Physico-chemical changes in rice bran oil during heating at frying temperature

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Abstract Rice bran oil was subjected to static heating at $180\pm 2^\circ\text{C}$ in a domestic fryer for 8 h in this process 150 ml of the heated oil samples were drawn, at intervals of every 2 h, to study the changes in the physico-chemical characteristics. Results indicated that the peroxide value and free fatty acid content increased gradually from 0.2 to 2.9 Meq. O₂/kg of oil and 0.25 to 0.63% respectively. The oil became darker as given by the colour value ($5R + Y$) 63 Lovibond units. Tocopherol content was found to decrease from 48 mg/100gram to 5 mg/100gram at the end of 8 h of heating whereas, oryzanol was fairly stable (1.59 to 1.40%). The *p*-anisidine value and Total polar compound (TPC) increased from 5.04 to 18.30 and 1.0 to 1.8% respectively, showing the formation of secondary oxidation products. Rice bran oil is a non-Newtonian fluids having shear thinning behavior. Heating was found to cause an increase in the flow behavior index. Fatty acid composition did not show significant changes except for the linoleic acid content which decreased from 29.4 to 27.1%.

Keywords Rice bran oil · Heating · Fatty acid · Oxidation

Introduction

Heat stability of the frying oil is mainly governed by two factors: the fatty acid composition and the presence of antioxidants and antioxidant precursors. Frying oil should have a low level of polyunsaturated fatty acids such as

linoleic or linolenic acids and high level of oleic acid with moderate amounts of saturated fatty acids. Rice bran oil has about 30% linoleic acid, 44% oleic and about 23% saturated fatty acids. Unsaturates are susceptible to oxidation or thermal degradation during heating or frying (Chen et al. 2001), leading to various chemical changes such as oxidation, polymerization, pyrolysis and hydrolysis (Yoon et al. 1987). Rice bran oil has high level of unsaponifiable matter and gamma oryzanol content (Gopalkrishna 2002). Unsaponifiables comprise of tocopherols, tocotrienols, phytosterols, polyphenols and squalene. It has been reported that presence of natural substances such as squalene, sterol fraction, quercetin. Oryzanol and ferulic acid enhance the stability of vegetables at a higher temperature (Gertz et al. 2000). Low viscosity of rice bran oil allows less oil uptake during frying (Chakrabarthy 1989). It is considerable to be good frying/cooking oil due to its high smoke point and delicate flavor (Ghosh 2007). Frying stability of oil can be assessed by monitoring the physicochemical changes occurring during heating the oil at temperatures in the range of $180\pm 2^\circ\text{C}$. The present study involves continuous heating of rice bran oil at frying temperature and determining the changes in certain physical and chemical parameters.

Materials and methods

Materials

Refined rice bran oil (RBO) edible grade was procured from local market in Mysore (India). All the chemicals and solvents were of analytical reagent grade obtained from the local chemical companies. Ethyl alcohol was refluxed with sodium hydroxide before distillation. *para*-Anisidine and

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Iso-octane were procured from Qualigens fine chemicals Ltd., Mumbai, India. *para*-anisidine was recrystallized, in hot water in the presence of sodium sulphite and activated carbon, before preparing reagent for estimation. Wij's solution was purchased from Nice chemicals, Kerala, India.

Rice bran oil (3 L) was taken in 5 l capacity of batch type hindalium domestic deep fryer and heated continuously under static condition. Oil temperature was maintained at $180 \pm 2^\circ\text{C}$. 150 ml of heated oil samples were drawn after 2, 4, 6, and 8 h of heating. The collected oil samples were labeled and stored at -20°C to be used for analysis.

Measurement of colour The colour value of the oils were measured by using a Lovibond Tintometer, model F, The Tintometer Ltd, Salibury, U.K. In a 1-inch (2.54 cm) cell in the transmittance mode and expressed as $5 \times \text{red} + 1 \times \text{yellow}$ Lovibond units.

