Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 2397-2407 | ISSN:2251-6727

A Physiological Impact: Understanding the Health Risks of Thermo-Oxidized Rice Bran Oil

Ravi Pratap[1,2], Rahul Kumar Maurya[1*], Ramesh Chandra Dubey[3], Mohammad Yasir[4], Alok Kumar Shukla[5]

[1]Amity Institute of Pharmacy, Amity University Uttar Pradesh, Lucknow Campus, Lucknow -226028, India

[2]Department of Pharmaceutical Sciences, Gurukul Kangri (Deemed to Be University), Haridwar, Uttarakhand -249404, India

[3]Department of Botany and Microbiology, Gurukul Kangri (Deemed to Be University), Haridwar, Uttarakhand - 249404, India

[4]School of Pharmacy, ITM University, Jhansi Rd, Turari, Gwalior, Madhya Pradesh -474001, India

[5]Babu Sunder Singh College of Pharmacy, Nigoha, Uttar Pradesh -227309, India

***Corresponding author-** Dr. Rahul Kumar Maurya, Associate Professor, Department of Chemistry, Amity Institute of Pharmacy, Amity University Uttar Pradesh, Lucknow Campus, Lucknow -226028, India

(Received: 28 January 2026 Revised: 16 March 2026 Accepted: 09 April 2026)

KEYWORDS

Oxidative stress, peroxide value, SOD, gas chromatography

ABSTRACT:
Thermal processing alters the chemical composition and health implications of vegetable oils, which are essential to the diet. This study investigates the compositional alterations and physicochemical characteristics of rice bran oil after 4 and 8 hours of thermal treatment at 180°C, utilizing gas chromatography. The observed values for normal and thermo-oxidized oil ranged as follows: acid value (0.36–2.39), specific gravity (0.9013–0.8893), refractive index (1.461–1.512), p-anisidine value (4.95–19.49), saponification value (182–194), peroxide value (0.56–8.5), and iodine value (104–89). Gas chromatography showed trans fats and substantial monounsaturated and polyunsaturated fatty acid alterations. In Wistar rats, a preclinical study showed alterations in oxidative stress indicators such as glutathione, catalase, SOD, and TBARS, as well as lipid profiles and hepatic enzyme levels. These results suggest that consumption of thermo-oxidized rice bran oil may have deleterious physiological effects, highlighting the importance of dietary oil quality in public health.

Introduction

Vegetable oils are essential to the human diet because they supply essential fatty acids, help absorb fat-soluble vitamins, and give us energy. Customer preferences are greatly impacted by the oil's stability and quality [1].

Rice bran oil (RBO) distinguishes itself from other edible oils due to its abundant natural antioxidants, which contribute to its extended shelf life and superior resistance to oxidation. Rice bran oil, after being processed, has a level of oxidative stability that is five times higher than that of groundnut oil [2]. Rice bran oil possesses a more potent cholesterol-lowering effect despite having fewer poly-unsaturated fatty acids than other common oils [3]. Using solvent extraction and cold pressing techniques, RBO is extracted from the outer layer of the grains of rice. Its mild flavour and smell make it useful in a variety of culinary applications. It has a neutral flavour, so it blends nicely with other foods [4].

RBO is suitable for high-temperature cooking methods like deep-frying, and stir-frying because of its smoke point, which is around 450°F/232°C. Rice bran oil has gained popularity owing to its extensive usage in cooking and well-documented health advantages, mostly attributed to its elevated levels of antioxidants [5]. Rice bran oil's health advantages include reducing inflammation, decreasing Low density lipoprotein (LDL) cholesterol, and improving heart health. The antioxidant properties may shield cells from free radical damage [6]. Skin and hair care products use rice bran oil, which is known for its ability to hydrate and moisturise. This

ingredient is often found in hair shampoos, lotions, creams, and makeup [7]. Rice bran oil is a sustainable byproduct derived from milling rice, as it utilizes a portion of the grain that would have been discarded otherwise. The environmental impact of rice farming and processing should not be disregarded [8]. Avoid oxidation and extend the shelf life of rice bran oil, it should be stored in a cool, dark location away from sunlight. Rice serves as a fundamental food source for almost half of the people in the area and is extensively cultivated. In 2014, the worldwide production of paddy rice amounted to 678 million metric tonnes [3]. Driven by the growing use of thermo-oxidized oil and rice bran oil in human diets, this study aims to address the knowledge gap with regards to their safety implications.

The toxicological effects of the oil need to be investigated following extended periods of heating. This study examines the toxicological effects of thermo-oxidized rice bran oil in animals using a comprehensive approach that includes physicochemical measurements and extensive preclinical analysis to replicate typical consumption scenarios.

Material and Methods

The investigation employed analytical-grade chemicals and rice bran oil that was acquired from Patanjali Ayurved Ltd. Sonipat, Haryana.

Generation of oil samples

The rice bran oil samples in measured amounts equal to 4 L were heated to 180 °C for 4 and 8 hours, respectively, prior to cooling to room temperature and being stored in amber-coloured bottles at 4°C in a refrigerator [8].

