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Gamma-oryzanol from rice bran oil – A review

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Rice bran oil is unique among edible oil due to its rich source of commercially and nutritionally important phytoceuticals such as, oryzanol, lecithin, tocopherols, and tocotrinols. However, most of these phytoceuticals are removed from the rice bran oil as waste byproducts during the refining process. γ-oryzanol is one of such component having the potential to be used in nutraceutical, pharmaceutical and cosmoceutical preparations. It is a mixture of ferulic acid esters of sterol and triterpene alcohols. It occurs in rice bran oil at a level of 1-2 per cent where it serves as natural antioxidant. The article describes the production of rice bran oil from rice bran and different methods of extraction of γ-oryzanol from rice bran oil. It also reviews the health care properties of γ-oryzanol.

Keywords: Rice bran oil, Edible oil, γ-oryzanol

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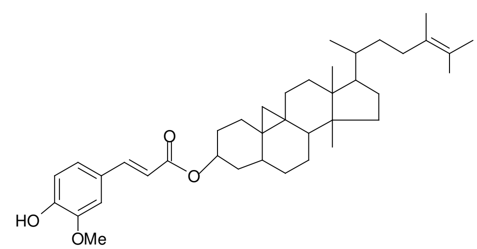
Introduction

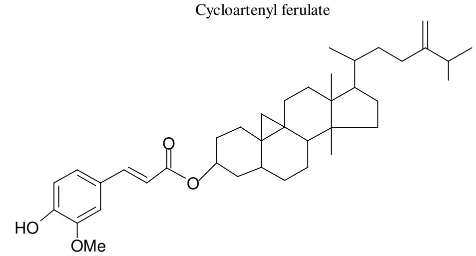
Rice bran obtained during milling of rice is gaining commercial importance in the world as it has many beneficial nutritive and biological effects. Rice bran oil (RBO) can be extracted from rice bran by solvent extraction using food grade *n*-hexane[1] or in solvent free process by using ohmic heating[2] or supercritical fluid extraction technology[3]. The crude rice bran oil obtained in the solvent extraction process is subjected to either chemical refining[4] or physical refining[5] to meet the specifications of edible grade vegetable oil.

Chemical refining of crude rice bran oil yields better product in terms of colour and cloud point[5]. However, this process also causes high refining losses in the form of wax sludge, gum sludge, and soap stock[4]. These residues, produced in the RBO refining industry, are the rich source of many nutraceutical like, oryzanols, tocopherols, vitamin E, ferulic acid, phytic acid, lecithin, inositol and wax[6].

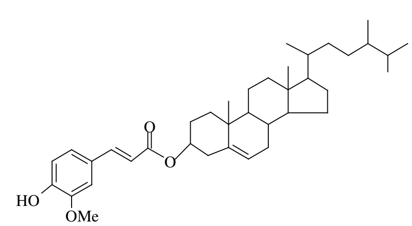
γ-oryzanol component of rice bran oil was first presumed to be a single component[7]. But later it was determined to be a fraction containing ferulate (4-hydroxy-3-methoxy cinnamic acid) esters of triterpene alcohols and plant sterols[8]. Cycloartenyl ferulate, 24-methylenecycloartanyl ferulate and campesteryl ferulate (Scheme 1) are the three major



Cycloartenyl ferulate



24 methylene Cycloartanyl ferulate



Campesteryl ferulate

Scheme 1—Shows cycloartenyl ferulate, 24-methylenecycloartanyl ferulate and campesteryl ferulate the three major components

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components and account for 80 per cent γ-oryzanol[9]. The γ-oryzanol component of rice bran oil can be simultaneously separated and quantified by high-performance liquid chromatography[8].

Oryzanol is an antioxidant compound and is associated with decreasing plasma cholesterol[10], lowering serum cholesterol[11], decreasing cholesterol absorption[11] and decreasing platelet aggrigation[12]. Oryzanol has also been used to treat hyperlipidemia[13], disorders of menopause[14] and to increase the muscle mass[15].

India is the second largest producer of paddy after China and contributes about 23 per cent of the total world production of paddy[6]. Japan contributes just 2 per cent of the total world production of paddy and produces dozens of high value chemicals and nutraceutical from the derivatives of paddy[6], but unfortunately in India the potential of rice bran oil as cooking oil still remains largely untapped. Table 1 gives the potential and actual exploitation of rice bran in India[6].

The article describes the extraction and refining method of RBO, and extraction of γ-oryzanol from RBO. It gives a detailed review of the health care properties of γ-oryzanol.

Extraction and Refining of Rice Bran Oil

Hexane is used as a solvent to chemically extract oil from rice bran[16]. However, hexane poses potential fire, health, and environmental hazards[17]. Therefore, short chain alcohols, especially ethanol and isopropanol have been proposed as the alternative extraction solvent due to their greater safety and reduced probability of regulation[16,18,19]. Many studies have been conducted using isopropanol as a solvent for extraction of soybean and cotton seed oil[20-26]. However, only a few studies have been conducted on rice bran using isopropanol or ethanol as an extraction solvent. Isopropanol has been used to extract rice bran oil, rich in B-vitamins[27] and ethanol has been used to extract rice bran oil rich in tocopherols and B-vitamins[28]. Weicheng *et al*.[29] have compared isopropanol and hexane as extraction solvents for recovery of vitamin E and oryzanol from stabilized rice bran and found that isopropanol is a promising alternative solvent to hexane for extraction of oil from stabilized rice bran. Shen *et al*.[30,31] have studied the solubility of rice bran oil and some constituents, in dense and supercritical CO_2. Kuk and Dowd[32] studied the solubility of rice bran oil and its classes of compounds in SC-CO_2. Various workers[3,33,34] have studied the extractability and the scaling up of SC-CO_2. Kim *et al.*[35] have studied the use of SC-CO_2 to enrich the rice bran oil in essential fatty acids. The use of SC-CO_2 to fractionate rice bran oil by supercritical fluid fractionation (SFF) for selective enrichment in sterols and lipid species of rice bran oil has also been studied[36,37,38] Giuseppe *et al*.[39] have evaluated the supercritical fulid extraction (SFE) technology for the recovery of all the rice by-products and novel conversion process to manufacture value

Table1— Rice bran potential and actual exploitation in India (1995-96 to 2001-02)