Estimation of Free fatty acid, peroxide value and iodine value Free fatty acid (FFA) content, peroxide value (PV), and iodine value of control and heated oil samples were determined as per (AOCS 2003), method No. Ca 5a-40, cd 8–53 respectively. Analyses were done in triplicates and average values were calculated.

Estimation of Free fatty acid, peroxide value and iodine value Total amount of intermediate polar compounds (peroxides and aldehydes) that result from lipid oxidation was measured as totox number (Wan et al. 2009).

$$\text{Totox number} = \text{AV} + (2 \times \text{PV})$$

Where,

AV Anisidine value

PV Peroxide value

Estimation of polar compounds Polar compounds were analysed according to the AOCS method, Cd11C-93 by column chromatography. The column was prepared using 30 g of silica gel slurry with petroleum ether. Accurately 0.9 g of fat samples was weighed and dissolved in 3 ml of chloroform. Dissolved sample was quantitatively transferred to the top of the column by repeated rinsing with 3 ml of chloroform. The sample was eluted using 250 ml solvent for each fraction, as shown below,

- Fraction-I (triglycerides)-250 ml 10% diethyl ether in petroleum ether.
- Fraction-II (diglycerides)-250 ml 25% diethyl ether in petroleum ether.

- Fraction-III (monoglycerides)-250 ml 100% diethyl ether.
- Fraction-IV (glycerol and/or polar material)-200 ml 100% ethyl alcohol.

The above effluents were collected separately and solvents were evaporated to dryness in a tarred 250 ml flasks on a steam bath under a stream of nitrogen. The flasks were dried until a constant weight was obtained.

Calculations:

- Fraction – I (triglycerides) = $\frac{\text{mass of fraction Ig}}{\text{mass of sample}} \times 100$
- Fraction – II (diglycerides) = $\frac{\text{mass of fraction IIg}}{\text{mass of sample}} \times 100$
- Fraction – III (monoglycerides) = $\frac{\text{mass of fraction IIIg}}{\text{mass of sample}} \times 100$
- Fraction – IV (glycerol and/or polar material) = $\frac{\text{mass of fraction IVg}}{\text{mass of sample}} \times 100$

Estimation of *p*-anisidine value *p*-anisidine value was determined following the AOCS method No. Cd 18–90. 1.0 ± 0.5 g of heated oil samples taken in a 25 ml volumetric flask was dissolved in iso-octane, to make up the volume. Optical density (O.D.) (Ab) of this was read at 350 nm using iso-octane as blank in UV-240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Further, from the oil solution 5 ml was pipetted into 10 ml graduated test tube, 1 ml of the *p*-anisidine reagent was added to the each tube and shaken. After 10 min of the reaction time the O.D was measured at 350 nm (As), using a mixture of iso-octane and *p*-anisidine solution as a blank.

$$p\text{-anisidine value} = \frac{25 \times (1.2A_s - A_b)}{(m) \text{ Weight of the sample}}$$

As Absorbance of the fat solution after reaction with the *p*-anisidine reagent.

Ab Absorbance of the fat solution

m mass, in g of the test portion (sample wt)

Estimation of fatty acid composition Fatty acid composition of the oil was determined using AOCS method No. Ce 2–66. Methyl esters were analysed in a Gas-Liquid Chromatograph (model GC-15A; Shimadzu Corporation), equipped with a data processor (model CR-4A; Shimadzu Corporation), FID detector, and a stainless steel column (3m x 3mm i.d.) packed with chromosorb W 60–80 mesh, precoated with 15% diethylene glycol succinate. The gas chromatograph was operated under the following conditions: nitrogen flow 40 ml per minute, hydrogen flow 40 ml per min, air flow 300 ml per min, column temperature 180°

C, and FID temperature 200°C. Fatty acids were identified based on their retention time compared with standard fatty acid methyl esters.

