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EFFECT OF HEAT ON PHYSICO-CHEMICAL AND THERMO-OXIDATIVE STABILITY OF REPEATEDLY HEATED RICE BRAN OIL (RBO)

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Rice bran oil was subjected to 3 cycles of alternate heating (2 h at 180 ± 2 °C) and cooling (24 h at room temperature). This was followed by another 3 cycles of alternate heating (4 h at 180 ± 2 °C) and cooling (24 h at room temperature). Physico-chemical parameters and oxidative deterioration of oil during first 2 h of heating caused rapid increase in colour from 12 to 69.9 units, PV 0.2 to 6.3 Meq.O_2/kg, Av 5.04 to 19.4 and TPM 1 to 4.1% respectively. After 2 h of heating, almost half of the tocopherol was last whereas oryzanol had better stability. An increase in conjugated diene and triene were also noticed. This was also reflected in increased viscosity of heated oil at the end of heating period. A gradual loss of in antiradical activity was seen after 18 h of heating about 40 percent of the activity was lost. Changes in the relative proportion of fatty acids was observed to lower the iodine value (IV) from 100.2 to 93 after 6 h and finally to 82 at the end of 18 h. p-anisidine value correlated negatively with tocopherol, oryzanol as well as IV contents.

Keywords: Deep fat frying, Free fatty acid, Peroxide value, Antioxdant activity, Tocopherols, Oryzanol

INTRODUCTION

Thermo-oxidation is a major cause of food quality deterioration, and has been a challenge for manufacturers and food scientists alike. Auto-oxidation is defined as the spontaneous reaction of atmospheric oxygen with lipids and it is the most common process leading to oxidative deterioration of oils and fats. This process of auto-oxidation gets accelerated at higher temperatures, such as those experienced during deep-fat frying, leading to changes in physico-chemical characteristics of the oil. Unsaturated fatty acids are the major reactants affected by such reactions, whether they are present as free fatty acids, triacylglycerols (as well as diacyglycerols or monoacylglycerols), or phospholipids. It is known that both auto-oxidation and thermal oxidation of unsaturated fatty acids occur via a free radical chain reaction. The use of fat or oil for frying still remains one of the most popular methods for the preparation of foods worldwide. The choice of frying fat depends on its frying performance, flavor and stability of the product during storage. Before selecting frying oil, it is necessary to examine some of the major changes which occur in the oil during deep frying (Sebedio *et al.*, 1996). As deep fat frying is normally carried at high temperature (between 160-190 °C) and in the presence of air and moisture, these frying oils and fats will undergo physico chemical deterioration which will affect their frying performance and the storage stability of the fried products (Fauziah *et al.*, 2000). The degradation products formed during deep frying include both volatile and non volatile compounds, although most of the volatiles are lost during the frying process. The non volatile compounds are produced primarily by thermal oxidation and polymerization of unsaturated fatty acids (Chang *et al.*, 1978; and Fritch 1981).

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Auto-oxidation leads to oxidative deterioration of oil due to spontaneous reaction of atmospheric oxygen with lipids. This process is accelerated at higher temperatures such as during deep-fat frying (180-190 °C). At these temperatures thermal oxidation of the oil will occur also with increase in free fatty acid and polar matter content, foaming, colour and viscosity. Heating in the presence of air causes partial conversion of fats and oils to volatile short chain-scission products, non-volatile oxidized derivatives, and dimeric polymeric or cyclic substances (Chang *et al.*, 1978). Besides, different oil has been found to behave differently during auto-oxidation leading to differences in the rates of formation of polar components and secondary oxidation products. Oxidation of polyunsaturated fatty acid is accompanied by an increase in the UV absorption; as well as isomerization and conjugation of double bonds. The main objective of the present work was to evaluate the effect of repeated heating and cooling on quality of Rice Bran Oil (RBO) and correlate the physico-chemical parameters.

MATERIALS AND METHODS

Edible grade refined Rice Bran Oil (RBO) was procured from a local super market in Mysore (India). All the chemicals and solvents were of analytical reagent grade. Ethyl alcohol was refluxed with sodium hydroxide before distillation. *para*-anisidine and iso-octane were procured from Qualigens Fine Chemicals, Mumbai, India. *para*-anisidine was recrystallized in hot water in the presence of sodium sulphite and activated carbon. Wij's solution was purchased from Nice Chemicals, Kerala, India.

