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Methodical Characterization and Quantitative Estimation of Crude *Oryza sativa* Bran Oil

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ABSTRACT

Oils, a rich source of dietary energy are recognized as essential nutrients in human diets and contain more than twice the caloric value of equivalent amount of sugar. Furthermore, oils contribute significantly in maintaining the agricultural economy of the country, considering the fact that India is the largest producer of rice bran oil (RBO) with a theoretical potential of 1.2 MMT per year. For the last couple of decades, there has been a surge of global interest pertaining to the beneficial nutritive effects of bioactive phytochemicals such as, oryzanol, lecithin, tocopherols, and tocotrinols, obtained from crude rice (*Oryza sativa*) bran oil (RBO), manufactured from rice bran, a by-product of rice processing. The present research work was designed to methodically characterize and quantitatively determine the various physicochemical properties of crude RBO, extracted through solvent extraction from rice bran, using standard estimation methods. The parameters evaluated are useful in determining the suitability of the oil for edible or other industrial purposes.

Keywords: Crude Rice Bran Oil, Characterization, Fatty Acids, Oryzanol, Tocopherols, Tocotrienols

1. INTRODUCTION

In recent times, there has been an emerging interest in the use of naturally occurring phytochemicals for their potential therapeutic usage in minimizing the risk of major chronic diseases like cardiovascular disease, cancer, diabetes, Alzheimer disease, cataracts, and age-related functional decline.[1-3] Rice (*Oryza sativa* L.) is by far the most economically important food crop in many developing countries, providing two third of the calorie intake of more than 3 billion people in Asia, and one third of the calorie intake of nearly 1.5 billion people in Africa and Latin America.[4] Rice bran obtained during milling of rice (*Oryza sativa*) is gaining surmount commercial importance in the world on account of its innumerable beneficial nutritive and biological effects with 16 –22% lipid, 12 –16 % protein, 8-12 % crude fiber and high levels of other vitamins and minerals.[5]

From a commercial perspective, the most available and adequately investigated rice bran derived product is the oil made from the pericarp and germ of the *Oryza sativa* seeds. India is the largest producer of *Oryza sativa* (Rice) bran oil (RBO) with a potential of 1.2MMT per year. According to the estimates, the annual production of RBO in 2006 was 0.75MMT, out of which 0.73MMT was utilized for edible purpose and the remaining for non-edible purposes.[6] With reference to a WHO report, RBO consumption towards edible purpose increased from 78% to 97% during 2001–2006 owing to the awareness of its health benefits and increased use in hydrogenated fats as well as in blended oils.[7] RBO is unique among edible oils due to its rich source of commercially and nutritionally important phytoceuticals such as, oryzanols (a mixture of steryl ferulates and triterpenalcohols), tocopherols, vitamin E, phytic acid, lecithin, inositol and wax, majority of which exhibit anti-oxidant potential. The research institutes in India and abroad have found RBO as a "Heart Friendly Health Oil" with unique properties for maintaining good health. Its prolonged use reduces the risk of coronary heart disease in humans by reducing total cholesterol and triglycerides. It also possesses anti-cancer properties which help in restoring all-round health.[8] As a result of developments in the stabilization of rice bran and the increase in knowledge about health benefits associated with rice bran oil (RBO), extraction of RBO by solvent extraction using food grade n-hexane or in solvent free process by using ohmic heating or supercritical fluid extraction technology has received greater attention.[9, 10]

In contrast to most common refined vegetable oils, crude RBO contains a rich unsaponifiable fraction (up to 5%) mainly constituting of sterols (43%), triterpene alcohols (28%) 4-methyl-sterols (10%) and less polar components (19%).[11] The so-called gamma-oryzanol often identified as the physiologically active moiety of RBO, is a natural mixture of ferulate (4-hydroxo-3-methoxycinnamic acid) esters of sterols (campesterol, stigmasterol and ß-stigmasterol) and triterpene alcohols (cycloartenol, cyccloartenol, 24-methylenecycloartanol, cyclobranol).[12] Gamma-oryzanol, the medicinally important antioxidant, has professed health benefits including decrease of hepatic cholesterol biosynthesis, and plasma cholesterol, growth promotion, development of lean muscles and stimulation of hypothalamus.[13,14]

In view of the emerging public demand and scientific interest about the overall composition, nutrition profile, functional characteristics of oils, the quality assessment and composition of non-conventional oilseed crops is of prime concern to cope up with the existing challenge. Presently, there is a dearth of information on the entire physico-chemical characterization and quantitative estimation of crude RBO indigenous to the subcontinent, particularly in India. In the light of the above perspective, the present investigation was undertaken to provide baseline information on the detailed physico-chemical parameters and simultaneous quantitative estimation of crude RBO, indigenous to the state of Gujarat, India.

