The ricebran oil has gained significant position in the Indian edible oil scenario in the past decade. The edible ricebran oil is being used mainly in shortening manufacture and a part of it is being refined for making refined, bleached and deodorised oil for direct edible consumption. The major fatty acids and triglycerides of ricebran oil are oleic, linoleic and palmitic. In recent studies it has been proven beyond doubt that ricebran oil scores over the other refined oils produced in the country due to the presence of antioxidants, vitamin E and other nutritionally important constituents. It contains sterols, squalene, tocols, oryzanols etc. A brief literature review on the nutritionally important minor components of rice bran oil is given hereunder:

2.1 MINOR COMPONENTS

2.1.1 Sterols: Sterols are the major part of the unsaponifiable fraction of ricebran oil. Itoh et al. (35) isolated, identified and quantitated sterols from the unsaponifiable fraction of the oils. Unlike other vegetable oils, ricebran oil has higher percentage of unsaponifiable matter (4.2%) which predominantly contains sterols (around 43%). They divided the unsaponifiable fraction in four fractions:

(i) less polar components (hydrocarbons, aliphatic alcohols etc.) - 19%
(ii) triterpene alcohols - 28%
(iii) 4-methyl sterols - 10%
(iv) sterols - 43%

Sterol fractions were analysed by GLC and mass spectroscopy and ricebran oil was found to contain eight sterols viz. β-sitosterol 49%, campesterol 28%, stigmasterol 15%, Δ^5-avenasterol 5%, Δ^7-avenasterol 2%, Δ^5-stigmasterol 1%, and traces of brassicasterol and cholesterol. Triterpene alcohols and sterols in different vegetable oils were separated by Fedeli et al. (36). They
reported the oils to contain triterpene alcohols, cycloartanol and 24-methylene cycloartanol in most of the vegetable oils.

The 4-methyl sterol fraction of ricebran oil was found to contain 13% obtusifoliol, 47% gramisterol, 40% citrostadienol; and 24-methylene cycloartanol 42%, cycloartenol 41%, cycloartanol 9% and others 8% in the triterpene alcohol fraction of the unsaponifiable matter (37). Gaydou et al. (38) found traces of ergosterol (0.5%) and cholesterol (0.4%), 1.5% of total sterol but no brassicasterol in addition to other major sterols while analysing unsaponifiable matter of six Malagasy ricebran oils.

2.1.1.1 Nutritional values: The unsaponifiable matter of ricebran oil showed cholesterol suppressive action. Since in unsaponifiable matter the maximum percentage is found to be of sterols, it was presumed that sterols had cholesterol lowering effect. Experiments showed that adding plant sterols to the diet prevents the hypercholesterolemic effect of dietary cholesterol. This is due to the fact that phytosterols interfere with the absorption of cholesterol as a result of competition among these sterols in the absorption process, either in the intestinal lumen or at the intercellular site of esterification. In the dietary fat containing 1% cholesterol, the addition of twice this amount of plant sterol resulted in 30% decrease in cholesterol absorption. It was proposed that in the intestine the sterol decreased the solubility of cholesterol in the oil as well as miscellar phase with a consequent decrease in cholesterol absorption (39).

The average diet provides a daily intake of plant sterols in the range of 250 - 300 mg per day and the effect of this intake on plasma cholesterol is perhaps negligible. A distinct lowering of cholesterol occurs when the sterols are fed at a level of 10 - 15 g per day. It was reported that sterols have hypolipidemic activity much less than other components of ricebran oil such as oryzanols (40).
2.1.2 Squalene: The squalene content of the ricebran oil varies from 202 to 289 mg per 100 gram, which is remarkably high when compared to groundnut oil (22.9 mg/100g) and cottonseed oil (27 mg/100 g). It is used in natural or hydrogenated form in cosmetic preparations as a moisturising agent for giving emollient property (41). Nutritionally it is important because it is a precursor of cholesterol synthesis. Upon ingestion it has a noticeable effect pharmaceutically, it is specially efficacious against skin diseases. It is also believed to be a remedy for tuberculosis (13).

2.1.3 Tocols: Ricebran oil contains high amount of tocopherols and tocotrienols. They are nutritionally important and provide oxidative stability to the oil. Various methods of extraction of tocopherols from vegetable oils are given in the literature. Some of them are as follows:

2.1.3.1 Extraction of tocopherols: Morozova et al. (1979) reported that the oil produced by pressing followed by solvent extraction contained tocopherol at 1.5 fold the level than the oil produced by pressing alone. They also observed that various steps of refining reduced the concentration of each component, the extent of reduction was reported to depend on the technique used (42).

95% pure tocopherols were isolated from low concentrated vegetable sludges by esterification followed by treatment with strongly alkaline resins and the process was patented by Ajinomoto Company, Japan (1981), (43).

Koman et al. (1982) studied the removal of unsaponifiable fractions in the industrial deodorisation process of plant oils. It was reported that rapeseed oil deodoriser distillate contains 16.2% sterol, 21.0% sterol esters, 13.8% alcohols, 8.6% α tocopherol and 3.2% β tocopherol (44).
Palm oil or ricebran oil deodoriser distillates containing high concentrations of fatty acids were extracted with Et₂O or hexane in presence of sodium carbonate. This extract was reported to contain higher concentrations of tocopherols and tocotrienols (45). This process was patented by Agency of Industrial Sciences and Technology (1982). They also patented another procedure in which crude palm oil or ricebran oil was extracted with MeOH, EtOH or 1:9 Me₂CO - EtOH to give an extract containing higher concentrations of tocopherols and tocotrienols (46).

Nisshin Oil Mills (1982) obtained a patent for the process in which tocopherols were obtained by esterification of the free fatty acids in the byproducts in the deodorisation step of soyabean with polyhydric alcohols and subsequent distillation (47).

Agency of Industrial Sciences and Technology, Japan (1982) esterified the wastes from vegetable oil refining into fatty acid methyl esters which were distilled off and the residue was extracted with organic solvents. The extraction was fractionated by column chromatography to yield food grade tocopherols (48).

The study of Kataoka (1985) on supercritical fluid extraction of soyabean distillate showed that the extraction of tocopherol from the deodorised soyabean oil by a supercritical gas is not more advantageous than molecular distillation (49).

Schulz et al. (1985) studied the isolation of tocopherols from scum by preparative HPLC. Oil refining wastes were dissolved in MeOH and cooled at -40°C to precipitate the sterols and free fatty acids. The resulting tocopherol concentrate was passed through a silica column (which was washed with petroleum ether and eluted with 6 - 12% Et₂O in petroleum ether) to remove more sterols and pigments. α, β, γ and δ tocopherol were preparatively
separated from the purified concentrate by HPLC on a LiChrosorb Si 60 column with iso-octane - isopropanol (99.5 : 0.5) as eluent. The method gave clear and reproducible separation of the components as shown by the subsequent gas chromatography analysis (50).