Impact of thermal oxidation on the physicochemical parameter of rice bran oil

Determination of acid value (AV) and percent free fatty acid

A titrimetric method was used to find the AV. In this method, 2 g of oil was carefully weighed into a 250 mL conical flask. Then, 50 mL of ethyl alcohol that had been neutralised to phenolphthalein was added, and the mixture was heated until it dissolved well. Using established formulas and titration data from 0.1 M potassium hydroxide (KOH) as the titrant and phenolphthalein as the indicator, we found the acid value (AV) and the percentage of free fatty acids.

$$Acid\ value = \frac{\mathrm{A \times M \times 56.1}}{\mathrm{W}} \quad \text{(i)}$$

Where W is the weight (g) of the oil sample, M is the molarity of KOH, and A is the amount (mL) of 0.1M KOH consumed by the sample [9].

$$\%\ Free\ fatty\ acids = \frac{\text{Acid Value}}{2} \quad \text{(ii)}$$

Specific gravity of the rice bran oil samples

A dry pycnometer was used to measure specific gravity. By comparing the density of oil and water, the specific gravity of the former was ascertained. The pycnometer was filled with distilled water, and an electronic balance was used to weigh it. The same method was used to measure the weight of oil. To stop air from leaking into the pyrometer, precautions were adopted. Using Equation (iii), the specific gravity value was calculated as follows. [10,11].

$$specific\ gravity = \frac{\text{weight of the oil (g)}}{\text{Weight of distilled water(g)}} \quad \text{(iii)}$$

Determination of refractive index

Using an Abbe refractometer, the refraction index was calculated using the AOCS Cc 7–25 method (AOCS, 1998) [12,13].

Determination of p-Anisidine value

The p-anisidine value is ascertained using the AOCS (1992) method. Cd 18-90 was analysed to determine the p-anisidine value. A 25 mL volumetric flask was filled with 1.0±0.5 g of thermo-oxidized oil, and iso-octane was added to make up the remaining volume. A shimadzu UV-1800 spectrophotometer, manufactured in Japan, was used to measure optical density at 350 nm using iso-octane as a blank. 5 mL of oil and 1 mL of p-anisidine reagent were combined in a 10 mL graduated test tube. Ten minutes following the reaction, the OD was measured [14].

$$p - \text{anisidine value} = \frac{25 \times (1.25\ \mathrm{As} - \mathrm{Ab})}{\mathrm{W \times m}} \quad \text{(iv)}$$

where m is the mass of the test sample in g, As is the absorbance of the fat solution after it has interacted with the p-anisidine reagent, and Ab is the absorption of the fat solution, w is the weight of the sample.

Determination of saponification value

The IUPAC standard method, seventh edition (1987), was used to compute the saponification value. For saponification, 1 g of oil or fat needs a certain amount of KOH (measured in milligrams). The alcoholic KOH solution, 0.5 N HCl, and phenolphthalein indicator are required. For one hour under reflux, 2.5 g of oil and 25 mL (0.5 N) of ethanolic KOH react. The pink colour vanished, signalling the end. The peroxide values of the oil samples were calculated using the titration method and the equation (v) [15].

$$Saponification\ value\ = \frac{56.1 \times N\ (X-V)}{W} \quad (v)$$

where X is the amount of HCI used in the blank (mL), V is the volume of HCl used in the sample, N is the HCI normality, and W is the sample weight in g.

Smoke point of the oil

Smoke point indicates the temperature at which it starts to release using the clevis and open cup method as described in AOCS Official Method Cc 9a-48, the smoke point was determined. Prior to heating, a thermometer was placed in the vertical centre, a beam of light was placed across the centre, and the sample was filled to the filling line in the cleveland Open Cup. The cup's temperature was raised to 50°C initially, then by 5°C every minute after that. When a thin, continuous stream of blue smoke emerged, the sample had reached its smoke point [16].

Determination of Peroxide value

The peroxide value is determined by measuring the amount of iodine generated when peroxides (in oil) react with iodide ions. The excess acetic acid in the process absorbs the base that is created. Sodium thiosulfate is used in a redox titration to detect the iodine. The peroxide value was calculated by using the following equation vi

$$Peroxide\ value\ = \frac{S \times N \times 1000}{W} \quad (vi)$$

S is titration value, N is normality of sodium thiosulphate, and W is weight of oil sample [15,16].

Determination of Iodine value

The standard IUPAC method (1987 edition) was used to determine the iodine value, which represents the degree of unsaturation in fats and oils. 20 mL of carbon tetrachloride were used to dissolve 0.2 g of the oil sample, which had been weighed into an iodine flask for the analysis. Next, 0.2 N Wij's reagent (25 mL) was added. After being stoppered and gently shaken, the flask was left in the dark for an hour. Following this time frame, 0.2 g of the oil sample were weighed into an iodine flask for analysis and dissolved in 15 mL of 10% carbon tetrachloride. Next, 0.2 N Wij's reagent (25 mL) was added. After being stoppered and gently shaken, the flask was left in the dark for an hour. After titrating the released iodine with 0.1 N sodium thiosulphate until the blue tint (from the starch-iodine complex) disappeared, 15 mL of a 10% potassium iodide solution was added. Equation (vii) was then used to determine the iodine value.

$$Iodine\ value\ = \frac{(B-S) \times N \times 12.69}{W} \quad (vii)$$

where S is the volume of 0.1 N $Na_2S_2O_3$ required to titrate the sample (mL), B is the volume of 0.1 N $Na_2S_2O_3$ required to titrate the blank (mL), and N is the normality of $Na_2S_2O_3$, W is the sample weight in gm [17].

Analysis of oil samples through GC-FID

In the procedure of Gas chromatography-flame ionization detector (GC-FID) take test tube, 50–100 mg of oil and 200 mg of anhydrous sodium sulphate are weighed. Next, 2.0 mL of n-Hexane, 200 mg of sodium chloride, and 5.0 mL of sulphuric acid in methanol are added. The mixture is incubated after 5 minutes at 80°C for 15 minutes. This process is preceded by extraction using petroleum ether or diethyl ether in either an alkaline or acidic environment. For ten minutes, this mixture was centrifuged at 5000 RPM. About two minutes were spent stirring the mixture. In order to prepare for GC analysis, the hexane fraction was extracted using a micropipette, filtered through a 0.22 µm syringe filter, and then transferred to a glass vial.