2.Material & Methods

The present study was conducted at the Department of Pharmacology, Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat. Rice bran was obtained from Suryodaya Rice Mills, Ahmedabad, Gujarat through their milling process. All solvents and reagents were either of HPLC grade or of analytical reagent grade and were obtained from commercial sources.

2.1 Extraction of RBO

Fresh rice bran obtained from the local rice mill was stored in a refrigerator before use. Crude RBO was extracted from rice bran (50 g) by soxhlet extraction for 3 h using hexane as the solvent. The oil percentage was calculated on the basis of following formula:-

Percent oil in rice bran= Weight of oil (g) / Weight of sample (g)*100

The extracted crude oil was subsequently analyzed for the following physico-chemical parameters, including the content of oryzanol, tocopherols, tocotrienols and free fatty acids.

2.2 Analysis of extracted RBO:

i)Organoleptic characters

The organoleptic characteristics of the crude RBO were determined by the visual appearance and assessment of the sample for color and flavor.

ii)Physical properties

a)Determination of Specific Gravity:[15]

The dry pycnometer was filled with the oil sample in such a manner to prevent entrapment of air bubbles after removing the cap of the side arm. The stopper was inserted, immersed in water bath at 30ºC±0.20ºC and held for 30 minutes. Any oil

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that came out of the capillary opening was carefully wiped off. The bottle was removed from the bath, cleaned and dried thoroughly. The cap of the side arm was removed and quickly weighed ensuring that the temperature did not fall below 30ºC.

The specific gravity of the oil was calculated from the following relationship:

Specific Gravity at 30ºC / 30ºC = A-B / C-B

Where, A = weight in gm of specific gravity bottle with oil at 30ºC
B = weight in gm of specific gravity bottle at 30ºC
C = weight in gm of specific gravity bottle with water at 30ºC

b)Determination of Viscosity:[16]
The relative viscosity of crude RBO was determined using Ostwald viscometer by measuring its time of flow at the temperature and the corresponding time of flow for the same volume of water in the same viscometer at 30ºC. The viscosity value of the oil was expressed in centipoise.

The relative viscosity of the oil was calculated from the following relationship:
Viscosity of oil at 30ºC (n_1) = $n_2 d_1 t_1 / d_2 t_2$

Where, n_1= Viscosity of oil at 30ºC
n_1= Viscosity of oil at 30ºC
d_1= Density of oil at 30ºC
d_2= Density of water (Reference liquid) at 30ºC
t_1= time of flow of oil
t_2= time of flow of water

c)Determination of Moisture Content: [17]
Moisture content of oils is the loss in mass of the sample on heating at 105±1ºC under operating conditions specified. The moisture content was determined by the Air-Oven Method. About 10g of oil was weighed in a previously dried and tared dish. The lid of the dish was loosened and heated, in an oven at 105±1ºC for 1 hour. The dish was removed from the oven and the lid was closed. The sample was cooled in a desiccator containing phosphorus pentoxide and weighed. It was then heated in the oven for a further period of 1 hour, cooled and weighed. This process was repeated until change in weight between two successive observations did not exceed 1 mg.

