Review
of
Literature
The term rice bran refers to the coating removed from brown rice during the process of milling. The bran constituted nearly 8.5% of total grain and is highly nutritious being rich in lipids, protein, minerals and vitamins. It is a major sources of oil varying from 12-25% depending upon quality of bran and the degree of polishing (Gupta, 1989).

2.1 Composition of rice bran

Rice bran composition is affected by the degree of milling. Amount of bran obtained and its oil content also depend on degree of polishing. In the rice grain, lipid content is highest in the embryo and the aleurone layers. The oil content of bran both incremental and average decreased with increased time of milling. After 10 seconds of milling, oil content ranged from 8.95 to 15.13% depending upon variety (Singh et al. 2002).

Saunders (1985) reported that rice bran contains 6.7-17.2% protein, 4.7-22.6% fat, 6.2-26.9% fiber and 8.0-22.2% ash. The wide variations are attributed to rice cultivar, treatment of grain prior to milling and fractionation processes operative during milling.

The samples of rice bran collected from different rice growing areas of West Bengal and Orissa were examined for their composition. The rice bran samples contained total lipids ranging from 19-23%, protein 15.2-18.5%, ash 13.3-18.6% and sand silica 3.7-8.8% (Sarker and Bhattacharya, 1989).
According to Orthoefer (1996), rice bran contains protein, oil, ash, carbohydrates approximately to the extent of 15, 18, 7 and 50%, respectively. It also contains about 7% crude fiber which is composed of about 28% true fiber consisting of 2.4% soluble fiber and 25.6% insoluble fiber.

Babcock (1987) analyzed the stabilized rice bran and found 7.0 to 11.4% crude fiber consistent of neutral detergent fiber, pentosans, hemicelluloses, cellulose and lignin amounting to 23.7-28.6%, 7.0-8.3%, 9.5-16.9%, 5.9-9.0% and 2.8-3.9%, respectively.

Prakash and Ramanathan (1995) found that the proteins of rice bran are rich in albumin (32%) and globulins (26%). Protein content of defatted, milled and sieved rice bran flour ranged from 16.5-18.2%. It was observed that full fat and defatted bran showed lower protein content when obtained from parboiled paddy but their ash and crude fiber contents were higher than the untreated rice bran samples.

Commercial rice bran contains a fairly good amount of starch due to the presence of endosperm in it. Reported values of total sugar content of rice bran ranged from 3-5% on moisture free basis. Non-reducing sugars are more abundant then reducing sugars, the ratio being 3.1 to 4.1. Glucose, fructose and sucrose have been reported to be present in rice bran. Lignin content ranged from 7.7 to 13.11% (Luh, 1991).

Fat content of rice bran ranged from 3.4% in defatted bran to 22.5% in bran milled after the rice was parboiled. Calcium content of the bran ranged from
0.09 to 4.86%, calcium to phosphorus ratio (Ca:P) of bran varied from 2.7:1 to 1:15.7 Phosphorus of content brans ranged from 1.41 to 2.19% (Davis et al., 2000).

2.2 Stabilization rice bran

One of the problems in incorporation of rice bran in food products is its high instability due to instant action of lipase on oil (Akazama, 1972). Upon milling of rice bran, neutral oil is exposed to lipases, which causes rapid breakdown of triglycerides to free fatty acids (Desikachar, 1974). According to Orthoefer (1996), during milling of rice the structure of the bran is disrupted and lipase is mixed with the oil. As a result, there is a rapid increase in free fatty acids content of oil in the bran. Stabilization of rice bran helps to overcome this problem (Desikachar, 1994).

Several methods have been developed to stabilize the rice bran and thus enhance its stability for much longer time than raw rice bran. Sayre et al. (1982) reviewed the methods of rice bran stabilization with special emphasis on extrusion cooking. They reported three general types of heat stabilization procedures viz. retained moisture heating, added moisture heating and dry heating at atmospheric pressure.

Randall et al. (1985) developed an extrusion cooking procedure which produces stable rice bran with no significant increase in its free fatty acids content during storage of at least 30-60 days. In the optimized process, 500 kg/hour of 12-13% moisture bran was extruded at 130°C and held at 97-99°C for
3 minutes before cooling. Stabilized bran contained 6-7% moisture and was in the form of small flakes with 88% larger than 0.7 mm (25 mesh).

Prabhakhar and Venkatesh (1986) reported a chemical method for stabilization of rice bran with concentrated hydrochloric acid. Ramezanzadeh et al. (1999) adjusted the moisture content of freshly milled raw rice bran to 21% and heated in a microwave oven at 850 watt for 3 minutes. Raw and stabilized rice brans were packed in Zyippedtop bags or vaccum sealed bags and stored at 4-5°C and 25°C for 16 weeks. Free fatty acids (FFA) values obtained in this study showed that microwave heat could be used as a method of inactivation of lipase with a view to extent the shelf life of rice bran.

Ismail et al. (2001) noted that heat stabilization of bran at 130°C for 15 minutes and extrusion stabilization at 130°C and 150 rpm decreased the acid value to 5.6 and 7.7 respectively after 55 days compared with control which has an acid value of 102.53. Extrusion stabilisation at 130°C and 150 rpm could effectively destroy the activity of lipase and produces a shelf stable rice bran which has an acid value of 8.7 after 3 months. Heat stabilization showed an increase in palmitoic and oleic acids, while linoleic and linolenic acid were decreased. Extrusion showed an increase of oleic and linoleic acids. Sharma and Chauhan (2002) also stabilized rice bran by dry heat 120°C for 45 minutes and wet extrusion cooking methods.

Lopez et al. (2003) suggested an alternative rice bran stabilization method of carbon dioxide (CO₂) aeration. IR 64 rice bran was captured in a
receiving chamber with a circulating food grade CO₂ and was heat stabilized (without moisture adjustment) using microwave heat, this method resulted in a very low increase in the free fatty acids content compared to untreated rice bran after four weeks of storage. The method was, therefore, found to be effective in lowering the enzymatic action of lipase that is responsible for the hydrolytic rancidity of rice bran.