Instrument condition for GC-FID

The GC-FID system was optimised using a 100 m x 0.25 mm ID x 0.20 µm HP-88 column, 1.0 mL injection volume, 20:1 split ratio, 200 °C intake temperature, 280 °C detector temperature, a maximum temperature of 260 °C, and an air flow rate of 400 mL/min. The oven operated at 40 mL/min for hydrogen gas and 25 mL/min

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 2397-2407 | ISSN:2251-6727

for makeup gas for 70.667 minutes. The peaks in the sample chromatogram (peaks on the same RT as observed in the FAME standard) were integrated following the completion of the chromatographic run [18].

Evaluation of thermally oxidized rice bran oil's toxicological effects in vivo

The 8–10-week-old Wistar rats, weighing between 100 and 120 g, were provided by an albino male from the animal house of Sri Satya Sai College of Pharmacy, SSSUTMS, Sehore, Madhya Pradesh, India. All animals were housed in plastic cages with iron lids and soft wood-chip bedding in a controlled environment with a temperature range of 22 to 26 °C, a humidity range of 40% to 70%, and a 12-hour light/12-hour dark cycle. They were also fed a typical laboratory pellet diet and had unlimited access to water [19].

Each group of six animals participated in the study. For 30 days, the animals were fed various oil samples combined with 15 g of regular pellet feed every day. The control group received normal oil, while groups 2-4 received oils that had been heated to 180°C for 4 and 8 hours, respectively. Blood samples were extracted into test tubes without anticoagulant for serum separation before being sacrificed under anaesthesia for biochemical tests, including SGOT, SGPT, Total Cholesterol, Triglycerides, High Density Lipoprotein (HDL), LDL, Glucose, and haematological parameters, including haemoglobin (Hb), RBC count, WBC count, platelet count, and antioxidant enzyme levels in organ tissues. These samples were examined using an auto-blood analyser (Biogen Scientific, India).

Assessment of activity of antioxidant enzyme in tissue organ

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity in tissue supernatant was assessed. After adding enzyme extract at 420 nm, the inhibition rate of pyrogallol auto-oxidation was measured using the Spectramax Plus spectrophotometer (Molecular Devices, USA). One enzyme unit results in a 50% inhibition of pyrogallol auto-oxidation. IU/mg protein units were used to measure the enzyme activity. [20]

Catalase

We used a spectrophotometer to test the catalase activity. The absorbance drop at 240 nm for one minute was noted. The definition of an enzyme unit is one µmol of H_2O_2 used per minute. The amount of protein was expressed in parts per million [20].

Glutathione peroxidase

The enzyme activity of glutathione peroxidase (GPx) is defined as the consumption of 1 µmol NADPH per minute. GPx activity was measured in U/mg protein units [21].

Thio barbituric acid reactive substances

0.1 mL of homogenate, 1 mL of 10% trichloroacetic acid, and 1 mL of 0.67 percent thio barbituric acid were added to each test tube. After that, the tubes were placed in a boiling water bath with a lid on and heated for 20 minutes. The test tubes were immersed in a bath of broken ice and centrifuged for ten minutes at 6000 rpm. The absorbance of the supernatant was measured at 540 nm [21].

Statistical analysis

The results of the physicochemical parameters for antioxidant enzyme levels, blood biochemistry, and haematological parameters were statistically analysed and displayed using Graph Pad Prism 10 (Graph Pad Software, Inc., La Jolla, CA, USA) and one-way ANOVA. The results were shown as mean ± SD. Every parameter was estimated twice for every sample, and the mean of the data was given for both the treatment group and each sample. $P < 0.05$ was set as the significant threshold.

Ethics statement

College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, Opposite Oil Fed Plant, Pachama, NH-18, Sehore, Madhya Pradesh, IAEC permission number 1587/PO/Re/S/11/CPCSEA.

Results

Comparison of untreated and thermo-oxidized rice bran oil's physical characteristics

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 2397-2407 | ISSN:2251-6727

To determine the effect of thermal degradation, the physicochemical characteristics of samples of normal and thermally oxidized rice bran oil were compared. The normal, 4-hour, and 8-hour heated oil samples' acid values were determined to be 0.36 ± 0.22, 1.12 ± 0.02, and 2.39 ± 0.19, respectively, while the corresponding percentages of free fatty acid were 0.185 ± 0.011, 0.56 ± 0.02, and 1.19 ± 0.21. Specific gravity values declined with heating, recorded at 0.92 ± 0.001, 0.9013 ± 0.003, and 0.8893 ± 0.002. Smoke points decreased from 232 ± 1.71°C in the normal sample to 210 ± 1.5°C and 202 ± 2.5°C in the 4-hour and 8-hour samples, respectively. Refractive index values increased progressively from 1.461 ± 0.004 to 1.5013 ± 0.003 and 1.5123 ± 0.002. The p-anisidine values, indicating secondary oxidation, also rose significantly from 4.95 ± 0.10 to 12.2 ± 0.30 and 19.49 ± 0.23. Saponification values were found to be 182 ± 0.39, 188 ± 0.41, and 194.45 ± 0.41, while peroxide values, a marker of primary oxidation, increased from 0.56 ± 0.34 to 4.5 ± 0.21 and 8.5 ± 0.34. A decreasing trend was observed in iodine values, reported as 104 ± 0.036, 95.8 ± 0.31, and 89.58 ± 0.05, indicating a reduction in unsaturation levels. These parameters, summarized in Table 1, serve as critical indicators of the extent of thermal oxidation in rice bran oil. The observed variations confirm the degradation of oil quality due to prolonged heating. (Table 1).