Heating Protocol and Oil Sampling

Refined rice bran oil (3L) was taken in a glass beaker of 5 liter capacity and homogenized for 30 minutes using Heidolph RZR 2020 homogenizer at a speed level of 8, power 1, range 1 and 260 rpm to uniformly distribute the constituents present in the oil. The homogenized oil was transferred to a hindalium domestic deep fryer and heated continuously under static condition till the oil reached 180 $\pm$ 2 °C. Heating was continued for 2 h followed by cooling for 24 h. This cycle was repeated 3 times on 3 consecutive days. In the second phase, the same oil was heated for 4 h followed by cooling for 24 h. This cycle was repeated thrice. During heating the oil was occasionally agitated manually to transfer the heat uniformly within the oil. At the end of the each heating period the fryer was put off and the oil was allowed to cool to ambient temperature (25$\pm$1 °C). Oil samples (150 ml) were drawn in triplicates after 2, 4, 6, 10, 14 and 18 h. Samples were labeled and stored at -20 °C for subsequent physico-chemical analysis.

Physico-Chemical Estimations
Measurement of Colour

Colour value of the oils were measured in a Lovibond Tintometer, model F (The Tintometer Ltd., Salisbury, UK) using a 1-inch (2.54 cm) cell in the transmittance mode. Colour value was expressed as (5 × red+1 × yellow) Lovibond units.

Estimation of Free Fatty Acid (FFA) Peroxide Value (PV) and Iodine Values (IV)

Free Fatty Acid (FFA) content, was estimated by AOCS Official Method (AOCS, 2002). Neutralized hot alcohol was added to the oil sample and titrated against sodium hydroxide solution (0.1 N) using phenolphthalein indicator. The FFA values were expressed as % oleic acid. Peroxide Value (PV) was estimated by (AOCS, 2002). The oil sample was dissolved in acetic acid/chloroform mix (3:2), taken in a conical flask followed by the addition of saturated potassium iodide solution. The flask was kept under the dark condition for 1 min. The reaction was terminated with addition of distilled water and the contents in flask were titrated against standard sodium thiosulfate using starch as indicator (1%) till the blue colour disappeared. PV was expressed as milliequivalents of oxygen/kg of oil. Iodine value was determined by following Wijs method (AOCS Cd-1-25) (5). 0.2 ± 0.1 g of oil samples was taken in an iodine flask and to that 20 ml of carbon tetra chloride was added followed 25 ml of Wij's solution. The flask was kept in a dark place for 1 h, followed by addition of 20 ml of potassium iodide solution (15%) and 25 ml of distilled water. This mixture was titrated with sodium thiosulphate (0.1 N) using 1% starch indicator till the blue colour disappeared.

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 with 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:

$$a.\ Fraction-I\,(triglycerides) = \left(\frac{Mass\ of\ \ fraction\ Ig \times 100}{Mass\ of\ \ sample}\right)$$

$$b.\ Fraction-II(diglycerides) = \left(\frac{Mass\ of\ \ fraction\ IIg \times 100}{Mass\ of\ \ sample}\right)$$

$$c.Fraction-III(monoglycerides) = \left(\frac{Mass\ of\ \ fraction\ IIIg \times 100}{Mass\ of\ \ sample}\right)$$

$$d.Fraction-IV(glycerol\ and\ /\ or\ \ polar\ material) = \left(\frac{Mass\ of\ \ fraction\ IVg \times 100}{Mass\ of\ \ sample}\right)$$

Estimation of p-Anisidine Value (AV)

p-Anisidine Value (AV) was determined following the AOCS method No. Cd 18-90. 1.0±0.5 g of heated oil sample 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 (0.25 g/ 100 ml of glacial acetic acid) was added to the each tube and shaken. After 10 minutes 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.

$$p-anisidinevalue = 25 \times \left(\frac{1.25As - Ab}{weight\ of\ the\ sample \times m}\right)$$

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 (3 m x 3 mm 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 ^{o}C, and FID temperature 200 ^{o}C. Fatty acids were identified based on their retention time compared with standard fatty acid methyl esters.

Estimation of Total Tocopherol

Total tocopherol content was determined by the IUPAC method no. 2.301 (Paquot *et al.*, 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 (16 g/10 ml of water) 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) were added to the sample and dried under nitrogen. The residue was dissolved in 1ml of hexane and evaporated completely under a stream of nitrogen. Finally 1ml of heptane was added for further analysis.

Colour Development

0.1 ml of heptane diluted sample was taken in test tube and 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 was carried out in the dark. The total tocopherol content was determined from a standard graph obtained by estimating different concentrations of á-tocopherol and the value expressed as mg/100 g oil.