Year	1995-96	1996-97	1997-98	1998-99	1999-00	2000-01	2001-02
Paddy production Million Mt	119.40	120.80	122.30	118.60	135.10	127.10	136.5
Rice production Million Mt	79.6	81.7	82.5	86.1	89.7	84.90	91.00
Total rice bran potential, million Mt (7.5 per cent of Rice)	6.00	6.13	6.19	6.46	6.73	6.37	6.83
Rice bran oil Potential, lakh Mt, (15 per cent recovery)	9.00	9.20	9.30	9.70	10.10	9.60	10.20
Actual rice bran Processing, lakh Mt	32.00	32.00	33.00	32.00	33.10	33.00	36.00
Actual production of Rice bran oil, Lakh Mt							
Edible:	3.10	3.10	3.50	3.50	3.70	3.80	4.30
Non edible:	1.70	1.70	1.50	1.30	1.30	1.20	1.20
Total	4.80	4.80	5.00	4.80	5.00	5.00	5.50
Untapped potential Rice bran oil, lakh Mt	4.20	4.30	4.30	4.20	4.20	4.80	4.70
Percentage of exploitation	53	52	53	50	54	50	54

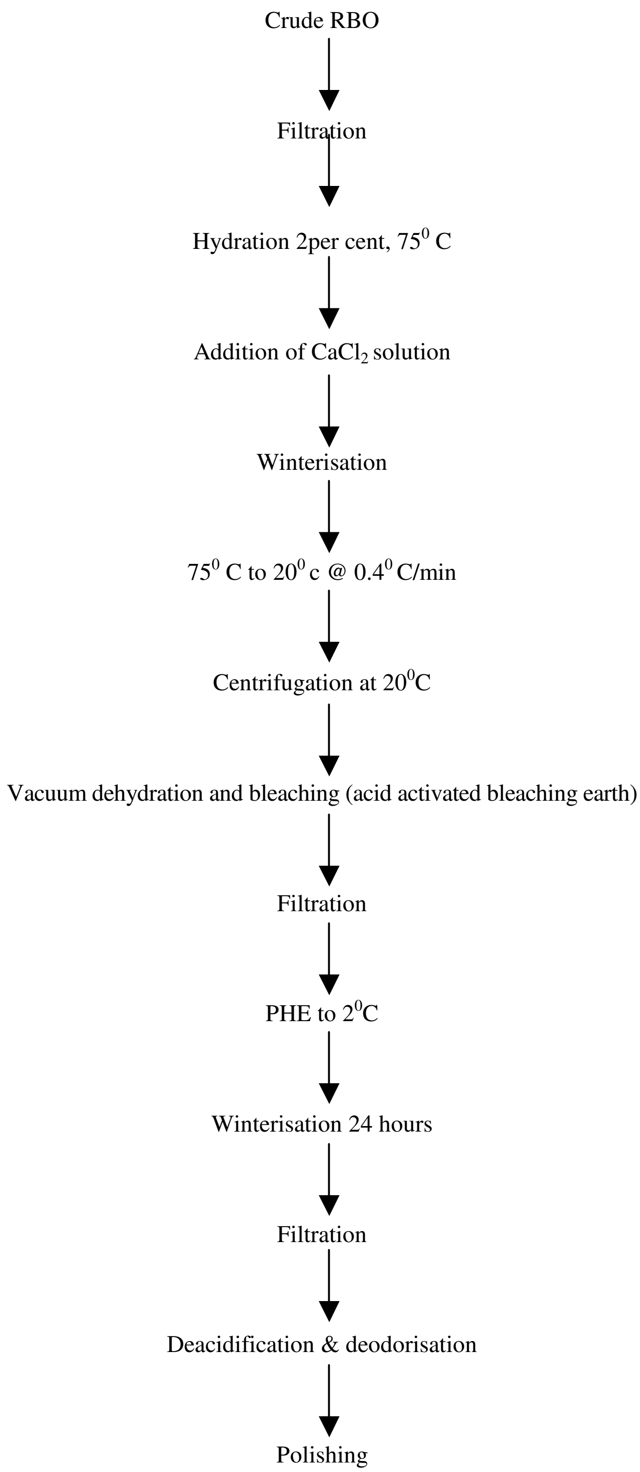

Fig. 1—Physical refining of rice bran oil

added food products. In their study they have reported the highest extraction yield of 4.93g of rice bran oil with only 100 g of CO_2 at 80°C and 10,000 psi pressure. According to their study SC-CO_2 can be

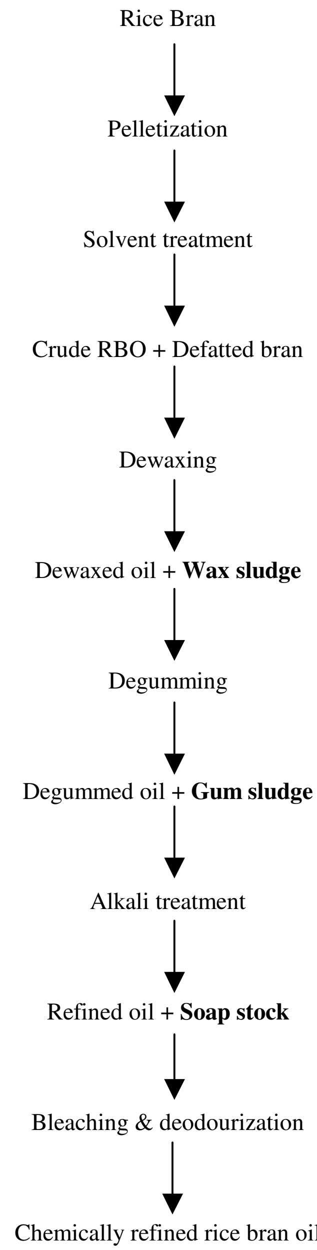

Fig. 2—Chemical refining of rice bran oil

used to extract oil efficiently and rapidly from all the byproducts of rice. But the high cost of the equipment to extract the oil is the only limiting factor for the proposed technology.