GC-FID analysis

Fig. 1 displays the GC-FID analysis of regular rice bran oil. It was determined that the fatty acids in RBO, RBO4, and RBO8 were found to be polyunsaturated (33.23%, 28%, 23.66%), monounsaturated (43.17%, 46.35%, 48.12%), and saturated (23.59%, 25.56%, 28.02%), respectively. The heating process changes the amounts of trans and saturated fatty acids (Table 2).

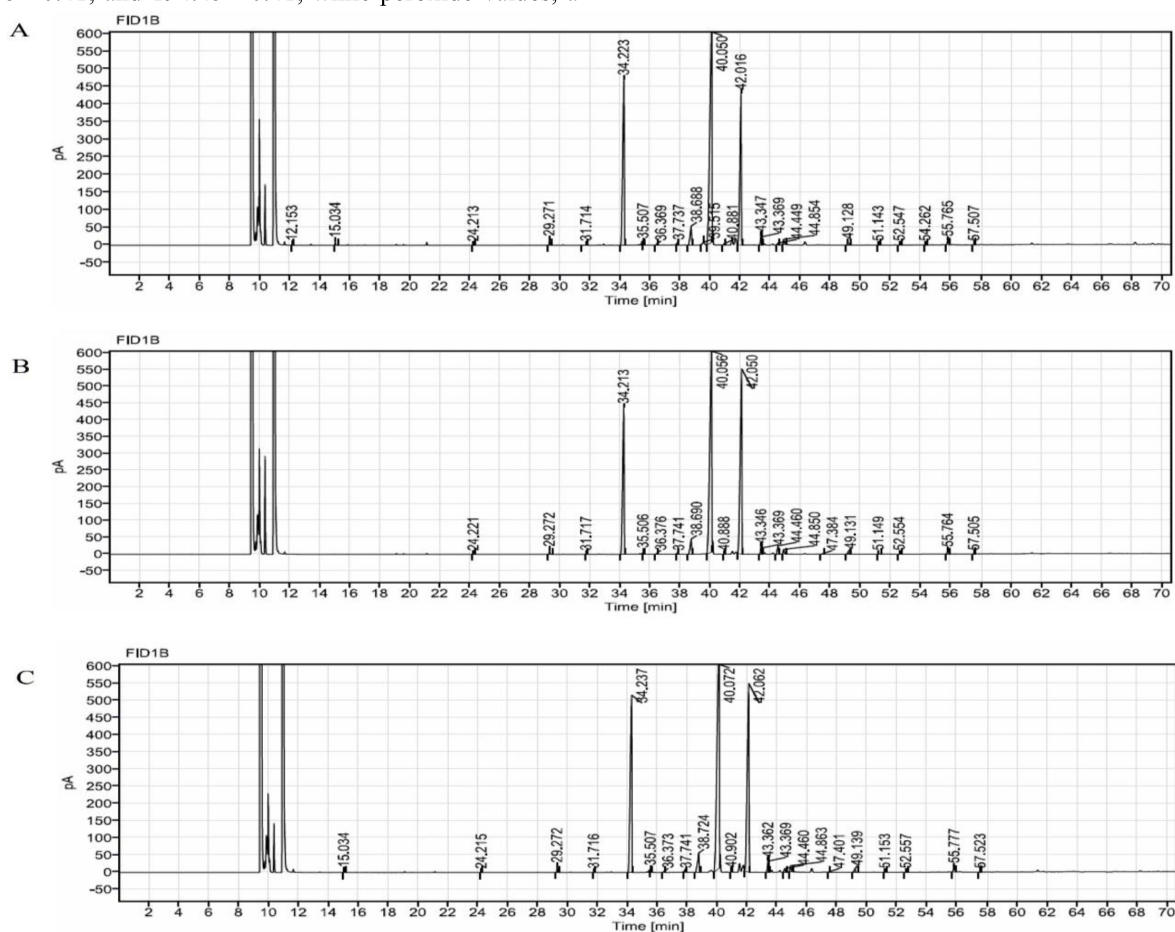

Fig. 1. GC-FID analysis of A) normal; B) 4 h thermally oxidized and C) 8 h thermally oxidized rice bran oil samples

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 2397-2407 | ISSN:2251-6727

Table 1. Physicochemical characteristics of normal and thermo-oxidized Rice bran oil samples

Oil sample	Acid value	% free fatty acid	Specific gravity	Smoke point	Refractive Index	*p*-Anisidine value	Saponification value	Peroxide value	Iodine value
RBO	0.36±0.220	0.185±0.011	0.92±0.001	232±1.71	1.461±0.004	4.95±0.10	182±0.39	0.56±0.34	104±0.03
RBO4	1.12±0.02	0.56±0.02	0.9013±0.003	210±1.5	1.5013±0.003	12.2±0.30	188±0.41	4.5±0.21	95.8±0.31
RBO8	2.39±0.19	1.19±0.21	0.8893±0.002	202±2.5	1.5123±0.002	19.49±0.23	194.45±0.41	8.5±0.34	89.58±0.05

Table 2: Percentage of saturated, monounsaturated and polyunsaturated fatty acids

Name of the oil	SFA	MUFA	PUFA	Trans fat
RBO	23.59	43.17	33.23	0.02
RBO4	25.56	46.35	28.00	0.10
RBO8	28.02	48.12	23.66	0.20