Rao et al. (2004) used ohmic heating to stabilize rice bran and to improve rice bran oil extraction yield as compared to microwave for rice bran stabilization with moisture addition. Free fatty acid concentration increased more slowly than the control for raw bran samples subjected to ohmic heating with no corresponding temperature rise, indicating that electricity has a non-thermal effect on lipase activity. Ohmic heating increased the total per cent of lipids extracted from rice bran to a maximum of 92%, while 53% of total lipids were extracted from control samples. Lowering the frequency of alternating current significantly increased the amount of oil extracted probably due to electroporation. This could have important implication for the enhanced extraction of non-polar constituents.

2.3 Rice Bran Oil

Rice bran oil is seen as a superior oil which is rich in vitamins and low in ingredients responsible for cholesterol. Rice bran oil is extensively used in Japan, Korea, China, Taiwan and Thailand as a premium edible oil. In Japan, it is more popularly known as a “heart oil”. Recently, United State scientists have also
shown tremendous interest in the cholesterol lowering properties of rice bran oil. It has acquired the status of a “health food” in western countries. (www.seasofindia.com/002.htm).

2.3.1 Food Regulations.

According to regulation 202 (food regulation, 1985), rice bran oil shall be edible oil obtained from the rice bran of Oryza sativa. Rice bran oil shall have a specific gravity of 0.910 to 0.920 at 30°C, a refractive index of 1.4600 to 1.4700 at 40°C, a saponification value of 175 to 195 milligrams potassium hydroxide per gram and an iodine value of 90 to 105. Rice bran oil shall not contain more than 30 gram per kg of unsaponifiable matter (www.moh.gov.my/fqc/reference/food% Regulations/Regulation 202.htm).

2.3.2 BIS Specifications

The specifications of raw and refined rice bran oils as per BIS: 3348 (1984) are given in table 2.1.

2.3.3 Characteristics of rice bran oil

Mukherjee and Bhattacharya (1978) reported that the colour of the oil was deepened with increase in free fatty acid content and varied from 35-43 (1/4 inch cell, y+5R). The oil was found to contain phosphotides (0.4-3%) and chlorophyll (2 ppm). (Ramaswamy and Gopalakrishna, 1982). The saponifiable matter consisting of hydrocarbon such as squalene, fatty alcohols, sterols, tocopherols, triterpenoids alcohol etc. was estimated between 3.9-6.6% in raw oils (Bhattacharya and Bhattacharya, 1983).
Table 2.1: BIS specifications for rice bran oil (IS 3448, 1984)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Characteristic</th>
<th>Type of rice brain oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Raw grade I</td>
</tr>
<tr>
<td>1</td>
<td>Moisture insoluble Impurities (% max.)</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>Specific gravity at 30° C ( max)</td>
<td>0.91-0.92</td>
</tr>
<tr>
<td>3</td>
<td>Acid value</td>
<td>20 (Max.)</td>
</tr>
<tr>
<td>4</td>
<td>Flash point, penskey martens (closed) (°C min.)</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>Iodine value</td>
<td>85 -100</td>
</tr>
<tr>
<td>6</td>
<td>Unsaponifiable matter (% max.)</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>Refractive index at 40°C</td>
<td>1.46 -1.47</td>
</tr>
<tr>
<td>8</td>
<td>Saponification value</td>
<td>175-195</td>
</tr>
<tr>
<td>9</td>
<td>Colour/ in cell expressed as Y+ 5 R not deeper than ⅛&quot; cell</td>
<td>-</td>
</tr>
</tbody>
</table>

1. Raw grade I: used for making refined oil and hydrogenated oil.
2. Raw grade II: Used for non-edible industrial purposes
3. Refined grade: edible uses
Joshi (1992) reported the characteristics of raw rice bran oil which are given in table 2.2.

2.3.4 Composition of Rice bran oil

The rice bran oil contains many valuable components viz. wax (2-6%) vitamin E (0.10%), squalene (0.3-0.4%), oryzanol (1.2%), fatty acids (variable), soap (variable) and gum or phospholipids (1-3%) (Bhattacharya, 1988).

Srinivasan (1968) reported that rice bran oil contain medium carbon chain fatty acids viz. C\textsubscript{10}, C\textsubscript{12}, C\textsubscript{13} and C\textsubscript{15} to a low extent of 0.2, 0.2, 0.6 and 0.9%, respectively. It also contain monooenic fatty acids viz. C 14:1, 16:1, and 20:1 to the extent of 0.1, 0.5 and 0.30%, respectively, apart from the common fatty acids. Bhattacharya et al. (1985) reported that the rice bran oil contains a good proportion of linoleic acid. The ratio of various unsaturated fatty acids in rice bran oil is so well balanced that it does not have any problem of developing toxicity during deep fat frying. According to Gupta (1989), rice bran oil contain 15-20% saturated fatty acids and 80-85% unsaturated fatty acids.

The fatty acid composition of rice bran oil as reported by various researchers is given in Table 2.3.

Jeong et al. (1984) examined the sterol composition of rice bran oil. Ten sterols were confirmed as 4-dimethyl sterol, 9 as monomethyl sterol and 4 as 4, 4-dimethyl sterol. Such uncommon phyllosterols in higher plants as fucosterol were detected in rice bran oil.
<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Free Fatty acids</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(a) Edible oil</td>
<td>1.6-11.3%</td>
</tr>
<tr>
<td></td>
<td>(b) Non-edible oil</td>
<td>1.72-78.9%</td>
</tr>
<tr>
<td>2.</td>
<td>Spectrophotometric colour</td>
<td>8-35 units</td>
</tr>
<tr>
<td>3.</td>
<td>Chlorophyll content</td>
<td>2-4%</td>
</tr>
<tr>
<td>4</td>
<td>Wax content</td>
<td>3-6%</td>
</tr>
<tr>
<td>5.</td>
<td>Iodine value (Wifs)</td>
<td>99-108</td>
</tr>
<tr>
<td>6.</td>
<td>Saponification value</td>
<td>181-189</td>
</tr>
<tr>
<td>7.</td>
<td>Unsaponifiable matter</td>
<td>2.7-6.5</td>
</tr>
<tr>
<td>8.</td>
<td>Specific gravity (at 25°C)</td>
<td>0.916-0.921</td>
</tr>
<tr>
<td>9.</td>
<td>Refractive index (at 25°C)</td>
<td>1.470-1.473</td>
</tr>
<tr>
<td>10.</td>
<td>Moisture and volatile matters</td>
<td>0.32-1.10%</td>
</tr>
</tbody>
</table>