Calculation:
Moisture and volatile matter (Percent by weight) = W1 x 100 / W
Where, W1 = Loss in gm of the material on drying
W = Weight in gm of the material taken for test

iii)Chemical properties
a)Determination of Saponification value: [18]
The saponification value is the number of mg of potassium hydroxide required to saponify 1 gram of oil/fat. The oil sample is saponified by refluxing with a known excess of alcoholic potassium hydroxide solution. The alkali required for saponification is determined by titration of the excess potassium hydroxide with standard hydrochloric acid. The saponification value is an index of mean molecular weight of the fatty acids of glycerides comprising a fat. Lower the saponification value, larger the molecular weight of fatty acids in the glycerides and vice-versa. About 2.0 g of the crude oil sample was weighed into a 250 ml Erlenmeyer flask. 25 ml of the alcoholic potassium hydroxide solution was pipetted into the flask. A blank determination was conducted along with the sample. The sample flasks and the blank flask were connected with air condensers, kept on the water bath, boiled gently but steadily until saponification was complete, as indicated by absence of any oily matter and appearance of clear solution. Clarity was achieved within one hour of boiling. After the flask and condenser had cooled somewhat, the inside of the condenser was washed with about 10 ml of hot ethyl alcohol neutral to phenolphthalein. The excess potassium hydroxide was titrated with 0.5N hydrochloric acid, using about 1.0 ml phenolphthalein indicator.

Calculation:
Saponification Value = 56.1 (B-S) N / W
Where, B = Volume in ml of standard hydrochloric acid required for the blank.
S = Volume in ml of standard hydrochloric acid required for the sample
N = Normality of the standard hydrochloric acid and
W = Weight in gm of the oil/fat taken for the test.

b)Determination of Unsaponifiable Matter: [19]
The unsaponifiable matter is defined as the substances soluble in oil which after saponification is insoluble in water but soluble in the solvent used for the determination. It includes lipids of natural origin such as sterols, higher aliphatic alcohols, pigments, vitamins and hydrocarbons as well as any foreign organic matter non volatile at 100ºC. Light Petroleum or diethyl ether is used as a solvent but in most cases, results will differ according to the solvent selected and generally the use of diethyl ether will give a higher result.

5 gm of well mixed oil sample was weighed accurately into a 250ml conical flask. 50ml of alcoholic potassium hydroxide solution was added. The content was boiled under reflux air condenser until the saponification was complete. The condenser was washed with about 10 ml of ethyl alcohol.

The saponified mixture was transferred while still warm to a separating funnel. The saponification flask was washed first with some ethyl alcohol and then with cold water, using a total of 50 ml of water to rinse the flask and was subsequently cooled to 20 to 25ºC. 50 ml of petroleum ether was added to the flask, shaken vigorously, and the layers were allowed to separate.

The lower soap layer was transferred into another separating funnel and the ether extraction was repeated for another 3 times using 50 ml portions of petroleum ether.

The combined ether extract was washed three times with 25 ml portions of aqueous alcohol followed by washing with 25 ml portions of distilled water to ensure ether extract was free of alkali. The ether solution was transferred to 250 ml beaker, separator was rinsed with ether, rinsings were added to the main solution. About 5ml was evaporated and transferred quantitatively using several portions of ether to 50ml Erlenmeyer flask previously dried and weighed. The ether was evaporated. When all ether had been removed, 2-3 ml acetone was added and while heating on steam or water bath completely, solvent was removed under a gentle air. To remove last traces of ether, drying was carried out at 100ºC for 30 minutes till constant weight was obtained. The residue was dissolved in 50 ml of warm ethanol which had been neutralised to a phenolphthalein end point and titrated with 0.02N NaOH.

Calculation:
Step I: Weight in g of the free fatty acids in the extract as oleic acid = 0.282 VN

Where, V = Volume in ml of standard sodium hydroxide solution
N = Normality of standard sodium hydroxide solution
Step II: Unsaponifiable matter = 100 (A-B) / W
Where A = Weight in g of the residue
B = Weight in g of the free fatty acids in the extract
W = Weight in g of the sample

c)Estimation Of the Wax Content:
Wax content in the crude oil sample was determined in terms of acetone insoluble as proposed by Ramaswamy *et al.*[20]Chilled (5–7ºC) acetone was added to 5 ml of oil sample (1:1, v/v) and centrifuged at 5000 rpm for 20 min. Supernatant oil was decanted carefully and insoluble portion was washed with 5 ml of chilled acetone and centrifuged. The wax obtained was then dried in oven and weighed.

d)Determination of Iodine value: [21]
The iodine value of oil is the number of grams of iodine absorbed by 100 g of the oil, when determined by using Wij's solution. The iodine value is a measure of the degree of unsaturation of oil and gives an idea of its drying characters. It is also helpful in determining the adulteration in oils.