* SFA-Saturated fatty acids, MUFA-Monounsaturated fatty acids, PUFA -Polyunsaturated fatty acids

Analysis of biochemical parameters

In Blood biochemical examination of lipid showed that group IV (RBO8) had a considerably higher total cholesterol level (148.41 ± 3.00) than group III (RBO4) (108.13 ± 6.59) and group II (RBO) (92.20 ± 1.49). The cholesterol level in group IV was somewhat higher than in group II when compared to the normal control group I (85.13 ± 1.69). Additionally, group IV (RBO8) had greater triglyceride levels (150.66 ± 6.81) than group III (RBO4) (104.20 ± 6.05) and group II (RBO) (80.20 ± 1.52). However, group I exhibited the lowest triglyceride level (62.16 ± 2.33) compared to group II (RBO). HDL cholesterol was significantly lower in group IV (RBO8) (19.12 ± 2.09) compared to group III (RBO4) (31.13 ± 2.05) and group II (RBO) (34.72 ± 2.19). However, the HDL value in group I (43.17 ± 3.28) was higher than in group II (RBO). LDL levels were elevated in group IV (RBO8) (105.90 ± 4.15) compared to groups III (RBO4) (85.22 ± 4.04) and II (RBO) (60.12 ± 1.70). In contrast, group I had a slightly lower LDL value (51.32 ± 1.86) than group II (RBO). The VLDL value was higher in group IV (RBO8) (31.89 ± 1.57) compared to groups III (RBO4) (21.38 ± 1.20) and II (RBO) (16.39 ± 1.00). Group I had a slightly lower VLDL value (13.29 ± 0.91) than group II (RBO) (Fig. 2). Liver function tests indicated that group IV (RBO8) had significantly higher alanine transaminase (ALT) levels (91.32 ± 1.15) compared to group III (RBO8) (72.06 ± 1.50) and group II (RBO) (50.50 ± 0.99). Conversely, group I exhibited slightly lower aspartate transaminase (AST) levels (40.53 ± 5.84) compared to group II (RBO) (Fig. 3).

Effect on haematological parameter

The haemoglobin level in group IV (RBO8) was significantly decreased (9.63 ± 0.45 g/dL) compared to group III (RBO4) (11.22 ± 0.58 g/dL) and group II (RBO) (14.09 ± 0.37 g/dL). The control group I exhibited a higher haemoglobin value (16.63 ± 0.31 g/dL), with group II remaining within the normal range. Group I

showed a slightly higher haemoglobin concentration compared to group II (RBO) (Fig. 2). Red blood cell (RBC), white blood cell and platelet counts did not significantly change in any of the groups (Fig. 4 and 5). However, group IV (RBO8) had significantly higher blood glucose levels (203.34 ± 6.43 mg/dL) than group III (128.23 ± 2.44 mg/dL) and group II (103.44 ± 1.01 mg/dL). Additionally, this result was higher than the control group I's glucose level (94.71 ± 3.62 mg/dL) (Fig. 2).

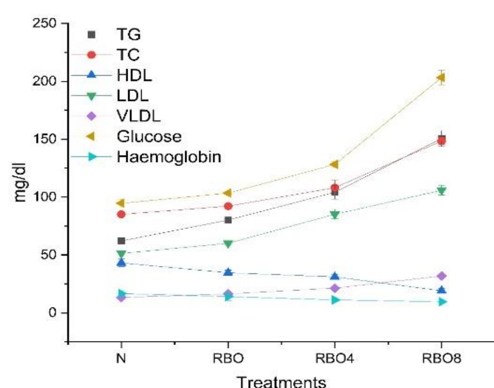

*Fig. 2. Thermo-oxidized rice bran oil (RBO) and regular fresh oil were subjected to biochemical analysis under various conditions. Oil samples were prepared by thermal oxidation at 180°C for 4 and 8 hours. Four animal groups—normal, RBO, RBO4, and RBO8—were created. Rats in the normal/control group received regular water and normal feed for 30 days, while the other groups were exposed to treated oil with the feed. Blood samples were taken following treatment, and haematological and biochemical parameters were assessed. The biochemical parameters of Triglycerides, total cholesterol, HDL, LDL, VLDL, Creatinine, glucose, and haemoglobin were examined with an Autoanalyzer. The outcome is represented by mean ± standard deviation (n=6). #P<0.001, *P<0.05, **P<0.01, relative to the control group.*

Antioxidant enzyme results in heart and liver

The heart's mean glutathione levels were significantly lower in the RBO8 and RBO4 groups than in the normal control group (P<0.001). The RBO group's GSH levels stayed the same.

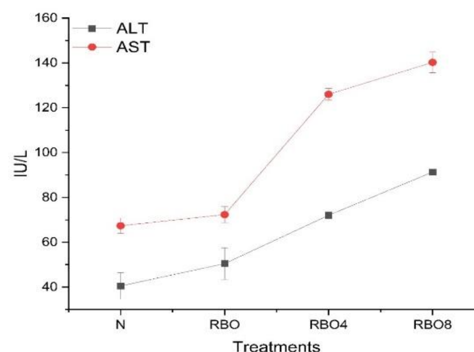

*Fig. 3. Liver enzymes from groups treated with thermo-oxidized rice bran oil (RBO) and regular fresh oil under various conditions. The outcome is given as mean ± SD (n=6). # P<0.001, * P<0.05, ** P<0.01 in comparison to the control group.*

In contrast to Fig. 6a, the normal control group. Figure 6b shows that the mean TBARS levels in the RBO8 and RBO4 groups were considerably (P<0.001) higher than those in the normal control group. In the RBO8 and RBO4 groups the mean SOD levels were significantly (P<0.001) lower than those in the control group (Fig. 6c). Catalase levels were significantly (P<0.001) lower in the RBO8 and RBO4 groups than in the normal control group (Fig. 6d).