Food grade rice bran oil is obtained by subjecting the crude rice bran oil (CRBO) to either physical refining[5] (Fig.1) or chemical refining[4] (Fig.2). Gum sludge, wax sludge and soap stock sludge are the waste byproducts produced in the chemical refining process and are underutilized presently. Lloyd *et al.*[40] have studied the effect of processing on the presence

Table 2— Comparison of γ-oryzanol yield by solvent extraction and SFE

	Solvent extraction[a]	SFE[b]	SFE[c]
Yield (mg/g of rice bran)	1.68± 0.02	5.39±0.43	1.11±0.07
Concentration (mg/g of extract)	11.8± 0.2	51.0± 5.5	674.6± 148.1

[a]Extraction with hexane/isopropanol (50:50) at 60^0C for 60 min
[b]Extraction under 680 atm at 50^0C for 25 min
[c]Extraction under 680 atm at 50^0C and collection between 15-20 min

of antioxidants in rice bran, while Yoon and Kim[41] have studied the effect of refining on γ-oryzanol content in hexane extracted rice bran oil. The effect of different processing steps of refining on retention or the availability of γ-oryzanol has been investigated by Krishna *et al.*[42] According to the study degumming and dewaxing of crude RBO removed only 1.1 and 5.9 per cent of oryzanol while the alkali treatment removed 93.0 to 94.6 per cent of oryzanol from original crude oil. The oryzanol content of oil extracted from the bran of 18 Indian paddy cultivars ranged from 1.1 to 1.74 per cent for physical refined RBO while for alkali-refined oil it was 0.19-0.2 per cent. The oil subjected to physical refining retained the original amount of oryzanol after refining whereas the chemical refined oil showed a considerable lower amount (0.19 per cent). Thus the Oryzanol which is lost during chemical refining process has been carried into the soap stock. The content of Oryzanol in the RBO, soap stock, acid oil and deodorizer distillate were in the range: 1.7-2.1per cent, 6.3-6.9 per cent, 3.3-7.4 per cent and 0.79, per cent respectively. These results show that the processing steps viz., degumming, (1.1 per cent), dewaxing (5.9 per cent), physical refining (0 per cent), bleaching and deodorization of the oil did not affect the content of oryzanol appreciably, while 83-95 per cent of it was lost during alkali refining.

Extraction and Purification of γ-Oryzanol

γ-oryzanol present in rice bran can be extracted by using organic solvents or by using supercritical CO_2 technique. Xu and Godber[43] have compared the supercritical fluid and solvent extraction method for extracting γ-oryzanol from rice bran. According to their study, a solvent mixture with 50 per cent hexane and 50 per cent isopropanol (vol\vol) at 600 ^{0}C for 45-60 min produced the highest yield (1.68 mg/g of rice bran) of γ-oryzanol among organic solvents tested. Saponification, during solvent extraction decreases the yield of γ-oryzanol. However the yield (5.39mg/g of rice bran) of γ-oryzanol in supercritical fluid extraction at 50^0C and 680 atm pressure for 25 min was approximately four times higher than the highest yield of solvent extraction. Also a high concentration of γ-oryzanol in extract was (50-80 per cent) obtained by collecting the extract after 15-20 min of extraction under optimized condition. Table 2 lists the yields of γ-oryzanol from rice bran and concentrations in extract, obtained by solvent extraction and SFE, respectively[37].

γ-oryzanol can be extracted from the raw oils of rice bran and ferment, maize and barley by distillation of these oils at comparatively low temperature. The residue obtained is then extracted with hydrosol solvents and alkalinized below 0.5*N* and the extract is neutralized with organic acids to pH 6. In this process, raw γ-oryzanol can be crystallized having melting point 135.7^0 C and UV bands maximum at 216, 231, 291 and 315 mμ. Yamamoto and Takeshi[44] stirred 1 kg of raw rice bran oil with a solution of 150 g 90 per cent NaOH in 150 ml water and stored it for one day at room temperature. Then it was acidified to pH 4 by H_2SO_4 and heated to 80^0C followed by addition of 50 g NaCl to give an oily layer which was washed with aqu. NaCl solution. Then some methanol was added to extract as much water as possible and then again 500 mL methanol was added. The mixture was cooled for 2 h at 0^0 C to precipitate the waxy components. The wax was then filtered and a solution of 5g H_2SO_4 in 300 mL methanol was added to the filtrate. After 24 h at 30-40^0C with periodical stirring methanol was evaporated and 2 per cent(of filtrate wt) acid clay added. The mixture was heated for 30 min. at 100^0 C in vacuo, filtered and the filtrate was distilled at 2 mm pressure. To 100 g residue, 100g *n*-hexane was added with stirring. The solvent was distilled and the same treatment repeated with 100 mL methanol. The residue was extracted 3- times with 50 mL 0.5*N* methanolic NaOH. Then the extracts were neutralized with methanolic acetic acid to *p*H 6.8-7 and stored to precipitate 3g raw crystallized γ-oryzanol.

γ-oryzanol can also be extracted from alkali treated rice bran oil by hydrolyzing it with dil. HCl and by refluxing the residue with a solution of NaOH in methanol. The saponified solution is treated with methanol and filtered. The filtrate is neutralized and the methanol is evaporated. The residue is treated with dilute HCl, dissolved in diethyl ether and treated with disodium trioxide to remove the fatty acid. Then it is washed with water, dried and evaporated to give a residue containing oryzanol[45].

Oryzanol can be precipitated from the alkaline oil foots of rice bran oil by treating it with methanol, acetone or isopropanol and fatty acids[46]. Dissolving RBO with methanol, acetone or isopropanol and treating with sodium dihydrogen phosphate, disodium citrate or disodium EDTA can also precipitate oryzanol[47]. γ-oryzanol can also be precipitated from alkaline oil cake of rice bran oil by treating the oil cake with methanol or ethanol followed by passing of CO_2 with stirring. It is then filtered and the residue is washed with methanol and dried to give yellow powder containing oryzanol[48].

Crude γ-oryzanol isolated from rice bran oil can be purified by treatment with the ketones and /or alcohols. Then it is filtered and cooled to give a crystal of purified oryzanol[49]. Shimizu[50] has given a method for separation of the highly concentrated oryzanol by two-step alkali treatment.

Crude rice bran oil contains 1.5 to 2.9 per cent of oryzanol and during refining, about 90 per cent of the oryzanol goes into the soapstock as a refinery waste[4]. The commercial products of rice bran oil industry had a varied oryzanol content of 1.8 to 2 per cent in crude oil, 0.19-0.22 per cent in chemically refined oil and 2.21-6.71 per cent in the soapstock[4]. Therefore, oryzanol needs to be recovered from the soap-stock of rice bran oil. Physically refined RBO has retained about 85-90 per cent of oryzanol in the oil itself as no soapstock is produced during removal of fatty acids. Processing of soap stock to recover oryzanol has been made difficult due to use of varied conditions of refining of RBO. Oryzanol has been isolated from the commercial soapstock of rice bran oil by using solvent extraction methods, as shown in Fig. 3. Kim and Kim[38] have also given a method for the separation of oryzanol from the refining byproducts of rice bran oil.