The separation of oil from wheat germ by extraction with supercritical CO₂ was reported by Taniguchi et. al. (1985). Oil extracted by this method had comparable contents of α tocopherol and β tocopherols (51).

Wastes from the deodorisation process in oil and fat refining were dissolved in ethyl alcohol and treated with cation exchange resins to form free fatty acids methyl esters in the wastes (52). After the removal of fatty acids methyl esters by conventional methods the residue containing tocopherols was obtained by Nishin Oil mills (1985).

Concentration of tocopherols from soy oil deodorisation scum was studied by Sheabar and Neeman (1988). The scum was reported to consist of tocopherols, sterols, free fatty acids, esters of sterols, mixed fatty acid glycerides and other materials. The end products of various concentration and purification methods were tocopherol concentrates. Crystallisation in hexane followed by crystallisation in acetone resulted in a product containing 35.5% tocopherols. In other process which consisted of crystallisation in hexane followed by crystallisation in acetone and treatment with sodium carbonate a concentrate with a tocopherol content of 45.5% was obtained (53).

Miyawaki et. al. (1988) studied the tocopherols in ricebran. Aqueous suspension of defatted ricebrans was stirred for 5h and freeze-dried to give a water soluble antioxidant containing tocopherols (54).

Mares et. al. (1989) reported the manufacture of tocopherol concentrate from vegetable oil by - products by a) two stage distillation in vacuum (b) dissolution
of the second distillation fraction in acetone and cooling to crystallise impurities which are removed and washed, and (c) evaporation of the mother liquor and a second two stage distillation in vacuum which yielded product containing 45 - 60% tocopherol (55).

Changes in the tocopherol contents of post deodorisation condensates from the refining of soyabean and rape oils were studied by Gogolewski (1989) and the results were correlated with the oil contents. The soyabean oil condensate was found to be more important source for extraction of tocopherols (56).

A study was made by Lanzani et. al. (1990) for the recovery of fatty acids, sterols and tocopherols from condensate obtained during deodorisation of soyabean oil. Three processes investigated were: methylation followed by distillation, dry fractionation with solvents with or without prior methylation and saponification which resulted in a fraction containing 44.3% tocopherol (57).

Goh et. al. (1990) studied the separation of tocopherols from palm oils or similar vegetable oils. Isolation of minor non-glyceride components of palm oil or similar vegetable oils containing free fatty acids comprised of four steps (a) esterifying the free fatty acid component of the oil with ≥1 monohydric alcohols to form an esterified oil with a very low FFA content, (b) converting the glycerides into monoesters by transesterification using ≥1 monohydric alcohol, (c) absorbing the non-glyceride components onto a selective adsorbent to separate them from esters and, (d) desorbing the non-glyceride components from the adsorbent (58).

A study was carried out by Lee et. al. (1991) to develop the extraction process for concentration of 17-18 % (w/w) tocopherols from soyabean sludge using supercritical carbon dioxide at temperature ranging from 45° - 70°C and pressure 200 - 400 bar. On the basis of results it was confirmed that a general counter-current extraction method could be properly used for the concentration of tocopherols from sterol removed soyabean sludge (59).
The possibility of enriching tocopherols from soyabean oil deodoriser condensates in a counter-current separation process with supercritical CO\textsubscript{2} as solvent (60) was investigated by Brunner et al. (1991). They concluded that counter-current separation was better for making enriched tocopherol concentrate.

Lee et al. (1991) applied a supercritical fluid extraction method to test the feasibility of tocopherol concentration from soyabean sludge with carbon dioxide. The supercritical solubility of esterified soyabean sludge was found to be 4 - 6 times greater than that of the original soyabean sludge. By a simple batch type one stage method the tocopherols in the esterified soyabean sludge could be concentrated up to 40\% (w/w). The overall result led to the conclusion that soyabean sludge initially containing about 13 -14\% (w/w) tocopherols may require a counter-current multistage column to be highly and effectively concentrated (61).

Melted deodorant distillate which is a by-product from processing animal and vegetable fats and oils was added to a heated alcoholic solution of urea, a crystallised urea fatty acid complex was formed and separated from the mother liquor. The crystals were dissolved in water to yield a fraction rich in tocopherols and sterols (62). The above study was carried out by Maza Aurelia (1992).

Preparation of tocopherol and sterol concentrates was studied by Cvengros et al. (1992). The condensate remaining after the vegetable oil deodorisation was run through a filter press and the filtrate was subjected to a three step wiped-film distillation. The second and third fraction from the distillation contained the tocopherols and sterols in increased concentrations. The second fraction may be further treated with CaO or Ca(OH)\textsubscript{2} and Me\textsubscript{2}CO to increase the concentration of the products (63).
Ramamurthi and McCurdy (1993) studied the separation of sterols and tocopherols from fatty acids in the deodoriser distillate, in which the separation was facilitated through lipase catalysed modification of fatty acids in canola and this distillate was mixed with soya deodoriser distillate. The fatty acid esterification with methanol catalysed by an immobilised non-specific lipase proceeded rapidly, with conversion of fatty acids to methyl esters. Simple vacuum distillation was employed by them to remove the volatile fraction of esterified deodoriser distillate leaving behind sterols, sterol esters and tocopherols. Overall recovery of tocopherols was reported to be over 90% (64).

The isolation of tocopherol and sterol concentrate from deodoriser distillate has been reported by Ghosh and Bhattacharyya (1996). The sunflower oil deodoriser distillate was composed of 24.9% unsaponifiable matter with 4.8% tocopherols. The isolation technique included process steps such as biohydrolysis, bioesterification and fractional distillation. The overall recovery of tocopherols after hydrolysis, esterification and distillation was reported to be around the original content in sunflower deodoriser distillate (65).

2.1.3.2 Identification and quantitation of tocopherols: Several methods for the analysis of tocopherols have been published. Methods for the determination of tocopherols can be classified as chemical, spectrometric, electrochemical and chromatographic. The procedure of Emmerie and Engel (66) is the most widely employed which uses ferric chloride solution for oxidation of tocopherol followed by measurement of ferric ion produced.

The second order derivative UV spectra of α, β, γ and δ tocopherols showed small differences that allowed development of derivative spectrophotometric method for determining individual tocopherols in a mixture (67). Polarographic technique had been successfully applied in measuring individual tocopherols (68).
The most successful techniques used in the past for the quantitation of individual tocopherols have been chromatographic techniques. Tocopherols and tocotrienols present in oils and fats were determined by one dimensional TLC using n-hexane / ethyl acetate system 92.5:7.5 (69). A simple and rapid procedure had been developed for the isolation, concentration, esterification and gas liquid chromatographic (GLC) quantitation for the vitamin E content of vegetable oils (70). They can also be determined by capillary gas chromatography (71).