Estimation of Oryzanol

Oryzanol content was estimated following a method given by Seetharamaiah *et al.* (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

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

Radical Scavenging Activity

The Radical Scavenging Activity (RSA) of the oils was assayed by reduction of 2, 2 diphenyl 1-picrylhydrazyl (DPPH) radicals. The RSA and the presence of hydrogen donors in the oil were determined by reduction of DPPH in toluene. A toluenic solution of DPPH radicals was freshly prepared at concentration of 10^{-4}M. The radical, in the absence of antioxidant compounds, was stable for more than 2 h of the normal kinetic assay. For evaluation, 10 mg of oil sample (in 100 µl toluene at room temperature) was mixed with 390 µl toluenic solution of DPPH radicals, and the mixture was vortexed for 20 s at ambient temperature (25 $\pm$ 1 °C). Against a blank of pure toluene without DPPH, the decrease in absorption at 515 nm was measured in 1-cm quartz cells after 10, 15, 20, 30, 40 and 60 min using a visible spectrophotometer (UV-1601-Shimadzu Corporation, Kyoto, Japan). The RSA toward DPPH radicals was estimated from the differences in absorbance of the toluenic DPPH solution with or without sample (control-unheated oil), and the percentage of inhibition was calculated from the following equation:

%RSA = [(O.D of control-O.D. of sample)/O.D. of control)] × 100

Conjugated Dines (CD), Conjugated Trienes (CT)

Conjugated dines and conjugated trines (oxidation products) (AOCS.O.M. No Tila-64,1 977) of the controlled (un heated/un fried) oil samples were analysed by using spectrophotometric method at the wave length of 232 and 270 nm, respectively. These oxidized products were also assessed for controlled heated and fried oil samples after deep fat frying of low and high moisture products.

Estimation of Trans fatty acid

(TFA) The oil samples converted to fatty acid methyl esters (FAME) using KOH in methanol (Kohn *et al.*, 2006). were analyzed by GLC (Fisons, 8000 series, CE Instruments, Rodano, Italy) with FID. Supelco, SP-2380 (0.25 millimeter × 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.

Opacity Measurement

The opacity of the oil samples (un heated/heated) were transferred in a glass petrick dish having 5.2 cm diameter and opacity was determined employing a colour measuring system (Model LabScan XE, Hunter Lab Instruments, Virginia, USA). The measurements were conducted in the reflectance mode with the CIE standard observer of 10° employing illuminant D65 and a port opening of 6 mm. Data were analyzed by Universal software (version 3.8), supplied by the equipment manufacturer. The white and black standard plates, supplied by the manufacturer, were employed to determine the opacity of samples as per cent basis.

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±0.1 °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 (3 triplicates) were subjected to analysis, of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was applied to differentiate among the means of different samples at a probability of $p \leq 0.05$ (Duncan, 1995).

RESULTS AND DISCUSSION

Colour

The colour values of repeatedly heated (18 h) RBO are shown in Table 1. Significant changes in red and yellow values were observed at the end of 2 h of heating. Comparatively the increase was found to be markedly more than that occurring in later stages of heating. Nearly four fold increase was seen in red and yellow units at the end of 2 h of heating. Subsequent heating did not cause as much damage to the colour as in the initial stage. After 18 h of heating, the total colour of the oil was found to increase from 12.0 units to 69.9 units. Presence of usaturated carbonyl compounds, and degraded oxidation compounds are known to darken the oil (Augustin *et al.*, 1983; and Gutierrez *et. al.*, 1988). Darkening can be considered as an useful index of quality that helps in monitoring oil deterioration, at elevated temperatures.

Table 1: Changes in Colour Values of Rice Bran Oil During Repeated Heating (180 ± 2 °C) and Cooling

Duration of Heating (h)	Red	Yellow	Blue	5R+Y*
0	1.3±0.05 [a]	5.5±0.05 [a]	0.1±0.01 [a]	12±0.05 [a]
2	5.2±0.05 [b]	20.3±0.01 [b]	0.2±0.05 [b]	46.3±0.05 [b]
4	5.3±0.02 [b]	22.4±0.02 [c]	0.2±0.05 [b]	48.9±0.05 [b]
6	6.3±0.05 [c]	22.4±0.05 [c]	0.2±0.02 [b]	53.9±0.02 [c]
10	6.5±0.01 [c]	22.4±0.05 [c]	0.2±0.01 [b]	54.9±0.05 [c]
14	6.5±0.02 [c]	32.4±0.01 [d]	0.2±0.02 [b]	64.9±0.02 [d]
18	6.9±0.01 [c]	35.4±0.02 [d]	0.2±0.01 [b]	69.9±0.01 [d]
0.0212	0.0363	0.0212	0.0034	0.0821

Note: *5R+Y-Refers to the total colour, *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.

Fatty Acid Composition

Rice bran oil has a balanced fatty acid ratio of 23:44: 33 of SFA (Saturated fatty acid) to MUFA (Mono unsaturated fatty acid) to PUFA (Poly unsaturated fatty acid). It was noticed that there was no significant change ($p>0.05$) in fatty acid composition during repeated heating of RBO (Table 2 a). However, as the heating time increased up to 18 h, contents of saturated and unsaturated fatty acids were slightly affected. Stearic acid, the major SFA of RBO, did not show significant change even after 18 h. Among the unsaturated fatty acids, only linoleic acid content was found to decrease from 29.4 to 27.6% whereas oleic acid was seen to increase from 43.7 to 46.0%. Commonly, fatty acids with double bonds are liable for faster oxidation during heating. Arroya *et, al.*, reported that heat treatment of the oil induces modifications of fatty acids with two or three double bonds. The decrease in linolenic acid during repeated heating for 18 h is due to the occurrence of oxidative and thermal degradation (Garrido Polonio *et al.*, 1994; and Arroya *et al.*, 1995).