*Joshi (1992)*
Table 2.3: Fatty acids composition of rice bran oil

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>% w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Myristic</td>
<td>0.3-0.5</td>
</tr>
<tr>
<td>Palmitic</td>
<td>15.9-21.6</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>0.3-0.7</td>
</tr>
<tr>
<td>Stearic</td>
<td>1.2-2.2</td>
</tr>
<tr>
<td>Oleic</td>
<td>39.9-46.7</td>
</tr>
<tr>
<td>Linoleic</td>
<td>30.1-33.4</td>
</tr>
<tr>
<td>Aradridonic</td>
<td>1.9-4.3</td>
</tr>
<tr>
<td>Linolenic</td>
<td>0.4-1.4</td>
</tr>
<tr>
<td>Eicosamonoenoic acid</td>
<td>-</td>
</tr>
<tr>
<td>Lignoceric acid</td>
<td>-</td>
</tr>
<tr>
<td>Behonic acid</td>
<td>-</td>
</tr>
<tr>
<td>Pentadecanoic acid</td>
<td>-</td>
</tr>
<tr>
<td>Heptadecanoic acid</td>
<td>-</td>
</tr>
<tr>
<td>Ficosadienoic acid</td>
<td>-</td>
</tr>
</tbody>
</table>

A Bhattacharya et al. (1985)
B Mani (1987)
C Gaydou and Raonizafinimanana (1980)
D Gupta (1989)
E Hwaug et al. (2002)
2.4 Extraction of rice bran oil

Oil extraction from rice bran is conventionally done by solvent extraction (Matill et al. 1964). In commercial production of rice bran oil, n-hexane is generally used as an extractant. However, n-hexane has been identified as an air pollutant (Rosenthal et al., 1996).

The application of enzymatic process for extracting rice bran oil have been reported by Sengupta and Bhattacharya (1996) and Hernandez et al. (2000). They observed that the oil yields were higher when rice bran was treated with cellulase and pectinase and then extracted with n-hexane.

Enzyme assisted aqueous oil extraction has emerged as an eco-friendly process of oil extraction. The addition of specific enzymes during extraction enhances the oil recovery by breaking the cell wall and lipid bodies (Rosenthal et al. 1996 and Singh et al. 1999) Recently, amylase has been employed to facilitate the extraction of rice bran oil. This approach yielded only 5% increase in oil recovery. A thermal treatment of rice bran was first applied to inactivate lipase as well as to gelatinize starch prior to reaction with amylase followed by a saccharifying step with glucose amylase to produce glucose. The resultant paste could be subjected to protease treatment for protein extraction or directly treated with solvent to obtain bran oil (Rosenthal et al. 2000).

Sharma et al. (2001) carried out enzyme assisted aqueous extraction of rice bran oil under optimized conditions using a mixture of protease, α-amylase
and cellulase. Centrifugation of the mixture at 10,000 X g for 30 minutes yielded a 77% recovery of the oil.

Mansoor et al. (2003) described the effect of temperature (20-60°C) on the aqueous extraction of emulsified rice bran oil from commercial rice bran. The total solids, protein, fat and carbohydrate content of the rice bran emulsions extracted at various temperatures were 4.82-6.99, 1.05-1.40, 0.82-1.65 and 2.63-3.36%, respectively.

Shen et al. (1996) studied the effect of temperature, pressure and flow rate of dense carbon dioxide on its ability to extract, refine and fractionate rice bran oil. Column beds (300 g) of rice bran were extracted with dense carbon dioxide at a flow rate of approximately 2.5 kg/hour, temperature of 0-60°C, and presence of 17-31 M Pa over a period of 6 hours. The extracted total oil, the free fatty acid, alpha tocopheral, sterols (campesterol) stigmasterol beta-sitosterol, and oryzanol components together with moisture were measured at intervals. Extraction was almost complete in 6 hours and rates of extraction were consistent with saturation of the carbon dioxide with rice bran oil throughout most of the process. Extraction of oil components was described by apparent partition coefficients provide a basis for refining and fractionation of rice bran oil. In their further studies. Shen et al. (1997) developed a pilot scale extraction and fractionation method of rice bran oil using supercritical carbon dioxide. In the first stage of this two stage process, crude rice bran oil was extracted with supercritical carbon dioxide (SC-CO₂) from a 300 g batch of rice bran. Oil-Laden SC-CO₂ from the extractor 24.1 MPa/40°C passed continuously to a second stage
column where an oil phase (raffinate) separated from the SC-CO$_2$ at various controlled temperatures and pressures. Measurement of the composition of raffinates and extracts allowed calculation of partition coefficients of triglycerides, free fatty acids (FFAS), alpha-tocopherol, sterols and oryzanol and hence, the selectivity's of the fractionation. Fractionation removed almost all water and reduced the FFA concentration in raffinate by up to 50%. Oryzanol and alphatocopherol concentrations in the raffinate were not reduced my fractionation, but the sterol concentration was reduced under conditions favored FFA removal. Under the flow rate conditions studied (3.5 kg of CO$_2$/h), the fractionation could be described by equilibra between oil and CO$_2$ phases.

Mamidipally et al. (2004) reported extraction of oil from rice bran by d-limonene and hexane (for compression) at their respective boiling point at various solvent-to-meal ratios and for various extraction times. The preliminary data suggested ratio and extraction time required for d-limonene extraction of rice bran oil to be 5:1 and 1 hour, respectively. The initial quality characteristics namely free fatty acid content, oil colour and phospholipids content of crude oil extracted under these optimum conditions were analysed and were found to be comparable to the oil extracted with hexane.