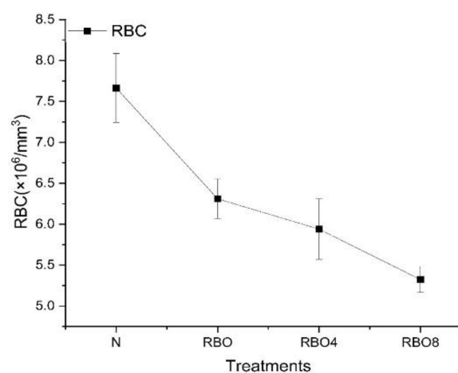

*Fig. 4. RBC count of the groups treated with thermo-oxidized rice bran oil (RBO) and regular fresh oil under various conditions. Mean ± SD (n=6) is the result's expression. * P<0.05, ** P<0.01, and # P<0.001 in comparison to the controlled group.*

GSH levels in the livers of the normal control group were significantly (P<0.001) higher than the GSH levels in the livers of the RBO8 and RBO4 groups. GSH levels in the

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 2397-2407 | ISSN:2251-6727

RBO group were significantly lower (P<0.05) than in the normal control group (Fig. 6 A). Compared to the control group, the RBO8 and RBO4 groups' mean TBARS values were significantly (P<0.001) higher (Fig. 6 B). The mean SOD levels in the RBO8 and RBO4 groups were significantly (P<0.001) lower than those in the normal control group (Fig. 6 C). Compared to the normal control group, the mean catalase levels in the RBO8 and RBO4 groups were significantly lower (P<0.001) (Fig. 6 D).

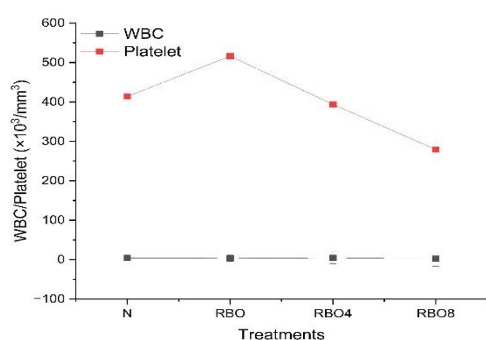

*Fig. 5. WBC and Platelets count of the groups treated with thermo-oxidized rice bran oil (RBO) and regular fresh oil under various conditions. The outcome is given as mean ± SD (n=6). In comparison to the Normal group, # P<0.001, * P<0.05, and ** P<0.01.*

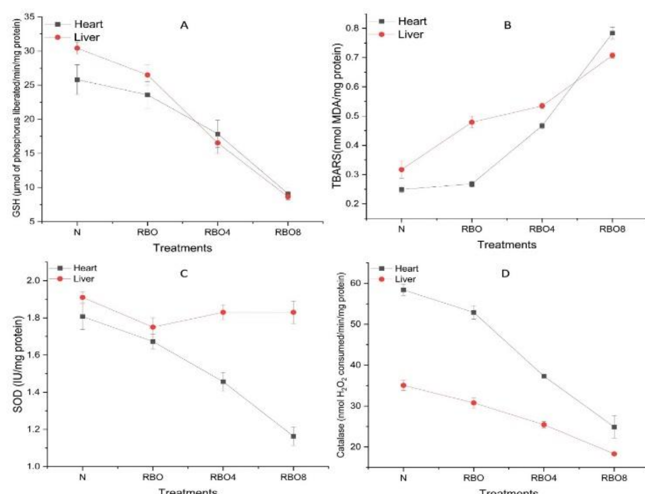

*Fig. 6. (A) GSH levels in the liver and cardiac tissue of groups treated with thermo-oxidized rice bran oil (RBO) and normal fresh oil under various conditions. Following therapy, the rats' liver and heart tissue GSH levels were assessed. GSH levels dropped, indicating tissue oxidative injury. (B) The amount of TBARS in the heart and liver tissue of the groups treated with thermo-oxidized rice bran oil (RBO) and regular fresh oil under various conditions. Following therapy, the rats' liver and heart tissue were examined for TBARS levels. (C) SOD levels in the liver and heart tissue of groups treated with thermo-oxidized rice bran oil (RBO) and conventional fresh oil under various conditions. TBARS values dropped, indicating tissue oxidative injury. (D) The amount of catalase in the liver and cardiac tissue of the groups treated with thermo-oxidized rice bran oil (RBO) and regular fresh oil under various conditions. Following therapy, the rats' liver and heart tissue's catalase levels were assessed. Decreases in catalase levels indicated oxidative tissue injury. All the result are expressed as mean ± SD (n=6). # P<0.001, * P<0.05, ** P<0.01, as compared to the normal group.*

Effect on histopathological studies in heart and liver

Rats subjected to RBO8 showed mild damage to their heart tissue, as evidenced by fewer fatty particle-containing lesions than normal rats, undamaged heart tissue. Following by RBO4, fatty particles were discovered in the treated group's heart cells. The rats which administered only RBO showed a consistent structure without any abnormal changes (Fig. 7: A-D). Rat hepatocytes treated with thermally heated RBO8 showed an increase in the size of lipid vacuoles and a decrease in the quantity of fatty particles in comparison to the normal control group. Fatty particles were seen in hepatocytes following RBO4 group. The liver morphology of the group given regular rice bran oil appeared to be normal in Fig. 7 (E-H).