Trans Fatty Acid

Even after 18 h of heating, *trans* fatty acids were not detected.

Table 2a: Fatty Acid Composition (Weight %) and Iodine Value of Rice Bran Oil During Repeated Heating (180 ± 2 °C) and Cooling

Fatty Acid		Duration of Heating (h)						
		0 h	2 h	4 h	6 h	10 h	14 h	18 h
14:00	Myristic	0.30±0.01 [a]	0.30±0.05 [a]	0.30±0.05 [a]	0.30±0.02 [a]	0.30±0.01 [a]	0.30±0.01 [a]	0.30±0.01 [a]
16:00	Palmitic	22.7±0.05 [b]	22.1±0.01 [b]	22.0±0.01 [b]	21.9±0.05 [b]	22.2±0.01 [b]	22.7±0.02 [b]	23.2±0.02 [b]
18:00	Stearic	1.8±0.05 [c]	1.2±0.01 [c]	1.2±0.05 [c]	1.2±0.01 [c]	1.3±0.05 [c]	1.4±0.01 [c]	1. 4±0.01 [c]
18:01	Oleic	43.7±0.05 [d]	42.4±0.01 [d]	43.4±0.02 [d]	43.6±0.02 [d]	44.5±0.01 [d]	45.2±0.05 [d]	46.0±0.05 [d]
18:02	Linoleic	29.2±0.01 [e]	31.5±0.05 [e]	31.1±0.01 [e]	31.1±0.02 [e]	29.9±0.03 [e]	28.7±0.01 [e]	27.6±0.01 [e]
18:03	Linolenic	1.3±0.05 [f]	1.7±0.01 [f]	1.2±0.02 [f]	1.1±0.05 [f]	1.0±0.01 [f]	0.90±0.02 [f]	0.70±0.02 [f]
20:00	Arachidic	0.90±0.01 [g]	0.80±0.01 [g]	0.80±0.05 [g]	0.80±0.01 [g]	0.80±0.01 [g]	0. 80±0.01 [g]	0.80±0.02 [g]
SEM		0.0017	0.0012	0.0054	0.0021	0.0040	0.0031	0.0030

Table 2b

Iodine Value	100.2 ±0.05 [d]	98.3 ±0.02 [d]	96.7 ±0.05 [d]	93.0 ±0.05 [d]	89.9±0.01 [d]	86.9 ±0.05 [d]	82.0±0.02 [d]
SFA (%)	25.6 ±0.01 [a]	24.5±0.01 [a]	24.3 ±0.01 [a]	24 .2±0.02 [a]	24.6±0.05 [a]	25.2±0.05 [a]	25.7±0.01 [a]
MUFA (%)	43.7±0.01 [c]	42.4±0.05 [c]	43.4±0.02 [c]	43 .6±0.01 [c]	44.5±0.05 [c]	45.2±0.01 [c]	46.0±0.02 [c]
PUFA (%)	30.7±0.01 [b]	30.1±0.02 [b]	32.3±0.01 [b]	32.2±0.01 [b]	30.9±0.01 [b]	29.6±0.05 [b]	28.3±0.01 [b]
SEM	0.0021	0.0040	0.0050	0.0021	0.0053	0.0065	0.0050

Note: *Estimations done in triplicates (n = 3), SFA - Saturated fatty acids, MUFA - Monounsaturated fatty acids, PUFA - Polyunsaturated fatty acids. Values in the same column with different superscripts are significantly ($p \leq 0.05$) different, SEM - Standard Error Mean.

Drastic heating or frying condition (>200 °C and >24 h) should be required to generate *trans* fatty acids formation in the oil. A study dealing with heating of sunflower oil at 220, 240 and 270 °C for 5 h reported (Mishra *et al.*, 2011) no *trans* fatty acid formation at 220 °C. But, at 240 and 270 °C, the levels of *trans* fatty acids rose by 3% and 11% respectively. In our earlier work it was seen that continuous heating of rice bran oil at 180 °C did not induce formation of *trans* fatty acids (Franbkel, 1998). Similarly the heating conditions employed in our experiment did not lead to formation of *trans* fatty acids even after 18 h of repeated heating and cooling.