Estimation of total tocopherol (Saponification and sample preparation) Total tocopherol content was determined by the IUPAC method no. 2.301 (Paquot and Havtffenne 1987). 4 ml of Ethanolic Pyrogallol solution was added to 1 g of the heated oil taken in a 250 ml flat bottom flask. The flask was attached with air condenser and brought to boil. At this stage 1 ml of potassium hydroxide solution was added and boiling was continued for 3 min. The flask was cooled under running water and then 25 ml of distilled water was added. The contents transferred quantitatively to a separating funnel were extracted (4 times) using 40 ml diethyl ether. Ether extract was repeatedly washed with distilled water until the water after washing did not turn pink on addition of phenolphthalein. Diethyl ether was evaporated using rotary evaporator. Ethanol (1 ml) and benzene (4 ml) was added to the sample and dried under nitrogen. The residue was dissolved in 1 ml of hexane and evaporated completely under a stream of nitrogen. Finally 1 ml of heptane was added for further analysis.

Colour development 0.1 ml of heptane diluted sample was taken in test tube to that 3.5 ml of alcoholic ferric chloride 0.2 ml (2 mg/ml) and 2,2'-dipyridyl 0.2 ml (2 mg/ml) was added and the total volume was made up to 4 ml. The absorbance at 520 nm in model UV-240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was read immediately after the colour developed (2 min) the reaction should be carried out in the dark. The total tocopherol content was determined from a standard graph obtained by estimating different concentrations of α -tocopherol and the value expressed as mg/100 g oil.

Estimation of oryzanol Orzanol content was estimated given by Seetharamaiah and Prabakar 1986. Accurately weighed oil samples (about 10 mg each) in replicates were dissolved in hexane, and made up to 10 ml. O.D. of the solution was recorded in a 1-centimeter cell at 314 nm in a Shimadzu UV-240 double beam recording spectrophotometer (solutions having OD more than 1.2 were further diluted before recording). The oryzanol content in the oil was calculated using the formula

$$\text{Oryzanol, g\%} = \frac{\text{O.D of hexane solution}}{\text{Weight of oil (g)/100ml}} \times \frac{100}{358.9}$$

Estimation of trans fatty acid (TFA) The oil samples converted to fatty acid methyl esters (FAME) using KOH in methanol (Andrew Kohn and Mitchell 2006) were analyzed by GLC (Fisons, 8000 series, CE Instruments,

Rodano, Italy) with FID. Supelco, SP-2380 (0.25 millimeter $\times$ 30 meter) capillary column was used, operating at programmed column temperature 50°C to 220°C at 5°C per minute. Other operational conditions were injection temperature 230°C, split ratio 1:20; detector temperature 240°C, and nitrogen flow, 15 ml per minute. The fatty acids were identified by using authentic standards and presented as relative percentage.

Viscosity measurement The rheological behavior of the oil samples was measured using a controlled stress rheometer (Rheostress 6000 Thermo Scientific, Karlsruhe, Germany) with a coaxial system attachment. The shear stress was progressively increased up to 20 Pas and 100 data sets were generated. A circulatory water bath was employed to keep the temperature of measurement at $25 \pm 0.1^\circ\text{C}$. The apparent viscosity of the oil samples is the ratio of shear stress and shear rate while the latter has been taken as 100 s^{-1} . Flow behavior index and consistency index were calculated according to the power law model, and suitability of this model was judged by calculating the correlation coefficient (r).

Statistical analysis Data (5 replicates) were subjected to statistical analysis (Duncan 1995), of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was applied to differentiate among the means of different samples at a probability of $p \leq 0.05$.

