

# Enzymatic Hydrolysis Of Vegetable Oils Versus Sardine Fish Oil By Hydrated Rice Bran With And Without Husk

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**Abstract:** Rice bran is a byproduct obtained by milling paddy to get rice. The fresh rice bran is mainly rich in proteins, vitamins, minerals, and oil. Rice bran oil has noticeable nutritional value in food industry. The percentage of oil content decreases with time due enzymatic hydrolysis forming free fatty acids and glycerol. Aqueous extracts of fresh rice bran hydrolysis sardine fish relatively more than that of sunflower and groundnut oil. The rice bran extract with husk shows relatively higher hydrolysis 1.63 % than that of rice bran extract without husk 1.45% hydrolysis on fifth day irradiation under sun light with respect to the control (1.30 %). When the sample incubated at 40°C, the hydrolysis was increased. The percentage FFA of sardine was recorded as 1.70 % on the first day of incubation and 1.87% on the fifth day incubation against the control with 1.30% in the presence rice bran extract with husk. However, the oil sample having rice bran extract without husk shows a relatively lesser percentage of free fatty acids as 1.68% on first incubation and 1.85% on fifth day incubation. Under sunlight, the sardine fish oil shows reluctant hydrolysis. The percentage of FFA on the first day irradiation was 1.50 % and 1.63 % on the fifth day irradiation for the sample having an aqueous rice bran extract with husk against control of 1.30% to that of sardine fish oil having aqueous rice bran extract without husk recorded 1.32 % on first day and 1.45% on fifth day respectively. The peroxide value under the sun light was increased rapidly to that of heat treatment. A maximum 254.44 meq O<sub>2</sub>/ kg of peroxide value was noted for sardine fish oil having hydrated rice bran (HRB) with husk under sunlight and 137.21 meq O<sub>2</sub>/ kg to that of samples under heat treatment. On correlating the percentage of enzymatic hydrolysis of sardine fish by HBR is more effective with heat treatment than irradiation by sunlight.

**Key words:** HBR, Husk, Enzymatic hydrolysis, Sardine fish oil, Sunlight, Heat treatment, FAA, Peroxide value.

## 1. INTRODUCTION

Rice (*Oryza sativa*) is one of the leading food crops worldwide followed by maizes (corn) and wheat. Rice is produced by milling paddy. Rice bran (RB) is a by-product obtained from the outer layer of the brown (husked) rice kernel during milling to produce white rice. Many researchers reported the nutritional values and chemical composition. The chemical composition of RB show that it composes of high nutritional value proteins [1] and may also be hypoallergenic [2]. It is rich in nutrients with 14%-16% protein, 12%-23% fat, and 8%-10% crude fiber. Pragasam A, Vinayak M Naik Research scholar and Guide. Research and Development Centre, Bharathiar University, Coimbatore, Tamil Nadu, India Pragasam A, Preeti N Tallur, Vinayak M Naik, Professors in chemistry and Vaishnavi V.Naik, Nivedita P. Naik, students. Government arts and Science college, Karwar, Karnataka, India RB is also a good source of vitamins and contains minerals such as iron, potassium, calcium, chlorine, magnesium, and manganese [3]. Moreover, recent United States Department of Agriculture (USDA) reports show that rice bran is a good or even better than oat bran in reducing serum cholesterol and reducing the risk of heart disease. Even, rice bran cost less and taste better than oat bran [4]. The chemical composition of rice bran was significantly affected by the milling process. Among the samples evaluated, rice bran produced at 8% milling degree (MD) had the lowest protein, fat and crude fiber contents. Mineral content was highest for rice bran produced at 6% M D, while the highest vitamin content was found in rice bran produced at 2% M D. The study also indicated that all types of rice bran offer healthier benefits [5]. Rice bran contains 12-23 % of oil typically an oleic linoleic-type fatty acid, and its physical-chemical properties qualify it for a good quality edible oil [6]. However, complete utilization of bran oil suffers for there is a large accumulation of free fatty acids (FFA), which has been attributed to the presence of lipase activity [7].