Free Fatty Acid

FFA was found to increase on heating. The percentage of FFA of fresh (unheated) RBO was 0.25%; subsequently it gradually increased and reached 0.93% after 18 h of heating (Figure 1). During high temperature heating of oils, the formation of FFA is due to the cleavage and oxidation of double bonds to form carbonyl compounds, which are subsequently oxidized to low molecular weight fatty acid (Baby Latha *et al.*, 2011). In the present study where the oil is heated in the absence of water/moisture, the hydrolysis of glycerides is negligible and the raise in FFA content is mainly due to oxidation. On the other hand, deep fat frying causes formation of FFA by hydrolysis as well oxidation. FFA is considered to be an indicator of oil quality in food industry as it leads to development of off-flavour in oils and fried foods. It has been reported that the increase in FFA level is not a very reliable parameter of degradation of frying oil, because it is difficult to differentiate between the FFA formed by oxidation and hydrolysis (Irwandi *et al.*, 2000).

Peroxide Value

The peroxide value of fresh RBO was 0.2 meq O_2/kg. After heating for 10 h it was found to increase to 7.5 meq O_2/kg, but, beyond 10 h there was a slight decrease. The values were 6.5, 6.3 meq O_2/kg for 14 and 18 h respectively (Figure 1). This indicates that the increase in PV is not related to duration of heating because peroxides are unstable and decompose at high temperature to carbonyls and other oxidation products. Peroxides are the prime initial reaction products of lipid oxidation. The oil initially forms hydroperoxide compounds, which are good indicator of lipid oxidation under normal condition. Lipid peroxides are relative stable at room temperature and in the absence of metals. However, at high temperatures; these compounds undergo further reaction to form alkoxy radicals which on further reaction form low molecular weight, ketones, acids, esters and short chain hydrocarbons. Hence, PV is not accepted as a reliable parameter to assess the frying oil quality (Che-Man *et al.*, 1998; and Ramadan *et al.*, 2006).