The extraction of rice bran oil from various brands of rice bran available in Sri Lanka was studied and data necessary for extraction equipment design and process prediction were determined. Experiments were conducted using a soxhlet apparatus and pilot-scale leaching unit, to extract rice bran oil using hexane as the solvent. The key factors controlling the extraction and optimal
operating conditions were identified. Analysis of free fatty acid content in the extracted oil showed that the steaming is the most effective method of bran pre-treatment. Bran obtained from parboiled paddy had a higher yield of rice bran oil compared with the raw rice bran, despite the oil's darker colour. Lenoleic, oleic and palmitic acids were the major fatty acid constituents in rice bran oil. Equilibrium data showed no preferential adsorption of oil or solvent to the inert bran and approximately constant solid-solution ratios in the under flow raffinate streams. Batch extraction tests showed that the rate of extraction decreased with time and the solution approaches saturation at an exponential rate. (Amarasinghe and Gangodavilage, 2004).

2.5 Refining of rice bran oil

The object of refining of rice bran oil is to remove impurities without altering the basic triglycerides of raw material so as to obtain an edible product which meets the nutritional requirements. Rice bran oil in common with other oils can be refined either by physical refining process or traditional caustic refining method or by both in combination (Singhal, 1987).

2.5.1 Dewaxing

Rice bran oil contain 2-6% wax. This wax has very high melting point. The wax causes problems in futher refining and have laxative action in human being. Therefore, wax must be removed prior to further step of refining of oil (Bhattacharya, 1988).
The wax and other insoluble matter in rice brain oil were removed by chilling the oil or miscella by treating the oil with a water solution of sodium silicate which caused the flocculation of the contaminant particles and thereafter separated the wax floc from the oil by centrifugation and filtered (Kinsey and Hunnel, 1969). Studies were conducted by Fan et al. (1972) to remove the wax like and other substances from rice bran oil by freezing out method. They reported that it was feasible to combine absorption refining with freezing out followed by filtration. Singhal (1987) suggested the process of winterization for removal of waxes from rice bran oil. The oil was cooled to 8-10°C to crystallize the high melting waxes which were then separated by centrifugation. Sirur (1987) dewaxed the oil by chilling the oil-hexane miscella to 3°C and after proper growth of the wax crystals, it was fed continuously to a special pressure super centrifuge where crystallized wax was separated from the miscella giving a wax free oil.

Sah et al. (1983) reported the separation of wax from rice bran oil by the emulsification and hydration of wax in the presence of aqueous solution of solubilizers viz. sodium dodecyl benzene sulphonate, tween 60, Triton X-100, sorbitan monooleate sodium stearate. They observed that the addition of 0.1% of electrolyte such as sodium chloride, potassium chloride etc. and mixture of an ionic and nonionic solubilizers further enhanced the separation of wax. Rice bran oil containing 1.3% wax was completely dewaxed by centrifugation in laboratory and pilot plant studies (Mallikarjuna et al. 1976).
Haraldsson (1983) reported the simultaneous dewaxing and alkali refining of the crude oil by heating to refining temperature (60°C) and treating with phosphoric acid (0.2-0.4%), then neutralized with lye and separated. The oil was then cooled to 8°C and mixed with 4-6% of water and heated to 18°C. During mixing a heavy suspension of wax was formed which was separated by centrifugation giving a wax free oil.

Belavadi and Bhowmick (1988) isolated soft and hard wax fractions form settling tank by solvent crystallization. The settling consisted mainly of wax esters of long chain fatty acids.

Bhattacharya (1987) employed double extraction method for dewaxing of rice bran oil in which oil was first extracted with hexane at 4-6°C and then extracted a second time at 60°C which on cooling yielded wax as a precipitate. Singh (1992) also dewaxed rice bran oil by hot extraction and reported an insignificant effect on per cent recovery of oil. While the amount of wax recovered by the cold-hot extraction method was significantly more than the hot extraction method.

Praditdoung et al. (1990) prepared a wax emulsion by using the purified rice bran wax recovered from the waste of rice bran oil refining plant. Rice bran wax was totally used as well as by replacing carnauba wax at 50, 40, 30, 20 and 10 per cent in the standard formula. The results showed that the formula of the stable mixed wax emulsion consisted of carnauba wax 60.5 and rice wax 60.5 grams, oleic acid 60 or 69 grams, triethanolamine 28g, paraffin wax 48 g and
water 950 or 941 CC. The percentage of total wax was 14. This wax emulsion could be coated on fresh limes and oranges, giving glossy film when dried. Munee (1991) investigated a suitable purification method of rice bran wax from the dewaxing waste and to formulate into fruit cooling wax emulsion. Using hexane isopropanol yielded 13-17% rice bran wax with lighter colour when compared with one that was extracted by benzene which yielded 7-8% wax.

2.5.2 Degumming

The rice bran oil contain phosphotides, protein, carbohydrates, mucilage's and resin substances and oil soluble organic matter as gummy substances. The gums vary in their content and composition and impart colour, turbidity and odour to the oil. Phosphotides, the major component of gum, increases refining losses, create foaming problem and generate more colour. However, phosphotides like lecithin are commercially used as emulsifying, wetting and dispersing agent (Bhattacharya and Bhattacharya, 1985).