Due to shortcomings of the above mentioned methods high performance liquid chromatography (HPLC) was investigated for quantitation of various tocopherols in different vegetable oils. Tocopherols were separated in three fractions of \( \alpha, \beta \) plus \( \gamma \) and \( \delta \) tocopherols (72). A rapid and selective method for measuring individual tocopherols found in vegetable oils have been developed using HPLC with ultraviolet absorption detection (73). The results indicated this method to be more reliable in measurements of samples with high tocopherol levels.

Analysis of tocopherols by HPLC was done by using evaporative light scattering detector (ELSD) and fluorescent detector (FD) and it was observed that FD was ten times more sensitive than ELSD (74,75).

Separation of tocopherols can be achieved by using both normal and reverse phase HPLC but normal phase HPLC allowed fast and easy separation of positional isomers. A method had been described for analysis of vitamin E vitamers of ricebran oil on normal phase HPLC with iso-octane / ethyl acetate / acetic acid / 2,2- dimethoxy propane (98.5 : 0.9 : 0.85 : 0.10) as mobile phase (76).
Tocols in ricebran oil were quantitated by HPLC. α, β and γ tocopherols and α, γ tocotrienols were identified. Tocotrienols were reported to account for 57% of tocopherols (77).

2.1.3.3. Therapeutic benefits of tocols: Tocopherol is one of the oil soluble vitamins in ricebran oil. It is reported that between 1 to 1.5% of tocopherol is present in crude ricebran oil and between 0.25 to 0.40% in refined oil. Tocopherol is closely related to the internal secretion of the hormones. It is a nutritive agent for brain as well as a valuable vitamin that maintains proper balance in nervous system. Despite a substantial quantity of tocopherol is lost during high temperature deodorisation, small quantity still remains in the oil which offers antioxidant qualities to the oil (13). The feasibility of tocopherols as antioxidants was found to be as follows in descending order, β, γ and α-d-tocopherols (78). As antioxidants, these tocols are important for the protection of unsaturated lipids against peroxidation particularly in bio-membranes. Vitamin E vitamers have attracted the attention of scientists, nutritionists, epidemiologists and clinicians because of their apparent functions in cardiovascular diseases, as possible anticarcinogenic agents and as compounds that lower the cholesterol level in blood (78). Vitamin E was also indicated as one of the most effective antioxidant in LDL (21).

α Tocotrienol had been shown to be anticholesterolemic. Possibly it suppresses HMG Co. A (3-hydroxy-3-methyl glutaryl- co enzyme A ) reductase activity, leading to the inhibition of cholesterol biosynthesis. Recently it has been shown to have antitumor activity in laboratory animals. γ - Tocotrienol also impedes the aggregation of blood platelets (79-81).

2.1.4 Oryzanols: One of the unsaponifiable ingredients in ricebran oil was identified by Tomataro Tsuchiya of Tokyo as oryzanol. The oryzanol content in Indian ricebran oil samples ranged from 1.1 - 2.6% (20).
2.1.4.1 Extraction of oryzanol from ricebran oil: Several methods of oryzanol extraction from ricebran oil are given in the literature but most of them are covered under patents.

2.1.4.1.1 Extraction of oryzanol from soap stock: Shimizu extracted oryzanol from ricebran oil by a two step alkali treatment in which the percentage of oryzanol was higher than in one step alkali treatment (82).

Tomotaro et al. extracted oryzanol from soap stock. After splitting washing and fractionation of soap stock, unsaponifiable matter was separated out which on extraction with diethyl ether gave oryzanol (83). In another method Tomotaro et al. (84) saponified the oil with 25% KOH - EtOH and then the soap stock was shaken with Et₂O which resulted in formation of two layers. Thereafter, from the ether layer, oryzanol was obtained by methanol extraction and recrystallisation with several solvents.

Shimizu (85) studied oryzanol extraction by washing ricebran oil with alkali and the extract was neutralised with crude or distilled fatty acids to isolate oryzanol. 10 kg. ricebran oil was neutralised with 33% NaOH at 55°C and the neutral oil layer was extracted with 3% aqueous NaOH at 60°C. The extract was washed with hexane and salted to yield sodium salt of oryzanol, which on hydrolysis and extraction gave oryzanol.

Azuma extracted oryzanol from ricebran oil by alkali washing and neutralisation of extract with acids. 200 g of an alkali extract in isopropanol was washed with hexane, heated with distilled fatty acids at 60°C and extracted with hexane. Evaporation of the extract gave crude oryzanol (86).

Seetharamaiah and Prabhakar had isolated oryzanol by extraction of soapstock with diethyl ether. The soapstock obtained after alkali refining contained 1.3 -
3.1% oryzanol and the ether extract contained 16% oryzanol which was further enriched by column chromatography. Pure oryzanol crystals were obtained by crystallisation and recrystallisation with different solvents (20).

2.1.4.1.2 Extraction of oryzanol from dark oil: The dark oil is reported to contain about 5% oryzanol (87). Dark oil obtained from ricebran oil was mixed with aluminium sulphate and other metallic salts that trap stearins. The mixture was reported to be stirred while heating to 85° - 100°C and while cooling to 45°C and centrifuged. The supernatent was allowed to stand at 30° - 45°C and high purity γ-oryzanol crystallised out. This method was patented by Baso Oil and Fat Co. Ltd. (88).

The residue of distillation of dark oil was extracted with MeOH and the solvent insoluble portions containing oryzanol was treated with acetone to separate acetone soluble portion. From the acetone soluble portion oryzanol was obtained by Tomotaro et al. (89).

In other method studied by Tomotaro and Osamu (90), dark oil was heated with MeOH containing 4% concentrate sulphuric acid and dehydrated. The product was passed through a column filled with 500 cc Amberlite. The column was washed with Et₂O and eluted with a mixture of MeOH and Et₂O containing small amount of HCl. The eluent after washing gave oryzanol.

Tomotaro et al. (91) treated ricebran oil with alkali followed by hydrolysis and distilled at 2 mm pressure to remove the fraction having boiling point below 198°C. The residue was saponified with methanolic NaOH and after saponification the solvent was evaporated. The residue after heating with HCl, dissolving in Et₂O and treatment with Na₂CO₃ gave oryzanol.
2.1.4.1.3 Extraction of oryzanol from methyl esters: Rice bran oryzanol was first extracted by Rhyohei and Tomotaro (92). They extracted the oil with acetone and the solute was replaced by diethyl ether. The FFA was removed by Na$_2$CO$_3$ solution and subjected to esterification. This ester after purification by repeated chromatography and crystallisation gave oryzanol.

In other method studied by Azume et. al.(93), rice bran oil methyl esters were dissolved in hexane. A solution of urea dissolved in methanol was added to the solution which resulted in the formation of urea addition products. It was filtered and decomposed by water to yield crude fatty acid methyl ester. The filtrate after removal of hexane and cooling at -10°C gave oryzanol.