Funatsu et al. reported the identifying and characterizing the lipases from bran using efficient methods to overcome rice bran oil instability. Two soluble lipases have been purified from the rice bran. Lipase I have a molecular mass of 40 k D and a pH optimum of about 7.5. It is activated by calcium and cleaves preferentially fatty acids from the sn-1 and sn-3 positions of triacylglycerols. Lipase II has a molecular mass of 32 kD, a pI of 9.1, and pH optimum of about 7.5 [8-9]. Rice bran has been used as a fermentation substrate for the production of enzymes such as lipase by *Candida* Sp. [10-11], with cassava starch and rice hulls for the production of gluco amylase by *Aspergillus* Sp. [12] and with wheat bran for the production of alkaline protease by *Trichoderma koningii* [13]. A thermally stable lipase (EC 3.1.1.3.) was first identified in rice (*Oryza sativa*) bran, and the enzyme was purified to homogeneity using octyl-Sepharose chromatography. The enzyme was purified to 7.6-fold with the final specific activity of 0.38  $\mu$  Mol min<sup>-1</sup> mg<sup>-1</sup> at 80°C using [9,10-3H] triolein as a substrate. The purified enzyme was found to be a glycoprotein of 9.4k D. Enzyme showed a maximum activity at 80°C and at pH 11.0 [14]. The extracted lipase was purified 4.61-fold immobilized in calcium alginate beads and comparative characterization of free and immobilized enzymes was performed. The entrapped lipase showed good thermo-stability and retained 77.96% of its activity upon exposure to a high temperature of 70°C when compared to 49.9% for the free enzyme. The immobilized and free enzymes were most stable in the acidic pH range of 5.0-7.0. They showed good stability towards metal ions at 1mM concentration. Polar organic solvents inhibited the enzyme stability to a lesser extent than non-polar solvents [15]. Hydrolysis of triglyceride to yield free fatty acids and glycerol from cooking palm oil have been studied for various parameters such as enzyme and oil loading, temperature, pH, and agitation speed. A maximum conversion was achieved in 90 min at oil concentration of 0.1 g/ml, enzymes loading of

7.46 kLU/ml, temperature of 45°C, pH 7.5 and 200 rpm [16]. Enzymatic hydrolysis of Indian tuna fish oil catalyzed by candida rugosa lipase (CRL) in biphasic solvent system was reported by Kriti Bhandari et al. [17]. Researchers reported that the extraction of rice bran oil was maximized by inactivating the enzymatic hydrolysis using different chemicals such as hydrochloric acid, Phosphoric acid, Acetic acid, Sodiummetabisulphite [18]. Present work was focused on the rice bran enzymatic hydrolysis of sunflowers and ground oil versus sardine fish oil. The enzymatic extract of rice bran was added to edible oils and free fatty acid value determined at the end of each day by weighing 5 gm of oil from the samples. The free fatty acid value was determined with sunlight and under heat treatment. The relative rate of enzymatic hydrolysis of sunflowers and groundnut oils was compared with sardine fish oil.

## 2 Materials and Methods

### 2.1 Chemicals

The chemicals used are of analytical and Lab grade which were purchased from Nice chemical supplier. Glacial acetic acid, Chloroform, Potassium iodide, Sodium thiosulfate, Potassium dichromate, soluble starch, Isooctane and Anisidine

### 2.2 Sampling

Fish oil samples were prepared as shown the table to determine the deterioration of the nutritional value of the oil.

SFO	Commercial sun flower oil available in local market
SFO + HRB	Commercial sunflower oil and hydrated rice bran
GNO	Commercial groundnut oil available in local market
GNO + HBR	Commercial groundnut oil and hydrated rice bran
FO	Sardine fish oil available in local extractor
FO + HRB	Sardine fish oil and hydrated rice bran

### 2.3 Percentage of Free fatty acid (% FFA)

Freshly extracted oil was used to determine acid value of oil. Acid value is an important indicator of oil quality. AV is expressed as the amount of KOH (in milligrams) required to neutralize free fatty acids contained in 1 g of oil. Now, AV determination is based on the international standard method [19-21]. The chemical used are analytical grade with 99% purity.

$$\text{FFA \%} = \frac{V_{\text{KOH}} \times N_{\text{KOH}} \times M}{10 \times W_{\text{sample}}}$$