The cost of oryzanol varies depending on the grade and the manufacturing method in the international market[4] (Rs 1000/kg - Rs 7000/kg prepared using chemical treatment process and Rs 15, 000/kg-75, 000/kg for solvent free processed products). With the production of 200 mkg of chemical refined oil, India has the potential of recovering 4 mkg of oryzanol.

Health Care Properties of Gamma Oryzanol

Antioxidant Property

At the molecular and cellular levels, antioxidants serve to deactivate certain particles called free radicals. Free radicals are the natural by-products of many oxidative metabolic processes within cells. These free radicals can cause damage to cell walls, certain cell structures and genetic material within the cells. Vitamin E is thought to be the most effective antioxidant due to its abundance in the body. γ-oryzanol found in rice bran oil is also a potent antioxidant. One test-tube study found that γ-oryzanol was more than four times as effective at stopping tissue oxidation as vitamin E[52]. The nutritional function of γ-oryzanol components may be related to their antioxidant property because of the ferulic acid structure. Ferulic acid is a phenolic acid antioxidant[53-56], and Ohat *et al.*[57] have identified ferulic acid sugar esters as active components in corn bran hemicelluloses fragments. However the antioxidant capacities of γ-oryzanol components were not known previously and were studied by Zhimin *et al.*[58] by using a linolenic acid model. According to the study the three major components of γ-oryzanol (24-methylene cycloartanyl ferulate, cycloartenyl ferulate

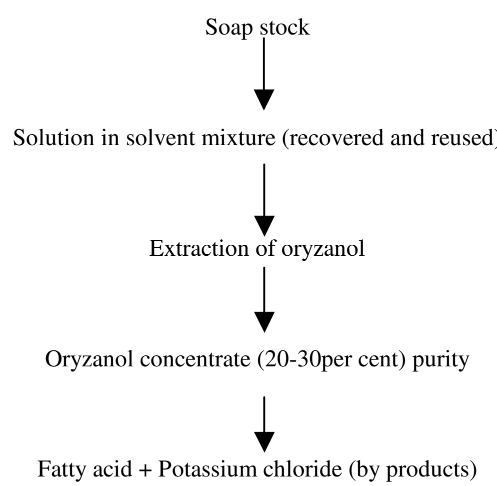

Fig. 3—Preparation of oryzanol concentrate from rice bran oil soap stock

and campesteryl ferulate) evidenced significant antioxidant activity when they were mixed with linoleic acid in a molar ratio of 1:100 and 1:250 but not in a molar ratio of 1:500. Antioxidant activity of tocopherols, tocotrinol, and γ-oryzanol components from rice bran against cholesterol oxidation were studied by Xu *et al*.[59] and highest antioxidant activity was found for the 24-methylenecycloartanyl ferulate. All the three γ-oryzanol components had activities higher than that of any of the four vitamin E components (α-tocopherol, α-tocotrinol, γ–tocopherol, and γ-tocotrinol)[59]. Inhibition of cholesterol autoxidation by the nonsaponifiable fraction in rice bran was studied Kim *et al*.[60] in an aqueous model system.

Effects on Cholesterol

One of the most important properties of γ-oryzanol is its cholesterol lowering property. There are several studies on humans and animals[61-65] showing that the rice bran oil (RBO) has the property of lowering low density lipoprotein cholesterol and total serum cholesterol and increasing the high density lipoprotein cholesterol to some extent either by influencing absorption of dietary cholesterol or by enhancing the conversion of cholesterol to fecal bile acids and sterols. Further studies confirm that the γ-oryzanol component of RBO is responsible for the hypocholesterolemia[66-68].

Recently, an experiment was conducted on human being to see the hypocholesterolemic property of RBO. In the experiment 66 persons were fed cooked rice, sprayed with brown rice extract for 30 d. The extract contained mainly inositol, γ-amino butyric acid (GABA) and, γ-oryzanol. Levels of lipid serum (total cholesterol, beta lipoprotein and LDL cholesterol) decreased significantly. Total cholesterol showed a remarkable decrease, especially in persons with total cholesterol level of more than 200mg/dL before study[69].

The mechanism of cholesterol lowering action of, γ-oryzanol was investigated by Rong *et al.*[70]. Hamsters were made cholesterolemic by feeding chow-based diets (containing coconut oil and 0.1 per cent cholesterol with or without oryzanol) for 7 weeks. Relative to control animals' oryzanol administration resulted in significant reduction of plasma total cholesterol levels (28 per cent), non-high-density lipoprotein cholesterol (non-HDL-C) 34 per cent, and 25 per cent reduction in percentage cholesterol adsorption. Aortic fatty streak formation was reduced by 67 per cent in the oryzanol treated animals. It was concluded that oryzanol was at least partly responsible for the cholesterol lowering action of RBO and is associated with the reduction in aortic fatty streak formation. Seetharamiah and Chandrasekhara[71] have found that the serum total, free esterified and (LDL+VLDL) cholesterol level of rats maintained at a 10 per cent RBO diet were lower than those maintained on a 10 per cent ground nut oil diet. Addition of 5 per cent oryzanol to the diet containing rice bran further reduced the serum cholesterol. The authors have concluded that the cholesterol lowering property of RBO might be due to oryzanol and /or other nonsaponifiable constituents present in RBO. Seetharamiah and Chandrasekhara[72] also found that hypertriglyceridemia induced by fructose was lower in animals maintained on a 0.5per cent oryzanol containing diet than the corresponding control. Sugano *et al*.[73] have observed that blending of rice bran oil with safflower oil in definite proportion (7:3 w/w) magnified the hypocholesterolemic activity compared with the effect of each oil alone. They have also reported similar results in their later studies[60]. γ-oryzanol can also lower the plasma cholesterol level[74]. Although the mechanism underlying this effect is not apparent at present the presence of oryzanol and tocopherols in the rice bran is thought to be responsible for this favorable effect. The blending may have a practical significance. Hiramatsu *et al.*[75] have studied the effect of γ-oryzanol on atheroma formation in hypercholestrolemic rabbits. Cicero and Gaddi[76] have studied the effect of rice bran oil and γ-oryzanol in the treatment of hyperlipoproteinamia. When added to a high cholesterol diet it also inhibits platelet aggregation, preventing heart attacks and strokes[7]. Nutritional and biochemical aspects of the hypolipidemic action of rice bran oil have been reviewed by Rukmini and Raghuram[77]. Scavariello and Arellano[78] have reviewed the physiological, antioxidant and hypocholestrolemic properties of γ-oryzanol.