Preparation of Wij's Iodine monochloride solution -10 ml of iodine monochloride was dissolved in about 1800 ml of glacial acetic acid and shaken vigorously. 5 ml of Wij's solution was pipetted, 10 ml of potassium iodide solution was added and titrated with 0.1N standard sodium thiosulphate solution using starch as indicator. The volume of the solution was adjusted till it was approximately 0.2 N.
Crude oil sample was weighed accurately following Table I given below:

Expected Iodine value	Weight to be taken for estimation (g)	
	Maximum	Minimum
5	6.3460	5.0770
10	3.1730	2.5384
50	0.6612	0.5288
100	0.3173	0.2538
150	0.2125	0.1700
200	0.1586	0.1269

Since the iodine value of crude RBO as per literature reports ranges between 99-108, 0.3 gm of the crude RBO sample was weighed accurately as indicated in Table I into a 500 ml conical flask with glass stopper, to which 25 ml of carbon tetrachloride was added. [22] The content was mixed well. 25 ml of Wij's solution was pipetted and the glass stopper was replaced after wetting with potassium iodine solution. The flasks were swirled for proper mixing and kept in dark for half an hour. A blank was carried out simultaneously. After standing, 15 ml of potassium iodide solution was added, followed by 100 ml of recently boiled and cooled water, rinsing in the stopper also. The liberated iodine was titrated with standardised sodium thiosulphate solution, using starch as indicator at the end until the blue color formed disappeared after thorough shaking with the stopper on.

Calculation:
Iodine value = 12.69 (B – S) N / W
Where, B = volume in ml of standard sodium thiosulphate solution required for the blank.
S = volume in ml of standard sodium thiosulphate solution required for the sample.
N = normality of the standard sodium thiosulphate solution.
W = weight in g of the sample.

a)Determination of Acetyl value: [23]
The acetyl value is the number which expresses in milligrams the amount of potassium hydroxide required to neutralize the acetic acid liberated by the hydrolysis of 1 g of the acetylated substance.

10 g of the crude oil sample was placed with 20 ml of acetic anhydride in a long necked round bottomed 200 ml flask attached to a reflux condenser. The flask was supported on a sheet of heat resistance material in which a hole of about 4 cm in diameter was cut and heated with a small naked flame not more than 25 mm in height and it did not impinge on the bottom of the flask. Boiling was carried out gently for 2 hours. The flask was then allowed to cool, poured into 600 ml of water contained in a large beaker; 0.2 g of pumice powder was added and boiled for 30 minutes. The solution was cooled, transferred to a separator and the lower layer was discarded. The acetylated product was washed with three quantities, each of 50 ml of a warmed saturated solution of sodium chloride until the washings were no longer acidic to litmus paper. Finally the solution was shaken with 20 ml of warm water and the aqueous layer was removed as completely as possible. The acetylated substances was poured into a small dish, 1 g of powdered anhydrous sodium sulphate was added, stirred thoroughly and filtered. The saponification value of the acetylated substance was determined using the following equation:

Calculation:
Acetyl value = 1335 (b-a) / (1335-a)
Where, a = Saponification value of the substance
b = Saponification value of the acetylated substance

b)Determination of Acid Value: [19]
The acid value is defined as the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. It is a relative measure of rancidity as free fatty acids are normally formed during decomposition of oil glycerides. The value is also expressed as per cent of free fatty acids calculated as oleic acid. The value is a measure of the amount of fatty acids which have been liberated by hydrolysis from the glycerides due to the action of moisture, temperature and/or lypolytic enzyme lipase.

About 10 g of crude oil sample was weighed accurately in a 250 ml conical flask and 50 ml of freshly neutralised hot ethyl alcohol and about one ml of phenolphthalein indicator solution was added. The mixture was boiled for about five minutes and titrated while hot against 0.1M potassium hydroxide until the solution remained faintly pink after shaking for 30 seconds. The weight of the oil taken for the estimation and the strength of the alkali used for titration was such that the volume of alkali required for the titration did not exceed 10 ml.