$V_{\text{KOH}}$  = Volume of KOH

$N_{\text{KOH}}$  = Normality of KOH

$W_{\text{sample}}$  = Weight of the sample

M = Molecular weight of the fatty acid

For, oleic acid, M = 282, lauric acid, M = 200, ricinoleic acid,

M = 298, palmitic acid, M = 256

### 2.4 Peroxide Value(PV)

Exactly, 5 gm fish oil was weighed into the Erlenmeyer flask with glass stopper. Acetic acid and chloroform mixture in the ratio 3:2 was added to the flask. To this 0.5 ml saturated KI solution was added and peroxide value was determined by titrating against standard 0.01 M sodium thiosulfate solution using starch as an indicator. The procedure was followed by officially recommended method by AOCS [19, 22].

$$\text{PV} = \frac{(\text{S}-\text{B}) \times \text{N} \times 1000}{\text{Weight of sample}}$$

B = Blank titre value. S = Sample titre value. N = Normality of sodium thiosulfate solution

## 3 Results and discussion

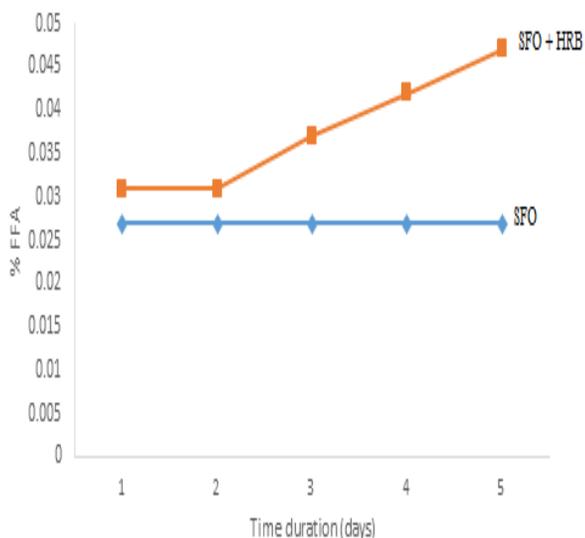
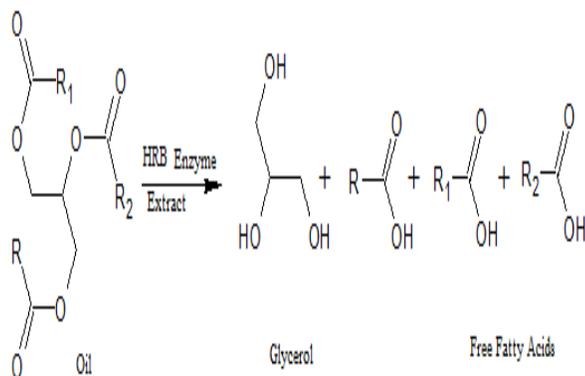
### 3.1 Determination of relative % of FFA of sunflower, groundnut and sardine fish oil under sun light.

The oil samples were irradiated with sunlight eight hours per day and the percentage of free fatty acid was determined at the end of each day by weight 5 gm of oil samples. The estimated FFA values were reported as in the Table 1.

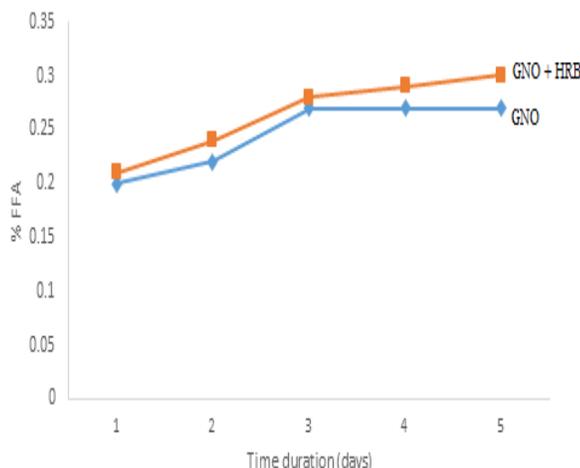
**Table 1:** % FFA value with time duration in presence of HRB under sun light