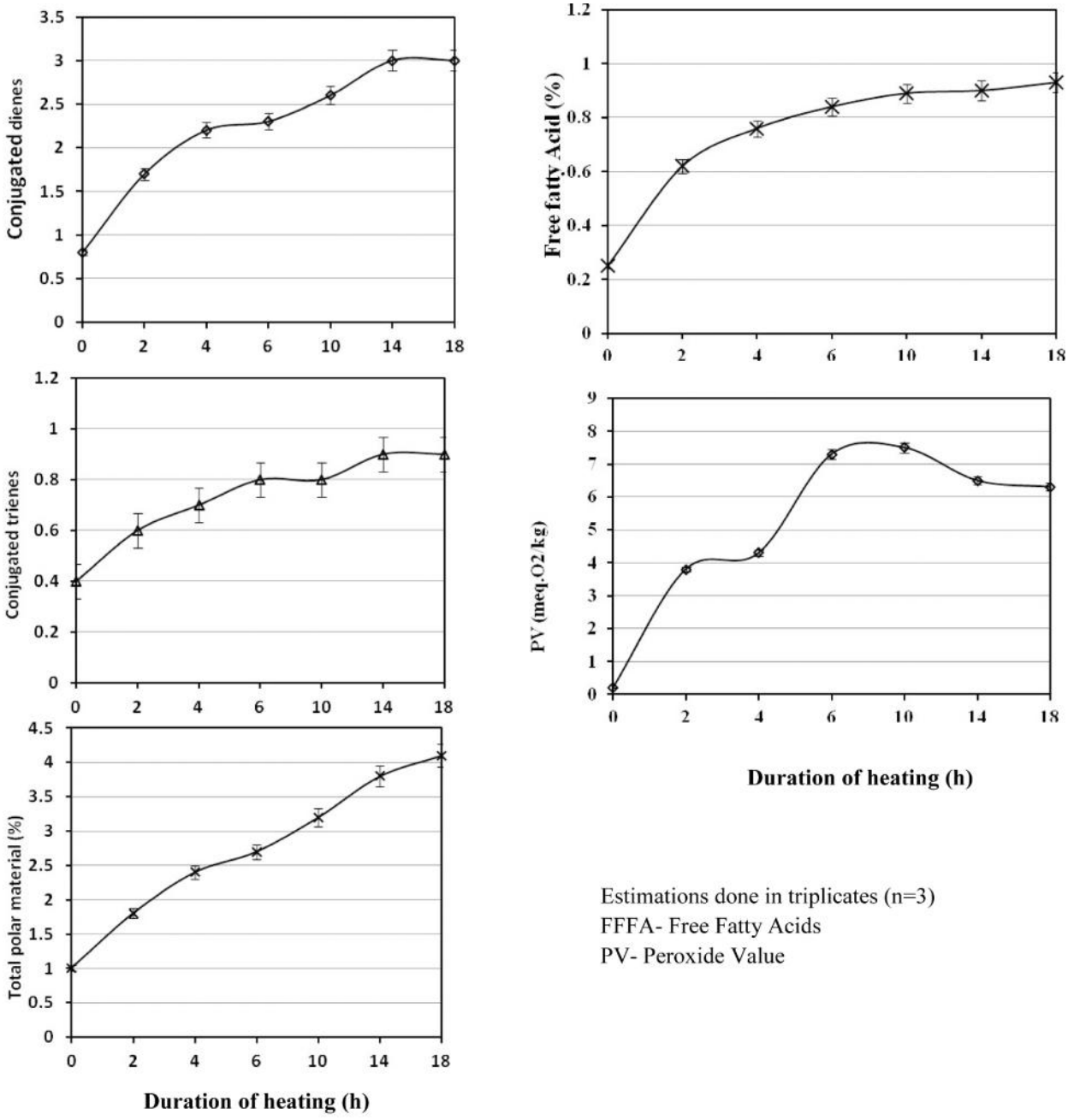
Anisidine Value

Secondary oxidation products are mainly non-volatile aldehydes, principally 2, 4-dienals and 2-alkenals (Sulieman *et al.*, 2006). Anisidine value is a measure of these oxidation products. AV was seen to increase from 5.04 to 19.41 during 18 h of heating. Initial stages of heating resulted in faster increase of AV and after 6 h of heating it was gradual. This could be due to further decomposition of the carbonyls and formation of polymers (Figure 2). However, aldehyde level is not the only parameter that indicates the extent of oxidation of the oil, the levels of various other oxidation products, such ketones and malonaldehyde also have to be taken into account.

Totox Value

Totox value [AV+2(PV)] represents the total oxidation of the oil. It is a measure of both peroxides and secondary oxidation products. It increased form 5.4-32.0 as the time of heating period increased form 2-18 h (Figure 2).

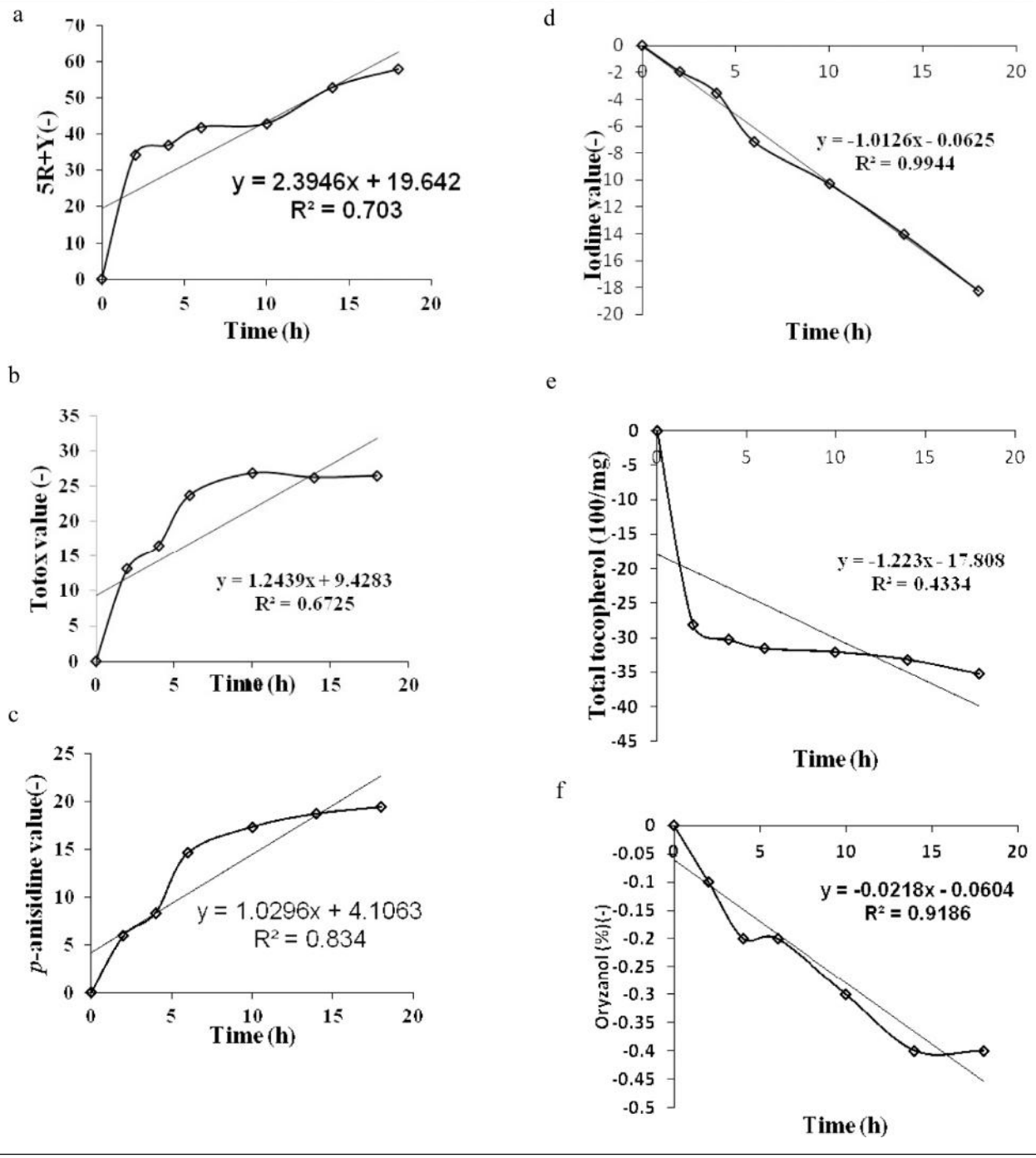
Figure 1: Thermo Oxidative Changes in Rice Bran Oil During Repeated Heating (180 ± 2 °C) and Cooling



