, homocatechol, propylgallate, nordihydroguariaric acid, resorcinol, phioroglucinol, hydroquinone, butylated hydroxyanisole, and various flavonoids and related compounds (Takahama, 1985; O'Connor and O'Brien, 1991). Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) inactivates soybean LOX-1 irreversibly (Mitsuda et al., 1967). Heat treatment can affect protein solubility and adversely change the functional properties of soy products. Brown et al. (1982) inactivated LOX by 99% at a temperature of 91 degrees C and above while 70% of protein solubility was retained. They adjusted soybean moisture to 16.3% with pH 9.8 buffer and then heated the samples with steam for 10 seconds. Graveland (1970) showed that oxidation of linoleic acid in flour-water suspensions leads to production of two isomeric hydroxy-octadecadienoic acids. He noticed that defatting flour with petroleum ether leads to an increase in production of optically active 9 and 13 hydroperoxide isomers. Defatting did not affect LOX activity.

Williams et al. (1986) reported that LOX was the primary cause of development of off-flavors in English green peas and green beans. LOX was responsible for aroma changes defined as unripe, banana, grassy, straw, and ammonia and partly responsible for the sour component. Pea and green bean LOX were more heat sensitive than peroxidase at 60 degrees C for 10 min. Therefore, a less severe heat treatment was required to inactivate LOX in English green peas and green beans. According to Ganthavorn et al. (1991), LOX in asparagus tips was more heat stable than peroxidase. Therefore, a heat treatment (50 degrees, 60 degrees, or 70 degrees C for 10 min) sufficient to inactivate peroxidase may not be sufficient to inactivate LOX. Differences in heat stability of LOX from asparagus and green peas indicate the importance of independently evaluating enzyme stability in different vegetables.

Yamamoto et al. (1980) observed a relatively high lipoxygenase activity in unfractionated fresh rice bran that was removed from brown rice. Dhaliwal et al. (1991) studied the LOX changes in milled rice obtained from different varieties of pappads stored for 1, 6, and 12 months with two different moisture levels. They concluded that LOX activity was not altered with drying. Sekhar and Reddy (1982) concluded that since LOX acts on polyunsaturated fatty acids, such as linoleic acids that are present in up to 40%-45% of the total fatty acids in rice, it can be assumed that the varieties with lower activities of this enzyme may have better storage qualities.

Esaka et al. (1986) found that microwave-heat may be effective for inactivation of LOX and trypsin inhibitor in whole soybean. The microwave heating could be considerably effective in inactivating the LOX and trypsin inhibitors of whole winged bean seeds and in increasing the rate of water absorption of the seeds (Esaka et al., 1987). Wang and Toledo (1987) concluded that microwave-heat treatment of soybeans at their natural moisture content (8.7%) for 4 min could provide suitable material for soy milk processing. Esaka et al. (1987) reported that LOX was completely inactivated in winged beans by microwave heating for 3 min. Soaking of the seeds before microwave heating decreased the heating time needed to inactivate the enzyme.

The microwave processing of rice bran results in inactivating lipase, the major enzyme responsible for hydrolytic rancidity for a storage period of 8 weeks (Tao, 1989; Malekian, 1992). No information is available in the published literature as to how the deleterious effect of oxidative rancidity on bran due to LOX can be controlled during storage.

Furthermore, there is no report on the effect of microwave heat on LOX activity in rice bran. Free unsaturated fatty acids act as substrate for oxidative deterioration. Lipid oxidation occurs through the action of the enzyme lipoxygenase found in the germ and also autoxidation in the presence of catalysts. This reaction leads to off-flavors (painty and/or cardboardy) found in the bran. Bran, after proper stabilization, is a good source of calories, essential fatty acids, and nutritionally interesting products such as tocopherol and ferulic acid derivatives. Protection of the unsaturated fatty acids of bran during storage, and consequently of the nutritional value of rice bran, promises wider markets to rice millers and farmers, in addition to providing a healthy food product to consumers.

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## **Materials and Methods**

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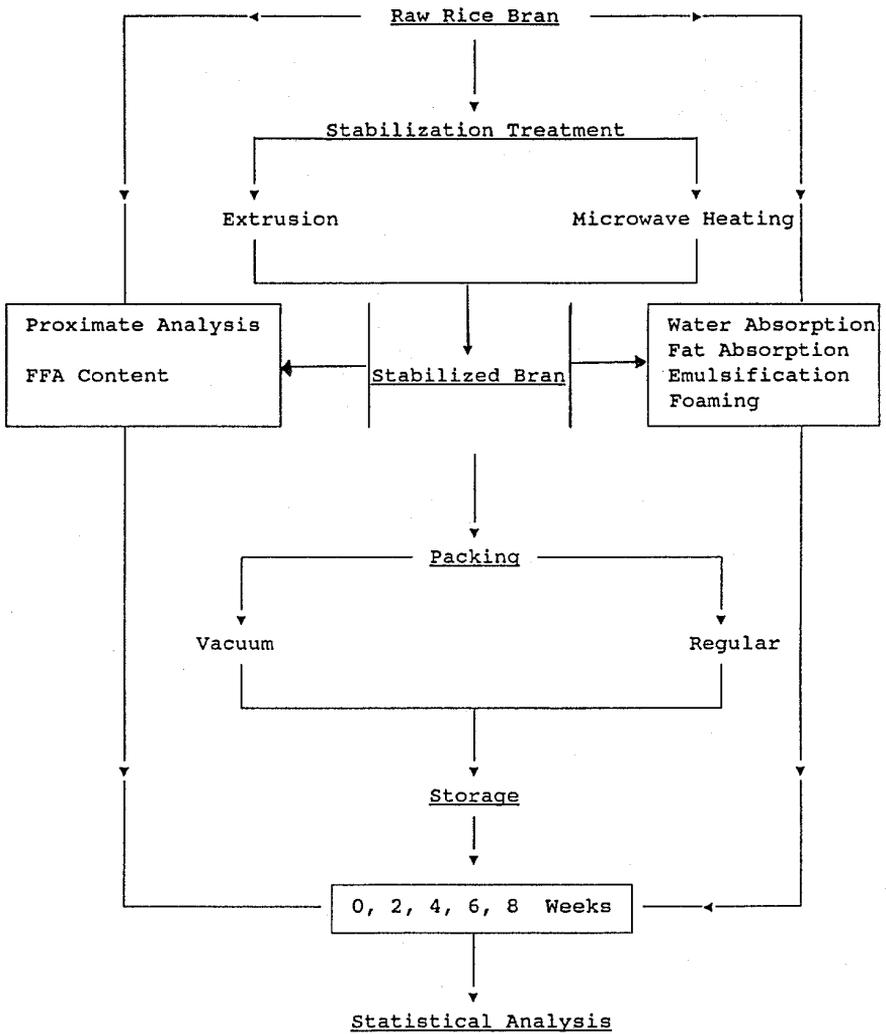
The first phase of this project involved the stabilization of freshly milled rice bran with microwave heat or extrusion cooking. The stabilized rice bran was subsequently packed in zipper-top bags or vacuum pack bags and stored in a refrigerator for 8 weeks. The effect of microwave heat compared with extrusion cooking on lipase activity and functional properties (fat absorption capacity, water absorption capacity, emulsification, and foaming capacity), of rice bran was examined. Figure 3 shows the schematic diagram of the first phase.

The second phase involved the stabilization of freshly milled rice bran with microwave heat after which the rice bran was packed in zipper-top bags and vacuum pack bags and stored at 4-5 degrees C and 25 degrees C over 16 weeks. The effect of microwave heat, two different packaging methods and two storage temperatures on lipase activity, LOX activity, fatty acid, and proximate composition was investigated. Figure 4 shows the schematic diagram of the second phase.

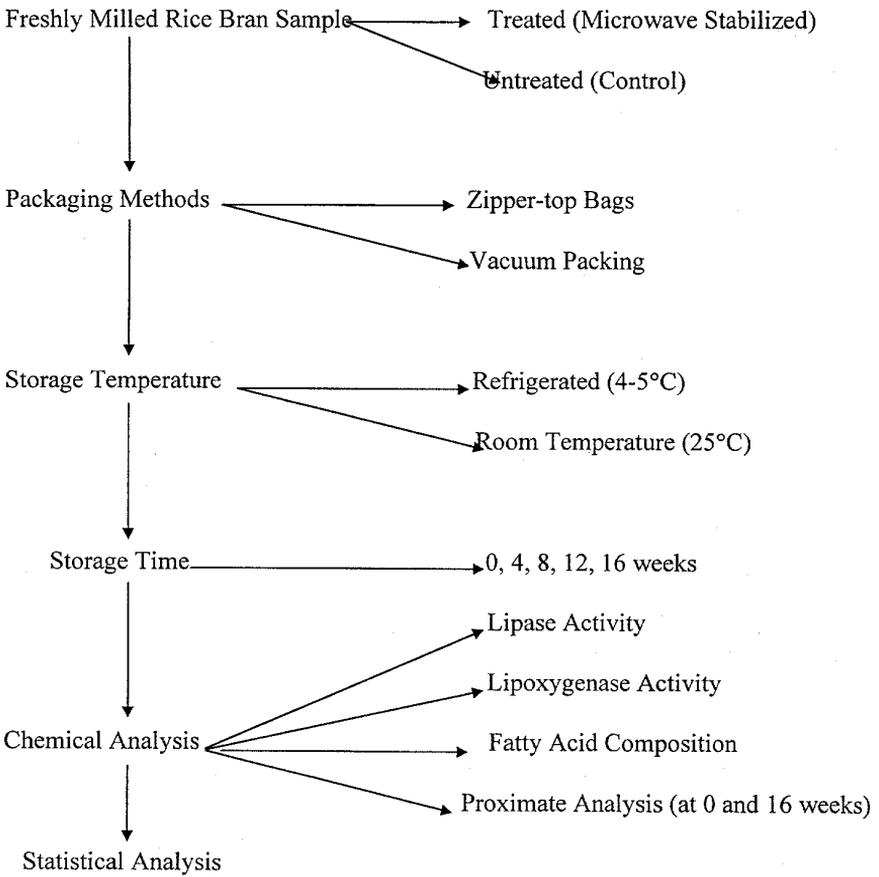
### **Phase I:**

#### **Sample Collection and Preparation**

Rice bran was obtained from Riviana Foods, Inc., Abbeville, La. Composite samples of fresh rice bran were collected in a ridged cardboard airtight sealed drum lined inside with a polyethylene bag. The drum was placed under the collecting bin, thus collecting the bran as the rice was milled. Each bag was tied, and the drum was sealed with its lid and stored in the cooler at 5 degrees C.



**Figure 3. Schematic diagram showing the phase I experimental procedure.**



**Figure 4. Schematic diagram showing the Phase II experimental procedure.**

## Stabilization Treatments

### Extrusion Stabilization

A Food-Ex (Houston, Texas) model 1002 L single-screw extruder was used for the stabilization process. The extruder was powered by a 30 hp A.C. motor with a fixed screw speed of 1200 rpm. The extrusion chamber measured 12 in with a screw pitch of 1 in, yielding a residence time of 1.2 sec. The extrudate was forced to pass through a cup-cone configuration. The temperature was adjusted by adjusting the spacing

between the cup and the cone. A 12-ft-long, 6-in diameter, all-purpose roof auger conveyed the bran from the extruder to the storage bin. A 0.75 hp variable speed motor was used to control the auger speed and to set the post-extrusion dwell time for 3 min (Martin et al., 1991).

Rice bran was stabilized in the extruder at a temperature ranging from 125-130 degrees C for 30 sec and then held in the holding/transport auger for 3 min. The bran temperature in the auger ranged from 97-99 degrees C. Stabilized rice bran was air cooled at room temperature and collected in polyethylene bags.

### **Microwave Stabilization**

A commercial Option 3 microwave oven (Thermador Division, Norris Industries, Los Angeles, Calif.) operating at 2,450 MHz and 550 W maximum output power was used as the microwave energy source. The oven was preheated for 3 min prior to loading the rice bran.

One-hundred-fifty grams of raw rice bran at 21% moisture content was placed in a polyethylene microwave-safe bag (zipper-top) and exposed to microwave heating for 3 min. The temperature of the heated bran was 107 degrees C. The bran was removed from the oven and cooled to room temperature (24 degrees C). This process was repeated until sufficient microwave-stabilized rice bran was prepared for the study.