Days	Percentage of FFA					
	Sunflower oil		Groundnut oil		Sardine fish oil	
	SFO	SFO + HRB	GNO	GNO+ HRB	FO	FO + HRB
1	0.027	0.031	0.20	0.21	1.30	1.32
2	0.027	0.031	0.22	0.24	1.30	1.39
3	0.027	0.037	0.27	0.28	1.30	1.42
4	0.027	0.042	0.27	0.29	1.30	1.43
5	0.027	0.047	0.27	0.30	1.30	1.45

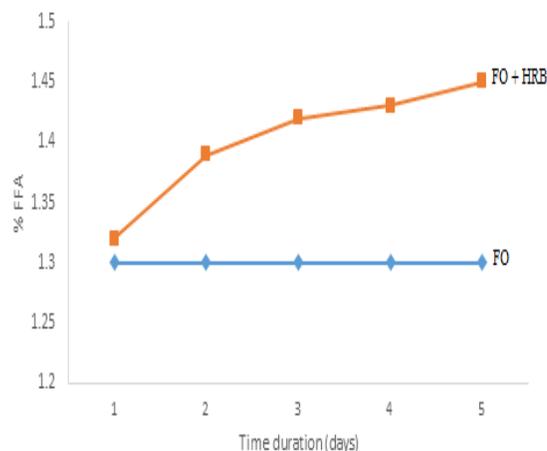
The plot of % FFA versus time duration clearly explains the enzymatic hydrolysis of oils forming free fatty acids and glycerol. Enzymatic hydrolysis increases with time duration of irradiation with oil samples containing hydrated extract of rice bran whereas oil samples without HRB show a constant percent of FFA. Sunflowers and groundnut oil samples showed a relatively low percentage of FFA attributing reluctant hydrolysis as compared with sardine fish oil. A maximum, of 0.047 percent FFA was observed for sunflower oil with HRB to that of plain sunflower oil (0.027 % FFA) on the fifth day of irradiation (Fig 1). The maximum percent of FFA for groundnut oil was recorded as 0.30 % against the groundnut oil without HRB having 0.27 % FFA (Fig 2). Sardine fish oil shown a conspicuous enzymatic hydrolysis producing relatively more FFA to that of the sunflower and groundnut oils. The relative maximum of 1.45 % of FFA was estimated for sardine fish oil with HRB to that of fish oil without HRB having 1.30 on the fifth day of irradiation under sunlight (Fig 3). The determination of FFA by sunlight induced rice bran enzymatic hydrolysis was estimated as oleic acid.



**Figure 1:** % FFA versus time duration of HRB enzymatic hydrolysis of sunflower oil under sunlight.



**Figure 2:** % FFA versus time duration of HRB enzymatic hydrolysis of groundnut oil under sunlight.



**Figure 3:** % FFA versus time duration of HRB enzymatic hydrolysis of sardine fish oil under sunlight.

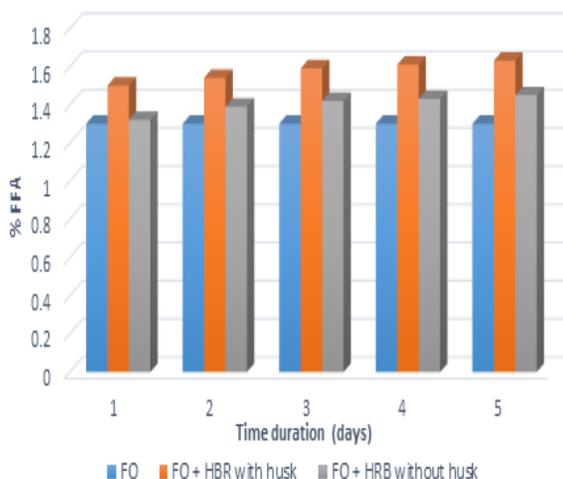
**3.2 Determination of % of FFA of sardine fish oil under sun light.**

Rice bran with and without husk was ground fine and weighed 1gm of each into a 250ml beaker. To this 1ml distilled water was added and mixed well to paste mass. 50ml sardine fish oil was added to each beaker, mixed thoroughly with a clean glass rod and kept in presence of sunlight for six hours with occasional mixing. 50ml sardine fish oil and 1ml distilled water was considered as control. The percentage of FFA was determined at end of each for five days as in the Table 2.