Results and discussion

Colour value In the early phase of heating, a drastic increase in both red and yellow units was observed. A threefold increase in red units and nearly fourfold increase in yellow units was found after 2 h of heating. Interestingly, there was less pronounced change between 2 and 4 h of heating. When heating was continued beyond 4 h the oil became darker. Generally, colour value of vegetable oils is represented as $5R + Y$. This value was found to increase from 12 to 43 in the first two hours of heating and to 63 at the end of 8 h of heating. Visually, this corresponded to change of colour from yellow to amber and finally to reddish brown. The darkening of the colour may be attributed to thermal and auto oxidation as well as due to the presence of phospholipids which darken on heating.

Estimation of primary and secondary oxidation products The rice bran oil samples drawn at 0 h (control) and at intervals of two hours were analysed for free fatty acid content, peroxide value, anisidine value and total polar components. These parameters are directly related to the oxidative status of the vegetable oils. The results given in Fig. 1(a) shows that the free fatty acid (FFA) percent of fresh rice bran oil

Fig. 1 Changes in oxidative parameters and flow characteristics of rice bran oil during heating at $180\pm 2^\circ\text{C}$. *Estimations done in triplicates ($n=3$)

was 0.25% which gradually increased during heating to reach 0.63% at the end of 8 h heating. Similar observation was noticed for peroxide value (PV) which rose gradually from 0.2 to 2.9 Meq.O₂/kg oil. Slow rate of increase in FFA and PV may be attributed due to the protective effect of oryzanol present in rice bran oil. Total polar compounds (TPC) which are generated due to thermal oxidation and auto-oxidation provide a reliable measure of the extent of oxidative degradation. TPC increased significantly from 1.0 to 1.8% in the course of 8 h of heating at $180\pm 2^\circ\text{C}$. *p*-anisidine value is a measure of aldehydes and ketones formed during thermo-oxidative degradation of unsaturated fatty acids. Initially the anisidine value was 5.04 which after 2, 4, 6, 8 h of heating increased to 8.05, 12.45, 16.75 and 18.31 respectively. Unsaturated fatty acids are the primary targets of thermal oxidation as well as autooxidations, leading to formation of secondary oxidation products. Breakdown of unsaturated fatty acids is reflected in

Table 1 Physical and chemical changes in rice bran oil during heating at $180\pm 2^\circ\text{C}$ *

Duration of heating(h)	Red	Yellow	Blue	5R + Y	<i>p</i> -anisidine value	Totox Value AV + (2 × PV)	Iodine value	Total Tocopherol (mg/100 g)	Oryzanol content (%)
0	1.2±0.05 ^a	5.5±0.05 ^a	0.1±0.01 ^a	12.1±0.05 ^a	5.0±0.01 ^a	5.4±0.01 ^a	100.2±0.05 ^a	48.0±0.05 ^e	1.6±0.01 ^b
2	4.0±0.05 ^b	20.0±0.05 ^b	0.1±0.03 ^b	42.8±0.05 ^b	8.0±0.01 ^b	13.4±0.01 ^b	100.1±0.14 ^a	19.1±0.10 ^d	1.5±0.03 ^a
4	4.5±0.05 ^b	23.0±0.05 ^b	0.1±0.01 ^a	51.1±0.05 ^c	12.5±0.05 ^b	17.8±0.01 ^c	99.2±0.01 ^b	15.1±0.10 ^c	1.5±0.01 ^a
6	4.9±0.05 ^b	30.0±0.05 ^c	0.2±0.01 ^b	54.5±0.05 ^c	16.8±0.01 ^c	22.6±0.01 ^d	97.0±0.02 ^c	8.9±0.05 ^b	1.5±0.10 ^a
8	6.2±0.05 ^c	40.5±0.05 ^d	0.2±0.02 ^c	63.0±0.05 ^d	18.3±0.01 ^d	24.1±0.01 ^d	95.0±0.01 ^d	5.1±0.05 ^a	1.4±0.01 ^a
SEM	0.00234	0.0054	0.0002	0.18016	0.0179	0.0018	0.0104	0.02193	0.00335

*Estimations done in triplicates ($n=3$)

AV Anisidine value, PV Peroxide value

Values in the same column with different superscripts are significantly ($p\leq 0.05$) different

SEM-Standard Error Mean

changes in iodine value which is a measure of unsaturation in fatty acids. Results in Table 2 show that iodine value was inversely proportional to anisidine value. The decrease in iodine value from 100 (0 h) to 95.4 (after 8 h) corresponded to increase in anisidine value from 5.04 (0 h) to 18.31 (after 8 h).