Calculation:
Acid value = 56.1VN / W
Where V = Volume in ml of standard potassium hydroxide used
N = Normality of the potassium hydroxide solution; and
W = Weight in g of the sample

c)Determination of Hydroxyl Value: [24]
The hydroxyl value is the number of milligrams of potassium hydroxide required to neutralise the acid combined by acylation in 1 g of the substance.

Crude oil sample was weighed accurately following Table II given below:

Presumed Hydroxyl value	Quantity of Substance (g)	Volume of pyridine acetic anhydride reagent (ml)
10 to 100	2.0	5.0
101 to 150	1.5	5.0
151 to 200	1.0	5.0
201 to 250	0.75	5.0
251 to 300	0.60 (or) 1.20	5.0 (or) 10.0
01 to 350	1.00	10.0
351 to 700	0.75	15.0
701 to 950	0.5	15.0

Since the hydroxyl value of crude RBO as per literature reports lies well within 100, 2.0 gm of the crude RBO sample was weighed accurately as indicated in Table II in a 150-ml acetylation flask fitted with a condenser and 5ml of pyridine acetic anhydride reagent was added. [25] It was boiled for 1 hour on a water-bath and subsequently cooled. 5 ml of water was then added through the top of the condenser. The condenser and the walls of the flask were rinsed with 5 ml of 95% ethanol, previously neutralized to dilute phenolphthalein solution. The resulting solution was titrated with 0.5M ethanolic potassium hydroxide using dilute phenolphthalein solution as indicator. A blank determination was also performed.

Calculation:
Hydroxyl value = Acid Value + 28.05 v/w
Where, v = difference, in ml, between the titrations;
w = weight, in g, of the substance.

a)Determination of Ester value: [24]
The ester value is the number of milligram of potassium hydroxide required to saponify the esters present in 1 g of the substance.
Calculation:
Ester value = Saponification value – Acid value

b)Determination of Peroxide value: [26]
Peroxide value is an indication of the extent of oxidation suffered by oil. It is the number of milliequivalents of active oxygen that expresses the amount of peroxide contained in 1000g of the substance.

5g of the crude oil sample was accurately weighed into a 250 ml glass-stoppered conical flask, 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform was added, swirled until dissolved and 0.5 ml of saturated potassium iodide solution was added. The solution was allowed to stand for exactly 1 minute, with occasional shaking, 30 ml of water was added and titrated gradually, with continuous and vigorous shaking, with 0.01M sodium thiosulphate until the yellow colour almost disappeared. 0.5 ml of starch solution was added and the titration was continued, shaking vigorously until the blue colour just disappeared (a ml). The operation was repeated omitting the substance being examined (b ml).

Calculation:
Peroxide value = 10 (a-b) / w
Where w = weight, in g of the substance.

i)Determination of Oryzanol Content: [27]
Crude RBO sample (about 10 mg) was accurately weighed and dissolved in hexane and the volume was made upto 10 ml and mixed well. The optical density (OD) was read in a 1 cm cell at 314 nm in a Shimadzu UV-2450 double beam recording spectrophotometer. The oryzanol content in the oil was calculated using the formula:

$$\text{Oryzanol, g\%} = \frac{\text{OD of hexane solution} * 100}{\text{Weight (g) of oil} * 10 \quad 358.9}$$

Where, 358.9 is the specific extinction co-efficient of oryzanol.

ii)Determination of Tocopherol and Tocotrienol Content:
Analysis of tocopherols and tocotrienols was carried out by normal-phase high-performance liquid chromatography (HPLC), following the method of Xu (2002). [28]

Sample Preparation
0.1 g of the crude oil sample and 0.05 g ascorbic acid (as an anti-oxidant to reduce oxidation reactions that could impact tocopherols and tocotrienols during saponification and extraction) was weighed into a test tube. 5 ml of 90.2% ethanol and