Methyl esters were dissolved in C$_6$H$_6$ - MeOH (7:5) by Park et. al. (94) and passed through a basic anion exchange column. The UV absorption spectra of eluent in 1% hexane was measured at 315 nm and it was observed by them that most of the oryzanol was absorbed on the resin and thus the methyl esters were reported to give the best recovery of oryzanol.

Methyl esters after distillation gave residue which on saponification and column chromatography with tetrahydrofuran as an eluting solvent gave oryzanol. The process was patented by Kawada et. al. (95).

2.1.4.1.4 Extraction of oryzanol from alkaline oil cake and pitch: Rice bran pitch was also used to prepare oryzanol by Kyokuto (96). Alkali bottom sediments from alkali refining of rice bran oil was extracted with methanol, ethanol or acetone. The residue was stirred with 0.1 - 0.3 N solution of alkaline substance in an organic solvent and filtered. The filtrate was mixed with acetic acid in methanol and oryzanol was separated out by adjusting the pH value to 6 - 9. This method was patented by Toyo Kaotsu Industries Inc. (97).
Alkaline oil cake was stirred with chlorinated solvent for 30 minutes at 40°C. The insoluble matter after mixing with water was again stirred while adding CO₂ gas which gave oryzanol. The residue was reported by Shibuya to be purified by extraction with methanol and ethanol (98).

The alkaline oil cake of ricebran oil dissolved in MeOH and CO₂ gas was introduced while stirring at 40°C and 1 atm pressure. The mixture was filtered and the residue after washing with MeOH was reported to give oryzanol by Nishihara and Tshibuya (99).

2.1.4.1.5 Extraction of oryzanol by molecular distillation: In literature molecular distillation was reported to be helpful for extraction of oryzanol. Akio and Tomotaro (100) studied molecular distillation of crude ricebran oil at <140°C. The residue was admixed with ethanol and aqueous alkali which after extraction with hexane and ether gave crude oryzanol.

Molecular distillation of ricebran oil, ricegerm oil, ricebran dark oil and their esters at 210 - 300°C are reported by Arawake et al. (101) to give concentrate oryzanol fraction. They gave oryzanol after extraction with hexane and furfural.

Watanebe et al. reported that ricebran dark oil on molecular distillation at 250°C - 300°C and 10⁻³ to 10⁻⁴ pressure gave purified oryzanol (102).

The molecular distillation of methyl ester of ricebran dark oil at 280°C -300°C gave concentrate fraction of oryzanol containing 20.2% oryzanol (103). This method was patented by Watanebe et al.

2.1.4.1.6 Extraction of oryzanol by other methods: A stimulated moving bed chromatography separator was tested by Saska and Rossiter (104) for recovery of γ- oryzanol from degummed and dewaxed ricebran oil that contained 1.0 -
1.6% oryzanol. A crude product with 12 - 15% oryzanol was obtained and a 90 - 95% pure product was recovered from the concentrate by crystallisation with heptane. With the recycling of the crystallisation liquor, the overall recovery of oryzanol was feasible and potentially higher than the recovery in the conventional soap stock based process. In other method reported by Chan (105), oil after extraction from ricebran by alkaline methanol was filtered and heated at 39°C. Oryzanol was crystallised by adding 20% citrate solution. The gummy matter of ricebran oil separated from the oil during washing with warm water by Tomotaro and Osamu showed presence of oryzanol (106). Two anion exchange resins were investigated by Hiroshi and Taksuo (107) for the separation of oryzanol from ricebran oil. In one process oryzanol and FFA absorbed by the resins were simultaneously eluted with a mixture of C₆H₆, MeOH and HCl. Oryzanol was recovered from the elute. In another method developed by Hiroshi and Noguchi (108), oryzanol was separated by the resin after esterifying the FFA and elution and was recovered from the effluent.

2.1.4.2 Purification of oryzanol: Oryzanol obtained by above methods was purified by treatment with ketones and alcohols (109). Individual fractions of oryzanol were reported to be isolated from oryzanol by multistage recrystallisation, multistage solid liquid extraction using higher alcohols, higher hydrocarbons and/or their mixtures and chromatography (110-112). They can also be obtained by synthetic means (113).

2.1.4.3 Identification, separation and quantitation of oryzanol: Identification and quantitation of oryzanol was done by UV and HPLC. They can be determined by electrophorosis in a fused silica tube (114), by combination differential matrix spectrophotometry (115), NMR of unsaponified fraction of ricebran oil (116) and mass spectroscopy (117). From UV Spectrophotometer identification was done at \( \lambda \) max. 315nm in petroleum ether (60° - 80°C) using specific extinction coefficient \( (E^{1%}_{1cm}) \) as 358.9 (20)
Quantitation of oryzanol was done by normal phase HPLC (118). Separation of oryzanols from vitamin E components was done by normal phase high performance liquid chromatography as vitamin E components elute as group before oryzanols. However, different fractions of oryzanols could not be separated from normal phase chromatography (19). Separation of different components of oryzanol was done by reverse phase chromatography using acetonitrile: methanol: isopropanol: water (45:45:5:5) as mobile phase. The elution order of oryzanols was cycloartenyl ferulate < 24-methylene cycloartanyl ferulate < campestryl ferulate < cycloartanyl ferulate and β-sitosteryl ferulate. Chemical ionisation mass spectrometry (CIMS) was used to identify these fractions and EI mass spectroscopy was used for quantitation of different fractions in oryzanol (119).

Different fractions of oryzanol were separated and quantitated by reverse phase HPLC using acetonitrile: n-butanol: acetic acid: water (82:3:2:13) as mobile phase at a flow rate of 1.2 ml/min. The eluent was monitored using diode array detector (120).

2.1.4.4 Therapeutic benefits of oryzanol: Ricebran oil is considered to be a good edible oil as it keeps the cholesterol level low due to its linoleic acid, tocopherols and oryzanol content. The most important biological activity of γ-oryzanol is its cholesterol lowering property. Studies indicated that oryzanol fed to humans and animals reduced LDL cholesterol and increased HDL cholesterol either by influencing absorption of dietary cholesterol or enhancing conversion of cholesterol to faecal bile acids and sterols. Recently the mechanism of cholesterol lowering action of oryzanol was investigated in hamsters by Nicolosi’s group (121). The hamsters were made cholesterolemic by feeding chow based diets containing coconut oil and 0.1% cholesterol with or without 1% oryzanol for 7 weeks. Relative to control animals, oryzanol administration
resulted in significant reduction of plasma total cholesterol level (28%), non HDL cholesterol (34%) and a 25% reduction in percentage cholesterol absorption. Aortic fatty streak formation was reduced by 67% in the oryzanol treated animals. It was concluded that oryzanol is at least partly responsible for cholesterol lowering action of ricebran oil (RBO).