Iodine Value

Changes in the IV during heating is shown in Ttable 2b. Initial value of 100.2 was found to gradually decrease to 98.3, 96.7, 93.0, 89.9, 86.9 and 82.0 after 2, 4, 6, 10, 14 and 18 h of heating respectively. A decrease in the IV is consistent with the decrease in double bonds due to oxidation. The greater the unsaturation (or higher IV), the more rapid the oil tends to be oxidized, particularly during deep fat frying (Tomkins *et al.*, 1999). Although RBO has high levels of unsaturated fatty acids the protective role of the natural antioxidants (Tocopherol, Tocotrienol and Oryzanol) resulted in a smaller decrease in the double bonds. The present results are in agreement with those reported in another study (Kim *et al.*, 2004).

Figure 2: Regression Equations Relating Time of Heating and Physico-Chemical Properties of Rice Bran Oil Due to Repeated Heating (180 ± 2 °C) and Cooling



Diene and Triene Value

Oxidation can shift the position of double bonds CD and CT structures are formed, which is indicated by UV absorption (Kun, 1990). The changes in the absorptivity at 230 nm and 270 nm are presented in Figure 1. When linoleic acid is oxidized to form hydroperoxides, a shift in one of the double bonds occurs, producing a conjugated diene that can be measured by UV absorbance at 232 nm. The

increment of absorptivity at 232 nm which is an indicator for the formation conjugated dienes, was significant in repeatedly heated RBO. UV absorbances at 232 nm substantially increased with heating time. The absorptivity at 270 nm, is an indicator for the formation of conjugated trienes, as well as unsaturated ketones and aldehydes. Changes in absorptivity at 270 nm presented a pattern similar to that of the absorptivity at 232 nm. As heating time continued the formation of hydroperoxides and their decomposition to conjugated triene is faster.

Total Polar Material

Figure 1 shows the increase in the TPM content of the repeatedly heated RBO. TPM content is an objective and valid criterion for the evaluation of deterioration of oils and fats during heating or frying. Oil breaks down to generate peroxides, acids and other free radicals, which may further cause some molecules to become polar reported by (Warner, 1996). Unlike AV, the raise in TPM was almost uniform indicating gradual build up of polar materials. The TPM content increased from 1.0% to 4.1% after 18 h and was correlated with the heating period. It was shown that build up of polar materials was concurrent to increase in viscosity during heating/frying (Xu *et al.*, 1999).

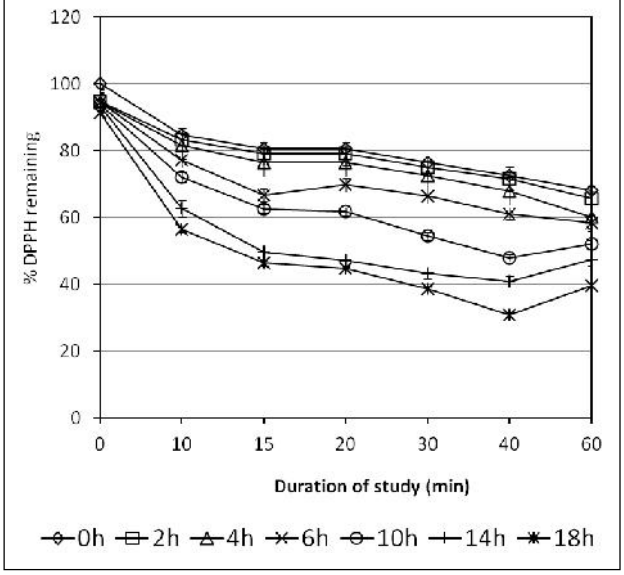
Regression

Linear regression method was applied to correlate attributes of oil with time of heating (Figure 2). An increase in time of heating increases colour value, totox and p-anisidine, while decrease occurs for iodine value, total tocopherol and oryzanol content. However, considering Figure 2 and the values of the correlation coefficients (r), it is concluded that time of heating has a good linear relation with iodine value and oryzanol content ($r = 0.91\text{-}0.99$, $p \leq 0.01$).