### **Packaging and Storage**

Samples of raw, extrusion, or microwave-heat stabilized bran were stored either in polyethylene zipper-top bags or in vacuum-packed polyethylene bags and marked for storage times of 0, 2, 4, 6, and 8 weeks. Samples were taken for proximate analysis at 0 and 8 weeks and at 2-week intervals for FFA, water and fat absorption, foaming, and emulsification capacity. All bags were stored at 4-5 degrees C until ready for use.

### **Free Fatty Acid Determination**

Free Fatty Acids (FFA) were determined by the method of Hoffpauir et al. (1947) with modification. Samples were removed at 2-week intervals and lipids were extracted for 4 hr with petroleum ether using a Gold Fish apparatus (Laboratory Construction Co., Kansas City, Mo.). FFA were determined by dispersing the lipid residue in a solution consisting of 25 ml of m-cresol purple and 10 ml of petroleum ether. The

amount of alcoholic NaOH to change the yellow color of the solution to grayish purple was recorded. A blank consisting of 25 ml m-cresol purple and 10 ml of petroleum ether was also titrated. FFA percent was calculated as oleic acid and expressed as a percentage of the total lipids.

## **Functional Properties of Rice Bran**

Functional properties of rice bran were determined by standard methods. These include water absorption capacity (Sosulski, 1962), fat absorption capacity (Lin and Humbert, 1974), emulsifying activity (Yasumatsu et al., 1972; Puski, 1975), and foaming activity (Lawhon et al., 1972).

## **Statistical Analysis**

A  $3 \times 2 \times 5$  factorial design was used. The factors were stabilization methods (extrusion, microwave heat, and no heat), packaging methods (vacuum and zipper-top bags), and storage duration (0, 2, 4, 6, and 8 weeks). Separate rice bran samples were marked for each one of the 30 treatment combinations. Rice bran samples subjected to each one of the factorial combinations were evaluated for FFA, water absorption capacity, fat absorption capacity, emulsification, and foaming capacity. All measurements were made in duplicate. Statistical analysis of the results was performed using the SAS<sup>®</sup> program (SAS, 1989). The differences were significant at  $p$ -value  $< 0.05$ .

## **Phase II:**

### **Sample Collection**

Rice bran from the variety 'Lemont' (long grain), cultivated at the Louisiana State University Agricultural Center, Rice Research Station at Crowley, La., was used. The rice samples were dehusked and milled by a Satake milling system (friction type) (Satake USA, Houston, Texas). Rice bran was collected in a barrel lined with a black plastic bag. Dry ice was added continuously to the rice bran in the barrel during milling to prevent the hydrolysis of fatty acids by lipase activity. The bags were placed in an ultra freezer (-78 to -80 degrees C) until the day of sample preparation (within 10 days). On the day of sample preparation, the samples were put through a 20-mesh sieve to remove husks and broken pieces of rice.

## **Microwave-heat Stabilization**

One-hundred-fifty grams per batch of raw rice bran at 21% moisture content was placed in one-gallon, zipper-top storage bags. The bran was stabilized for 3 min in a Sharp Carousel microwave oven (Sharp Electronic Corporation, Mahwah, N.J.) operating at 2450 MHz and 850 W maximum power output set at 100% power for 3 min. At the end of 3 min, the temperature of the sample was  $107 \pm 2$  degrees C. The sample was cooled to room temperature (25 degrees C). This was repeated until there was sufficient bran stabilized with microwave heat for the experiments. The samples were stored in an ultra freezer (-78 to -80 degrees C) until the day of packaging (within 2 days).

## **Packaging and Storage of Rice Bran**

Microwave-heat stabilized and raw rice bran samples were divided into two parts. One part was packed in polyethylene zipper-top bags and the other part was placed in non-permeable vacuum bags and vacuum packed. Half of each type of bag was stored at 4-5 degrees C, and the other half was stored at room temperature (25 degrees C). Both types of bags were marked for storage times of 0, 4, 8, 12, or 16 weeks. Samples were taken for proximate analysis at 0 and 16 weeks of storage and at 4-week intervals for FFA, fatty acid composition, and LOX activity.

## **Free Fatty Acid Determination (Lipase Activity)**

Free fatty acids were determined by using the method of Hoffpauir et al. (1947).

## **Lipoxygenase Activity (LOX) Determination**

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

## **Enzyme Extraction from Rice Bran Samples**

Ten grams of rice bran was mixed with 40 ml of 50 mM sodium phosphate buffer pH 7.0 for 30 min at room temperature. The sample was filtered using two layers of cheese cloth. The filtrate was collected and centrifuged at  $9000 \times g$  for 15 min at 5 degrees C. The supernatant was collected and its volume recorded. Solid ammonium sulfate was added to each sample to obtain 50% saturation (Cooper, 1942). The sample was mixed gently and centrifuged at  $9000 \times g$  for 10 min at 5

degrees 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. This solution was filtered through a 0.20-  $\mu$ m filter, and the filtrate was used as the source of enzyme.

### **Lipoxygenase Assay**

Soybean lipoxygenase was purchased from Sigma Chemical Co., St. Louis, Mo. The standard enzyme contained 110,600 units per mg solid. An enzyme solution was made by adding 11.6 ml of 0.01 M borate buffer, pH 8.5, to 1 mg standard enzyme to obtain 10,000 units of enzyme per ml of buffer.

To a 100-ml volumetric flask, 100  $\mu$ l of linoleic acid (99%+, NuChek Prep, Inc., Elysian, Minn.) and 60 ml of absolute ethanol were added. The mixture was mixed gently into an emulsion and then, with slow stirring, water was added to bring the volume to 100 ml. This was used as a stock solution. For the assay, 1 ml of stock solution was diluted with 6 ml of 0.01 M borate buffer, pH 8.5, for a concentration of 0.4571 mM linoleic acid. Substrate solutions were made using the substrate stock solution and 0.01M borate buffer.

Enzyme activity was measured with a thermostated Beckman DU 640 spectrophotometer (Beckman Instruments, Inc., Houston Texas) at 234 nm and 25 degrees C for 5 min. The cuvette contained 2.9 ml of substrate solution and was placed in the sample compartment of the spectrophotometer. One tenth ml of enzyme solution was rapidly added, mixed, and the increase in absorbance (A) versus the blank was recorded. One unit of LOX activity was defined as the change in absorbance of 0.001AU/min in 3 ml volume and 1-cm light path when linoleic acid was used as substrate (Shastri and Rao, 1975).

### **Optimum pH for Lipoxygenase Activity**

The pH of the solutions were adjusted to 5, 6, 7, 8, 8.5, 9, and 10 with HCL and NaOH. LOX activity at each pH was determined as described above.

### **Optimum Temperature for Lipoxygenase Activity**

LOX activity was measured at 15, 20, 25, 30, 40, and 50 degrees C by adjusting the thermostat in the spectrophotometer. The time was set

for 5 min, and the pH of the substrate solution was 8.5. LOX activity was determined as described above.

### **Lipoxygenase Kinetics**

Michaelis constant ( $K_m$ ) and maximum velocity ( $V_{max}$ ) of the reaction were determined in duplicate using linoleic acid stock solution diluted with 0.01 M borate buffer pH 8.5 to attain four different concentrations (0.1231, 0.0627, 0.0421, and 0.0317 mM). The reaction was followed in the spectrophotometer at 25 degrees C. LOX activity at each substrate concentration was determined as described above.

### **Protein Assay**

Protein concentration of the enzyme solution was determined using the Warburg-Christian method (Layne, 1957). The absorbance of each protein-containing solution was measured in duplicate at 280 and 260 nm. The ratio of these two values was determined and used to select the appropriate correction factor (Cooper, 1942; Layne, 1957). The absorbance at 280 nm was multiplied by the factor to yield the protein concentration in mg/ml:

$$(A_{280})(\text{correction factor}) = \text{mg/ml protein}$$

### **Specific Activity of Lipoxygenase**

From the observed changes  $A_{234 \text{ nm}}/\text{min}$  (correcting blank was used), the enzyme activity per ml of enzyme extract was calculated (Shastry and Raghavendra Rao, 1975; Aurand et al., 1987).

$$\text{Specific activity} = \frac{\Delta A_{234}/\text{min}}{\text{mg/ml protein}}$$

### **Fatty Acid Composition**

Fatty acid composition was determined by gas chromatography (GC) according to the American Oil Chemists Society method # Ce 1b-89 (AOCS, 1991) with modifications. After extracting the fat with petroleum ether and dissolving it in 10 ml of petroleum ether, 100  $\mu\text{l}$  was saved in a glass test tube in the freezer (-25 degrees C) until the day of analysis. To each sample, 100  $\mu\text{l}$  of internal standard (IS) was added. The solvent was evaporated, and 1.5 ml of 0.5 N NaOH was added to each sample. The sample was flushed with nitrogen, capped tightly, mixed, and heated at 100 degrees C for 5 min. The samples were cooled, and 2

ml of BF<sub>3</sub>/Methanol reagent (Boron trifluoride-methanol, 14% solution, Sigma Chemical Co., St. Louis, Mo.) was added to each sample to make methyl esters. The sample was flushed with nitrogen, capped tightly, mixed, and heated at 100 degrees C for 30 min. The samples were cooled to 30-40 degrees C and 1 ml of iso-octane was added to each sample. The samples were flushed with nitrogen, capped tightly, and mixed for 30 seconds. Five ml of saturated NaCl was added, the sample flushed 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 tube, and the samples were once again flushed 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 extract was transferred to a GC vial, capped, and then run immediately on the GC.

Internal standard (IS) used was tricosanoic acid methyl ester (C:23) (NuChek Prep Inc., Elysian, Minn.) and was prepared by weighing 25 mg of C:23 into a 25 ml volumetric flask and brought to volume with iso-octane. The calibration standard (NuChek Prep Inc., Elysian, Minn.) of fatty acid methyl esters was prepared by emptying the content of ampoule received (100 mg) in a 10 ml volumetric flask and bringing to volume with hexane. Further dilutions were made to obtain a concentration of 1 mg/ml.

The GC used was a Hewlett Packard 5890 (San Fernando, Calif.) with autosampler and Flame Ionization Detector (FID). The column used was a fused silica capillary column 30 m in length, 0.25 mm ID, and 0.20  $\mu$ m film thickness (Supelco, Inc., Bellefonte, Pa.). The initial column temperature was programmed at 50 degrees C, held for 1 min, then increased at 10 degrees C/min to 150 degrees C and held for 10 min, increased at 2 degrees C/min to 175 degrees C and held for 10 min, and increased at 5 degrees C/min to 225 degrees C and held for 7 min. The injector and detector port temperatures were maintained at 170 degrees C and 270 degrees C, respectively, and helium was used as a carrier gas. Fatty acids were identified by comparing their retention times with a standard mixture containing 30 fatty acid methyl esters.

Fatty acid contents of the samples were quantified based on peak areas of known concentration of respective standards obtained under identical conditions. Recovery (%) of internal standard was also determined.

## **Proximate Analysis**

Protein, fat, moisture, and ash were determined by standard AOAC methods (AOAC, 1991). Percent carbohydrate was determined by difference using the formula below:

$$\% \text{ Carbohydrate} = 100 - (\% \text{ protein} + \% \text{ fat} + \% \text{ moisture} + \% \text{ ash}).$$

## **Statistical Analysis**

A 2x2x2x5 factorial design was used. The factors were stabilization methods (microwave heat and no heat), packaging methods (vacuum and zipper-top), storage temperature (25 degrees C and 4-5 degrees C), and storage duration (0, 4, 8, 12, and 16 weeks). Separate rice bran samples were marked for each one of 40 treatment combinations. Rice bran samples subjected to each one of the factorial combination were evaluated for LOX activity, LOX specific activity, free fatty acid, fatty acid composition, and proximate composition. Proximate composition (% fat, % protein, % ash, % moisture, and % carbohydrate) was determined for fresh rice bran samples and after 16 weeks of storage only. Pairwise comparison of the means was done using Student-Newman-Kelus (SNK). A statistical analysis of variance (ANOVA) was performed on all values using the Statistical Analysis System (SAS<sup>®</sup>) program version 6.12 (SAS, 1997). Differences were considered significant at the p-value < 0.05. Whenever interactions were significant, cell mean comparison was made.

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# **Results and Discussions**

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## **Phase I:**

### **Free Fatty Acids**

Rice bran stabilized by microwave heating and extrusion cooking was tested for FFA content over an 8-week storage period at 2-week intervals. In Table 1, the FFA values are presented for microwave-heat and extrusion stabilized rice bran in vacuum packs and zipper-top bags during storage. In the control, the FFA content increased during storage from an initial value 3.7% to a final value of 26.7% in vacuum packs and 22.2% in zipper-top bags. These increases were significant (p-value < 0.05). The rate of FFA formation was even faster in vacuum-packed samples after 4 weeks of storage.

