CHAPTER 3

ENZYME IN BRAN AND STABILIZATION OF THE BRAN

From chapter 2, it has been explained that rice bran is a potentially important raw material in the edible oil and compound feed stuff industry but a major difficulty of rice bran utilization as a raw material for the production of high quality edible oil is associated with its deterioration during storage. Major causes of rice bran deterioration should be mentioned.

a) Enzymes
b) Microorganisms
c) Insects

ENZYMES

A lipolytic enzyme present in the rice bran becomes active when bran is separated from the rice, causing the hydrolysis of the rice bran oil into glycerol and free fatty acids.[1,5] This reaction is due to the action of moisture in the presence of enzymes which acts as a catalyst. An extensive study has been undertaken by funatsu et al[27] on the lipase of rice bran. Gas-chromatographic analysis has shown that during storage of rice bran, awaked liberation of free fatty acid occurs, possibly due to the hydrolytic breakdown of glycerides(neutral fats) in rice bran.[27] Lipase catalyizes the enzymatic reaction depicted in Fig[2.1] Funatsu's group used tributyrin as substrate.

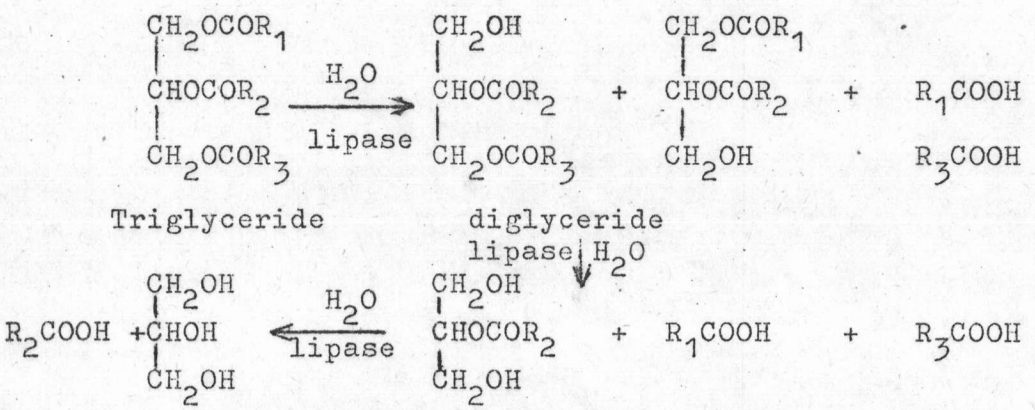

Fig 2.1 Reaction of Glyceride to Glycerol and Free Fatty Acid

The free fatty acid, ordinarily below 3 % immediately after milling, increases at the rate of about 1 % an hour[28] following removal of the bran.

The location of lipases in rice kernel and its characteristics has been reviewed by Desickachar.[13] There is definite evidence that the lipases are present mostly in the layer just peripheral to the aleurone layers or in the testa or cross layer of the rice germs[2,35] when the bran is scoured and esolated from the grain during rice milling the enzyme and substrate are brought together and the lipolytic enzyme is triggered into activity.

Rice bran lipase is a naturally occuring with a molecular weight of 41,000.[27,29] Optimum pH for activity is 7.4 to 7.6 . Only small amounts of calcium could inhibit the enzyme. Heavy metal such as Cu^{++}, Zn^{++}, EDTA, CN^{-} and F^{-} and also alcohol and acetone inactivate the enzyme. Heating of the enzyme solution at 60°C for 10 minutes has been found to inactivate the enzyme.[30]

MICROORGANISMS

Rice bran is found to be highly contaminated by microflora, with reported counts for bacteria and mold, respectively, 3,770,000 and 110,000 per gram.[31] The microbe contaminant are found to be species of Endomycopsis, Aspergillus, Mucor, Penicillium, Fusarium and Bacillus.[12] These microorganisms produce the lipases which increases the free fatty acid content of rice bran while also producing the dangerous mycotoxins.

INSECTS

Other contaminants are found to be insects which included the rice weevil Sitophilus oryzae L., granary weevil Sitophilus granarius L., and rice moth Corcyra Ciphalonica Staint.

3.1 STABILIZATION OF RICE BRAN

Several processes developed for the stabilization of

rice bran can be classified into two groups.