Effects on Serum TSH

A single oral dose (300 mg) of γ-oryzanol extracted from rice-bran oil produced a significant reduction on the elevated serum TSH level in hypothyroid patients. Similarly, chronic treatment with γ-oryzanol resulted in decreased serum TSH level in 6 of 8 patients. There was no change in the serum level of thyroxine-iodine and triiodothyronine during the study. In addition, there was no difference in the serum, thyroid stimulating hormone (TSH) response to thyroid releasing hormone (TRH) in hypothyroid patients and normal subjects. These observations suggest that γ-oryzanol inhabits serum TSH levels in patients with primary hypothyroidism, possibly by a direct action at the hypothalamus rather than the pituitary[79].

Effects on Menopause

Two uncontrolled studies have investigated the use of γ-oryzanol for menopause. A Japanese study in the 1960s gave 13 women who had hysterectomies, also called surgical menopause, 100 mg γ-oryzanol three-times daily for 38 d and found that it halved menopausal symptoms such as, hot flashes in more than 67 per cent of the women[14]. In a later Japanese study, 40 perimenopausal women received 300 mg γ-oryzanol daily for four to eight weeks. Ninety per cent of the women improved and 40 per cent experienced "excellent effectiveness" in reducing menopausal symptoms. Yamauchi *et al.*[80] have reported the inhibition of LH secretion in rat by γ-oryzanol. Ishihara *et al.*[81] have studied the clinical effect of γ-oryzanol on climacteric disturbance on serum lipid peroxidase.

Effects on Muscle

γ-oryzanol /ferulic acid, inposine, chromium and medium chain triacyl glycerol are used as ergogenic aids by strength/power athletes[82]. The effect of γ-oryzanol Supplementation during resistance exercise training has been explained by Fry *et al.*[83]. Very preliminary evidence suggests that γ-oryzanol may increase endorphin release and aid muscle development[15, 84]. These findings have created an interest in using γ-oryzanol as a sports supplement.

Carcinogenic Effects

The carcinogenic potential of γ-oryzanol, a drug mainly used for the treatment of hyperlipaemia was studied in F344 rats[85] and $B6C3F_1$ mice[86]. The findings indicate that under the experimental conditions described, γ-oryzanol was not carcinogenic in F344 rats and $B6C3F_1$mice. Inhibitory effect of cycloartenol ferulate, a component of rice, on tumor promotion in two-stage carcinogenesis in mouse skin was studied by Yasukawa *et al.*[87]. According to their study the active components of rice bran, sitosterol ferulate, 24-methylcholesterol ferulate, cycloartenol ferulate and 24-methylenecycloartanol ferulate inhibited markedly the TPA-induced inflammation in mice. Cycloartenol ferulate, a component of γ-oryzanol in rice bran oil shows marked inhibition on the tumour promoting effect of TPA in 7, 12-dimethylbenz [a] anthracene-initiated mice. Hirose *et al.*[88] have studied the modifying effects of phytic acid and γ-oryzanol on the promotion stage of rat carcinogenesis[75]. Sugano *et al.*[89] have studied the health benefit of rice-bran oil and its anticancer property.

Uses of γ-oryzanol

γ-oryzanol has a protective role in UV-light induced lipid peroxidation and hence it is used as a sunscreen agent. A cosmetic sunscreen composition containing ferulic acid and γ-oryzanol has been patented[90]. Ferulic acid and its esters stimulate hair growth and prevent skin aging[91]. Such preparations are claimed to accelerate cell differentiation and to reduce wrinkles in aged women. The eyebrows, eyelashes, and surrounding skin are protected from oxidative damage, due to environmental influence or chemical treatment by use of compounds containing ϒ-oryzanol[92].

ϒ-oryzanol is insoluble in water and various ways of solubilizing the compound for cosmetic, pharmaceutical, and food applications have been described. Oryzanol becomes water-soluble when treated with urea, nicotinamide and/or thioctamide dissolved in acetone or etanol-acetone mixture[93]. Another method involves conversion to a cyclodextrin inclusion compound[94]. Solubilization of γ-oryzanol into medicinal drinks is achieved by using sucrose fatty acid ester and ethoxylated HCO (ref. 95 and 96). The drinks are useful as revitalizing tonics. Solubilization of γ-oryzanol in water has also been studied by Hiroshi[97]. Ricebran oil containing inositol and/or γ-oryzanol is claimed to be useful for improving the quality of cooked rice[98]. Apart from all the health benefits, it is light and delicate in salads and unlike olive or canola, has very low flavour which makes it excellent for both frying and baking without changing the taste of the recipe. Rice bran oil has a very high heat tolerance or smoke point (around 475°F or higher) and one can fry, sauté and stir fry without thickening, smoking, foaming or breakdown.

Conclusions

India has become the second largest producer of rice bran oil which uniquely contains γ-oryzanol. This high value compound can be isolated from physical refined oil and from the residual soap stock produced during chemical refining of the oil. Therefore, processors of rice bran oil should exploit this situation for their economic advantage to produce high value products for pharmaceutical and nutraceutical use. The new separation process like supercritical and fluid extraction can be utilized for γ-oryzanol extraction. Also, more R&D work should be undertaken for development of a low cost refining process (for RBO) retaining maximum percentage of γ-oryzanol in the refined oil.

References

1 Johnson L A & E W Lusas, Comparison of alternative solvents for oil extraction, *J Am Oil Chem Soc,* **60** (1983) 229-242.

2 Lakkakula Rao N, Marybeth Lima & Walker Terry, Rice bran stabilization and rice bran oil extraction using Ohmic heating, *Bioresource Technol,* **92**(2004) 157-161.

3 Ramsay M E, Hsu J T, Novak R A & Reightler W J, Processing rice bran by supercritical fluid extraction, *Food Technol,* **30** (1991) 98-104.

4 Gopalakrishna A G, Value-added Products from Rice Bran Oil Industry, *SAARC Oils Fats Today* (August 2003) 20-23.

5 Arumughan C, Soban kumar, Rajam L & Sundaresan A, Integrated technology for RBO refining and byproducts recovery, *SAARC Oils Fats Today,* (August 2003) 10-14.