Discussion

Vegetable oil is a vital dietary component in everyday use. Thermally oxidised oil is often used in food preparation as a means of cost reduction. The advantages of vegetable oil may be negated by prolonged heating that triggers lipid oxidation. The quality of dietary oils and fats has a considerable impact on the development of harmful health disorders [21]. High cooking temperatures and fat content boost the production of mutagens in meals, while heated lipids increase membrane macromolecules' peroxidation, mutagenicity, genotoxicity, and potential cancer development. In food preparation, reusing oil is a widespread practice. Large city restaurants, like roadside food vendors, use this method to reduce costs [22].

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 2397-2407 | ISSN:2251-6727

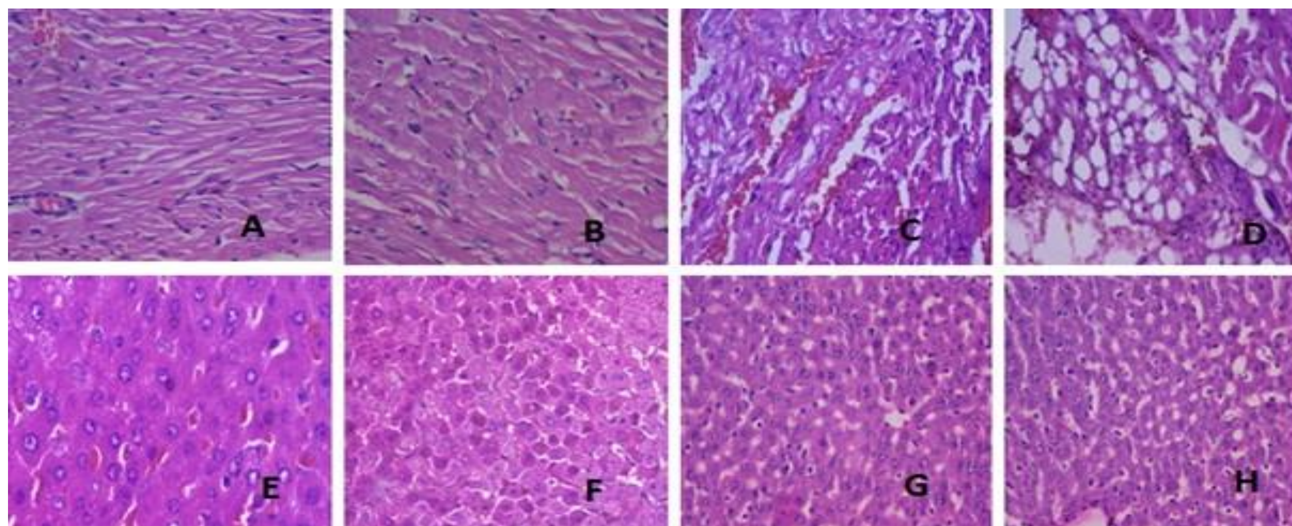

Fig. 7. Histopathological analysis of the albino wistar rat's heart (A-D and liver tissues (E-H)) after normal and thermo-oxidized oil treatment.

The study aimed to reveal the potential harm on rat health from consuming thermally oxidized rice bran oil. The physicochemical characteristics of oil determine its nutritional and physical attributes. Iodine value, peroxide value, saponification value, free fatty acid, and color appearance are characteristics of fats and oils. To preserve the oil's quality, vegetable oils must be stored correctly to prevent deformation. This is because their shelf life may be shortened due to their susceptibility to oxidative degradation. Oils and fats are a common component of daily life. As a major source of energy, fats and oils are vital components of human diets. In addition to their use in traditional medicine and cooking, edible oils are vital sources of non-synthesized fatty acids that maintain the integrity of cell membranes. They aid in the synthesis of prostaglandins, which are essential body chemicals. Vegetable oils are favourably received for their cholesterol-lowering properties. Unsaturated vegetable oils contrast unsaturated animal fats by being notably more chemically reactive. Depending on regional conditions, vegetable oils are vital for global nutrition and come in various qualities [23].

Acid value, free fatty acid, refractive index, saponification value, p-anisidine, and iodine value, peroxide value of RBO changed when heated at 180±2 °C, potentially due to oil degradation. The physicochemical attributes of the thermally oxidized oil underwent substantial alterations.

According to the GC-FID analysis, MUFA (palmitoleic acid methyl ester (C16:1), cis-10-Heptadecenoic acid methyl ester (C17:1), oleic acid methyl ester (C18:1n9c), cis-11-Eicosenoic acid methyl ester (C20:1), nerveonic acid methyl ester (C24:1n9), and trans fats (trans-9-octadecenoic methyl ester (C18:1n9t) as well as linolelaidic acid methyl ester (C18:2n6t) are formed. Lipid peroxidation in rice bran oil was indicated by the synthesis of trans fats.

Rat blood samples from the RBO4 and RBO8 groups showed significantly higher levels of glucose, total cholesterol, LDL, triglycerides, VLDL, AST, and ALT than those from the RBO and control groups. The need to improve these detrimental health factors warrants serious consideration [24]. No previous research has examined blood biochemistry in rats administered thermally oxidized rice bran oil. In this study, rat blood cells hematological levels were altered following consumption of heated vegetable oil. Hageman et al., reported similar results [25]. According to reports, there is no influence on the proportions of blood cells in animals that consume heated maize and peanut oils. In tissues and plasma, antioxidant capacity is diminished by obesity [26].

The current study reported reduced glutathione levels in rats given RBO8. Free radicals and reactive oxygen species are neutralized by enzymatic antioxidants like SOD and Catalase. Thermally oxidized RBO

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 2397-2407 | ISSN:2251-6727

significantly damages the liver and heart by reducing the activity of antioxidant enzymes.