The hydrated rice bran oil when treated with 20% citric acid solution at 45°C removed some additional quantity of phospholipids and oil deodorized in the presence of citric acid and proved more stable in storage (Volotovskaya et al., 1974). Further the use of oxalic acid instead of phosphoric acid as a degumming agent prior to alkali refining of the oil, alleviated the pollution problem caused by the presence of phosphate waste in streams (Ohlson and Svensson, 1976).
Bhattacharya and Bhattacharya (1985) degummed the rice bran oil containing 4.6-30.2% free fatty acids using water (20 kg/ton oil) at 70°C for 25 minutes. Degumming was also reported with surface active agents like lauryl sulphate, sodium oleate, alkylated phenol ethylene condensate (Lissapol) and alkyl aryl sulphonate in water (20 kg/ton oil). The study showed that phosphoric acid, mixture of sulphuric acid and phosphoric acid, citric acid and calcium chloride were effective for removing phospholipids and colouring matters. Water alone was not an effective agent for degumming rice bran oil. The ionic and non-ionic surface active compounds were effective as degumming agents since they reduce the phospholipid content. Process loss was 2.5% for water, 2% for inorganic and organic acids and about 3% for surface active compounds. Joshi (1992) also reported that non-ionic surfactants were more suitable as degumming agents when used between 0.5 to 1.0% by weight, with regards to the extent of gum, colour and unsaponifiable matter removable and process loss.

Kim et al. (1985c) reported that 20 ml of 4% oxalic acid/kg oil gave the same degumming efficiency with slightly higher yield than 2 ml of the widely used 85% phosphoric acid. Dijkstra and Opstal (1989) suggested a degumming process which involved dispersion of a nontoxic acid such as phosphoric or citric acid into the oil, allowed a contact time and then mixed a base such as casuistic soda or sodium silicate into a acid-in-oil emulsion. Subsequently the oil was passed to a centrifugal separator where the gum was removed from the oil with minimum oil loss. They also observed an increase in oil yield by 0.5% in comparison with classical refining process. The laboratory refined rice bran oil
showed lower hydroxyl, acetyl and peroxide values and higher saponification and iodine values than industrially refined and unrefined oils. Citric acid was most effective in facilitating degumming and dewaxing. Peroxide value decreased with citric acid and phosphoric acid. Most of the phospholipids were removed at the stage of degumming (Munshi et al. 1990).

Bhattacharya and Bhattacharya (1987a) developed a degumming process for the rice bran oil by passing the steam into it at a temperature of 80-100°C. However, Singhal (1987) suggested that the insolubles obtained after the process of degumming could be left as such in the oil and latter removed during the caustic refining along with the soap stock.

2.5.3 Neutralization

Neutralization is the process of removal of the free fatty acids from crude fats and oils (Anderson, 1962). It was found that the high refining losses of rice bran oil accounts for its acidity and presence of hydroxylated compounds. The hydroxyl number of rice bran oil was reduced by progressive acetylation with acetic anhydride. This was accompanied by gradual reduction of refining losses (Hartman and Reis, 1976). According to Mallikarjuna et al. (1978) incorporation of molasses prior to alkali refining reduced the refining loss from about 20 to 14%, representing a 30% reduction.

Castro Romos (1969) neutralized rice bran oil samples containing 2-25% free fatty acids in 2 steps by sodium hydroxide followed by sodium carbonate with a loss of only 2% oil per degree of acidity. The colour of neutralized oils
varied with the method of neutralization. El-khalafy (1971) studied the refining of high acid rice bran oil (acid value 123) using 30-75% sodium hydroxide solution. Theoretically, 75% sodium hydroxide reduced acid value to 59.05 with 35% refining loss. He also used aluminum oxide-sodium carbonate column and Fullers earth for refining oil miscella, oil-petroleum-ether and oil hexane mixtures of high and low acid values. Abd-EL-Hafez et al. (1973) used different concentration of steaic, oleic and palmitic acids along with urea in the rice bran oil to form acid-urea abducts. He reported that the per cent extraction of fatty acids was higher at higher initial acid concentration and increasing chain length and saturation favoured the extraction.

Chakraborty et al. (1978) studied three different methods of refining of rice bran oil viz. (a) degumming alkali refining and bleaching with activated earth and carbon (b) degumming treatment with sodium silicate and bleaching and (c) treatment of oil with a surface active agent followed by degumming alkali refining and bleaching. They observed that the method (c) was most advantageous with low refining loss, better removal of unsaponifiable matter and better filtration characteristics of oil. Method 'B' was more efficient than C in removal of colour. They suggested method 'C' for refining, if the rice bran oil was winterized in tall tanks, for a much better colour and less of unsaponifiable matter.

Bhattacharya and Bhattacharya (1985) neutralized the high free fatty acids containing rice bran oil by the mixed solvent alkali neutralization process at ambient temperature after degumming and dewaxing by phosphoric acid (1 kg/ton) at 75°C for 30 minutes followed by centrifugation. The mixed solvent
included hexane and ethanol in 4:1 ratio, Kim et al. (1985b) neutralized the rice bran oil by caustic solvent and steam refining process. The colour and acid value of steam refined oil were not as good as those of caustic refined oil. But steam refined oil showed better retention of natural antioxidants than caustic and solvent refining. In addition there was less loss of neutral oil and no soap formation in steam refining. Caustic refining of degummed rice bran oil resulted in satisfactory acid value and colour but result in more loss of neutral oil and produced large amount of soap stocks. They further reported that solvent refining was not efficient because of poor neutralization, high losses of neutral oil and darkening of colour. Steam refining was less effective then caustic refining in neutralization and bleaching.

Bhattacharya and Bhattacharya (1985a) refined the high free fatty acids rice bran oil to cooking oil by a combination of miscella dewaxing and miscella refining using binary solvent viz. hexane and ethanol at ambient temperature. They observed low refining losses. Bhattacharya et al. (1986) reported the refining of rice bran oil containing 15 to 55% free fatty acids by ambient two stage alcohol extraction of an oil with low free fatty acids was suitable for subsequent alkali refining and bleaching. Bhattacharya et al. (1987) studied the refining of high free fatty acids rice bran oil by isopropanol extraction and neutralization. They reported the optimum condition of neutralization by azeotropic isopropanol (91%) at ambient temperature 30°C and 2 stage extraction.