Effect of heating on fatty acid composition Changes in fatty acid composition during heating are given in Table 2. Rice bran oil has palmitic acid (22.7%) as the major saturated fatty acid. It is high in oleic acid (43.9%), and linoleic acid in the largest component (29.6%) of or of polyunsaturated fatty acids, followed by low level (1.25%) linolenic acid. Heating did not cause any appreciable change in myristic and palmitic acids. Oleic acid content was found to slightly increase from 43.7 to 44.5%. On the other hand, nearly 9% loss in linoleic acid was observed, indicating its oxidative degradation leading to formation of secondary oxidation products and polar compounds. Table 2 summarises the changes in saturated fatty acids (SFA) monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Effect of heating on Tocopherol and oryzanol content, it was found that heating caused drastic reduction in tocopherol content (Table 1). Decrease in tocopherol content was very rapid in the early phase (up to 2 h) of heating during which period more than 50% of the tocopherol lost. Similar findings were reported for palm oil (Valantina et al. 2010). Tocopherols are known to be antioxidants at room and moderate temperature. It has been shown that α -tocopherol degrades much faster in oils at frying temperature and produces four oxidation products, only one of these α -tocopherol ethane dimer shows antioxidants activity. On the other hand γ -tocopherol is

superior to α -tocopherol because it oxidizes to more stable compounds which are still effective as antioxidants (Kochar 2000). But the oryzanol content was rather unaffected. The oryzanol which is a methyl ester of ferulic acid is a high melting solid was found to heat resistant. However, its level did decrease from 1.59% to 1.4% at the end of 8 h heating. Similar findings have been reported by Mishra et al. (2011) where the oryzanol content decreased with respect to time of heating in microwave. It is known the γ -oryzanol together with tocopherols are responsible for the high antioxidative strength rice bran oil (Gopalkrishna 2002). As loss of oryzanol is marginal it is expected that the oil retains part of its antioxidant activity. In a study a stability of blends of oil during deep fat frying it is shown that rice bran oil present in the blend could significantly reduce oxidative and thermal degradation taking place in unsaturated fatty acids (Farhoosh and Kenari 2009).

Development of trans fatty acids during frying conditions Trans fatty acid, namely elaidic acid forms during hydrogenation of fat, and microbial action on fat molecule. Also trans fatty acids are formed when oil is exposed to heat for a longer period of time. But, under the heating conditions employed in this study, trans fatty acid was not formed even after 8 h of heating (Table 2).

Viscosity Figure 1(b) shows the flow characteristic of the heated rice bran oil. The flow behavior index for unheated oil (control) was 0.687 whereas it was between 0.835 and 0.867 for heated oils indicating that all these samples were non-Newtonian fluids having shear thinning behavior. An increase in time of heating markedly increased the flow behavior index indicating that the heated oils were closer to

Table 2 Fatty acid composition (weight %) of rice bran oil subjected to heating at $180 \pm 2^\circ\text{C}^*$