**Table 1. Effect of stabilization and packaging methods on free fatty acids (FFA) content of rice bran during storage**

| Treatment       | 0                | Storage Time (weeks)  |                   |                   |                   | 8 |
|-----------------|------------------|-----------------------|-------------------|-------------------|-------------------|---|
|                 |                  | 2                     | 4                 | 6                 |                   |   |
|                 |                  | FFA (% of oleic acid) |                   |                   |                   |   |
| Raw (Control)   |                  |                       |                   |                   |                   |   |
| vacuum-packed   | 3.7 <sup>a</sup> | 8.5 <sup>a</sup>      | 11.1 <sup>a</sup> | 18.7 <sup>a</sup> | 26.7 <sup>a</sup> |   |
| zipper-top bags | 3.7 <sup>a</sup> | 8.5 <sup>a</sup>      | 11.1 <sup>a</sup> | 17.0 <sup>b</sup> | 22.2 <sup>b</sup> |   |
| Microwave       |                  |                       |                   |                   |                   |   |
| vacuum-packed   | 3.2 <sup>a</sup> | 3.5 <sup>b</sup>      | 3.4 <sup>b</sup>  | 3.6 <sup>c</sup>  | 3.9 <sup>c</sup>  |   |
| zipper-top bags | 3.2 <sup>a</sup> | 3.5 <sup>b</sup>      | 3.5 <sup>b</sup>  | 3.7 <sup>c</sup>  | 3.9 <sup>c</sup>  |   |
| Extrusion       |                  |                       |                   |                   |                   |   |
| vacuum-packed   | 2.8 <sup>a</sup> | 2.9 <sup>b</sup>      | 2.9 <sup>b</sup>  | 3.2 <sup>c</sup>  | 3.2 <sup>c</sup>  |   |
| zipper-top bags | 2.8 <sup>a</sup> | 3.2 <sup>b</sup>      | 3.3 <sup>b</sup>  | 3.4 <sup>c</sup>  | 3.3 <sup>c</sup>  |   |

Means between columns with different letters are different (p-value < 0.05).

Comparison within each row is discussed in the body of the text.

All the means had Coefficient of Variation of less than 10%.

FFA in microwave-heat stabilized bran increased from an initial value of 3.2% to 3.9% in both packs and bags throughout the storage period, and this change was not significant. Vacuum packing did not show any advantage over zipper-top bags.

In extrusion stabilized bran, the FFA increased from an initial value of 2.8% to 3.2% and 3.3% in vacuum packs and zipper-top bags, respectively, but this increase was not significant.

Interaction between stabilization methods and packaging methods showed that in raw samples FFA increased steadily throughout the storage period, and vacuum-packed samples showed more increase in FFA level than samples in zipper-top bags. Microwave-heated and extruded samples did not show any significant increase regardless of packaging methods. Since the storage time showed significant interaction with other factors, comparison of means were performed within each level of storage time (Table 1).

## Functional Properties

### Water Absorption

Water absorption capacity depends on factors such as size, shape, hydrophilic-hydrophobic balance of amino acids in protein molecules, lipids and carbohydrates associated with protein, thermodynamic properties of the bran, physico-chemical properties, and environmental condi-

tions (pH, ionic strength, vapor pressure, temperature, presence or absence of surfactant). However, polar amino groups of protein molecules are the primary part of protein-water interactions that bind different amounts of water at cationic, anionic, and nonionic sites (Han and Khan, 1990).

Water absorption in control, microwave-heated, and extrusion stabilized rice bran is shown in Table 2. The percentage reductions in the control samples were from an initial value of 182% to 151% and 140% in vacuum-packed bran and bran packed in zipper-top bags, respectively, during storage and were significantly different from one another (p-value < 0.05).

In microwave-heat stabilized bran, the water absorption decreased significantly (p-value < 0.05) from an initial value of 210% to 198% and 177% in vacuum-packed and zipper-top bags, respectively, during storage.

In extrusion stabilized bran, water absorption was greater than the control and microwave-heat stabilized bran at 0 week, but it decreased significantly (p-value < 0.05) from an initial value of 229% to 189% and 177% in vacuum-packed bran and bran packed in zipper-top bags, respectively. Vacuum packing seems to protect the water absorption capacity of extruded samples. James and Sloan (1984) reported that water absorption of extruded rice bran was 213%.

**Table 2. Effect of stabilization and packaging methods on water absorption capacity of rice bran during storage**

| Treatment     | 0               | Storage Time (weeks) |                  |                  |                  |                   |
|---------------|-----------------|----------------------|------------------|------------------|------------------|-------------------|
|               |                 | 2                    | 4                | 6                | 8                |                   |
|               |                 | Water Absorption (%) |                  |                  |                  |                   |
| Raw (Control) | vacuum-packed   | 182 <sup>c</sup>     | 172 <sup>c</sup> | 161 <sup>b</sup> | 150 <sup>b</sup> | 151 <sup>d</sup>  |
|               | zipper-top bags | 182 <sup>c</sup>     | 178 <sup>c</sup> | 163 <sup>b</sup> | 163 <sup>b</sup> | 140 <sup>d</sup>  |
| Microwave     | vacuum-packed   | 210 <sup>b</sup>     | 183 <sup>b</sup> | 177 <sup>b</sup> | 188 <sup>a</sup> | 198 <sup>a</sup>  |
|               | zipper-top bags | 210 <sup>b</sup>     | 190 <sup>b</sup> | 199 <sup>a</sup> | 186 <sup>a</sup> | 177 <sup>b</sup>  |
| Extrusion     | vacuum-packed   | 229 <sup>a</sup>     | 210 <sup>a</sup> | 168 <sup>b</sup> | 187 <sup>a</sup> | 189 <sup>a</sup>  |
|               | zipper-top bags | 229 <sup>a</sup>     | 219 <sup>a</sup> | 179 <sup>b</sup> | 173 <sup>a</sup> | 157 <sup>cd</sup> |

Means between columns with different letters are different (p-value < 0.05). Comparison within each row is discussed in the body of the text. All the means had Coefficient of Variation of less than 10%.

Water absorption in microwave-heat and extrusion stabilized bran was higher than in the control. This observation may be due to heat dissociation and denaturation of protein, which unmask the nonpolar residues from the interior of the protein molecules (Abbey and Ibeh, 1987). Heat denaturation does not lower the water-binding capacity of sunflower protein; instead it improves the properties (Lin and Humbert, 1974).

### Fat Absorption

The effect of untreated and stabilized bran on fat absorption capacity of rice bran during storage is summarized in Table 3. Fat absorption capacity decreased significantly ( $p$ -value  $< 0.05$ ) in the control samples from an initial value of 218% to 180% in vacuum packs and from 218% to 214% in zipper-top bags.

Microwave-heat stabilized bran started at 0 week with a lower fat absorption capacity value than the control, but capacity increased significantly during storage ( $p$ -value  $< 0.05$ ) in both packaging methods.

Fat absorption capacity was lower in extruded samples than both the control and the microwave-heat stabilized bran at 0 week. The initial value was 160%, which did not change significantly during storage in either packs or bags of 155% and 160%, respectively.

**Table 3. Effect of stabilization and packaging methods on fat absorption capacity of rice bran during storage**

| Treatment     | 0               | Storage Time (weeks) |                  |                  |                  |                  |
|---------------|-----------------|----------------------|------------------|------------------|------------------|------------------|
|               |                 | 2                    | 4                | 6                | 8                |                  |
|               |                 | Fat Absorption (%)   |                  |                  |                  |                  |
| Raw (Control) | vacuum-packed   | 218 <sup>a</sup>     | 189 <sup>b</sup> | 180 <sup>b</sup> | 200 <sup>b</sup> | 180 <sup>b</sup> |
|               | zipper-top bags | 218 <sup>a</sup>     | 229 <sup>a</sup> | 200 <sup>b</sup> | 199 <sup>b</sup> | 214 <sup>a</sup> |
| Microwave     | vacuum-packed   | 208 <sup>b</sup>     | 220 <sup>a</sup> | 239 <sup>a</sup> | 210 <sup>b</sup> | 224 <sup>a</sup> |
|               | zipper-top bags | 208 <sup>b</sup>     | 200 <sup>a</sup> | 239 <sup>a</sup> | 238 <sup>a</sup> | 224 <sup>a</sup> |
| Extrusion     | vacuum-packed   | 160 <sup>c</sup>     | 179 <sup>b</sup> | 140 <sup>c</sup> | 160 <sup>c</sup> | 155 <sup>c</sup> |
|               | zipper-top bags | 160 <sup>c</sup>     | 180 <sup>b</sup> | 140 <sup>c</sup> | 139 <sup>d</sup> | 160 <sup>c</sup> |

Means between columns with different letters are different ( $p$ -value  $< 0.05$ ).

Comparison within each row is discussed in the body of the text.

All the means had Coefficient of Variation of less than 10%.

The application of heat exposes the lipophilic residues of protein (Lin and Humbert, 1974). The higher fat absorption capacity in microwave stabilized bran could be caused by exposure of lipophilic residues of rice bran protein by microwave heating. In sunflower products, high fat absorption capacity (207.8% for the flour and 256.7% for the isolate) is believed to occur because of proteins containing numerous nonpolar side chains that bind the lipophilic chains (hydrocarbon chains) of fats (Lin and Humbert, 1974).

### Emulsification

The formation and stability of an emulsified oil droplet depend on the formation of a protein film around the droplet (Kinsella, 1976). Hydrophobic regions of protein molecules associate at the lipid interface, while polar and ionic regions associate with the aqueous phase (Johnson and Brekke, 1983).

Stabilization methods had varying effects on emulsification of rice bran (Table 4). In the control sample, emulsification did not change significantly during the storage period of 8 weeks.

In microwave-heat stabilized bran, emulsification also did not change significantly with time, regardless of the method of packaging. These values were generally similar to control values.

**Table 4. Effect of stabilization and packaging methods on emulsification of rice bran during storage**

| Treatment     | 0               | Storage Time (weeks) |                 |                 |                  |                 |
|---------------|-----------------|----------------------|-----------------|-----------------|------------------|-----------------|
|               |                 | 2                    | 4               | 6               | 8                |                 |
|               |                 | Emulsification (%)   |                 |                 |                  |                 |
| Raw (Control) | vacuum-packed   | 46 <sup>a</sup>      | 56 <sup>a</sup> | 49 <sup>a</sup> | 45 <sup>a</sup>  | 45 <sup>a</sup> |
|               | zipper-top bags | 46 <sup>a</sup>      | 54 <sup>a</sup> | 49 <sup>a</sup> | 45 <sup>a</sup>  | 45 <sup>a</sup> |
| Microwave     | vacuum-packed   | 41 <sup>a</sup>      | 41 <sup>b</sup> | 24 <sup>b</sup> | 40 <sup>ab</sup> | 43 <sup>a</sup> |
|               | zipper-top bags | 41 <sup>a</sup>      | 45 <sup>b</sup> | 51 <sup>a</sup> | 38 <sup>b</sup>  | 42 <sup>a</sup> |
| Extrusion     | vacuum-packed   | 22 <sup>b</sup>      | 31 <sup>c</sup> | 13 <sup>c</sup> | 7 <sup>c</sup>   | 7 <sup>b</sup>  |
|               | zipper-top bags | 22 <sup>b</sup>      | 31 <sup>c</sup> | 29 <sup>b</sup> | 7 <sup>c</sup>   | 6 <sup>b</sup>  |

Means between columns with different letters are different (p-value < 0.05). Comparison within each row is discussed in the body of the text. All the means had Coefficient of Variation of less than 10%.

In extrusion stabilized bran, the initial emulsification value of 22% increased to 31% in the second week of storage, but then showed a significant ( $p$ -value  $< 0.05$ ) reduction to 7% in vacuum-packed and 6% in zipper-top bags. This could be due to protein denaturation in extruded samples. Heat denaturation decreases protein solubility of soybean, and this decreases the emulsifying action (Pomeranz, 1985).