Anand and Vasishtha (1978) re-esterified the rice bran oil of high acid value with equivalent proportion of glycerol with or without catalyst, at various
temperatures under reduced pressure. They reported that free fatty acids reduced from its original value of 64.7% to 3.4% and 2.8% at 190 and 200°C, respectively, for the uncatalysed reaction. Whereas, 3.5, 1.2 and 0.9 at 180, 190 and 200°C, respectively, for the catalysed reaction. The re-esterification of degummed rice bran oil (containing 40% free fatty acids) with glycerol reduced the free fatty acid content to 5.0 to 8.9% (Millwala and Shitole, 1987). According to Bhattacharya and Bhattacharya (1987) re-esterification could be combined with conventional alkali neutralization to produce a good quality edible rice bran oil.

Bhattacharya and Bhattacharya (1989) studied the potential of enzymatic neutralization process for refining of high free fatty acids rice bran oil by examination of the enzymatic esterification variables such as enzymes concentration, reaction temperature and reaction time glycerol concentration and amount of moisture in the reaction mixture. By adopting the biorefining process, they reduced the major portion of the free fatty acids by converting them into neutral glycerides with the aid of 1, 3-specific lipase under optimum condition and later neutralized the residual free fatty acids of the biorefined oil by alkali neutralization.

Soap stock from the neutralization of rice bran oil was characterized through the most important parameters with direct influence on the acidulation process. The responses of trials were gamma-oryzanol concentration in the acid oil and process time necessary for total hydrolysis of the soap present. The optimal process conditions were determined using the response surface
methodology. Adjustments were made to 2\textsuperscript{nd} order mathematical models and validity was verified by analysis of variance using the statistical programme. The process variable studied were molar ratio, sulphuric acid/soaps ratio (0.55-0.95) and process temperature (70-100\textsuperscript{0}C). The results indicated that the process temperatures did not significantly interfere in the concentration of gamma-oryzanol of the acid oil, but it did effect process time. In different concentrations of sulphuric acid added, represented by molar ratio of sulphuric acid/soaps, the super positions of the areas of the response surface contour curves, the optimal process parameters were determined. Process temperature of 95\textsuperscript{0}C and molar ratio of sulfuric acid/soap of 0.74 resulting in a gamma-oryzanol concentration of 3.4 per cent and a process time of 90 minutes. \textit{(Scavariello and Barrera, 2004)}.

\subsection*{2.5.4 Bleaching}

The rice bran oil extracted from rice bran using acetone and ethyl ether was separated into methanol soluble and insoluble fractions. The insoluble fraction contained chlorophyll ‘a’ and carotenoid without hydroxyl moiety and the soluble fraction contained a carotenoid containing hydroxyl group and various oxidation and decomposition products \textit{(Mani, 1987)}. The bleached rice bran oil during storage might develop colour which could be permanent or difficult to remove. \textit{(Joshi, 1992)}.

\textbf{Fahn and Fenderl (1974)} used bentonite treated with 280-1680 ml vol Hcl/100 g to bleach the oil. They reported that oil retention increased from 34 to 54\%, filtration time increased from 30 to 45 seconds and the decolourizing power
of bentonite increased with concentration of acid to a maximum for 600-1000 m vol/100 g treatment. The earth with optimum bleaching power also produced best colour stability. Mironova et al. (1974) studied the effect of askanite or Bulgarian clay on the changes in oxidation products. They reported that the carbonyl compounds and compounds with 3 conjugated double bonds were accumulated in the decolourized oils because they were weakly absorbed with the clays.

Gupta (1987) recommended higher proportions of acid activated bleaching earth ranging between 3 to 4% and activated carbon ranging from 10-20% of earth used in bleaching process with a temperature of 90-110°C, a vacuum of 27-29 inches of mercury and a residence time of 15-20 minutes for the bleaching of rice bran oil. However, Mathur (1987) used low proportion of activated earth 1% and activated carbon (0.25%) for bleaching of rice bran oil and filtered by plate and frame type of filter presses in the holding tanks.

Singhal (1987) reported that during bleaching the primary oxidation products namely peroxides were converted into secondary oxidation compounds and parts of which are observed on the activated earth. Bhattacharya et al. (1987) reported that most of the pigments were readily absorbed by bleaching earth or destroyed by heat but oxidation particularly, in the presence of iron or copper lead to fixing of colour which develop resistance to bleaching. He also observed that heating in presence of gum lead to darkening and difficulties in bleaching of rice bran oil.
2.5.5 Deodorization

About 146 compounds consisting of alkenes, aromatic hydrocarbons, pyrazines, quinolenes, thiozoles, thiopenes, 7 phenols and 17 carboxylic acids were identified in steam volatile neutral, acidic and basic constituents of rice bran oil. Some of them viz. 4 vinylguaicol, 4 vinylphenol, 2-acetylthiazole and benzothiazole compounds impart the typical rice bran oil odour which is retained during processing (Mani, 1987).

Moser (1965) noted that there was a definite relationship between temperature and time of deodorization to produce the most stable oil with respect to flavour and peroxide value Bhattacharya and Bhattacharya (1987) deodorized the rice bran oil by heating the oil to 200-250°C under high vacuum with dry steam to get an oil of characteristic odour of refined rice bran oil.

Joshi (1992) reported that if physical refining was used for neutralization no further deodorization was required. However, he found that a temperature of 170-190°C with a very high vacuum for 4 to 6 hours was sufficient to deodorize the rice bran oil in batch deodorization.

2.5.6 Interesterification of rice bran oil

The interesterification reaction in a natural oil resulted in its acyl groups randomly distributed on the glycerides which changed the physico-chemical properties of oil. It depends upon how far the original oil varied from a random distribution initially. Interest erification is important because the properties of all
oils depends not only on its fatty acid composition but also on the distribution of fatty acids in the glycerides (Hustedt, 1976).

Anand and Vasistha (1978) re-esterified the rice bran oil of high acid value with equivalent proportion of glycerol with or without catalyst at various temperatures under reduced pressure. They reported that free fatty acids reduced from its original value 64.7 to 3.4 per cent and 2.8 per cent at 190°C and 200°C, respectively for uncatalysed reaction whereas 3.5, 1.2 and 0.9 at 180, 190 and 200°C, respectively for the catalysed reaction.