Fatty acid		Duration of heating(h)					
		0	2	4	6	8	
14:0	Myristic	0.30 $\pm$ 0.01 ^a	0.30 $\pm$ 0.02 ^a	0.31 $\pm$ 0.05 ^a	0.31 $\pm$ 0.03 ^a	0.30 $\pm$ 0.02 ^a	
16:0	Palmitic	22.6 $\pm$ 0.05 ^c	22.8 $\pm$ 0.01 ^d	22.9 $\pm$ 0.01 ^d	23.6 $\pm$ 0.58 ^d	23.5 $\pm$ 0.04 ^d	
18:0	Stearic	1.8 $\pm$ 0.05 ^b	2.5 $\pm$ 0.01 ^c	2.7 $\pm$ 0.05 ^c	2.6 $\pm$ 0.10 ^c	2.6 $\pm$ 0.10 ^c	
18:1	Oleic	43.7 $\pm$ 0.05 ^d	44.0 $\pm$ 0.05 ^e	44.1 $\pm$ 0.01 ^e	44.5 $\pm$ 0.23 ^e	44.5 $\pm$ 0.05 ^f	
18:2	Linoleic	29.2 $\pm$ 0.01 ^c	28.4 $\pm$ 0.01 ^d	28.1 $\pm$ 0.05 ^d	27.1 $\pm$ 0.05 ^d	27.1 $\pm$ 0.12 ^e	
18:3	Linolenic	1.3 $\pm$ 0.05 ^b	1.2 $\pm$ 0.01 ^b	1.1 $\pm$ 0.01 ^b	1.2 $\pm$ 0.01 ^b	1.2 $\pm$ 0.02 ^b	
20:0	Arachidic	0.90 $\pm$ 0.01 ^a	0.80 $\pm$ 0.05 ^a	0.80 $\pm$ 0.01 ^a	0.80 $\pm$ 0.02 ^a	0.80 $\pm$ 0.01 ^a	
SEM	—	0.0047	0.0081	0.0082	0.0086	0.0084	SEM
SFA	—	25.6 $\pm$ 0.05 ^a	26.4 $\pm$ 0.05 ^b	26.7 $\pm$ 0.01 ^b	27.3 $\pm$ 0.01 ^c	27.2 $\pm$ 0.02 ^c	0.00745
MUFA	—	43.7 $\pm$ 0.01 ^a	44.0 $\pm$ 0.05 ^b	44.1 $\pm$ 0.01 ^b	44.5 $\pm$ 0.06 ^b	44.0 $\pm$ 0.01 ^b	0.00968
PUFA	—	30.7 $\pm$ 0.01 ^b	29.6 $\pm$ 0.05 ^a	29.2 $\pm$ 0.05 ^b	28.2 $\pm$ 0.58 ^a	28.3 $\pm$ 0.01 ^a	0.00232

*Estimations done in triplicates ($n=3$) Values in the same column with different superscripts are significantly ($p \leq 0.05$) different

SEM Standard Error Mean, SFA Saturated fatty acid, MUFA Monounsaturated fatty acid, PUFA Polyunsaturated fatty acid

the Newtonian behavior. On the other hand, the consistency indices of heated oils were only half that of unheated oils. The apparent viscosity was almost constant up to 2 h of heating but increase in viscosity was found at the end of the heating period (8 h) due to oxidation, isomerization and polymerization reaction. The power law model appears to be suitable for rice bran oil as the correlation coefficient values were between 0.994 and 0.999 (significant at $p \leq 0.01$). The results show that rice bran oil (unheated and heated) is a non-Newtonian shear-thinning fluid with flow behavior index well below one rheological characteristics of the oil can be explained by the power law model.

Conclusion

Efforts have been made to assess changes in physicochemical quality and the stability of the rice bran oil and inherent nutraceuticals like tocopherol and oryzanol at frying temperature. Based on the chemical parameters it can be concluded that rice bran oil has fairly good stability at frying temperature ($180 \pm 2^\circ\text{C}$). Not much change in fatty acid composition but for about 9% loss in PUFA was observed. Iodine value which is directly related to unsaturated fatty acids showed a relative decrease as expected. No *trans* fatty acid was generated during the heating period in frying conditions. Significant decrease was observed in tocopherol content but the oryzanol content changed only marginally. Viscosity of oil was found to increase on heating, due to the formation of oxidation products.

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