0.5 ml of 80% KOH was added to the test tube and vortexed for 30 secs. The test tube was incubated in a 70°C water bath for 30 min and vortexed periodically. It was then placed in an ice bath for 5 min. 3 ml deionized water and 5 ml hexane was then added and vortexed for 30 secs. It was then centrifuged for 10 min at 1000 × g at room temperature. The hexane layer was then transferred to another test tube. 5 ml hexane was added to the residual and aqueous layer and vortexed for 30 secs to re-extract. The residual layer indicates the solid phase that consists of aggregated sample particles on the bottom of the test tube, while the aqueous layer is the aqueous phase above the sample particles. They were extracted together by hexane a second time and centrifuged for 10 min at 1000 × g at room temperature. The hexane layer was transferred to the test tube containing the previous hexane layer. The hexane was then evaporated from the tube and 1 ml mobile phase was added and vortexed for 30 sec to redissolve the extract. The extract was transferred to a HPLC sample vial and injected into the normal-phase HPLC system. A Shimadzu Prominence System-HPLC unit coupled with a Shimadzu SPD-20A UV detector was used for the analysis. A 25-µL sample was injected into an analytical column (250 x 4.6 mm) packed with silica (e.g., Supelcosil LC-Si, Supelco) with 5-µm particle size. A mobile phase of ethyl acetate/acetic acid/ hexane (1:1:198 (v/v/v)) was used at the rate of 1.5 mL/min. Detection was performed at a wavelength of 295 nm. Pure standard of DL-a-Tocopherol acetate, containing the identical amounts of all the tocopherol and tocotrienol isomers, was used for identification and calibration.

iii) Determination of fatty acid composition: [29]

Fatty acid methyl esters were prepared according to standard IUPAC method 2.301 and analyzed on a SIGMA gas chromatograph model- 606, fitted with a methyl lignoserate coated (film thickness = 0.20 mm), polar capillary column SPTM-2330 (30 m x 0.32 mm), and a flame ionization detector. Oxygen free nitrogen was used as a carrier gas at a flow rate of 4.0 mL/min. Other conditions were as follows: initial oven temperature 140°C; ramp rate 4°C/min; final temperature 230°C; injector temperature, 250°C; detector temperature, 260°C; and temperature hold, 2 min. before the run and 4 min. after the run. A sample volume of 1.0 µL was injected. Fatty acids were identified by comparing their chromatographic retention times to those of authentic standards.

RESULTS AND DISCUSSION

About 19.64 percent oil was recovered from rice bran in this study, which was comparable to that reported by Lee *et al.,* (2002), Absar *et al.,* (1998), Marshall and Wadsworth, (1994) and Saunders (1990).[30-32, 5] The percentage oil content in general varies with the different varieties of rice bran and might be attributed to the diversity in natural soil texture of their derivation and other man-made cumulative effects along with the possible changes in environmental and geological conditions of the regions.[33]

The data for the analysis of various characteristics of the extracted crude RBO are summarized in Table III.

Table III: Characteristics of the extracted crude RBO

Sr. No.	Parameters	Values obtained
1	Organoleptic characters	
(a)	Appearance (30°C)	Opaque Liquid
(b)	Color	Brown
(c)	Flavor	Nutty
2	Specific Gravity (30°C)	0.9272
3	Viscosity (centipoise)	54.359
4	Moisture Content (%)	0.5
5	Saponification value (mg of KOH/g of oil)	193.54
6	Unsaponifiable Matter (%)	5.45
7	Wax Content (%)	1.66
8	Iodine value (g of 1/100g of oil)	108.29
9	Acetyl value	11.52
10	Acid value	27.49
11	Hydroxyl value	29.62
12	Ester value	166.05
13	Peroxide value (meq/kg of oil)	3.2
14	Oryzanol content (g%)	1.78