Screenivasar (1978) reported that by the process of interesterification there is a change in the existing non-random glycerides structure of oils and fats that produced by random distribution of the fatty acids among the glycerides. The fatty acids may also be added to a fat by interesterification with another fat or oil. Interesteerification can be used effectively to produce tailor made fats and margarine oils.

Sonntag (1979) reported that a variable and large groups of acids esters are prepared from monohydroxy alcohols, triols (glycerols, teteraols, and polyglycerols) carbohydate materials (sorbitol, sorbiton sucrose and others). The two most direct type of esterification of fatty acids are those done with monohydroxy alcohols (methanol and butanol) and glycerol itself.

Grace and Sen (1983) reported that interesterification is also used to prepare vanaspati like fats. For this purpose, they used the blends of sal (Shorea robusta) fat and groundnut oil (30:70 w/w) (ii) vanaspati-groundnut oil
(40:60) (iii) sal fat and safflower oil (50:50) (iv) cotton seed oil alone could be modified to give plastic fat with slip point of 30-34°C by interesterification using sodium methoxide as a catalyst. Randomization at 65°C for 30 minutes was found sufficient for blends of sal and groundnut oils products involving vanaspati-groundnut oils and groundnut oil. Sal : safflower oil and cotton seed oil required subsequent direct interesterification.

Freedman et al. (1984) studied the variables affecting the yield and purity of fatty esters from transesterified vegetable oils. Some main variables were molar ratio of alcohol to vegetable oil, type of catalyst (alkali Vs acidic) temperature and degree of refinement of the vegetable oils. Interesteerification by acid catalyst was much slower than alkali catalyst. Although, the crude oil could be transesterified and esters yields were reduced because of germ and extraneous material present in the crude oil. Laning (1985) observed that the chemical interesterification process are responsible for selected physical and functional changes. These interesterified oils and fats are utilized worldwide in a growing varieties of food products. The demands of these applications provide an endless need for fats and oils with varying physical and functional characteristics. Interesteerification alone and in combination with other processes such as hydrogenation and fractionation significantly extends the range of the products, other than, limited physical and functional characteristics of naturally occurring fats and oils.

Koritala (1988) carried out interesterification of fatty aicds by Aspergellus flavus and noted that the triglycerides thus produced were found intracellularly
and considerable amounts were also found extracellularly. The later was originated most likely from esterification of fatty acids by a cell bound lipase. Although, the fatty acids of these fungal triglycerides were the same as those of soybean fatty acids. Polyunsaturated fatty acids content was greater in these glycerides than expected from the added substrate. Khatoon (1991) applied biotechnological approach for the modification of fats and oils. She further explained that the conventional process was better than bioesterification process. In conventional process of interesterification, she used 0.2 per cent sodium methoxide as a catalyst and in bioesterification process 10 per cent lipase and 2 per cent water at 50°C under vacuum.

Bhattacharya and Bhattacharya (1987) applied interesterification to produce a light coloured edible oil and combined it with conventional alkali neutralization and bleaching. By this method, rice bran oils containing 15 to 30 per cent free fatty acids were successfully deacidified with glycerol after degumming and dewaxing.

Bhattacharya and Ghoshray (1992) prepared long chain alkyl ricinoleates by enzymatic esterification or by enzymatic alcoholysis with high yield of oil and without undesirable side reactions. On sulfonation to the hydroxyl group, the alkyl ricinoleates produce surface active compound. The teteadecyl ricinoleates showed the best surface active behaviour and seems to be much better than that of sulfonated castor oil commonly known as turkey red oil.
Zeitoun et al. (1993) studied the physical properties of interesterified fat blends. These fat blends subjected to interesterification with sodium methoxide as a catalyst. Fatty acid composition and triglyceride molecule species of each fat blend and the interesterified product were determined and correlated with the following physical properties namely melting point, crystallization characteristics and solid fat content. The differences in the endothermic and exothermic peak temperature, total heat of fusion and crystallization and solid fat content among the fat blends clearly showed the effect on the composition of each oil and its physical properties. Oils that contained a considerable amount of palmitic acid had a favourable influence on the crystallization and polymorphic form of interesterified blend.

Konishi et al. (1993) reported that chemical interesterification provides regioselectivity to fatty acid position in glyceride, sodium methoxide catalysed ester interchange between oil and methyl stearate was performed in hexane at low reaction temperature i.e. 30 to 60°C. The results indicated that the regioselectivity was obtained at 30°C. The ester interchange at 1-3 carbon progressed 1.7 times faster than at 2 carbon of glycerol moiety of triglyceride. Pre-heating of the mixture of reactant and catalyst at 60°C for 15 minutes promoted catalyst activation to accelerate the interesterification while maintaining regioselectivity. The method is believed to be feasible for modification of edible fats and oils.
2.6 Frying characteristics of rice bran oil

Fried foods are one of the major items in Indian dishes. Frying oil used in the preparation of fried foods are exposed to elevated temperatures in the presence of moisture under these conditions number of changes take place like hydrolysis, oxidation, thermolysis and polymerization. As these reactions proceed, they trigger formation of volatile and non-volatile decomposition products which have an effect on the flavor, colour and texture of fried foods as well as the life of frying oil. The decomposition products and the rate of their formation vary with the nature of the fat used, the surface-volume ratio, the excess of air, the nature of the fried food and interval of heating (Chang et al. 1978).

Karnalanathan et al. (1971) studied the acceptability of 6 commonly used food preparation utilizing rice bran oil. Ground nut oil was used as a standard for comparison. All samples were presented to a taste panel and scores were recorded for appearance, colour, texture, flavour and taste. Results showed that the flavour and taste of rice bran oil influence the food preparations adversely but colour and texture were acceptable.

Kim and Yeom (1985) studied the properties and frying performance of domestic rice bran oil. They reported that because of low contents of linoleic acid, domestic rice bran oil was estimated stable on frying, whereas other oil was easily polymerized. Oils with serious foaming and low smoke point on frying
caused a substantial quality loss in terms of flavour and appearance of fried materials.