Animal studies demonstrated the hypolipidemic effect of oryzanol. The reduction in serum and liver lipids in RBO fed rats was higher than groundnut oil (GNO) fed rats. There was a significant decrease in the total cholesterol, LDL and VLDL cholesterol. The triglycerides were also decreased but HDL cholesterol fraction was not decreased significantly. It was reported in the literature that hypertriglyceridaemia induced by fructose was significantly lower in animals which had been maintained on a 0.5% oryzanol containing diet (122).

It was observed that oryzanol reduced elevated serum TSH levels in hypothyroid patients possibly by a direct action at the hypothalamus rather than the pituitary (123). Oral administration of γ-oryzanol to the patients with primary hypothyroidism lowered serum TSH by 20 - 30 % in 1 - 4h (124).

γ-Oryzanol was effective to prevent experimental coronary atherosclerosis in rats (125-126). Sonic stress as one of the cardiac risk factors for atherosclerosis was studied in animal experiments. Results showed that γ - oryzanol may inhibit the development of atherosclerosis induced by sonic stress (127). Addition of 5% oryzanol to the diet containing RBO showed a decrease in total cholesterol. The cholesterol lowering ability of RBO appeared to be due to oryzanol and some other components of the unsaponifiable matter (128). Effect of γ -oryzanol as well as cycloartenol ferulic acid ester on the cholesterol diet induced hyperlipidemia was studied (129). I.V. injection of oryzanol accelerated the excretion of free cholesterol and glyceride (130).
It was observed that plasma LH response to LH-RH in rats was suppressed by an injection of \( \gamma \)-oryzanol (131). Oryzanol is a substance that acts directly on the hypothalamus correcting disturbances in the function and removing nervous complaints accompanied by whiplash syndrome. It also acts directly on the interbrain hypothalamus stimulating the nerves and relieving various nervous complaints. It is thus effective in correcting certain symptoms due to ageing such as vegetative stigmata and gastrointestinal neurosis (16).

Electroencephalographical study of experimental whiplash injury showed the effect of \( \gamma \)-oryzanol as therapeutic agent for unidentified clinical syndrome (132). Changes in the concentration of catecholamines in various nuclei in the brain was examined after administration of oryzanol. It was found that oryzanol increased the concentration of norepinephrine and dopamine in the median eminence but not in other nuclei (133). The norepinephrine content of the brain was increased when oryzanol was given by inhibiting degradation or release of norepinephrine (134).

Platelet aggregation was measured during platelet rich plasma prepared from rats fed oryzanol in the control diet and those fed oryzanol in a 1% cholesterol diet. On the other hand oryzanol fed along with high cholesterol diet significantly inhibited platelet aggregation (135-136).

Oryzanol inhibited ion induced lipid peroxidation (137), countered the estrous cycle in rats (138), was effective against lipogenic liver cirrhosis in the spontaneously hypersensitive rats (139), inhibited gastric lesions induced by both conditional emotional stimuli and rapid eye movement sleep deviation in mice (140), suppressed plasma prolactin response to thyrotropsin releasing hormone (141-142), gastric and ideal movements were enhanced after I.V. administration of \( \gamma \)-oryzanols in dogs (143).
For studying the carcinogenic potential of γ-oryzanol, a drug mainly used for the treatment of hyperlipidemia was studied in rats. The finding indicated that under the experimental conditions described γ-oryzanol was not carcinogenic (144-146).

Study on the biological effect of oryzanol on the growth of rats fed thermally oxidised oil. The result indicated that oryzanol had some antioxidative activity for the oxidation of polyunsaturated fatty acids in the tissues but this activity was weaker than tocopherol (147-148).

It was observed that oryzanol at 0.5 - 1% w/w retard thermal oxidation of oil. Oryzanol was found to be an effective antioxidant other than tocopherols in ricebran oil. The antioxidant action of oryzanol was marked at above 0.5% (149). It was observed that antioxidative effect of α-tocopherol disappeared with heating but that of oryzanol was retained. Ricebran oil was heated for 8h at 160°C and it was found that 12.4% tocopherol was retained but oryzanol was retained 83.8% (150).

Oryzanol suppressed the pituitary hormone secretion (151). Oryzanol and its metabolite can be effective agents for prostaglandin metabolism in the vascular wall and platelets, preventing the development of vascular lesions such as atherosclerosis (152). It suppressed the facilitation of propulsion of charcoal meal in the large intestine. Oryzanol has therapeutic effects on the gastric ulcers (153).

2.1.4.5 Uses of oryzanol: Oryzanol due to its marvellous therapeutic benefits and antioxidant properties, is used in various formulations mainly in pharmaceutical and cosmetic industries (154-155).
Oryzanol is used in the preparation of soft capsules (156-157). These capsules contain campesterol (32.0%), \( \beta \)-sitosterol (25.73%), cycloartenol (24.47%) and 24-methylene cycloartenol (14.60%).

It is widely used as an antioxidant (158). Oryzanol was dissolved in squalene and then added to foods as antioxidant (159). It is dissolved in ethyl alcohol and mixed with a surfactant and optionally tocopherol and oils and fats. The solution is further mixed with an aqueous amino acid solution and homogenised to yield a food antioxidant that prevents discolouration (160). It is also used as substance for modifying the effect of naturally occurring antioxidant (161-162).

Ointment formulations (163), and skin preparations (164-165) contains oryzanol as one of the ingredients. It has sebum secretion stimulating function on the dry skin and hence used in skin care products (166). Cosmetics containing antioxidants to delay the ageing of skin (167) and preventing wrinkles also contain oryzanol. Pharmaceuticals and cosmetic preparations containing squalene for the treatment of skin disorders uses oryzanol (168), and pharmaceuticals for nervous system disorders contain oryzanol (169).

It is used in hair preparations as dandruff controlling agents (170-171). Antidandruff hair preparations and hair creams which are effective in the control of dandruff and itching scalp (172) uses oryzanol as an ingredient. Preparations containing oryzanol have hair growth stimulating activity (173-174).

Pearly compositions containing \( \gamma \)-oryzanol are used as stabiliser and therapeutic agents (175-176). Oryzanol, ferulic acid and other related substances are dissolved in alcohols or in non ionic surfactants and are used as preservative for food (177-178). It is also used as an additive in foods to lower metabolisable cholesterol content (179).
A nutritional oil high in oryzanol and vitamin E is manufactured by trans-esterification of ricebran oil with other vegetable oils (180).

Cycloartenol ferulate isolated from oryzanol was found to be effective anticholesterolemic (181). γ-Oryzanol on hydrolysis gave ferulic acid which is useful as raw material medicine, agricultural chemicals, cosmetics, pigments and food additives (182). Hydrolysis and biotransformation of oryzanol also gave sterols (183). Oryzanol by chemical reaction can be converted to sodium 4-methylene cycloartanosuccinate which has an autonomic nerve stabilising activity with good water dispersity (184).