It is generally assumed that the heat processing leads to a decrease in protein functionality. In this study, the emulsification was decreased in extruded samples but not in microwave-heat stabilized samples. This observation could be caused by the type of heating process with less effect of microwave heat (less exposure time) on protein than extrusion cooking. Yasumatsu et al. (1972) reported that emulsifying properties correlated positively with protein and negatively with fiber content. By processing the capacity to lower the interfacial tension between hydrophobic and hydrophilic components in food, many proteins are effective surface-active agents. Various factors and conditions influence the measurement of emulsifying capacities of proteins, among them equipment design, rate of oil addition, temperature, pH, protein source, solubility and concentration, kind of oil used, salt, sugar, and water content. Therefore, emulsifying capacity is not solely a property of the protein under test but is rather a property of the emulsion system and equipment (Han and Khan, 1990).

## **Foaming**

The effect of bran stabilization and packing methods on foaming capacity is given in Table 5.

The non-stabilized control bran had a great increase in foaming capacity during storage period. The initial value of 9% increased to final values of 293% and 425%, respectively, in vacuum-packed bran and samples packed in zipper-top bags. The foam was stable even after 60 minutes.

The microwave-heat and extrusion stabilized bran exhibited little foaming capacity throughout the 8-weeks storage in the two different types of packaging.

The above observation could be due to heat denaturation of proteins in both stabilization methods. Foams are colloidal (not dissolved, but suspended) dispersions of gases in very viscous liquids. There is an interface between the liquid and the gas. The surface tension at the

**Table 5. Effect of stabilization and packaging methods on foaming capacity of rice bran during storage.**

| Treatment     | 0               | Storage Time (weeks) |                 |                  |                  |                  |
|---------------|-----------------|----------------------|-----------------|------------------|------------------|------------------|
|               |                 | 2                    | 4               | 6                | 8                |                  |
|               |                 | Foaming Capacity (%) |                 |                  |                  |                  |
| Raw (Control) |                 |                      |                 |                  |                  |                  |
|               | vacuum-packed   | 9 <sup>a</sup>       | 79 <sup>a</sup> | 372 <sup>a</sup> | 282 <sup>a</sup> | 293 <sup>b</sup> |
|               | zipper-top bags | 9 <sup>a</sup>       | 54 <sup>a</sup> | 350 <sup>a</sup> | 343 <sup>a</sup> | 425 <sup>a</sup> |
| Microwave     |                 |                      |                 |                  |                  |                  |
|               | vacuum-packed   | 5 <sup>a</sup>       | 6 <sup>b</sup>  | 6 <sup>b</sup>   | 6 <sup>ab</sup>  | 5 <sup>c</sup>   |
|               | zipper-top bags | 5 <sup>a</sup>       | 5 <sup>b</sup>  | 6 <sup>b</sup>   | 3 <sup>b</sup>   | 5 <sup>c</sup>   |
| Extrusion     |                 |                      |                 |                  |                  |                  |
|               | vacuum-packed   | 4 <sup>a</sup>       | 4 <sup>b</sup>  | 5 <sup>b</sup>   | 2 <sup>b</sup>   | 1 <sup>c</sup>   |
|               | zipper-top bags | 4 <sup>a</sup>       | 4 <sup>b</sup>  | 4 <sup>b</sup>   | 2 <sup>b</sup>   | 4 <sup>c</sup>   |

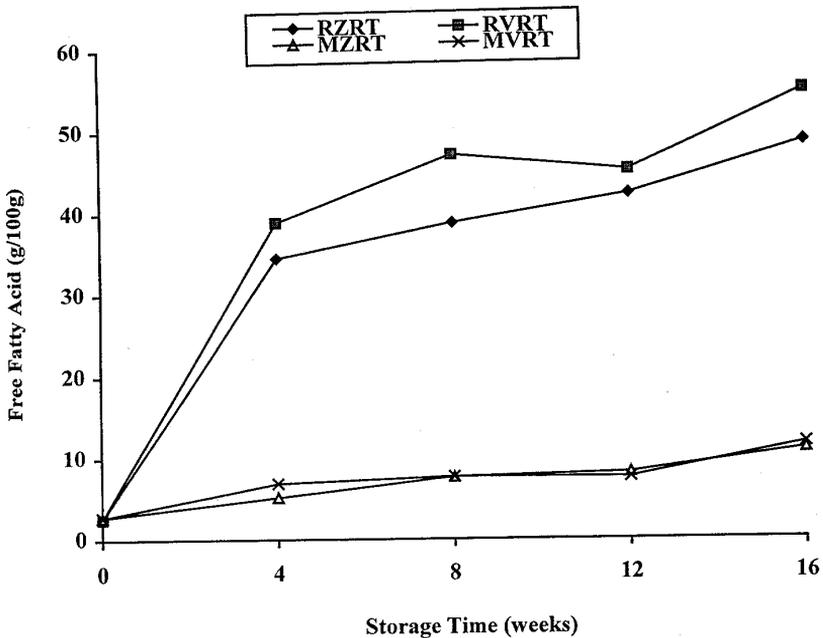
Means between columns with different letters are different (p-value < 0.05).  
Comparison within each row is discussed in the body of the text.  
All the means had Coefficient of Variation of less than 10%.

interface is lowered with an increase in the concentration of dissolved material. If the viscosity at the interface is sufficiently increased by the increase in dissolving material, the foam will be stabilized (Lee, 1975). Colloidal substances, concentrated at the interface, give stable foams, as do proteins. Han and Khan (1990) concluded that roasting beans (navy, pinto, and chickpea) decreased foamability. Protein insolubilization and denaturation are believed to be responsible for this effect. Another possible explanation for the observed formation of foaming could be that the method used to measure the foaming capacity involved the mixing of rice bran with water and agitating the mixture. The free fatty acids present in the mixture would readily cause soaps to form and subsequently cause foaming.

## Phase II:

### Lipase Activity

The effects of microwave heat on bran stability in terms of increase in free fatty acids (% of oleic acid) in raw (R) rice bran packed in vacuum packs (V) and zipper-top bags (Z), stored at room temperature (RT) and refrigerator (Ref) during 16 weeks of storage are shown in figures 5 and 6. 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,



**Figure 5. Free fatty acid changes in raw (R) (control) and microwave-heat stabilized (M) rice bran packed in zipper-top bags (Z) or vacuum pack (V) stored at room temperature (RT).**

respectively, in zipper-top bags at room temperature (RZRT) and in vacuum packs at room temperature (RVRT) (Figure 5). After the initial rapid rise, the FFA level rose much less rapidly between 4 and 16 weeks of storage until value of 48.0% and 54.3% for RZRT and RVRT were reached, respectively. In contrast, microwave treatment inhibited the formation of FFA. Microwave-heated bran stored in zipper-top bags (MZRT) or vacuum packs (MVRT) showed only a small FFA increase from 2.8% to 10.9% (MZRT) and from 2.8% to 11.6% (MVRT). Therefore, microwave heat significantly inhibited FFA formation, and the type of storage container (zipper-top vs. vacuum) had no significant effect on FFA level development. In the control bran samples, however, the vacuum packed consistently exhibited higher FFA values than the zipper-top bag samples at all storage times.

Interaction between storage time and packaging methods showed that FFA increased steadily throughout the storage period, and vacuum samples showed more increase in FFA level than samples packed in zipper-top bags, regardless of the storage temperature. Interaction

between storage temperature and packaging had a significant effect on FFA levels. FFA increased steadily in two different packaging methods as the storage temperature increased. Samples in zipper-top bags had less increase in FFA levels than vacuum-packed samples.

Both raw and microwave-heated rice bran samples were also stored at 4-5 degrees C in both zipper-top and vacuum-pack containers and their FFA levels measured over a 16-week period (Figure 6). For refrigerated raw bran in zipper-top bags (RZRef) and in vacuum packs (RVRef), FFA formation showed a steady increase over the 16-week storage period. RVRef samples exhibited greater FFA development than RZRef samples after 4 weeks of storage. In contrast, microwave-heat stabilized bran packed in zipper-top (MZRef) or vacuum packs (MVRef) showed essentially no FFA development over 16 weeks of storage. The data in figures 5 and 6 showed the effect of storage temperature on FFA content in both raw and microwave-heated bran. FFA formation was suppressed

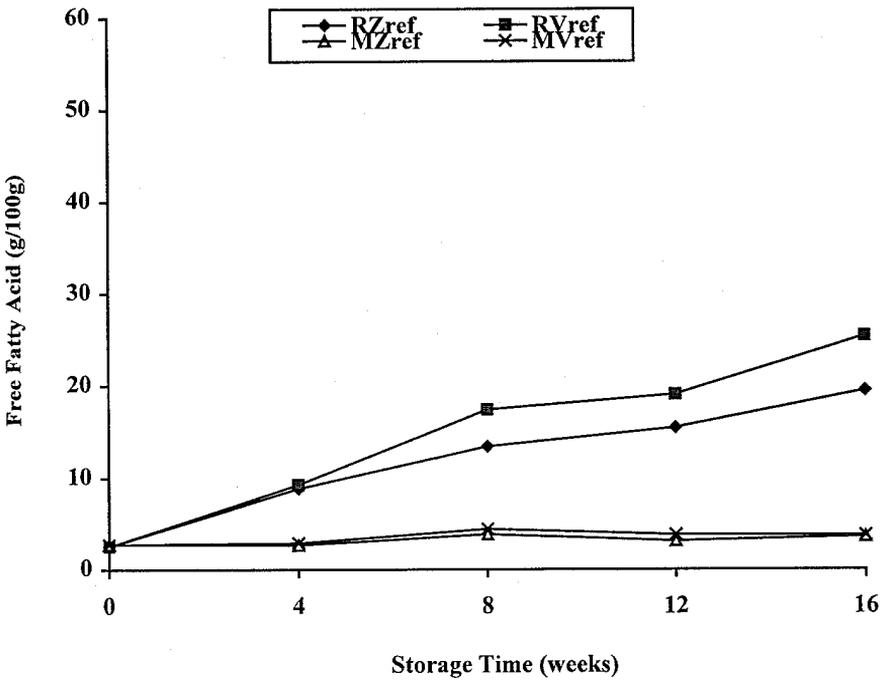


Figure 6. Free fatty acid changes in raw (R) (control) and microwave-heat stabilized (M) rice bran packed in zipper-top bags (Z) or vacuum packed (V) and stored in the refrigerator (Ref).

at the lower storage temperature of 4-5 degrees C for both treatments and methods of storage.

The increase in FFA content of raw rice bran 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 rapid development of hydrolytic rancidity in unstabilized rice bran, which makes this product unsuitable for human consumption (Martin et al., 1993; Tao et al., 1993). Previous studies (Tao, 1989; Malekian, 1992) have shown that FFA content of microwave-heat stabilized rice bran increased from 4.0% to 4.9% in long-grain rice bran (Lemont) and from 4.6% to 6.2% in medium-grain rice bran (Nato) during 4 weeks of storage. In untreated bran, the FFA content changes ranged from 4.0% to 68.3% and 4.6% to 56.8% for long- and medium-grain, respectively. Malekian (1992) showed that the FFA level, in raw rice bran samples placed in zipper-top bags and vacuum packs and stored in the refrigerator, increased from an initial value of 3.7% to 22.2% and 26.7%, respectively, and there was a significant difference between samples vacuum packed and samples packed in zipper-top bags. These findings confirm the results obtained in this study.

Although storage of raw bran at 25 degrees C greatly increased the FFA level in the zipper-top bag samples with an increase of storage time, the increase was greater in the vacuum-packed samples (Figure 5). Sharp and Timme (1986) noticed the same pattern in brown rice stored in bags and vacuum-packed samples. This could be because the removal of air and oxygen by vacuum processing activates anaerobic microorganisms with higher lipolytic enzyme activity present in raw rice bran. Lipases, both endogenous to the bran and of microbial origin, initiate hydrolytic deterioration of kernel oil (Champagne et al., 1992). Surface damage during dehulling disrupts the aleurone and germ (where oil is located), and lipase-producing mold and bacteria found on kernel surfaces would interact with bran oil (Champagne et al., 1992) and FFA increases.

In this study, in microwave-heat stabilized rice bran, the FFA level increased slightly above 10% in samples stored at room temperature (25 degrees C) (Figure 5) but only slightly above 3% for samples stored in the refrigerator (4-5 degrees C) (Figure 6) after 16 weeks of storage. There was not a significant difference in FFA content between vacuum packing and zipper-top bags. This could be because microwave heat destroyed bacteria (especially anaerobic) present in rice bran. Bran oil with an excess of 10% FFA and bran with more than 5% FFA are considered 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 converted to FFA within a week under high humidity and temperature conditions (Champagne and Hron, 1992).