3.1.1 Unthermal processes

3.1.2 Thermal processes

3.1.1 UNTHERMAL PROCESSES

Several references had refered to unthermal processes, that can be divided into two types;

1) Chemical Processes

2) Gamma radiation

3.1.1.1 CHEMICAL PROCESSES

Chemical treatments such as with 0.31 % ethylene chlorohydrin, 0.03 % sodium cyanate, 0.14 % propylene glycol diproprionate, 0.03 % 1,3 dimethyl 4,6 bis(chloromethyl) benzene, 5-20 % sodium bromide, 5-10 % sodium chloride, 5-10 % potassium iodine, 0.5-1.0 % Topanal OF and Topanal OC are ineffective in preventing the development of free fatty acid.(23,32,33)

Storage of rice bran under carbon dioxide, nitrogen gas, or vacuum are also ineffective in preventing deterioration.(23)

Azeemoddin, G, et al(38) used metabisulfite, which is known to be a good preservative used in food industries and used as a disinfectant and antiseptic in stabilizing the bran. He used 2% by weight in stabilizing the rice bran

It was found that the free fatty acid in from untreated bran stored for 0,10,20 and 30 days were 2.2,12,20 and 24 respectively and the corresponding figures for chemically treated rice bran were 2.2,2.5,2.5 and 3.5, respectively. It would appear that chemical treatment had immobilized lipase activity in the bran and stabilized it for safe storage over a peroid of one month. It was also observed that the oil yield from the bran was not reduced by treatment with the chemicals.

3.1.1.2 GAMMA RADIATION

Saad Ibrahim El-Hinnaway(35) had investigated the effect of gamma radiation on the activity of lipolytic enzymes and oil in rice bran. He subjected the bran with different doses of gamma

radiation from 100,000 R to 1,200,000 R, the samples of rice bran, after irradiation with different doses, were extracted with petroleum ether and the oils produced showed no effect on the constants of rice bran oil (such as the refractive index, acid value, saponification value and iodine value). It is clear from fig(3.2) that gamma radiation had a marked effect on the activity of the lipolytic enzymes in the rice bran. In other words the activity of the lipolytic enzymes in the rice bran was diminished by the increase in the dose of gamma radiation. Samples of rice bran which was irradiated with gamma rays at the rate at 1,200,000 R, showed no marked increase in its mixture with the neutral oil, i.e, this dose of gamma radiation seem to inhibit completely the activity of the lipolytic enzymes in the rice bran. This pricess could be used industrially to stop the lipolytic effect of the enzyme when that dose of irradiation could be cheaply produced.

3.2.2 THERMAL PROCESSES

Of all the methods of enzyme inactivation, thermal process has found favour with various workers though none of these has ever reached a commercial acale. Thermal processes of stabilizing rice bran can be divided into two subgroups;

1) Dry heat processes
2) Moist heat processes

3.2.2.1 DRY HEAT PROCESSES

Possibilities of dry heat treatment have been extensively investigated. In general, dry heat does not inactivate lipases totally. Many papers state that only partial inactivation is achieved.[18,21,22] The temperature used inactivating lipased should be about 105°C.[18]

Browne[22] reported that lipase is present in rice bran and that heating the rice bran to 90°C partially inhibited lipolytic action.

West and Cruz[18] reported that the formation of free fatty acids resulted from the action of moisture catalyzed by enzymes

1. bran irradiated at rate of 100,000 R
2. " " " 150,000 R
3. " " " 200,000 R
4. " " " 250,000 R
5. " " " 300,000 R
6. " " " 600,000 R
7. " " " 1,200,000 R
8. Untreated bran