6 Sharma A R, Value-addition in paddy processing, *SAARC Oils Fats Today*, (June-July 2002) 25-26.

7 Kaimal T B N, γ-oryzanol from ricebran oil, *J Oil Technol Aassoc India*, **31** (1999) 83-93.

8 Roger E J, Rice S M, Nicolosi R J, Carpenter D R, McClelland C A & Romanczyk L J, Identification and quantification of ϒ-oryzanol components and simultaneous assessment of tocols in rice bran oil, *J Am Oil Chem Soc,* **70** (1993) 301-307.

9 Xu Zhimin, Godber J S & Xu Z, Antioxidant activities of major components of gamma-oryzanol from rice bran using a linolenic acid model, *J Am oil Chem Soc,* **78** (2001) 465-469.

10 Yoshino G, Kazumi T, Amano M, Tateiwa M, Yamasaki T, Takashima S, Iwai M, Hatanaka H & Baba S, *Curr Ther Res,* **45 (**1989**)** 975.

11 Gerhardt A L & Gallo N B, Full fat rice bran and oat bran similarly reduced Hypercholesterolemia in humans, *J Nutr,* **128** (1998) 865-869.

12 Seetharamaiah G S, Krishnakantha T P & Chandrasekhara N, Influence of oryzanol on platelet aggregation in rats, *J Nutr Sci Vitaminol,* **36** (1990) 291-297.

13 Nakayama S, Manabe A, Suzuki J, Sakamoto K & Inagaki T, Comparative effects of two forms of gamma-oryzanol in different sterol compositions on hyperlipidemia induced by cholesterol diet in rats, *Japan J Pharmacol,* **44** (1987) 135-143.

14 Murase Y & Iishima H, Clinical studies of oral administration of gamma-oryzanol on climacteric complaints and its syndrome, *Obstet Gynecol Prac,* **12** (1963) 147-149.

15 Bonner B, Warren B & Bucci L, Influence of ferulate supplementation on postexercise stress hormone levels after repeated exercise stress, *J Appl Sports Sci Res,* **4** (1990) 10.

16 Johnson L A & Lusas E W, Comparison of alternative solvents for oil extraction, *Am Oil Chem Soc,* **60** (1983) 229-242.

17 Health and Safety Guide No.59, *n-hexane health and safety guide* (World Health Organization, Geneva) 1991

18 Lusas E W, WatkinsL R & Koseoglu S S, Isopropyl alcohol to be tested as solvent, *INFORM,* **2** (1991) 970-976.

19 Watkins L R, Koseoglu S S, Rhee K C, Hernandez C E, Riza M N, Johnson W H (Jr) & Doty S C, New Isopropanol system shows promise, *INFORM ,* **5** (1994) 1245-1253.

20 Beckel A C, Belter P A & Smith A K, The non-distillation alcohol extraction process for soybean oil, *J Am Oil Chem Soc,* **25** (1948) 10-11.

21 Harris W D, Bishop F, Lyman F C M & Helpert R, Isopropanol as a solvent for extraction of cottonseed oil, preliminary investigations, *J Am Oil Chem Soc ,* **24** (1947) 370-375.

22 Rao R K, Krishna M G, Zaheer S H & Arnold L K, Alcohol extraction of vegetable oils, Part I. Solubilities of cottonseed, peanut, sesame, and soybean oils in aqeous ethanol, *J Am Oil Chem Soc ,* **32**(1955) 420-423.

23 Rao R K & Arlnold L K, Alcoholic extraction of vegetable oils. Part I. Solubility of vegetable oils in aqueous 2-propanol, *J Am Oil Chem Soc,* **34** (1957) 401-404.

24 Rao R K & Arlnold L K, Alcoholic extraction of vegetable oils, Pilot plant extraction of cottonseed by aqueous ethanol, *J Am Oil Chem Soc ,* **35**(1958) 277-281.

25 Baker E C & D A Sullivan, Development of a pilot -plant process for the extraction of soy flakes with aqueous isopropyl alcohol, *J Am Oil Chem Soc* , **60**(1983) 1271-1277.

26 Hron R J & Koltun S P, An aqueous ethanol extraction process for cottonseed oil (Biorenewable alternative to hexane), *J Am Oil Chem Soc ,* **61**(1984) 1457-1460.

27 Meinke W W, Holland B R & Harris W D, Solvent extraction of rice bran: production of B-vitamins concentrate and oil by isopropanol extraction, *J Am Oil Chem Soc ,* **26** (1949) 532-534.

28 Talwalkar R T, Garg N K & Krishnamurti C R, Rice bran-a source material for pharmaceuticals, *J Food Sci Technol,* **2** (1965) 117-119.

29 Weicheng Hu, John Henry Wells, Tai-Sun Shin & Samuel J Godber, Comparison of isopropanol and hexane for extraction of vitamin E and oryzanols from stabilized rice bran, *J Am Oil Chem Soc,* **73** (1996) 1653-1656.

30 Shen Z, Palmer M V ,Ting S S T and Fairclough R J, Pilot scale extraction of rice bran oil with dense carbon dioxide, *J Agric Food Chem*, **44**(1996) 3033.

31 Shen Z, Palmer M V ,Ting S S T & Fairclough R J, Pilot scale extraction and fractionation of rice bran oil using supercritical carbon dioxide, *J Agric Food Chem,* **45**(1997) 4540.

32 Kuk M S & Dowd M K, Supercritical CO_2 extraction of rice bran, *J Am Oil Chem Soc,* **75**(1998) 623-625.

33 Taniguchi M, Tsuji T, Morimoto H, Shibata M & Kobayashi T, Treatment of rice bran with supercritical carbon dioxide, *Nippon Shokuhin Kogyo Gakkaishi,* **34**(1987) 227-231.

34 Zhao W, Shishikura A, Fujimoto K, Arai K & Saito S, Fractional extraction of rice bran oil with supercritical carbon dioxide, *Agric Biol Chem,* **51** (1987) 1773-1776.

35 Kim H, Lee S, Park K & Hong I, Characterization of extraction and separation of rice bran oil rich in EFA using SFE

process, *Sep Purif Technol,* **5** (1999) 1-5.

36 Saito N, Ikushima Y, Hatakeda K, Ito S, Asano T & Goto T, Fractional extraction of rice-bran oil and its esters with supercritical carbon dioxide, *Int Chem Eng*, **33** (1993) 307.

37 Dunford N T & King J W, Phytosterol enrichment of rice bran oil by a supercritical carbon dioxide fractionation technique, *J Food Sci*, **65** (2000) 1395.