## Lipoxygenase Activity

### Optimum pH for Lipoxygenase Activity

The pH profile of LOX activity is shown in Figure 7. Optimum activity occurred at pH 8.5 at room temperature (25 degrees C). Shastry and Rao (1975) noticed an optimum pH of 8.5 and some detectable activity at pH 6.5 in LOX extracted from fresh raw rice bran. Sekhar and Reddy (1982) performed a study on LOX from eight scented and two unscented rice varieties. They found that all the varieties with the exception of IR-8 had optimal activity around pH 8. The variety IR-8 showed a different type of activity, with maximum activity around pH 6. Yamamoto et al. (1980) showed an optimum pH of 6.5-7 for partially purified rice germ LOX. Ida et al. (1983) reported three distinct peaks of LOX activity (L1, L2, L3) in embryos of rice, each enzyme having a

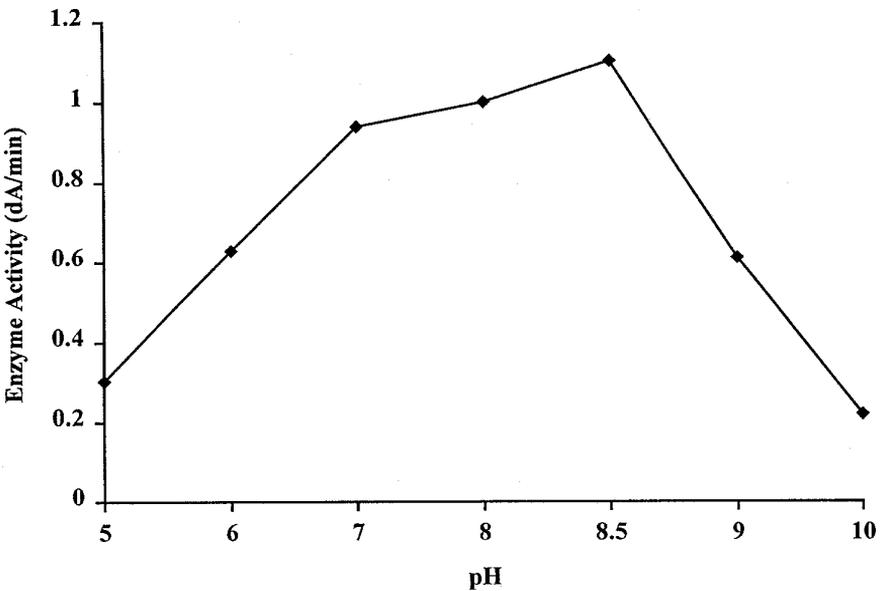


Figure 7. Optimum pH for lipoxygenase activity in rice bran at room temperature (25°C).

different pH optimum of 4.5, 5.5, and 7, respectively. The results obtained in this study are very similar to the data obtained by Shastry and Rao (1975) and Sekhar and Reddy (1982).

### Optimum Temperature for Lipoxygenase Activity

The study of rice bran enzyme extract at different temperatures (15, 20, 25, 30, 40, and 50 degrees C) revealed that enzyme activity increased with temperature from 15 degrees C until it reached 25 degrees C and then started decreasing sharply at temperature higher than 30 degrees C (Figure 8). The temperature of 25-30 degrees C was optimum at pH 8.5. Temperatures of 25-30 degrees C have been used for studying LOX activity by a number of researchers (Surrey, 1964; Wallace and Wheeler, 1972; Shastry and Rao, 1975; Sekhar and Reddy, 1982).

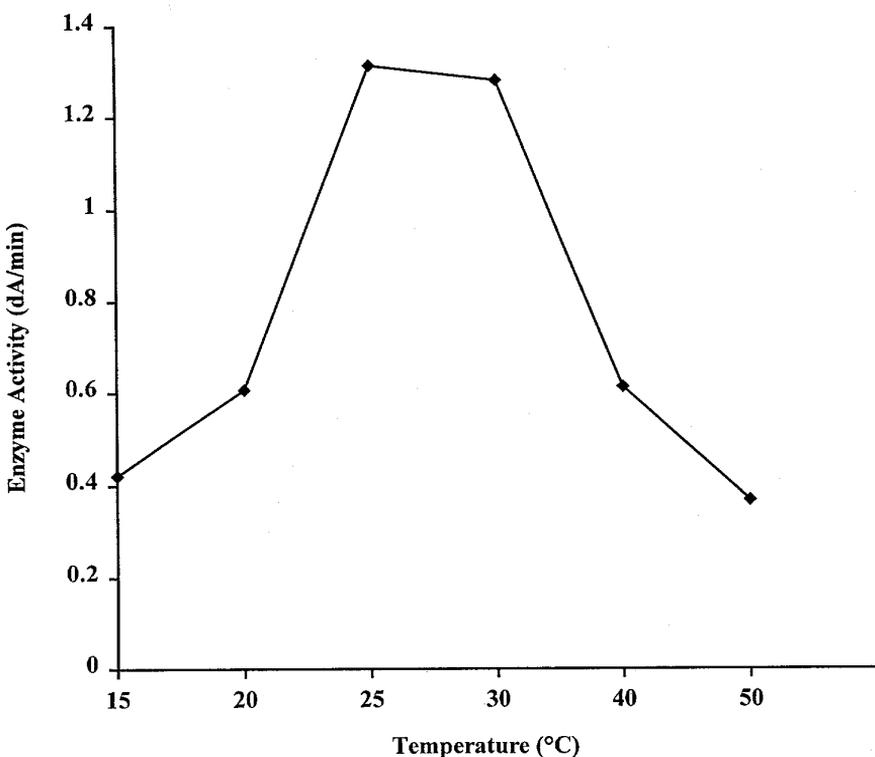
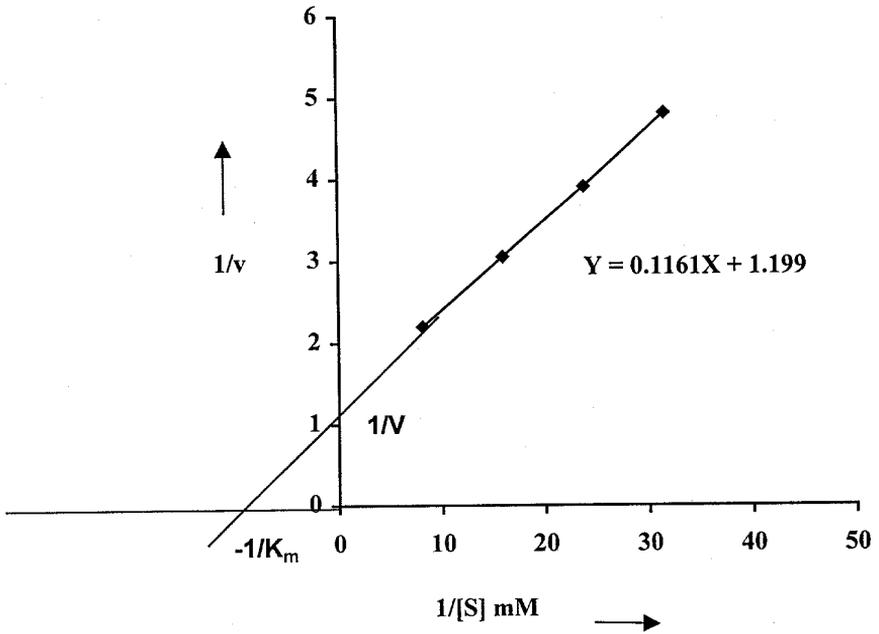


Figure 8. Optimum temperature for LOX activity in rice bran at pH 8.5.



**Figure 9. Determination of  $K_m$   $V_{max}$  according to the method of Lineweaver and Burk (1934) for LOX activity in rice bran. Activity was measured spectrophotometrically.  $K_m$  = Michaelis constant, S= substrate, v= velocity, V= maximum velocity.**

### Lipoxygenase Kinetics

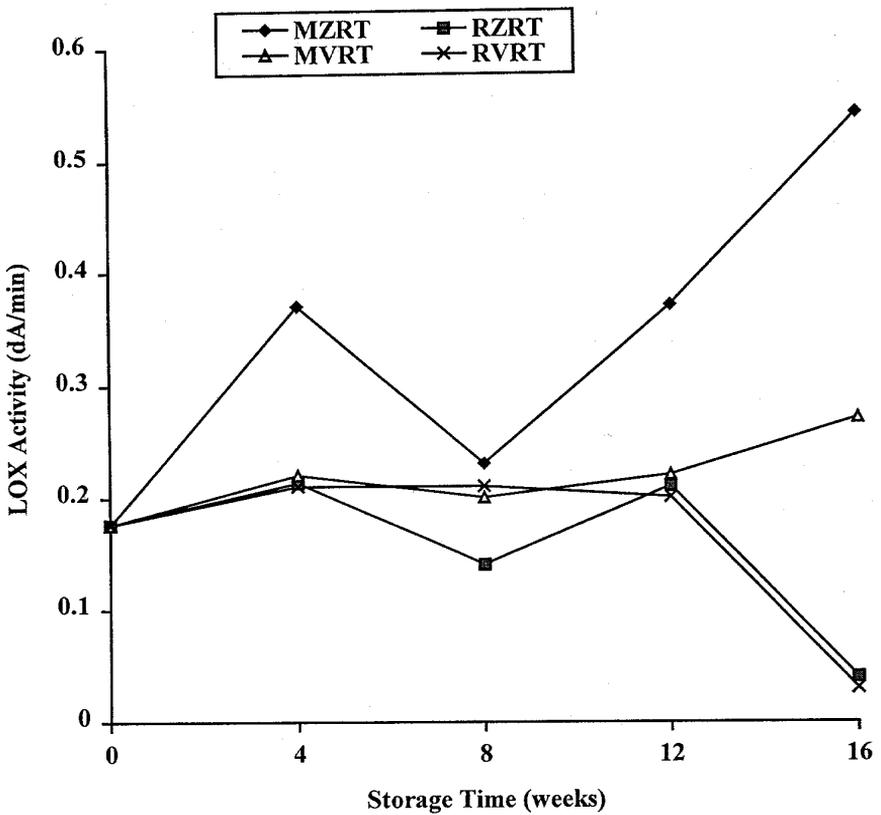
Enzyme activity was measured with different concentrations (0.1231, 0.0627, 0.0421, and 0.0317 mM) of linoleic acid. The results are presented using the Lineweaver-Burk plot (Figure 9). The  $K_m$  value was 0.097 mM and  $V_{max}$  was 0.834 A/min at pH of 8.5 and 25 degrees C. These two parameters ( $K_m$  and  $V_{max}$ ) can be used to give a quantitative measure of the specificity of an enzyme for a given substrate and the efficiency of an enzyme. The higher the ratio of  $V_{max}/K_m$ , the greater the preference (or specificity) of the enzyme toward that substrate (Price and Stevens 1991). Yamamoto et al. (1980) concluded that the rice germ enzyme reaction was highly specific for linoleic and linolenic acids, with a  $K_m$  value of 0.1 mM at pH 7 and 25 degrees C. They suggested that the initial velocity of the reaction was proportional to the enzyme concentration, and a linear relationship was characteristic of the LOX-catalyzed

reactions. Shastry and Rao (1975) reported a  $K_m$  value of 0.35 mM for LOX extracted from rice bran with linoleic acid as substrate at pH 8.5 and 25 degrees C. They suggested that this value was tentative since a technical grade linoleic acid was used, and the enzyme was not homogeneous.  $K_m$  values of 1 and 5 mM have been reported for soybean and wheat, respectively, and  $K_m$  values of 0.3, and 0.8 for potatoes have been reported (Shastry and Rao 1975).

When linoleic acid was dispersed in TritonX-100 or Brij, LOX had  $K_m$  values of 0.175 mM and 0.157 mM and  $V_{max}$  values of 0.225 and 0.532  $\Delta A/\text{minute}$ , respectively, at pH 7.0 (Boyes et al., 1992) for kiwifruit lipoxygenase. Marci et al. (1994) reported a  $K_m$  value of 0.2 mM at pH 5.5-6.0 for both linoleic and linolenic acids in highly purified soybean plasma membranes. The plasma membrane also showed a LOX activity in the alkaline (pH 9.0-9.5) range with a lower  $K_m$  value of 0.06 mM.  $K_m$  is by no means an absolute constant, but depends on pH, temperature, effectors, buffer, etc. (Michal, 1978).