**Table 2:** % FFA value with time duration in presence of HRB with and without husk under sun light

Percentage of FFA			
No. Days	FO	FO +HRB with husk	FO + HRB without husk
1	1.30	1.50	1.32
2	1.30	1.54	1.39
3	1.30	1.59	1.42
4	1.30	1.61	1.43
5	1.30	1.63	1.45

A bar graph was plotted percent of FFA against time duration in days as in the Figure 4. The control sample gives a constant 1.3 percent of FFA for a duration of five days. A gradual increase in percentage of the formation of FFA from the first day to the fifth day for the sample having HRB with and without husk. Relatively, percentage FFA was recorded for fish oil having HRB with husk from 1.50 % on the first day to 1.63 % on the fifth day whereas the fish oil having HRB without husk recoded relatively low percentage of FFA from 1.32 % on the first day to 1.45 % on the fifth day. The gradual increase in the percent of FFA formation is due to the enzymatic hydrolysis of the enzymes in HRB with and without husk. The enzymatic hydrolysis was increased significantly with HRB having husk. The HRB with husk, acts as a conspicuous enzymatic substrate to hydrolyze the sardine fish. Chemical content of rice husk consists of 50% cellulose, 25-30% lignin, and 15-20% silica [23]. These might be responsible for enhanced enzymatic hydrolysis sardine fish in compare to control and oil sample having HRB without husk.



**Figure4:** % FFA versus time duration for fish oil sample in the presence of sunlight

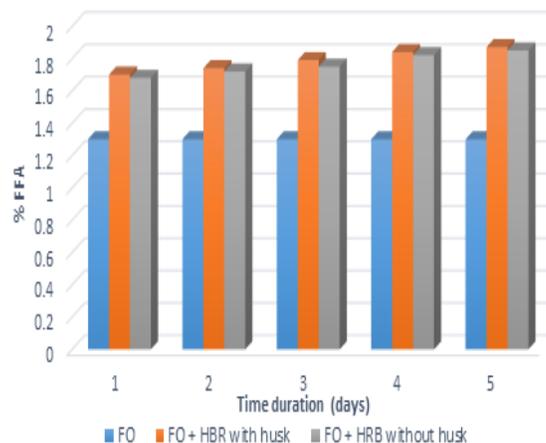
**3.3 Determination of % of FFA of sardine fish oil under heat treatment**

Rice bran with and without husk was ground fine and weighed 1gm of each into a 250ml beaker. To this 1ml distilled water was added and mixed well to paste mass. 50ml sardine fish oil was added to each beaker, mixed thoroughly with a clean glass rod and incubated at 40°C for six hours with occasional mixing daily. 50ml sardine fish oil and 1ml distilled water was considered as control. The percentage of FFA was determined at end of each day for five days as shown in the Table3.

**Table 3:** % FFA value with time duration in presence of HRB with and without husk under heat treatment

Number of heating	Percentage of FFA		
	FO	FO + HRB with husk	FO + HRB without husk
1	1.30	1.70	1.68
2	1.30	1.74	1.72
3	1.30	1.79	1.75
4	1.30	1.84	1.82
5	1.30	1.87	1.85

A bar graph was plotted percent of FFA against time duration in days as in the Figure 5. The control sample gives a constant 1.3 percent of FFA for a duration of five days. A gradual increase in percentage of the formation of FFA from the first day to fifth day for the sample having HRB with and without husk. The Relative percentage was recorded for fish oil having HRB with husk from 1.70 % FFA on the first day to 1.87 % on fifth day whereas the fish oil having HRB without husk recoded relatively low percentage of FFA from 1.68 % on the first day to 1.85 % on the fifth day. The gradual increase in the percent of FFA formation is due to the enzymatic hydrolysis of the enzymes in HRB with and without husk. The enzymatic hydrolysis was increased significantly with HRB having husk. The HRB with husk, acts as conspicuous enzymatic substrate to hydrolyze the sardine fish.