Highly concentrated solutions of oryzanol are prepared by dissolving it in substantially water free oils. The oil solution so obtained does not precipitate oryzanol even after prolonged storage at -20°C. Addition of squalene increased the solubility of oryzanols in oils (185-186). Oryzanol useful as an antioxidant and pharmaceutical is made water soluble by converting it into a oryzanol cyclodextin inclusion compound (187).

Bath compositions for dry skin was prepared from oryzanol (188-189). Oryzanol is added to soaps to prevent discolouration (190). Oryzanol was incorporated in detergent formulation which are used to treat dry skin conditions (191-192). It is used as an ingredient in detergent composition which form a protective coating on the skin (193).

Application of a cream containing 1% oryzanol to the skin of forearm of healthy human subjects resulted in an increased skin lipid content (194). It increased the sebaceous secretion and showed therapeutic effects on dermal xerosis and ichthyosis (195).
Soluble oryzanol is used for pharmaceutical cosmetics and food preparation (196-197), deodorant for controlling body odours (198), nail lacquers (199) and transdermal tapes in which it acts as stabiliser (200).

Medicinal drinks useful as revitalising tonics (201-202), health foods and drugs for preventing motion sickness (203-204), and drugs for atherosclerosis (205-206) contains oryzanol.

2.2 EFFECT OF CONDITIONS OF EXTRACTION AND PROCESSING ON MINOR COMPONENTS:

The effect of solvent to bran ratio (2:1 and 3:1 w/w), extraction temperature 40° and 60° C and time 5, 10, 15, 20 and 30 minutes were studied for hexane and isopropanol extraction. It was found that extraction time of 15 min. was sufficient for optimum crude oil, vitamin E and oryzanol extraction (207). In other experiment it was reported that oil extracted with isopropanol was significantly more stable to heat and induced oxidation than hexane extracted oil. Antioxidants that are more easily extracted by isopropanol than hexane may be responsible for the increased stability (208). Extraction of oryzanol was performed with supercritical carbon dioxide and it was found that maximum recovery of oryzanol was feasible with this method (209-211).

Characteristic natural antioxidant in ricebran oil such as tocopherol and oryzanol impart longer storage stability considering the oil's high degree of unsaturation. The levels of antioxidants were measured after refining and the results showed that refining reduced the contents of both tocopherols and oryzanols but 97.7% tocopherols and 91.7% oryzanols were retained after steam refining. In solvent refining oryzanol was mostly retained but tocopherol was diminished considerably (212).

The contents of natural antioxidants and the oxidation stability of ricebran oil at different stages of refining steps were determined and the maximum weight gain,
peroxide value and anisidine value were obtained from alkali refined oil during storage. The order of oxidation stability was crude \( \geq \) degummed \( > \) bleached \( \approx \) deodorised \( > \) alkali refined oil (213).

Components of ricebran oil had been assessed for their effect on refining losses. It has been reported in literature that waxes and oryzanol increased the refining losses substantially. When wax and oryzanol were present together in the oil, the effect was synergistic. The refining losses were higher than the sum of their individual effects (214).

2.3 NUTRITIONAL ASPECTS OF RICEBRAN OIL:

It has been reported that vegetable oils generally have no rising effect on serum cholesterol level, moreover safflower, corn, sunflower oils lowered distinctly its level. In ricebran oil lowering effect was found to be much more than the above referred oils. This effect may be attributed to its unsaponifiable matter since its linoleic acid content is much less than the mentioned oils (215).

Experimental as well as human studies had demonstrated the hypolipidemic effect of ricebran oil. Further, it was established that minor components present in the unsaponifiable fraction of ricebran oil were responsible for this effect. Nutritional evaluation studies carried out with 10% ricebran oil and 20% protein indicated that growth feeding efficiency and mineral balance were comparable in groundnut oil fed rats. Toxicological studies showed that there were no abnormalities in animals fed either GNO or RBO (216). In addition neither RBO nor the foods deep fried in it showed any mutagenicity as judged by AMES test (217).

Ricebran oil and groundnut oil had been found to contain similar amounts of saturated and essential fatty acids but different amounts of unsaponifiable matter. Addition of unsaponifiable matter in amounts equal to that present in
RBO to hypocholesterolemic inducing diet resulted in reduction of serum cholesterol, clearly demonstrated that unsaponifiable matter was the active fraction responsible for the hypocholesterolemic action of RBO. Supplementation with the unsaponifiable matter of RBO to the hypercholesterolemic diet fed to rats, produced lowering of total serum cholesterol and LDL and VLDL cholesterol fractions but did not effect significantly the HDL cholesterol fraction (38,218).

Rats fed ricebran oil at 10% level for a period of eight weeks have been reported to significantly lower levels of total cholesterol, LDL and VLDL cholesterol both on cholesterol containing and cholesterol free diet. HDL cholesterol was increased but triglycerides decreased, that was statically significant. Liver lipids were also reduced. Faecal excretion of neutral sterols and bile acids was increased after ingestion of RBO (219).

A comparative nutritive study was made to show that the extent of purification markedly influenced the nutritive characteristics of RBO. The coefficient of digestibility was 93.8% when RBO purified by degumming, deacidifying, bleaching and deodorisation was fed to rats, whereas it was 94.8% when extremely pure RBO which was achieved by inducing an additional dewaxing step was used. RBO without deodorisation but purified by other treatments showed a 96.2% coefficient of digestibility which was somewhat lower than GNO (220).

2.4 RICEBRAN OIL AS A COOKING MEDIUM:

Ricebran oil contained 15 - 20% unsaturated fatty acids and 80 - 85% saturated fatty acids. The edibility of refined RBO is comparable to other refined oils including groundnut oil, cottonseed oil, soyabean oil and rapeseed oil. Moreover RBO has better keeping quality due to the presence of natural antioxidants. The
food cooked in RBO was found to be very delicious and the oil has longer shelf life.

Refined RBO is possibly the most excellent cooking medium available today. Although it is a recent addition in the edible oil market in our country as a cooking oil, it is a major cooking media in Japan since long. Its nutritional value is superior to most vegetable oils and it is easily digestible. The modern medical science prescribes that a good cooking oil should be rich in PUFA to avoid the increase of cholesterol level in blood. The fatty acid profile of RBO is unique in this regard. Experiments revealed that serum cholesterol level in RBO fed rats was much lower than those of safflower oil fed groups indicating that RBO is even better than safflower oil, which is the best known cholesterol level reducer available in the market today. It does not have any problem of toxicity development during deep fat frying particularly in Indian style. Foods deep fried in RBO absorbs less oil compared to foods fried in other media and in fact cooks 15% more food compared to other oils. It has been observed the products deep fat fried in RBO retain their taste and blandness for a longer period without any deterioration. Ricebran oil fried the food faster having more golden brown color. None of the deep fat fried food in RBO showed any mutagenecity either with or without metabolic activation thereby, showing that deep fat fried food in RBO is as safe as food fried in GNO (221).