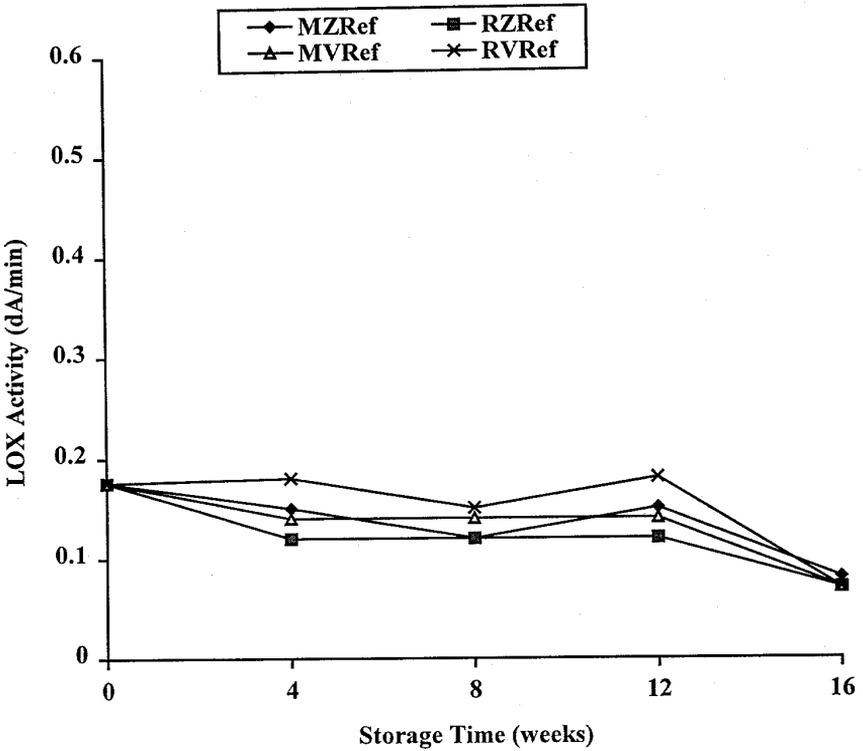
### **Effect of Microwave Heat on Lipoxygenase Activity**

Figure 10 shows that LOX activity increased slightly at week 4 in raw rice bran in zipper-top bags and stored at room temperature (RZRT) and then decreased at week 8, increased again at week 12, and decreased to almost zero activity at week 16. Raw rice bran vacuum packed and stored at room temperature (RVRT) showed a pattern similar to the zipper-top bags over the 16-week storage period, but the changes were not significantly different from each other. For microwave-heated samples in zipper-top bags (MZRT) or vacuum packs (MVRT), LOX activity varied depending on the type of packaging. There was consistently more activity in MZRT samples than MVRT samples over the entire storage period. LOX activity for both raw and microwave-heated rice bran packed in zipper-top or vacuum packs was generally the same when refrigerated at 4-5 degrees C (Figure 11). In fact, there was a slight downward trend in activity with increasing storage time. There was a significant ( $p\text{-value} < 0.05$ ) difference in the LOX activity between samples stored at room temperature and samples stored in the refrigerator (Figure 10 and Figure 11). During weeks 12 and 16, there were no significant changes in LOX activity for microwave-heated samples, except for a significant ( $p\text{-value} < 0.05$ ) increase of LOX activity in samples packed in zipper-top bags and stored at room temperature (Figure 10).



**Figure 10. Lipoxygenase activity in raw (R) (control) and microwave-heat stabilized (M) rice bran packed in zipper-top bags (Z) or vacuum packed (V) and stored at room temperature (RT).**

Interaction between storage temperature and treatment showed (Figure 10) a difference in LOX activity levels between microwave-heated samples and control. Extended storage resulted in the fluctuation of LOX activity in control samples. There was a decrease in LOX activity for samples stored at room temperature at week 8, a significant decrease at week 12, and a significant decrease at week 16 (Figure 10). LOX activity did not change significantly in samples stored in the refrigerator at week 12 and decreased significantly at week 16. LOX activity increased for samples stored at room temperature, while there was not much change in samples stored in the refrigerator. Microwave-heat stabilized samples showed fluctuation with a peak at 4 weeks, then a



**Figure 11. Lipoxygenase activity in raw (R) (control) and microwave-heat stabilized (M) rice bran packed in zipper-top bags (Z) or vacuum packed (V) and stored in the refrigerator (Ref).**

decrease at 8 weeks, and a sharp increase throughout the rest of the storage period (Figure 10). This fluctuation has been observed by Sharp and Timme (1986). They used long-grain (Starbonnet) brown rice and packed it in sealed polyethylene bags, sealed bags, metal cans, and punctured sealed bags sealed in a metal can under vacuum. They stored the samples at three different temperatures (3 degrees C, 25 degrees C, and 38 degrees C) for 9 months. LOX activity was expressed as the concentration of conjugated diene hydroperoxides (CDHP) in the samples. They showed that CDHP increased between months 1 and 2 and decreased between months 2 and 3, regardless of storage temperature.

LOX activity in rice that was microwave-heat stabilized and stored at

room temperature did not increase as sharply as did samples packed in zipper-top bags (Figure 10). This could be because of lack of oxygen, the co-substrate (Berry et al., 1997), which was removed during vacuum packaging. LOX catalyzes the oxidation of methylene-interrupted unsaturated fatty acids and their esters such as linoleic and linolenic acid (Richardson and Hyslop, 1985). The native enzyme, a relatively inactive form, contains an  $Fe^{++}$  cofactor, which is converted to the active ferric form ( $Fe^{+++}$ ) by oxidation, possibly with atmospheric oxygen (Gardner, 1988).

The results of this study showed that, overall, the samples stored at room temperature had higher LOX activity than samples stored in the refrigerator. An increase in CDHP level because of higher storage temperature has been previously reported (Mitsuda et al., 1972; Sowbhagya and Bhattacharya, 1976). 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. Oxidative rancidity was much higher in samples placed under light all the time compared with the same samples kept in containers and placed in the dark. The light caused an increase in oxidative rancidity in samples stored at room temperature. Sowbhagya and Bhattacharya (1976) concluded that a relatively high moisture content, storage in the dark, and low temperatures 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 because of 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 that was reduced either upon milling or upon autoclaving (120 degrees C, 30 min). Rice bran and rice bran oil contain a large amount of potent antioxidants 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).

Microwave heating for various periods of time destroyed  $\alpha$ -tocopherol. The loss of activity in tocopherols was found to increase in the order  $\gamma$ -,  $\delta$ -,  $\beta$ -, and  $\alpha$ -tocopherol (the most potent) with microwave

heating of the oils. The effectiveness of tocopherols as lipid antioxidants has been attributed to their ability to break chain reactions by reacting with fatty acid peroxy radicals. The findings in this study agree with the above conclusions. As a result, the LOX activity increased. Also, the increase in LOX activity in microwave-heat stabilized rice bran stored at room temperature observed in this study may be caused by an increase in the concentration of transition metals such as copper, cobalt, chromium, and especially iron.

This was demonstrated by the study of Rao and Artz (1989), who extruded a corn starch/soybean oil mixture. They reported that most of metals, especially iron, were present at concentrations that were highly catalytic with respect to LOX oxidation. An increase in iron concentration for the samples extruded at 135 degrees C and 175 degrees C was nearly 3 and 6 times, respectively, that of the unextruded samples (Rao and Artz, 1989). This iron was considered to contribute to higher lipid instability in extruded samples at higher temperature. Also, Rao and Artz (1989) noted that some of the increase in oxidation may be due to the surface area associated with thermal expansion of corn starch/soybean oil mixture. Shastry and Rao (1975) reported that partially purified LOX from unfractionated rice bran of an indica variety was activated by Fe<sup>++</sup>. Hiroyuki et al. (1986) indicated that rice LOX-3 was inactivated gradually in storage because of the participation of metal ions and linoleic hydroperoxide. Champagne et al. (1992) reported that increased susceptibility of ethanol-extracted brown rice kernel to oxidative deterioration could be attributed to ethanolic and heat denaturation of the homoproteins catalase and peroxidase found in the kernel. They suggested that unfolding the enzymes to bring about greater exposure of the heme groups to the substrate results in the initiation of oxidation. Microwave-heat employed in this study (107 ± 2 degrees C, for 3 min) could be a contributing factor to increase in LOX activity in microwave-heat stabilized bran compared with untreated samples.

Increase in LOX activity during storage has been demonstrated by Dhaliwal et al. (1991). They concluded that drying the paddy rice before storage did not affect LOX activity in milled rice, but activity increased significantly while samples were stored at room temperature for 12 months. A greater increase in activity was noticed for long-grain rice (Basmati-370) compared to short- (IR-8) and medium- (PR-108) grain varieties. Since LOX acts on unsaturated fatty acids like linoleic acid, which comprises up to 40%-45% of the fatty acids in rice, it can be

assumed that the varieties with lower activities of this enzyme may have better storage qualities (Sekhar and Reddy, 1982).

The moisture content of microwave-stabilized rice bran packed in zipper-top bags and stored at room temperature at the end of the storage period was significantly ( $p < 0.05$ ) lower than the samples at 0 weeks (Table 6). The vacuum-packed microwave-heat stabilized samples (MVRT) and the samples packed in the zipper-top bags (MZRef) had the same amount of moisture. Although the moisture content was nearly equal (6.3%-6.4%), the microwave-heat stabilized bran samples kept refrigerated (MZRef) had significantly lower LOX activity than those kept at room temperature, regardless of the packaging types (MZRT and MVRT). These samples had been dehydrated, which may have led to lower water activity in the rice bran samples. The moisture content of rice bran after drying in an open Petri dish for 24 hours was 2%-3%, which caused the increase in oxidation of lipids (Sowbhagya and Bhattacharya, 1976). A decrease in water activity accelerates the oxidation of lipids in food systems (Koch, 1961; Lee, 1975).

Raw rice bran samples (control) did not show significant ( $p > 0.05$ ) change in LOX activity except for raw samples packed in zipper-top bags and stored at room temperature. There was a significant decrease ( $p$ -

**Table 6. LOX activity 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**

|               |          |      | LOX Activity<br>(dA/min) | Moisture<br>(%)         |
|---------------|----------|------|--------------------------|-------------------------|
| Raw (Control) | 0 week   |      | 0.18 ± 0.00 <sup>b</sup> | 7.5 ± 0.1 <sup>b</sup>  |
|               | 16 weeks | ZRT  | 0.04 ± 0.01 <sup>c</sup> | 7.0 ± 0.0 <sup>bc</sup> |
|               |          | Zref | 0.07 ± 0.00 <sup>c</sup> | 9.3 ± 0.1 <sup>a</sup>  |
|               |          | VRT  | 0.03 ± 0.00 <sup>c</sup> | 6.5 ± 0.2 <sup>c</sup>  |
|               |          | Vref | 0.07 ± 0.02 <sup>c</sup> | 8.5 ± 0.2 <sup>a</sup>  |
| Microwave     | 0 week   |      | 0.18 ± 0.00 <sup>b</sup> | 8.4 ± 0.4 <sup>a</sup>  |
|               | 16 weeks | ZRT  | 0.54 ± 0.11 <sup>a</sup> | 6.4 ± 0.2 <sup>c</sup>  |
|               |          | Zref | 0.08 ± 0.01 <sup>c</sup> | 6.3 ± 0.1 <sup>c</sup>  |
|               |          | VRT  | 0.26 ± 0.10 <sup>b</sup> | 6.3 ± 0.6 <sup>c</sup>  |
|               |          | Vref | 0.07 ± 0.03 <sup>c</sup> | 7.6 ± 0.7 <sup>b</sup>  |

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

value  $< 0.05$ ) after 8 weeks, an increase at week 12, and a highly significant decrease ( $p < 0.001$ ) after 12 weeks of storage (Figure 10).

Sowbhagya and Bhattacharya (1976) observed that even with rapid production of FFA in raw rice, the rate of oxidation remained low. They suggested that either the oxidation-retarding factors in raw rice bran were active or the specificity of the hydrolytic enzyme(s) and the pattern of release of the acids were such that did not favor oxidation. The low LOX activity in raw rice bran samples also could be caused by the action of 9(S)-hydroperoxyoctadecatrienoic acid [9(S)-HPOT] derived from linoleic acid, which inactivated the LOX. Dai-Eun and MeeRee (1989) reported that the incubation of  $\alpha$ -linoleic acid with LOX initially at 4 degrees C and subsequently at 25 degrees C was required for maximal production of the conjugated triene acids, the condition required for the maximal inactivation of LOX activity. They reported that soybean LOX was inactivated gradually during the conversion of 9(S)-HPOT to 9,16-dihydroperoxy conjugated triene acid; therefore,  $\alpha$ -linoleic acid or 9(S)-HPOT was used as a substrate to form an unstable peroxide that could inhibit the soybean LOX irreversibly, following a suicide substrate mechanism (Williams et al., 1986). LOX-3 is the major enzyme in rice grain (Hiroyuki et al., 1986) that specifically produces 9(S)-HPOT (Ida et al., 1983).