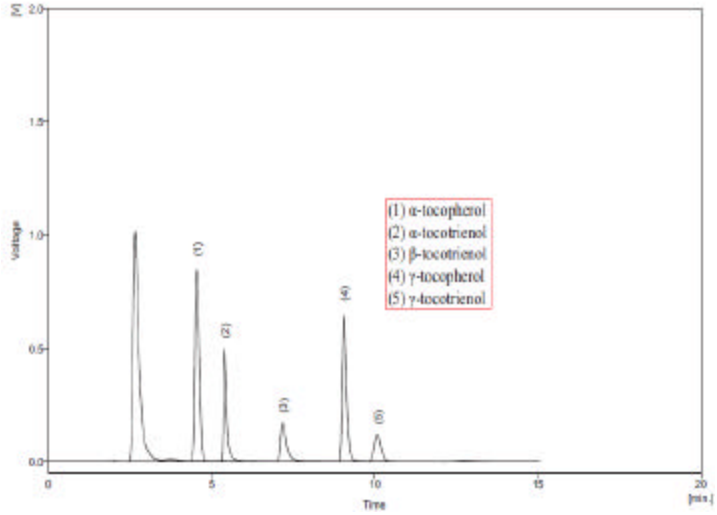
The values reported for specific gravity, moisture content, saponification, iodine value, unsaponifiable matter, acid value and hydroxyl value of the oil was in close agreement with those reported in the literature.[25,34-36] The peroxide value, which measures the hydroperoxides and aldehydic secondary oxidation products of the oils, was in line with that determined by Anwar *et al.,* (2005), who reported a range from 1.5-3.0 meq/kg of oil for RBOs extracted from different varieties of rice bran in Pakistan.[37] The percentage wax content determined in this study was in close agreement with that reported in the literature, wherein the content varies from less than 1% to more than 4%, depending on the origin of the rice bran and method of extraction.[38] A recent study by Manjula *et al.,* (2009), reported the wax content of crude RBO extracted samples from parboiled and raw rice bran to be 1.02% and 0.66%, respectively.[39] The acetyl value and ester value as determined in the present analysis could not be compared as to the best of our knowledge; there are no previously reported data of crude RBO to compare the results with our present work.

The percentage content of oryzanol (1.78%) in the crude RBO analyzed in the present study was in accordance to that reported (1.8-2.0%) in commercial products of RBO industry[22]. Oryzanol (OZ), although presumed to be a single component initially, was shown to be a mixture of ferulic acid esters of triterpene alcohols and plant sterols.[40,41] The nutritional function of the oryzanol components may be related to their antioxidant property because of the ferulic acid structure. A number of potentially therapeutically useful biological activities have been reported for oryzanol, in terms of improvement of the plasma lipid pattern of rodents, rabbits, non-human primates and humans, reducing total plasma cholesterol and triglyceride concentration and cholesterol absorption from cholesterol-enriched diets and aortic fatty streaks and simultaneously increasing the high density lipoprotein cholesterol level. [42,43] Other potential properties of oryzanol, that have been extensively investigated are the modulation of the pituitary secretion, inhibition of the gastric acid secretion, treatment of menopausal disorders, inhibition of the platelet aggregation and increasing the muscle mass. [44-47]

Table IV shows the content of different tocopherols and tocotrienols in the crude RBO as determined by a normal phase HPLC analytical system.

Sr. No.	Tocopherol and Tocotrienol Content	Values (%)
1	α-tocopherol	38.09
2	α-tocotrienol	15.66
3	β-tocotrienol	10.90
4	γ-tocopherol	25.62
5	γ-tocotrienol	9.73

Figure I illustrates a normal-phase chromatograph of tocopherols and tocotrienols in crude rice bran oil in the present study



Tocopherols are monophenolic anti-oxidants consisting of eight chemically distinct naturally occurring homologues, namely α-,β-,γ- and δ- tocopherol, characterized by a saturated side chain consisting of three isoprenoid units and their corresponding unsaturated tocotrienols (α-,β-,γ- and δ-).[48] In normal-phase HPLC on a silica column, the order of elution of tocopherols and tocotrienols is α-tocopherol, α-tocotrienol, β-tocopherol,β-tocotrienol, γ-tocopherol, γ-tocotrienol, δ-tocopherol, and δ-tocotrienol, although in our study, β-tocopherol, δ-tocopherol and δ-tocotrienol were below the level of detection. The percentage content of tocopherol in the investigated oil in our study was higher than that reported for palm kernels, coconut and palm oils, but well in sync to those reported for soybean, ground nut, high and low erucic acid rapeseed oils.[34] Improved tocopherol content is expected to contribute to substantial oxidative stability and protection to the oils during storage and processing. Moreover, tocopherols are considered to be the major chain-breaking antioxidants, preventing the propagation of oxidative stress, especially in biological membranes.[49] Epidemiologic and retrospective studies have further suggested that supplementa-