2.5 DEEP FAT FRYING:

Blumenthal et al. (1976) studied the effect of deep fat frying on the flavour of different fats and oils. The organoleptic properties of different samples were evaluated and the volatile decomposition products were determined by GLC after their quantitative isolation by high vacuum cryogenic entrainments (222).

Chang et al. (1978) studied the chemical reactions involved during the deep fat frying of foods. The volatile decomposition products produced by corn oil,
hydrogenated cottonseed oil, triolein, trilinolein, under stimulated frying conditions were collected and characterised. At 185°C after deep fat frying for 74h, triolein yielded 10.8%, trilinolein yielded 26.3% and tristearin yielded 4.2% non-urea adduct forming ester (223).

Graziano (1979) studied the oxidative deterioration of frying oils by measuring dielectric constant at constant temperatures and reported that the rate of increase in dielectric constant during frying correlated with the increase in polar substances (224).

Pai et al. (1979) studied the effect of heat treatment on the volatile composition of coconut oil after heating the oil under different conditions for 48h at 180°C. The values obtained were analysed by GCMS. In all heated samples a series of n-alkanes, l-alkenes, n-alkanals, methyl alkanones, alkenals, and lactones, methyl and ethyl esters and free fatty acids were identified (225).

Elder and Guhr (1979) fried potato chips in different oils for deep and shallow frying at 200°C and estimated the decrease in linoleic acid content which was found to be decreased 24 - 45% during deep frying and 13 - 34% during shallow frying (226).

Sultana and Sen (1979) observed the deterioration in groundnut oil, safflower oil and vanaspati during heating. There was a considerable damage of test oils through the formation of oxirane-oxygen conjugated double bond, oxides and non-urea adduct forming fatty acids during heating. An increase in lovibond colour units, viscosity, foaming, free fatty acids, refractive index and decrease in smoke point and iodine value was found (227).

De Plessis et al. (1981) studied the characteristics of cottonseed and peanut oil for frying of potato chips at various intervals and found that during first 20h frying the free fatty acid content and peroxide value of cottonseed oil increased rapidly.
as compared to peanut oil. Peanut oil frying lost 55\% of its total tocopherol whereas cottonseed oil frying retained these compounds at original level. Tocopherols were also better retained in cottonseed oil than in peanut oil (228).

The effect of BHA and BHT in retarding the deterioration of RBD palmolein during static heating (180°C) and the frying operation was assessed by determining the peroxide, anisidine, acid and iodine values, absorbances at 232 and 268 nm and the fatty acid composition of the oil by Augustin and Berry (1983). It was found that during static heating BHA was more effective antioxidant than BHT whereas during intermittent frying both the antioxidants were relatively effective in retarding the deterioration of the oil (229).

Smith et al. (1985) analysed the lipid content and fatty acid profiles of various deep fat fried foods. The total lipid content of each type of food varied among different commercial sources (230).

Effect of TBHQ on the quality characteristics of RBD olein during frying was studied by Asap and Augustin (1986). It was observed that addition of TBHQ reduced the level of polar components in the oil, decreased the rates of change of iodine value, dielectric constant and oxidation of C18:2 (231).

Kupranycz et al. (1986) studied the thermal oxidation behaviour of butter fat and selected vegetable oils heated at 185°C in presence of air (30 ml/min) and found that butter fat was more stable to thermal oxidation than canola, sunflower and soyabean oils (232).

Yoon et al. (1987) heated ricebran oil and double fractionated palm olein at 180°C for 50h to measure the lipid deterioration of the oil. Free fatty acids increased while iodine value and smoke point decreased during frying. Absolute content of polyunsaturated fatty acids reduced more than that of monounsaturated fatty acids (233).
An industrial production of pre-fried trench fries using palm oil as a frying medium was studied by Sebedia et al. (1991) over a period of 12 days. The quality of both the oil and the french fries were assessed (234).

Hassan (1991) conducted a survey of the quality of used frying oils from the restaurants and the discarded oil appeared to be heat damaged to a varying extent according to the degree of quality control applied by the corresponding restaurants (235).

Chung and Chen (1992) heated soyabean oil by deep fat frying at 200°C for 1h with the addition of varying proportions of water and then stored at 55°C for 26 weeks. The volatiles and peroxide value of these samples were monitored. All the samples were found to contain aldehydes as major volatiles (236).

Sen and Sen (1993) studied the oxidised fatty acid content of heat damaged frying oils and Indian deep fat fried products. Analysis of ten samples of heat damaged oils used for deep fat frying indicated that these samples had oxidised fatty acid content ranging from 0.4 - 2.8%, eight having more than 1.0% (237).

The alterations of sunflower oil were evaluated by column, gas, high performance size exclusion chromatography after being used for deep fat frying by Sanchez-Muniz et al. (1993). Polar compounds are reported to increase significantly, at the same time, linoleic acid decreased while oleic acid remained unaltered after 15 fryings. Triglyceride polymers, triglycerides dimers and oxidised triglyceride increased significantly in the oil (238).

Chu and Luo (1994) studied the effect of sugar, salt and water on soyabean oil quality during deep fat frying. Soyabean oil heated under static conditions for 80h was found to contain 29% total polar components (TP) which exceeds acceptable limit (27%) proposed by German Society of Fat Research. Among
the effect of salt, sugar and water the rate of oil deterioration was found to be highest for water followed by salt and sugar (239).

The behaviour of high oleic sunflower oil used for 75 repeated deep fat frying of potatoes were evaluated by Romero et al. (1995) by measuring the total polar component isolated by column chromatography. Data from this study indicated that there was a slight increase in the level of polar materials in the fryer during first frying followed by minor changes and a tendency to reach a near steady state in successive fryings (240).

Tyagi and Vashishtha (1996) quantitatively determined the polymeric fractions formed during the deep fat frying of potato chips using RBD soyabean oil with and without antioxidants and vanaspati by urea fractionation. The frying experiments were conducted at 170\(^0\), 180\(^0\) and 190\(^0\)C for 70h. The percentage of non-urea adduct-forming esters indicative of polymeric fractions were found to have their highest values ranging from 20 - 30% in case of soyabean oil whereas these values correspond to 14 - 18% with vanaspati. In another study soyabean oil was used for deep fat frying at 200\(^0\)C and the changes in physicochemical characteristics and fatty acid composition were determined which showed faster deterioration of soyabean oil as compared to vanaspati (241).

Dutta and Appelqvist (1997) fried potato chips in palm oil, sunflower oil and high oleic sunflower oil (HOSO) and studied the content of different phytosterol oxides during 0 - 25 weeks of storage. No considerable increase in sterol oxides was observed in samples of chips fried in palm oil and sunflower oil. The chips samples fried in HOSO had slightly higher level of sterol oxide (242).