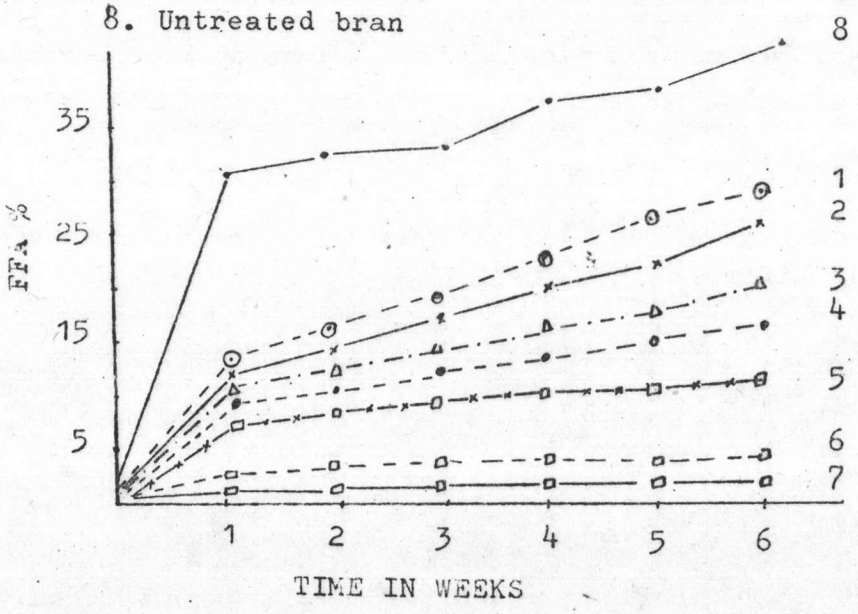

Fig 3.2 Average percentages of free fatty acid liberated in mixtures of the irradiated rice bran with neutral cotton seed oil (34)

and that the most convenient method to preserve rice bran was to heat it at about 105°C, for three hours and store in moisture-proof packages.

Loeb et al[23] found that dry heat up to 110°C, for two hours effectively inhibited the formation of free fatty acids in the oil, by removing the moisture required for hydrolysis, since the activity recurred where moisture was added.

Shcherbakov and Erokhima[24] treated rice bran by heating it at 105°C for 35 minutes. A rapid increase in the concentration of the free fatty acid was found in the untreated bran while the rate of free fatty acid increase was relatively small in the case of treated bran.

Dry heat stabilization in a revolving drum[25] provided with a tight, fitting lid, was also carried out. After charging the rice bran, the material was heated until steam began to emerge from a certain valve. Heating(110-115°C) was continued for 5 minutes after allowing the air to escape. It found that storage of the dry heat stabilized bran had free fatty acid content below 10 % even after more than 120 days indicating that the treatment in the roaster is effective in destroying the lipase.

Another method which claims stabilization of rice bran has been reported by Zachariassen.[26] Fresh rice bran was heated at 105°C for 2 hours, stored for 10 days, reheated. After a period of 40 days storage, it was observed that the percentage of free fatty acids in the treated bran was about 40. Although the stabilization is fairly good, the large treatment time necessary for this process, makes it unattractive.

3.2.2.2 MOIST HEAT PROCESSES

Enzyme inactivation processes in general have involved the application of heat with an initial addition of moisture. This procedure has been adopted because the heat sensitivity of enzymes[47] is greater at moisture content levels some what above those found in freshly milled rice bran.

Loeb indicated that in rice bran sterilized by autoclaving, no free fatty acids were formed in storage until microbial contamination occured.

Robert et al[8] showed that the formation of free fatty acids in stored bran was inhibited by preliminary steam treatment at 100°C for as little as one minute in a continuous blancher, but they did not report the moisture content nor the microflora present during storage. However, they commented on the high count of microorganisms in unheated rice bran and the possibility that these might furnish some or all of the lipase activity.

B.N. Srimani. D, Challopadhyay and A.N. Bose[9] reported that inactivation of the enzyme by dry heating the time of heating should be 15-20 minutes depending on the temperature of heating. The heating with live steam is more efficient to destroy the enzyme in rice bran.

Burn and Cassidy[10] increased the moisture content of rice bran and then subsequently dried it at temperatures about 100°C. In another patented process[11] for rice bran stabilization bran is subjected to humid hot air for a short period and then dried to a moisture content of 10-15 %.

Moist heat has been proved to be more effective than dry heat in stabilizing rice bran.[10] Moist heat treatment on the appropriate condition of moisture content, temperature and time achieved total irreversible destruction of all enzymes in rice bran also, destroying infestations by microorganisms and insects. However, when rice bran is injected with live steam, the moisture content of the rice bran will increase, thus, the steamed bran should be dried to liss than 10 % moisture content,[10,11] especially when it is to be stored in tropical conditions.

Even though, dry heat was claimed to be a reversible destruction of enzyme activities compared to moist heat, yet, dry heat treatment appears applicable and dependent upon the shelf life required, and the process is rather uncomplicated.