The reduction in LOX activity while the substrate concentration was increased to 0.2 mM linoleate in the absence of calcium ion could be caused by substrate inhibition, which is often attributed to saturation of the catalytic site by substrate (Yamamoto et al., 1970). This information supports data obtained in this study, in that the reduction in LOX activity in untreated samples may be due to an increase in the amount of substrate by the action of lipase, presence of an antioxidant, and also to an increase in microwave-heat stabilized samples that may be due to lack of substrate, more exposure of metal ions such as iron and calcium, and lack of antioxidants.

### **Specific Activity of Lipoxygenase**

The specific activity expressed as  $\mu\text{A}/\text{min}/\text{mg}$  of sample, of microwave-heat stabilized and untreated rice bran is shown in Table 7. The changes in specific activity are not significant at 4 weeks of storage. After 8 weeks there was a significant increase in microwave-heat stabilized samples vacuum packed and stored at room temperature. At week 12, microwave-heat stabilized samples stored at room temperature in a

**Table 7. LOX-specific activity 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**

|               | 0 week                                | 4 weeks                    | Storage Time                |                            |                            |
|---------------|---------------------------------------|----------------------------|-----------------------------|----------------------------|----------------------------|
|               |                                       |                            | 8 weeks                     | 12 weeks                   | 16 weeks                   |
|               | Specific Activity<br>units /mg sample |                            |                             |                            |                            |
| Raw (Control) | 1085.9±14.2 <sup>a</sup>              | 1085.9 ±14.2 <sup>a</sup>  | 1085.9 ±14.2 <sup>ab</sup>  | 1085.9 ±14.2 <sup>b</sup>  | 1085.9 ±14.2 <sup>b</sup>  |
| ZRT           |                                       | 1464.4 ± 30.6 <sup>a</sup> | 1164.6 ± 40.5 <sup>ab</sup> | 816.0 ± 25.7 <sup>bc</sup> | 165.4 ± 9.6 <sup>d</sup>   |
| Zref          |                                       | 1001.1 ± 82.9 <sup>a</sup> | 943.6 ± 13.9 <sup>b</sup>   | 464.0 ± 11.9 <sup>c</sup>  | 504.3 ± 26.3 <sup>c</sup>  |
| VRT           |                                       | 1454.1 ± 11.9 <sup>a</sup> | 1676.1 ± 39.9 <sup>ab</sup> | 856.8 ± 16.5 <sup>bc</sup> | 170.5 ± 41.4 <sup>b</sup>  |
| Vref          |                                       | 1153.1 ± 17.6 <sup>a</sup> | 870.7 ± 10.4 <sup>b</sup>   | 787.2 ± 56.3 <sup>bc</sup> | 640.1 ± 92.6 <sup>c</sup>  |
| Microwave     | 1119.1±10.5 <sup>a</sup>              | 1119.1 ±10.5 <sup>a</sup>  | 1119.1 ±10.5 <sup>ab</sup>  | 1119.1 ±10.5 <sup>b</sup>  | 1119.1 ±10.5 <sup>b</sup>  |
| ZRT           |                                       | 1204.2 ± 10.8 <sup>a</sup> | 1259.5 ± 98.9 <sup>ab</sup> | 1535.3 ± 21.5 <sup>a</sup> | 2008.7 ± 35.7 <sup>a</sup> |
| Zref          |                                       | 928.5 ± 12.6 <sup>a</sup>  | 1094.4 ± 88.0 <sup>ab</sup> | 579.9 ± 74.6 <sup>bc</sup> | 612.2 ± 68.0 <sup>c</sup>  |
| VRT           |                                       | 1273.4 ± 32.7 <sup>a</sup> | 1754.6 ± 65.1 <sup>a</sup>  | 836.0 ± 21.6 <sup>bc</sup> | 1271.4 ± 55.9 <sup>b</sup> |
| Vref          |                                       | 932.5 ± 19.5 <sup>a</sup>  | 917.8 ± 16.8 <sup>b</sup>   | 852.2 ± 15.5 <sup>bc</sup> | 532.1 ± 10.7 <sup>c</sup>  |

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

zipper-top bag showed a significant ( $p$ -value  $< 0.05$ ) increase, and the rest of the samples decreased but not significantly. At each storage interval, the specific activity of the microwave-heat stabilized bran kept refrigerated either in the zipper-top bags or vacuum packs showed no significant difference. For the microwave-heat stabilized bran, the specific activity of the sample in the zipper-top bags kept at room temperature (MZRT) increased with increased storage time; the opposite effect was observed for the samples in the vacuum-packed bags kept at refrigerated temperature (MVRef). Data (Table 7) also showed that the specific activity of microwave-heated bran in zipper-top bags kept refrigerated (MZRef) drastically dropped after 8 weeks of storage as opposed to after 12 weeks of storage observed for VRef. However, the specific activities of both MZRef and MVRef were similar (532.1-612.2 units/mg sample) (Table 7).

Hafez et al. (1985b) observed that there was a negative correlation between the dose of irradiation and the enzyme-specific activity in soybean. The highest inactivation was at doses of 65 KGy irradiation and moisture content of 30.47% ( $173.03 \pm 3.23$  A/min/g of defatted sample). The moisture content for inactivation of LOX activity was important. Esaka et al. (1987) suggested that microwave heat was more effective in inactivating LOX in winged bean seeds with higher moisture (soaking the seeds in water for 15 hr required 30 sec of microwave heating) to completely inactivate LOX. They concluded that microwave heating inactivated LOX of winged bean seeds in much less time than conventional heating. The same conclusion was obtained for soybean (Esaka et al., 1986). Wang and Toledo (1987) reported that LOX activity in microwave-heated soybean samples, with higher moisture content (38.8%, 47.0%, and 56.8%), were completely inactivated. The soybean temperature was around 100 degrees C. This confirms that not only is the temperature of the sample important for LOX inactivation, but the moisture content of the sample plays an important role, since it results in higher energy absorption.

In our study, the microwave heat did not inactivate LOX activity, and this could be mostly because of the moisture content of the samples. The moisture content in rice bran samples was adjusted from an initial 7.5% to 21% before the stabilization process. The temperature in rice bran samples reached  $107 \pm 2$  degrees C during microwave-heat processing. The amount of moisture in excess of 21% resulted in the bran becoming too clumpy, and moisture less than 21% resulted in the bran being too dry and burned in some areas (Tao, 1989; Malekian, 1992). It appears the

moisture content or duration of time was not enough to inactivate the LOX activity. On the other hand, lipase was inactivated under the above conditions.

## **Nutrient Losses**

### **Fatty Acid Content**

Distribution of fatty acids did not show significant differences between raw and microwave-heat stabilized rice bran at 0 week and during 16 weeks of storage except for the C18:1 content of raw ZRT and ZRef at 16-week storage. Oleic acid (C18:1), linoleic acid (C18:2), and palmitic acid (C 16:0) are dominant fatty acids (ca. 48%, 32%, and 14% respectively) (Table 8) in raw and microwaved rice bran. The level of these fatty acids depends on the variety and the location of the rice (Saunders, 1990; Gupta, 1989). The distribution of fatty acids agreed with data reported by Saunders (1990).

Palmitic acid content of raw and microwave-heat stabilized bran increased from about 14% at week 0 to 17% at week 16, except for the microwave VRT samples (Table 8). Stearic acid and linolenic acid did not change significantly. Oleic acid content decreased after 16 weeks of storage in both raw and microwave-heat stabilized samples. Significant decreases were observed for raw rice bran stored in the zipper-top bags, irrespective of storage temperature. There was a decrease in linoleic acid, but these changes were not significant. Linoleic acid, the best substrate for LOX, decreased from an initial value of 32.6% to 29.9% in microwave-heat stabilized samples after 16 weeks of storage, but there was no significant change in linoleic acid at each storage interval (Table 8). Hafez et al. (1985a) noticed there were no quantitative differences in fatty acid composition of raw and microwave-heat stabilized soybeans after microwave treatment for 15 min, but protein digestibility decreased.

Hafez et al. (1985b) noticed no significant changes in fatty acids (C16:0, C18:0, C18:1, and C18:2) at different radiation doses of 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. Gamma irradiation of rice seeds with intact hulls minimized the increase in the amount of unsaturated fatty acids in the FFA fraction, which are prone to oxidation in the presence of oxygen radicals generated by irradiation.

$\gamma$

**Table 8. Fatty acid composition of microwave (M) heat stabilized and raw (R ) rice bran at 0 and 16 weeks of storage**

|               |          | $C_{16:0}$              | $C_{18:0}$             | Fatty acid %             |                          |                         |                        |
|---------------|----------|-------------------------|------------------------|--------------------------|--------------------------|-------------------------|------------------------|
|               |          |                         |                        | $C_{18:1}$               | $C_{18:2}$               | $C_{18:3}$              |                        |
| Raw (Control) | 0 week   | 14.5 ± 0.5 <sup>a</sup> | 2.3 ± 0.2 <sup>a</sup> | 47.9 ± 0.2 <sup>a</sup>  | 32.4 ± 0.5 <sup>a</sup>  | 1.1 ± 0.0 <sup>a</sup>  |                        |
|               | 16 weeks | ZRT                     | 19.9 ± 0.1             | 2.7 ± 0.0 <sup>a</sup>   | 42.0 ± 0.2 <sup>c</sup>  | 32.4 ± 0.1 <sup>a</sup> | 1.2 ± 0.0 <sup>a</sup> |
|               |          | Zref                    | 17.1 ± 3.0             | 2.6 ± 0.5 <sup>a</sup>   | 45.0 ± 4.0 <sup>b</sup>  | 30.5 ± 4.3 <sup>a</sup> | 1.1 ± 0.2 <sup>a</sup> |
|               |          | VRT                     | 17.0 ± 2.0             | 2.7 ± 0.3 <sup>a</sup>   | 45.5 ± 2.0 <sup>ab</sup> | 33.4 ± 0.7 <sup>a</sup> | 1.1 ± 0.2 <sup>a</sup> |
|               | Vref     | 18.5 ± 0.2              | 2.5 ± 0.0 <sup>a</sup> | 45.9 ± 0.1 <sup>ab</sup> | 30.2 ± 0.1 <sup>a</sup>  | 1.1 ± 0.0 <sup>a</sup>  |                        |
| Microwave     | 0 week   | 14.3 ± 0.1 <sup>a</sup> | 2.2 ± 0.1 <sup>a</sup> | 48.0 ± 0.1 <sup>a</sup>  | 32.6 ± 0.2 <sup>a</sup>  | 1.1 ± 0.0 <sup>a</sup>  |                        |
|               | 16 weeks | ZRT                     | 18.1 ± 0.5             | 2.5 ± 0.1 <sup>a</sup>   | 46.7 ± 0.5 <sup>ab</sup> | 29.9 ± 0.3 <sup>a</sup> | 1.1 ± 0.0 <sup>a</sup> |
|               |          | Zref                    | 18.1 ± 0.4             | 2.5 ± 0.1 <sup>a</sup>   | 46.0 ± 0.3 <sup>ab</sup> | 30.3 ± 0.2 <sup>a</sup> | 1.2 ± 0.0 <sup>a</sup> |
|               |          | VRT                     | 14.7 ± 0.3             | 2.8 ± 0.0 <sup>a</sup>   | 44.2 ± 0.2 <sup>ab</sup> | 34.9 ± 0.1 <sup>a</sup> | 1.3 ± 0.0 <sup>a</sup> |
|               | Vref     | 18.3 ± 0.0              | 2.4 ± 0.0 <sup>a</sup> | 45.9 ± 0.0 <sup>ab</sup> | 30.4 ± 0.0 <sup>a</sup>  | 1.2 ± 0.0 <sup>a</sup>  |                        |

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

## Proximate Composition

Proximate composition of raw and microwave-heat stabilized rice bran was obtained and analyzed (Table 9). There were no significant changes in protein and fat during storage. These data agree with earlier findings of Wadsworth and Koltun (1986) and Yeo and Shibamoto (1991). Moisture content was significantly ( $p$ -value  $< 0.05$ ) higher in microwave-heat stabilized samples than raw samples, 8.4% and 7.5%, respectively, at 0 week. Raw samples with moisture content of 7.5% 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 caused by nonenzymatic browning. 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 showed significant browning compared with 14% moisture content. They explained the reduction in the moisture content, before and after microwaving, and production of darker color as follows. In the initial stage of microwave irradiation, the energy is predominately from microwaves. These waves cause dipolar substances, such as water, to undergo rotation and absorb microwave energy, resulting in an increase in temperature and thereby reduction in moisture. As the microwave irradiation proceeds, however, 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 favors the formation of brown color and loss of moisture in our samples.