Yoon et al. (1987) evaluated the physico-chemical changes in rice bran oil during heating at 180°C for 50 hrs. They reported an increase in free fatty acids and decrease in iodine value of rice bran oil during heating. They also observed a gradual change in colour to dark brown with increased heating time and the iodine value correlated very well with linoleic acid content. Rukmini (1988) reported that none of the deep fat frying foods prepared in rice bran oil showed any mutagenicity either with or without metabolic activation, thereby showing that deep fat fried food in rice bran oil is as safe as frying foods in ground nut oil. Singh et al. (2001) prepared blends of rice brain oil with refined ground nut oil in the ratio of 20:80 40:60, 60:40 and 80:20 and subjected to deep fat frying of potato chips at 180°C for 36 hours. Physico-chemical and frying properties of these blended oil samples were analyzed at an interval of 6 hours and it was observed that the stability of rice bran oil was comparable to ground nut oil. Ravi et al. (2005) investigated the effect of frying on the viscosity and sensory properties of oil blends. Different oil blends were used to deep fat fry poori. Frying was found to decreased the intensity of the typical aroma notes of the oil blends. During frying, changes in colour parameters occurred to a lesser extent in blends containing rice bran oil and sunflower oil than in those containing red palm oil. Deep fat frying caused a decrease in apparent viscosity for all blends. Principal component analysis segregated the oil into distinct groups and
traced the pathway of changes occurring during frying. It was concluded that the aroma profiles of blended oils are distinguishable even after seven frying cycles.

Ravi et al. (2005) studied the effect of frying on the viscosity and sensory properties of oil blends containing 80 parts sunflower oil (SNO), groundnut oil (GNO) or mustard oil (MO), and 20 parts rice bran oil (RBO), red palm oil (RPO) or sesame oil (SO). The different oil blends were used to deep fat fry poori. Seven (50 g) portions of poori were fried successively, and oil samples were withdrawn for analysis following frying of 1st, 4th and 7th portions. Aroma and colour were evaluated by 10-12 trained panelists and colour was also evaluated instrumentally using the CIE system. Apparent viscosity was calculated using a Heake RT 10 rheometer. Frying was found to decrease the intensity of the typical aroma notes of the oil blends. During frying changes in colour parameters occurred to a lesser extent in blends containing RBO and SO than in those containing RPO. Deep fat frying caused an increase in apparent viscosity for all blends. Principal component analysis segregated the oils into distinct groups and traced the pathway of changes occurring during frying. It was concluded that the aroma profiles of blended oils are distinguishable even after seven frying cycles.

2.7 Changes during storage of rice bran oil

Sidhom et al. (1975) detected very slight changes in the moisture, free fatty acids level, iodine value, saponification value and unsaponifiable matter during storage of rice bran oil at room temperature for 60 days. Oil stored in tin cans showed a relatively higher rate of increase in free fatty acid concentration
than the oil stored in PVC or polyethylene bags. Aoyogi et al. (1985) reported that utilization of rice bran as a source of oil is limited by its deterioration in storage due to enzymic hydrolysis of triglycerides. Rice bran stored at high temperature (30-40°C) showed a considerable increase in acid value while peroxide value remained unchanged in brown rice similarly stored, both acid value and peroxide value remained stable. Levels of free fatty acids (palmitic, oleic and linoleic acids), the main constituents of bran oil triglycerides increased during storage with increasing time. Successive fractions of rice grains obtained by abrasion were stored separately and it was found that outer layers were more strongly hydrolysed than inner one.

Shini and Chung (1998) studied the oxidative stabilities of rice germ oil, dried rice germ oil and crude and refined rice bran oils. The oils were evaluated by measuring acid value, peroxide value and fatty acid composition during 0-31 days of storage at 40 and 60°C. Acid value of all lipids were slightly altered during storage, while peroxide values were greatly dependent on storage temperature. Peroxide value of the dried rice germ oil and refined rice bran oil were 146.2 and 15.1 meq/kg oil, respectively, after 31 days of storage at 60°C, Peroxide value of the dried germ oil and the refined rice bran oil were 151.7 and 219.6 meq/kg oil, respectively. Major fatty acid of rice germ oil were linoleic (39.8%) and oleic acid (34.7%) while oleic acid (40.1%) and linoleic acid (38.1%) were predominant in rice bran oil. Major fatty acid compositions were not greatly influenced by drying or storage temperatures although linoleic acid decreased by approximately half during storage.
Semwal and Arya (2001) studied the storage stability of 2 edible oil blends (refined rice bran oil: unrefined ground nut oil and refined soybean oil, unrefined groundnut oil 80:20 proportion for both blends) in sealed tin containers at room temperature (15-34°C) was studied. Both oil blends remained stable for 15 months with out development of perceptible off flavour or odour. Peroxide, TBA, total carbonyls and anisidine values increased during storage. These changes correlated linearly with storage period. Increase in the free fatty acids content during storage was pronounced in unrefined oils than in refined oils. In general, the oil blends showed good stability. However, the food fried in the blend containing soybean oil showed lower sensory scores than those fried in the blend containing rice bran oil. Sheila et al. (2004) investigated the storage stability of blends of ground nut oil, sunflower oil and mustered seed oil with plamolein, rice bran oil and sesame oil. Physicochemical properties and level of minor potentially health promoting components of the blended oils packaged in pouches were studied during storage for 6 months at 27°C and 65% RH or 40°C and 30-40% RH. Colour, peroxide value (0.6-20.7 meq 02/kg) and content of free fatty acids (0.08-2.1%), tocopherol (360-170 ppm), oryzanol (460-2000 mg%) and same antioxidants (400-200mg%) did not change in either unblended or blended oils. Oils and oil blends containing a higher initial peroxide value (18.9-20.7 meq 02/kg) showed a slight reduction in peroxide value at 40°C, whereas peroxide value of oils having lower peroxide value (5-10 meq 02/kg) slightly increased during storage. B-carotene contents decreased by 8.9 to
65.2% at 27° C and 48 to 71% at 40° C. Results suggest that the packaged oil blends tested were stable under the conditions used.