**Figure 5:** % FFA versus time duration for fish oil sample under heat treatment

**3.4 Peroxide value of fish oil samples in the presence of sunlight and heat treatment**

Rice bran with husk was ground fine and weighed 1 gm of into two 250 ml beakers separately. To this 1 ml distilled water was added and mixed well to paste mass. 50 ml sardine fish oil was added to each beaker, mixed thoroughly with a clean glass rod and one sample was incubated at 40°C for six hours with occasional mixing daily. The same procedure was followed for five days. The other sample was irradiated for six hours in the presence of sun light every day and the same was repeated for five days as shown in the Table 3.

**Table3:** Time duration versus peroxide for fish oil samples under sunlight and heat treatment

Time duration (day)	PV of control		PV Under sunlight	PV under heat treatment
	Heat treatment	Under sunlight		
1	6.40	45.42	139.15	122.45
2	8.12	69.23	142.86	128.94
3	9.50	92.41	146,25	131.72
4	11.82	119.67	249.35	133.51
5	13.43	137.52	252.44	137.21

The enzymatic extract of rice bran with husk, shows relative high activity in hydrolyzing sardine fish oil. Incubating fish oil with 2 ml water at 40°C recorded a peroxide value 6.40 meq O<sub>2</sub> per kg on the first day and 13.43 meq O<sub>2</sub> per kg on fifth day whereas with sunlight it was recorded 45.42 meq O<sub>2</sub> per kg on first day and 137.52 on fifth day. Relative higher peroxide values were recorded for the samples with 2 ml enzymatic extract of rice bran having husk in the presence of sunlight and at incubation temperature. The sample in the presence of sunlight recorded the peroxide value 139.15 meq O<sub>2</sub> per kg on the first day and 252.44 meq O<sub>2</sub> per kg on fifth day. A steep increase in PV was observed from the first day irradiation to fifth day irradiation. The sample under incubation recorded the peroxide value 122.45 meq O<sub>2</sub> per kg on the first day and 137.21 on fifth day. A gradual increase in peroxide value was observed from the first day to fifth day. The change peroxide value shows more affinity for peroxidation with sunlight than that incubation. But, the relative % of FFA were more sample

under incubation compare to those with sunlight. This observation clarifies that enzymatic hydrolysis of rice bran extract as the enzyme like lipase show maximum activity at 37-40°C. Thus sardine fish oil could be hydrolyzed more effectively under heat treatment rather than in presence of sunlight to form free fatty acids.

#### 4 Conclusion

Rice bran is a rich nutritional byproduct on milling of paddy to get rice. The stability of before liberating from its sources has been conspicuously affected by enzyme activities. Sardine fish oil is rich source of poly unsaturated fatty acids like omega fatty acids. Hydrated rice bran hydrolysis the sardine fish much faster than sunflower and groundnut oil under heat treatment over irradiation by sunlight. A maximum, of 0.047 percent FFA was observed for sunflower oil with HRB to that of plain sunflower oil (0.027 % FFA) on fifth day of irradiation. The maximum percent of FFA for groundnut oil was recorded as 0.30 % against the groundnut oil without HRB having 0.27 %. Sardine fish oil shown a conspicuous enzymatic hydrolysis producing relatively more FFA to that of sunflower and groundnut oils. The relative maximum of 1.45 % of FFA was estimated for sardine fish oil with HRB to that of fish oil without HRB having 1.30 on fifth day of irradiation under sunlight. A gradual increase in percentage of the formation of FFA from the day one to the day five for the sample having HRB with and without husk. Relative percentage of FFA was recorded for fish oil having HRB with husk from 1.50 % on the first day to 1.63 % on fifth day whereas the fish oil having HRB without husk recorded lower percentage of FFA from 1.32 % on the first day to 1.45 % on the fifth day. The same fish oil samples HRB with husk recorded a higher value under heat treatment showing 1.70 % FFA on day one to 1.87 % on day five, whereas the fish oil having HRB without husk recorded relatively low percentage of FFA from 1.68 % on day one to 1.85 % on day five. The higher peroxide value sardine fish oil indicates the accessibility for peroxidation that inversely related to hydrolysis. Thus, the HBR with husk more efficiently hydrolysis sardine fish oil compare to sunflower and groundnut oil under heat treatment at 37-40°C.

#### 5 Acknowledgement

I would like to acknowledge the support of our beloved Principal and group members for their invaluable cooperation to work on this topic.

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