38 Dunford N T & King J W, Thermal gradient deacidification of crude bran oil utilizing supercritical carbon dioxide, *J Am Oil Chem Soc*, **78** (2000) 121.

39 Giuseppe P, Miniati E, Montanari L & Fantozzi Paolo, Improving the value of rice by-products by SFE, *J Supercrit Fluids,* **26**(2003) 63-71.

40 Lloyd B J, Siebenmorgen T J & Beers K W, Effect of commercial processing on antioxidants in rice bran, *Cereal Chem,* **77**(2000) 551-553.

41 Yoon S H & Kim S K, Oxidative stability of high-fatty acid rice bran oil at different stages of refining, *J Am Oil Chem Soc ,* **71**(1994) 227-231.

42 Krishna A G Gopala , Khatoon S, Shiela P M, Sarmandal C V, IndiraT N & Mishra A, Effect of refining of crude rice bran oil on the retention of oryzanol in the refined oil, *J Am Oil Chem Soc,***78**(2001) 127-131.

43 Zhimin Xu & Godber Samuel J, Comparison of supercritical fluid and solvent extraction methods in extracting ϒ-oryzanol from rice bran, *J Am Oil Chem Soc,***77** (2000) 547-551.

44 Yamamato T, ϒ- oryzanol, *DE Pat.1301002*(to Toyo Koatsu Industries, Inc,Ger) 14 August (1969); *Chem Abstr,* **71** (1969) 128704 .

45 Tsuchiya T, Kato A & Okubo O, Extraction of nutritive fractions from rice-bran oil, *Jap Pat 34009600*(to Bureau of Industrial Technics, JP), 27 october 1959; *Chem Abstr,* **54** (1960) 76011.

46 Nishihara M & Katsumata K, Oryzanol from alkaline oil foots of rice bran oil, *Jap Pat 47033123*(to Hokusen Chemical industry Co. Ltd Jpn) 23 August 1972, *Chem Abstr*, **77** (1972) 130584.

47 Nishihara M & Katsumata K, Oryzanol from alkaline oil foots of rice bran oil, *Jap patent 47033124* (to Hokusen Chemical industry Co. Ltd, Japan) 23 August 1972; *Chem Abstr*, **77** (1972) 130581.

48 Nisihara M & Shibuya Y, Oryzanol from alkaline oil cake of rice oil, *Jap Pat* 43012730(to Hokusen Kagaku Koyo Co. Ltd., Japan) 29 May 1968; *Chem Abstr*, **69**(1968) 107773.

49 Kubota Y & Sekine I, Purification of ϒ- Oryzanol, *Jap Pat 53130412*(to Kanebo Ltd, JP),4 november 1978; *Chem Abstr*, **90** (1979) 109948.

50 Shimizu H, Highly concentrated separation of Oryzanol by two-step alkali treatment, *Jap Pat 51123811*(to Okayasu Co. Ltd., Japan) 28 October 1976; *Chem Abstr*,**75**(1977) 104664.

51 Kim I & Kim C, Separation of oryzanol from the refining by-products of rice bran oil, *Korean J Food Sci Technol*, **23** (1991) 76-80.

52 Hiramitsu T & Armstrong D, Preventive effect of antioxidants on lipid peroxidation in the retina, *Ophthalmic Res*, **23** (1991) 196-203.

53 Cuvelier M E, Richard H & Berset C, Comparison of the antioxidative activity of some acid-phenols: Structure-activity relationship, *Biosci Biotech Biochem,* **56** (1992) 324-330.

54 Marinova E M & Yanishlieva N V, Effect of Temperature on the antioxidative action of the inhibitors in lipid autoxidation, *J Food Sci Agric,* **60** (1992) 313-318.

55 Marinova E M & Yanishlieva N V, Effect of lipid unsaturation on the antioxidative activity of some phenolic acids, *J Am Oil Chem Soc,* **71** (1994) 427-434.

56 Pratt D E, Water-soluble antioxidant activity in soybeans, *J Food Sci,* **37** (1972) 322-323.

57 Ohta T , Yamasaki S, Egashira Y & Sanada H, Antioxidative activity of corn bran hemicelluose fragments, *J Agric Food Chem,***42** (1994) 653-656.

58 Xu Zhimin, Godber J S & Xu Z, Antioxidant activities of major components of gamma-oryzanol from rice bran using a linolenic acid model, *J Am oil Chem Soc,* **78** (2001) 465-469.

59 Xu Zhimin, Hua N & Godber J S, Antioxidant activities of tocopherols, tocotrinols, and gamma-oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2´-azobias(2-methylpropionamidine) dihydrochloride, *J Agric Food Chem,* **49**(2001) 2077-2081.

60 Kim J S, Godber J S, King J M & Prinyawiwatkul W, Inhibition of cholesterol autoxidation by nonsaponifiable fraction fine rice bran in an aqueous model system, *J Am oil Chem Soc,* **78** (2000) 685-689.

61 Sharma R D & Rukmini C, Rice bran oil and hypocholesterolemic in rats, *Lipids,* **21** (1986) 715-717.

62 Rukmini C, Chemical nutritional and toxicological studies of rice bran oil, *Food Chem,* **30** (1988) 257-268**.**

63 Nicolosi R J, Ausman L M & Hegsted D M, Rice bran oil lowers serum total and low density lipoprotein cholesterol and Apo B levels in nonhuman primates, *Artherosclerosis,* **88** (1991) 133-142.

64 Kahlon T S, Saunders R M, Sayre R N, Chow F I, Chiu M M & Betschart A A, Cholestrol-lowering effects of rice bran and rice bran oil fractions in hypercholestrolemic hamsters, *Cereal Chem,* **69** (1992) 485-489.

65 Hegsted M & Windhuser M M, Reducing human heart disease risk with rice bran, *Louisiana Agric***, 36** (1993) 22-24.

66 Sugano M & Tsuji E, Rice bran oil and cholesterol metabolism, *J Nutr,* **127** (1997) 5215-5245.

67 Sasaki J, Takada Y & Handa K, Effects of gamma-oryzanol on serum lipids and apolipoproteins in dyslipidemic schizophrenics receiving major tranquilizers, *Clin Therap*, **12** (1990) 263-268.

68 Lichtenstein A H, Ausman L M & Carrasco W, Rice bran oil consumption and plasma lipid levels in moderately hypercholesterolemic humans, *Arterioscler Thromb,* **14** (1994) 549-556.