Kaur et al. (1997) determined the acid value and peroxide value of desi ghee, vanaspati, sunflower, groundnut and mustard oil before and after frying. These values both for residual fats and extracted fats were within the acceptable range.
High peroxide values were found in sunflower oil and high acid values in desi ghee and vanaspati after frying (243).

Romero et al. (1998) studied the effect of replenishment during deep fat frying of frozen foods in sunflower oil and high oleic sunflower oil. Data from this study indicated that HOSO performed more satisfactorily than sunflower oil in repeated frying of potato chips (244).

2.6 BLENDING:

Much work has not been carried out on blended oils in India. It is only since 1986 reports started coming in. Millwalla and Subrahmanyam (1986) studied the thermal stability of blended oils. Groundnut, cottonseed and safflower oil when blended with sesame oil showed better thermal stability at 180°C (245).

Murthi et al. (1987) studied the storage stability of edible oils and their blends. The storage stability of vegetable oil blends containing 25% raw edible oil and 75% refined oil was investigated. Edible oil blend showed a lower increase in free fatty acids than did the individual oils. The acceptability of raw edible oil became poor after 120 days of storage at 37°C when its peroxide value in eq/Kg reached between 5.9 - 16. In refined oils acceptability was poor after 90 days at the peroxide value between 4.4 - 9.0. Blends containing 25% of raw oils developed rancidity after 120 days of storage. The results indicated that incorporation of 25% raw edible oils in refined oils prolonged the shelf life over that of refined oils alone (246).

Nassirulah et al. (1991) studied the quality characteristics and stability studies on edible vegetable oil blends. Edible oil blends containing groundnut oil (GNO) : ricebran oil (RBO) and mustard oil (MO) : ricebran oil (RBO) were prepared in varying proportions and stored for a period of three years. Their physicochemical characteristics were determined at regular intervals. It was
observed that MR blend was quite stable upto two years whereas GR blend showed string rancidity just after few months (247).

El-Shami et. al. (1992) compared the dielectric properties to the conventional methods of analysis for evaluating the frying quality of a blend of cottonseed and sunflower oil. The apparent relaxation time, the activation energy (H) and the entropy change (S) was determined after frying. The results indicated that dielectric constant and dielectric loss are useful tools for predicting deterioration occurring during the heating of oil (248).

Properties of groundnut - mustard (GNMU) and sunflower - mustard (SFMU) oil blends were also studied by Handoo et. al. (1992). Studies showed a small but steady rise in FFA and fall in iodine value (IV) and colour in these blends but this rise was faster in SFMU blend as compared to GNMU blends. Pure safflower oil is reported to show poor shelf life and thermal stability as compared to its blends. Organoleptically 70:30 blend ratios of both GNMU and SFMU had greater acceptability (249).

Handoo et. al. (1992) studied the properties of groundnut (GNO) and cottonseed (CSO) oil blends. They found increase in PV, FFA, and fall in IV and colour in pure GNO and CSO. The similar trend was also observed in oil blends. The shelf life of 50:50 blend ratio of CSO : GNO was comparable to pure GNO. The thermal stability of blended oils was higher than pure CSO oils (250).

Goro et. al.(1992) studied the influence of blend ratio of vegetable oils on their thermal oxidation and decomposition of tocopherol. Blending of oil (soyabean oil + palm oil, rapeseed oil + palm oil, cottonseed oil + olive oil, safflower oil + high oleic safflower oil) enhanced auto and thermal oxidative stabilities at 60°C, but also enhanced thermal decomposition of tocopherol at 180°C (251).
Handoo et al. (1994) studied the storage properties of cottonseed - mustard oil (CSMU) and corn - mustard oil (COMU) blends. The results showed a rise in FFA and decrease in IV and colour in both the blends. The PV is increased in both cases but faster in CSMU blend. The shelf life of cottonseed oil was improved after blending with mustard oil. Blending of mustard oil with corn oil did not alter the shelf life of COMU blends significantly as compared to pure oils. Both blends showed acceptability upto four months (252).

The oxidative stability of polyunsaturated vegetable oils was improved by blending with high oleic sunflower oil (HOSO). Mixing different proportions of HOSO with polyunsaturated vegetable oils provided a simple method to prepare more stable edible oils with a wide range of desired fatty acid composition. Oxidative stability of soyabean, canola and corn oils blended with different proportions of HOSO to lower the respective levels of linoleate and linolenate, was evaluated at 60°C (253). This study was carried out by Frankel and Haung (1994).

Lakshmi and Sarojini (1994) studied the changes in total carotenoids in red palm oil (RPO) and its blend with groundnut oil during storage and heating. The result of the study showed that there was loss of 75% carotenoids from stored RPO and 78% during deep frying. The results obtained from the blended oils were similar to those obtained for RPO (254).

Thejappa and Subramanyam (1995) studied the changes in the stability of cottonseed and coconut oil blend due to inter-esterification. Interesterified blend had higher stability than simple blend which inturn had better stability than cottonseed oil, which is higher in unsaturation (255).

Effect of fatty acid composition of frying oils on intensities of fried food flavour and off flavours in potato chips and fresh fried potatoes were determined by Warner et al. (1997). Commercially processed cottonseed oil and high oleic
sunflower oil were blended to produce oils with 12 - 25% linoleic and 16 - 78% oleic acid (256).

Lakshmi and Sarojini (1998) tested the acceptability of blended oils i.e. red palm oil (RPO) blended with different proportions of groundnut oil (GNO) for deep fat frying. The results showed that RPO as well as 70:30 ratio of RPO and GNO were not suitable for deep fat frying. Blends of 50:50 and 30:70 were found suitable for deep fat frying as the products prepared with them were highly acceptable (257). They also studied (1996) the changes in α-tocopherol content of these blends during storage and heating. The results indicated that during heating there was a loss of 89% α-tocopherol from RPO as well as blended oils (258).

Nasirullah et. al. (1998) observed the changes in repeatedly heated oils during deep fat frying. Refined groundnut oil (GNO), raw GNO and refined GNO blended with raw sesame oil in the ratio 3:1 were used for deep fat frying and analysed for their % triglycerides, % di and monoglycerides, % FFA, UV spectrum of tri, mono and diglycerides at regular interval. The result showed that incorporation of sesame oil in refined GNO retards the process of oxidation (259).

Tyagi et. al. (1998) subjected refined, bleached and deodorised soyabean oil its blend with raw mustard and sesame oil to deep fat fry potato chips at 180°C for 36h. It is reported that deterioration in soyabean oil was much faster as compared to other two blends. However, blend of soyabean plus mustard showed higher stability. Similar observations were also made during static heating of soyabean oil (260).