In raw samples, the moisture content increased significantly ( $p$ -value  $< 0.05$ ) in the samples packed in zipper-top bags and vacuum packs and stored in the refrigerator, while the samples stored at room temperature had a slight decrease in the amount of moisture. In the microwave-stabilized samples, however, the moisture content decreased significantly ( $p$ -value  $< 0.05$ ) for all samples, which could also be a contributing factor for increase in LOX activity of samples stored at room temperature. Ash and carbohydrate showed significant fluctuations. The percent ash increased from 7.6 to 8.2-8.4 in microwave-heat stabilized samples. Microwave heat had a slight effect on mineral concentration of microwave baked potatoes (Klein and Mondy, 1981). Carbohydrate showed significant ( $p$ -value  $< 0.05$ ) decrease from an initial value of 51.8% to 48.2% in raw samples packed in zipper-top bags and stored in the

**Table 9. Proximate composition 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**

|               |          | Protein (%)                                                                                                                            | Fat (%)                                                                                                  | Moisture (%)                                                                                          | Ash (%)                                                                                                | Carbohydrate (%)                                                                                            |
|---------------|----------|----------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|
| Raw (Control) | 0 week   | 17.1 ± 0.6 <sup>a</sup>                                                                                                                | 16.4 ± 0.0 <sup>a</sup>                                                                                  | 7.5 ± 0.1 <sup>b</sup>                                                                                | 7.4 ± 0.1 <sup>bc</sup>                                                                                | 51.7 ± 0.7 <sup>a</sup>                                                                                     |
|               | 16 weeks | ZRT<br>17.4 ± 0.1 <sup>a</sup><br>Zref<br>17.0 ± 0.2 <sup>a</sup><br>VRT<br>17.5 ± 0.2 <sup>a</sup><br>Vref<br>17.0 ± 0.1 <sup>a</sup> | 16.9 ± 0.0 <sup>a</sup><br>17.6 ± 1.1 <sup>a</sup><br>16.9 ± 0.2 <sup>a</sup><br>16.9 ± 0.2 <sup>a</sup> | 7.0 ± 0.0 <sup>bc</sup><br>9.3 ± 0.1 <sup>a</sup><br>6.5 ± 0.2 <sup>c</sup><br>8.5 ± 0.2 <sup>a</sup> | 8.3 ± 0.2 <sup>a</sup><br>7.9 ± 0.1 <sup>ab</sup><br>8.4 ± 0.2 <sup>a</sup><br>7.8 ± 0.3 <sup>bc</sup> | 50.4 ± 0.0 <sup>b</sup><br>48.2 ± 0.6 <sup>c</sup><br>50.8 ± 0.4 <sup>b</sup><br>49.8 ± 0.7 <sup>bc</sup>   |
| Microwave     | 0 week   | 17.5 ± 0.4 <sup>a</sup>                                                                                                                | 17.5 ± 0.4 <sup>a</sup>                                                                                  | 8.4 ± 0.4 <sup>a</sup>                                                                                | 7.6 ± 0.1 <sup>bc</sup>                                                                                | 48.9 ± 0.3 <sup>bc</sup>                                                                                    |
|               | 16 weeks | ZRT<br>17.7 ± 0.4 <sup>a</sup><br>Zref<br>17.9 ± 0.3 <sup>a</sup><br>VRT<br>18.0 ± 0.1 <sup>a</sup><br>Vref<br>17.5 ± 0.3 <sup>a</sup> | 17.9 ± 0.3 <sup>a</sup><br>17.3 ± 0.0 <sup>a</sup><br>17.0 ± 0.1 <sup>a</sup><br>17.1 ± 0.1 <sup>a</sup> | 6.4 ± 0.2 <sup>c</sup><br>6.3 ± 0.1 <sup>c</sup><br>6.3 ± 0.6 <sup>c</sup><br>7.6 ± 0.7 <sup>b</sup>  | 8.4 ± 0.1 <sup>a</sup><br>8.2 ± 0.1 <sup>a</sup><br>8.4 ± 0.1 <sup>a</sup><br>8.3 ± 0.0 <sup>a</sup>   | 49.7 ± 0.5 <sup>bc</sup><br>50.3 ± 0.3 <sup>b</sup><br>50.4 ± 0.6 <sup>bc</sup><br>49.5 ± 0.9 <sup>bc</sup> |

Means within a column with different letters are different (p < .05).

refrigerator. However, the microwave-heat stabilized sample (0 week) started at significantly lower carbohydrate content than the raw samples and did not change significantly during 16 weeks of storage (Table 9). The results from this study agree 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 packs and stored in the refrigerator for 8 weeks (Malekian, 1992).

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## Summary and Conclusions

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In the first phase of the present study, microwave heat and extrusion were used for stabilization of rice bran, and the effect of these two methods on functional properties was studied. Commercially milled, fresh rice bran was used. Half of the raw bran (control) and stabilized samples were vacuum packed in polyethylene bags, and the other half was packed in polyethylene zipper-top bags. The samples were stored in a walk-in cooler (4 degrees C). For testing the efficiency of treatment and effect of microwave heat, the samples were analyzed for proximate composition (at the beginning and the end of storage period), FFA, water and fat absorption capacity, emulsification, and foaming capacity after 0, 2, 4, 6, and 8 weeks of storage.

Statistical analysis of the data showed no significant change in proximate composition of rice bran stabilized by microwave energy. The moisture content of bran increased in the vacuum-packed extruded samples. Microwave heating was as effective as extrusion in terms of bran stability. Control samples showed a highly significant increase ( $p < 0.01$ ) in FFA throughout the storage period. Vacuum-packed raw samples showed a higher increase in FFA after 6 weeks of storage. The microwave heat stabilized and extrusion-stabilized samples showed high water absorption capacity with a significant decrease ( $p < 0.05$ ) during storage and vacuum-packed samples showed less decrease compared with samples packed in zipper-top bags. Fat absorption in microwave-heat stabilized bran was significantly higher than in extrusion-stabilized samples and increased significantly ( $p < 0.05$ ) during storage in both packing methods. Emulsification in microwave-heat stabilized samples was significantly higher than in extrusion-stabilization samples with no significant changes during the storage period in both packaging methods. Both microwave-heat stabilized and extrusion stabilized rice bran did not form foam.

In the second phase of this study, microwave heat only was used to stabilize the rice bran. Freshly milled raw rice bran from rice variety 'Lemont' was exposed to microwave heat for 3 min, and the moisture content of the sample was adjusted from 7.7% to 21% before microwave heating. Half of the raw bran (control) and stabilized samples were vacuum packed in non-permeable polyethylene bags, and the other half were packed in polyethylene zipper-top bags. Half of the samples were stored in the refrigerator (5 degrees C), and the other half were stored at room temperature (25 degrees C). For testing the efficiency of treatment and effect of microwave heat, the samples were analyzed for free fatty acid (FFA), lipoxygenase (LOX) activity, LOX-specific activity, and fatty acid composition after 0, 4, 8, 12, and 16 weeks of storage. Samples were analyzed for proximate composition at week 0 and 16. FFA level increased significantly ( $p$ -value  $< 0.05$ ) in raw rice bran samples stored at room temperature in samples packed in zipper-top bags and vacuum packed. FFA level in raw rice bran samples increased significantly ( $p$ -value  $< 0.05$ ) regardless of packaging and storage temperature. Microwave-heat stabilized samples had a significant ( $p$ -value  $< 0.05$ ) increase in FFA level in samples stored at room temperature and packed in zipper-top bags and vacuum packed, but the FFA level in microwave-heat stabilized samples stored in the refrigerator did not increase significantly during 16 weeks of storage. Data collected show that vacuum packing did not show any advantage over zipper-top bags, and the best temperature for storage of microwave-heat stabilized rice bran was refrigeration. LOX activity in raw rice bran samples decreased significantly ( $p$ -value  $< 0.05$ ) for samples stored at room temperature and packed in zipper-top bags and vacuum packed during storage. LOX activity was reduced from the 0 week value for samples stored in the refrigerator in both types of packaging.

Microwave-heat stabilized samples stored at room temperature in zipper-top bags and vacuum packed had a significant ( $p$ -value  $< 0.05$ ) increase in LOX activity during the 16-week storage period. Samples stored in zipper-top bags showed a higher increase. LOX activity in microwave-heat stabilized samples stored at 4-5 degrees C decreased significantly, however, regardless of packaging. From the data obtained, it can be concluded that a storage temperature of 4-5 degrees C and zipper-top packaging were best for controlling LOX activity in microwave-heat stabilized samples.

Fatty acid composition, specifically linoleic acid, did not change significantly in microwave-heat stabilized rice bran samples packed in either zipper-top bags or vacuum packed and stored in the refrigerator (4-5 degrees C) during 16 weeks of storage. Proximate composition of microwave-heat stabilized rice bran samples did change significantly for samples packed in zipper-top bags or vacuum packed and stored in the refrigerator during storage. The moisture content decreased significantly ( $p < 0.05$ ) in samples stored in the refrigerator and packed in zipper-top bags or vacuum packed.

Data collected from the two phases of this experiment indicate that microwave stabilization of rice bran has advantages over extrusion. The advantages include shorter processing time and no apparent effect on nutritional value. Also, data showed that use of vacuum packing does not have any advantage over zipper-top bags for storage, and 4-5 degrees C is the suitable temperature (compared only 4-5 degrees C and 25 degrees C) to extend the shelf life in terms of prevention of hydrolytic rancidity and reducing oxidative rancidity in microwave-heat stabilized rice bran samples during 8 and 16 weeks of storage.

## **Recommendations**

The following recommendations are made:

1. Incorporate microwave-heat stabilized rice bran in products such as breads, muffins, meatballs, cookies, etc., and perform sensory evaluations with highly trained panelists.
2. Study the effect of storage temperature and packing materials and methods for longer periods of time (6 months to 1 year).
3. Determine vitamin E and oryzanol (antioxidant) content in microwave-heat stabilized vs. control samples and study changes in two different packaging methods and two different storage temperatures.
4. Determine the specific oxidation products from LOX activity and autoxidation in microwave-heat stabilized samples and study the effect of packaging methods and temperature during storage on these oxidation products.

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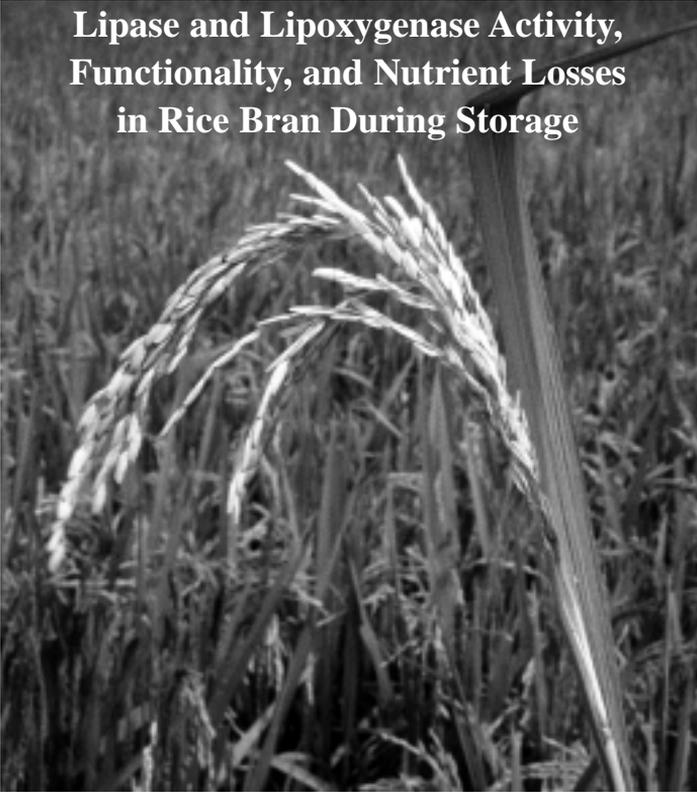
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**Lipase and Lipoxygenase Activity,  
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