Anti Radical Activity

An antioxidant can be defined as any substance that when present at low concentration compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substrate (Stier, 2001). RBO contains natural antioxidants such as oryzanol (esters of ferulic acid), tocopherol and tocotrienol, which were able to quench free radicals in the heated oil samples to some extent (Figure 3). There are many tests/assays to express the antioxidant potency/ability to inhibit lipid oxidation under accelerated conditions. However, the model of scavenging stable free radicals is widely used to evaluate the antioxidant properties in a relatively short time, as compared to other methods.

Figure 3: Antiradical Activity of Rice Bran Oil During Repeated Heating (180 ± 2 °C) and Cooling



The loss of antioxidant activity of the heated oil may be attributed to the volatilization of antioxidant present in it through evaporation and/or decomposition (Augustin *et al.*, 1983). Tocopherol content was found to decrease from 48 to 12.1 mg, whereas the more stable oryzanol decreased from1.6 to 1.2% (Figure 2).

Opacity

Transparency of sample is an attractive feature of many foods. Table 3 shows the rheological changes of the repeatedly heated RBO and the opacity of the heated and un heated oil samples. It is an indicator of changes in performance of particulate in control systems. This was seemed to increase from 6.2 to 12.3% after 18 h of heating.

Viscosity

Viscosity is the resistance of a liquid to flow, and k- value is consistency index. Both these physical parameters were found to increase from 75.2 mPas to 135.3 mPas and 0.078 $mPas^n$ to 0.14 $mPas^n$ respectively. In the last stage of heating that is, from 14 to 18 h the viscosity was found to markedly increase from 117.3 to 135.3 indicating rapid build up of polymers and high molecular weight compounds. Increase in viscosity of heated oil is reported to be due to formation of higher molecular weight compounds involving carbon to carbon and carbon to oxygen to carbon bonds between oxidized fatty acids Ramadan *et al.* (2004). If n-value which gives the flow behavior index of the fluid

Table 3: Physical Changes in Rice Bran Oil During Repeated Heating (180 ± 2 °C) and Cooling

Duration of Heating (h)	Mean Values			
	Opacity (%)	Viscosity, η (mPas)	Consistency Index, k ($mPas^n$)	Flow Behavior Index, n (Dimensionless)
0	6.2±0.02 [a]	75.2±0.01 [a]	0.078±0.05 [a]	0.99±0.05 [a]
2	7.9±0.05 [b]	85.7±0.02 [b]	0.095±0.02 [c]	0.98±0.02 [b]
4	8.5±0.01 [c]	86.3±0.02 [b]	0.087±0.01 [b]	0.99±0.01 [c]
6	9.5±0.03 [d]	92.8±0.01 [c]	0.094±0.02 [d]	0.99±0.02 [c]
10	10.6±0.05 [e]	104.4±0.05 [d]	0.105±0.02 [e]	0.99±0.02 [c]
14	11.2±0.02 [f]	117.3±0.05 [e]	0.119±0.01 [f]	0.99±0.01 [c]
18	12.3±0.05 [g]	135.3±0.02 [f]	0.139±0.01 [g]	0.99±0.02 [c]
SEM	0.0023	0.0058	-0.0349	0.0011

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

Table 4: Upper-Half Correlation Matrix for Oxidative Changes in Rice Bran Oil Due to Repeated Heating and Cooling

	Colour Value 5R+Y	p-anisidine	Totox Value	Iodine Value	Total Tocopherol
p-anisidine	0.97**				
Totox value	0.95*	0.97**			
Iodine value	0.84*	-0.91**	-0.84*		
Total tocopherol	0.96**	-0.89**	-0.91**	0.66^{NS}	
Oryzanol	0.91**	-0.98**	-0.91**	0.95**	0.79*

Note: * Significant at $p \leq 0.05$, ** Significant at $p \leq 0.01$, NS - Non-significant at $p = 0.05$.

is <1, then the fluid is considered as pseudoplastic, or that it has shear thinning property. Our results showed (Table 3), that the n-values are 1 for the all heated oil samples indicating that the oil has similar results were observed by Lalas *et al.* (2006).

Correlation

The upper-half correlation matrix for oxidative changes in rice bran oil due to repeated heating and cooling is shown in Table 4. The parameter p-anisidine possesses a high correlation with totox value and colour value ($r = 0.97$, $p \leq 0.01$). However, p-anisidine has a high negative r value with tocopherol, iodine value and oryzanol meaning that an increase in p-anisidine decreases these three parameters.

CONCLUSION

The results of this study suggest that early stages of heating caused more deterioration in colour and chemical parameters such as free fatty acids, peroxide value, total polar compound, CD, and CT. Antiradical activity decreased due to degradation of antioxidant at heating temperature employed in this study. *p*-anisidine value was negatively correlated with IV and contents of tocopherol and oryzanol. Oryzanol was more heat stable than tocopherol. The polar materials correlated with the increase in viscosity. Thermal flow behaviour showed that the oil is Newtonian fluid having shear thinning behavior. The marginal changes were observed in fatty acid composition mainly in unsaturated fatty acids.

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