69 Moriyama N, Shinozaki T, Kanayama K & Yamoti S, Development of the processing rice which added new functionality, *Nippon-Nogeikagaku-Kaishi*, **76** (2002) 614-620.

70 Rong N, Ausman L M & Nicolosi R J, Oryzanol decreases cholesterol absorption and aortic fatty streaks in hamsters, *Lipids*, **32** (1997) 303-309.

71 Seetharamaiah G S & Chandrasekhara N, Studies on hypocholesterolemic activity of rice bran oil, *Atherosclerosis,* **78** (1989) 219-23.

72 Seetharamaiah G S & Chandrasekhara N, Effect of oryzanol on fructose induced hypertriglyceridaemia in rats, *Indian J Med Res*, **88** (1988) 278-81.

73 Sugano M, Koba K, Tsuji E, Ishikawa T, Ogawa S & Shamsuddin A K M, Proceedings of the first international symposium on disease prevention by IP_6 and other rice bran components, *Anticancer Res*, **19** (1999) 3651-3658.

74 Nestel P J, Dietary fibre, *Medi J Austr*, **153**(1990) 123-124.

75 Hiramatsu K, Tani T, Kimura Y, Izumi S & Nakane P K, Effect of gamma-oryzanol on atheroma formation in hypercholesterolemic rabbits, *Tokai J Exp Clin Med*, **15** (1990) 299-305.

76 Cicero A F & Gaddi A, Rice bran oil and gamma-oryzanol in the treatment of hyperlipoproteinaemias and other conditions, *Phytother Res,* **15** (2001) 277-89.

77 Rukmini C & Raghuram T C, Nutritional and biochemical aspects of the hypolipidemic action of rice bran oil: a review, *J Am Coll Nutr,* **10** (1991) 593-601.

78 Scavariello E M S & Arellano D B, Gamma Oryzanol: Un importante componente del aceite de salvado de arroz, *Arch-Latinoameri-de Nutricion*, **48** (1998) 7-12.

79 Shimomura Y, Kobayashi I, Maruto S, Ohshima K, Mori M, Kamio N & Fukuda H, Effect of gamma-oryzanol on serum TSH concentrations in primary hypothyroidism, *Endocrinol Jap*, **12** (1980) 83-86.

80 Yamauchi J, Takahara J, Uneki T & Ofuki T, Inhibition of LH secretion by gamma-oryzanol in rat, *Horm Metab Res*, **13** (1981) 185.

81 Ishihara M, Ito Y, Nakakita T, Maehama T, Hieda S, Yamamoto K & Ueno N, Clinical effect of gamma-oryzanol on climacteric disturbance -on serum lipid peroxides, *Nippon Sanka Fujinka Gakkai Zasshi,* **34** (1982) 243-251.

82 Rosenbloom C, Millard Stafford M & Lathrop J, Contemporary ergogenic aids used by strength/power athletes, *J Am Diet Assoc,* **92** (1992) 1264–1265.

83 Fry AC, Bonner E, Lewis DL, Johnson R L, Stone M H & Kraemer W J, The effects of gamma-oryzanol supplementation during resistance exercise training, *Int J Sport Nutr,* **7** (1997) 318–329.

84 Bucci L R, Blackman G & Defoyd W, Effect of ferulate on strength & body composition of weightlifters, *J Appl Sports Sci Res,* **4** (1990) 110.

85 Tamagawa M, Shimizu Y, Takahashi T, Otaka T, Kimura S, Kadowaki H, Uda F & Miwa T, Carcinogenicity study of gamma-oryzanol in F344 rats, *Food Chem Toxicol*, **30** (1992) 41-48.

86 Tamagawa M, Otaki Y, Takahashi T, Otaka T, Kimura S & Miwa T, Carcinogenicity study of gamma-oryzanol in B6C3F1 mice, *Food Chem Toxicol,* **30** (1992) 49-56.

87 Yasukawa K, Akihisa T, Kimura Y, Tamura T & Takido M, Inhibitory effect of cycloartenol ferulate, a component of rice bran, on tumor promotion in two-stage carcinogenesis in mouse skin, *Biol Pharma Bull,* **21** (1998) 1072-1076.

88 Hirose M, Fukushima S, Imaida K, Ito N & Shirai T, Modifying effects of phytic acid and gamma-oryzanol on the promotion stage of rat carcinogenesis, *Anticancer Res,* **19** (1999) 3665-3670.

89 Sugano M, Koba K & Tsuji E, Health benefits of rice bran oil, *Anticancer Res*, **19** (1999) 3651-3657.

90 Brigitte K, Cosmetic sunscreen composition containing ferulic acid and gamma-oryzanol, *DE Pat 4421038* (to Goldwell GMBH, DE), 21 December 1995; *Chem Abstr*, **123** (1995) 296279.

91 Tatsu S, Cell differentiation promotor, *Jap Pat5310526* (to EISAI Co Ltd, JP) 22 November 1993; *Chem Abstr* ,**120** (1994) 143661.

92 Riedel J H & Petsitis X, Mascara and eyebrow pencils containing ϒ-oryzanol and calcium salt, *Eur Pat 945120*(to Beiersdorf A-G, Germany), 29 September 1999; *Chem Abstr*, **131**(1999) 248032

93 Kenji M, Production of treated oryzanol solubilized in water, *Jap Pat 60105695* (to Ichimaru Fuarukosu KK, JP), 11 June 1985.

94 Kenji M, Production of oryzanol solubilized in water, *Jap Pat 60105694* (to Ichimaru Fuarukosu KK, JP), 11 June 1985.

95 Hiroshi N, Solubilization of gamma-oryzanol for drink, *Jap Pat 2149597* (to Dai Ichi Kogyo Seiyaku Ltd, JP), 8 June 1990; *Chem Abstr*, **113** (1990) 198007.

96 Shimizu M, Solubilization of ϒ-oryzanol in water, *Jap Pat 07041422* (to Nissui Seiyaku Co, Japan) 10 February 1995; *Chem Abstr,* **122**(1995) 274110.

97 Hiroshi N, Solubilization of gamma-Oryzanol in medicinal drink, *Jap Pat 2149598* (to Dai Ichi Kogyo Seiyaku Ltd, JP), 8 June 1990; *Chem Abstr*, **113** (1990) 198006.

98 Haruyasu M, Rice bran oil for improvement of the quality of cooked rice, *Jap Pat 7255393* (to Minami Haruyasu, JP), 9 January 1995; *Chem Abstr* **124** (1995) 28692