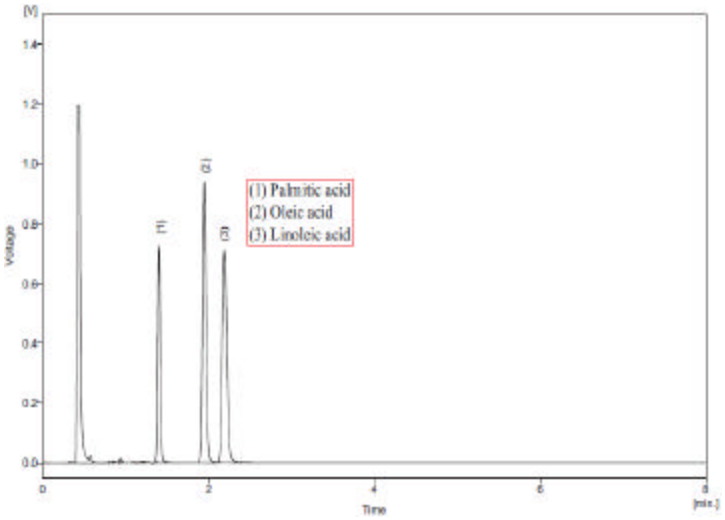
tion with tocopherols is a nutrition-based strategy to prevent diseases and to promote healthy aging.[50] They are also useful in protection against skin damage and aging by UV radiation.[51]

The tocotrienol content in the investigated crude RBO was higher than that of conventional vegetable oils[33]. However, the amount of γ-tocotrienol was found to be lower than palm oil (49%) and comparable to that of maize oil (8%).[52,53] The tocotrienols have been claimed to give protection against heart attack on account of their anti-thrombotic properties and have a comparatively greater antioxidant potential than the tocopherols.[34,45] The tocotrienols also possess powerful hypocholesterolemic, anti-cancer and neuroprotective properties that are often not exhibited by tocopherols. Moreover, oral tocotrienols have also been reported to offer substantial protection against stroke-associated brain damage *in vivo*.[54]

Table V shows the fatty acid (FA) profile of the crude RBO analyzed by a gas chromatograph.

Sr. No.	Fatty Acid	Values (%)
1	Palmitic (C16:0)	18.18
2	Oleic (C18:1)	60.29
3	Linoleic (C18:2)	21.53

Figure II illustrates the gas chromatogram of fatty acid composition of crude rice bran oil in the present study.



The results indicated that oleic and linoleic acids were about 82 percent in the crude RBO sample analyzed. Edwards and Radcliff (1994) and Sugano and Tsuji (1997) reported that oleic and linoleic fatty acids constitute more than 80 percent of fatty acids of glycerides.[55,56] Additional literature reports suggest that palmitic, oleic and linoleic fatty acids constitute more than 90% of the fatty acid portion of the glycerides in RBO[39]. Moreover, RBO is similar to peanut oil in fatty acid composition constituting of 49.9 and 35.4 percent oleic and linoleic acid, respectively.[57] The concentration of major fatty acids, C18:2, C18:1, C16:0 of the investigated oil was in close agreement to that reported by Hemavathy and Prabhakar (1987), for the rice bran oils indigenous in India.[58] Lee *et al*. (1991), also reported a higher percentage of oleic, linoleic and palmitic acids in rice bran oil, similar to that observed in the current study.[59] The fatty acid composition of the investigated oil was again quite similar in contents of C18:1 with the samples indigenous to Korea[30]. The fatty acid analysis of the crude RBO in the present work showed that oleic acid was the predominant fatty acid followed by linoleic and palmitic acid.

4.Conclusion

In India, RBO is the most important non-conventional oil in terms of its potential to augment the availability of oils, although little attention has been bestowed so far towards its phytochemical, physico-chemical properties, standardization and biological screening. The potential of this "Health Oil", if fully realized, would help in reducing the gap between demand and supply of indigenous oils in the Indian market. Hence, the need of the hour is to promote this "Heart Friendly Oil" in India with concerted efforts from the government, industrial sectors and other associated agencies.

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