Conclusion

Heating has a significant effect on the physical and chemical properties of oil. This study emphasises the importance of making informed dietary choices and the need for more investigation into the intricate relationship between nutrition and health. The chemical and physical characteristics of the oil are eventually impacted by the various oxidation products that are created during prolonged, intense heating. Heat-treated oils have been shown to have detrimental effects on the heart and liver. It has been demonstrated that heated oils raise the risk of cardiovascular disease by raising blood pressure, altering lipid profiles, and producing vascular sickness. These results highlight how important accurate dietary analysis is for the general public's health. Furthermore, research is required to examine the long-term impacts of heating oil on people and raise public awareness using empirical data.

Research on how thermo-oxidized RBO affects the human body has shown that it might be harmful to health. The oxidation products generated during the thermo-oxidation process have been linked to inflammation, oxidative stress, and an increased risk of chronic diseases such as cardiovascular disease. Future studies might concentrate on developing safer substitutes or stream lining the manufacturing procedure to reduce the generation of toxic substances.

Conflict of interest

The work presented in this study was not influenced by any conflicts of interest, according to the authors.

Source of funding

No funding was received for this research.

Author contribution statement

Conceptualization, RP, and RKM; methodology, RP; software, RP, and RKM; formal analysis, RP, MY, and AKS; validation, RP, MY, and RCD; investigation, RP; resources, AKS; data curation, RP; writing—original draft preparation, RP, MY and RKM; writing—review and editing, AKS; MY, RKM, RCD; visualization, RCD, RKM; supervision, RKM; project administration, RP, and RKM. The published version of the manuscript has been read and approved by all authors. Only those who have made significant contributions to the work reported should be listed as authors.

References

1. Deen, R. Visvanathan, D. Wickramarachchi, N. Marikkar, S. Nammi, B.C. Jayawardana, R. Liyanage, Chemical composition and health benefits of coconut oil: an overview, J Sci Food Agric 101 (2021) 2182–2193.
2. S. Rani, M.L. Joy, K.P. Nair, Evaluation of physiochemical and tribological properties of rice bran oil – biodegradable and potential base stoke for industrial lubricants, Ind Crops Prod 65 (2015) 328–333.
3. K. Sawada, H. Rahmania, M. Matsuki, H. Hashimoto, J. Ito, T. Miyazawa, K. Nakagawa, Absorption and Metabolism of γ-Oryzanol, a Characteristic Functional Ingredient in Rice Bran, J Nutr Sci Vitaminol (Tokyo) 65 (2019) S180–S184.
4. M. Zou, Y. Chen, C. Hu, D. He, P. Gao, Physicochemical properties of rice bran blended oil in deep frying by principal component analysis, J Food Sci Technol 59 (2022) 4187–4197.
5. N. Jolfaie, M. Rouhani, P. Surkan, F. Siassi, L. Azadbakht, Rice Bran Oil Decreases Total and LDL Cholesterol in Humans: A Systematic Review and Meta-Analysis of Randomized Controlled Clinical Trials, Hormone and Metabolic Research 48 (2016) 417–426.
6. Y.P. Pal, A.P. Pratap, Rice Bran Oil: A Versatile Source for Edible and Industrial Applications, J Oleo Sci 66 (2017) 551–556.
7. R. Baby Latha, Nasirullah, Physico-Chemical Changes and Stability of Nutraceutical in Rice Bran Oil During Simulated Domestic Frying, Curr Nutr Food Sci 9 (2013) 144–150.
8. R. Mishra, H.K. Sharma, Effect of frying conditions on the physico-chemical properties of rice bran oil and its blended oil, J Food Sci Technol 51 (2014) 1076–1084.
9. L.S.A. Deepam, C. Arumughan, Effect of saponification on composition of unsaponifiable matter in rice bran oil, J Oleo Sci 61 (2012) 241–247.

10. L.E. Bultum, S.A. Emire, L.T. Tufa, Physicochemical characterization of microwave-stabilized rice bran oil from Ethiopian small-scale rice-processing plants, Frontiers in Food Science and Technology 2 (2022).
11. A. El-Refai, M. Domah, M. Askar, Physicochemical Characterization of Rice (Oryza sativa) Bran Oil from Some Egyptian Rice Varieties, Journal of Food and Dairy Sciences 8 (2017) 201–206.
12. N. Idun-Acquah, G.Y. Obeng, E. Mensah, Repetitive Use of Vegetable Cooking Oil and Effects on Physico-Chemical Properties-Case of Frying with Redfish (Lutjanus fulgens), Science and Technology 6 (2016).
13. N. Rahman, S. Hashem, S. Akther, J.S. Jothi, Impact of various extraction methods on fatty acid profile, physicochemical properties, and nutritional quality index of Pangus fish oil, Food Sci Nutr 11 (2023) 4688–4699.
14. O.V. Santos, N.C.F. Corrêa, F.A.S.M. Soares, L.A. Gioielli, C.E.F. Costa, S.C.S. Lannes, Chemical evaluation and thermal behavior of Brazil nut oil obtained by different extraction processes, Food Research International 47 (2012) 253–258.
15. D. Wei, P. Deng, X. Tian, S. He, W. Li, Z. Wu, Accelerating the oxidation of pork fat by illumination and fat oil for the production of Baijiu beverage, J Sci Food Agric 101 (2021) 918–926.
16. M.E. Ojewumi, A.B. Ehinmowo, O.R. Obanla, B.M. Durodola, R.C. Ezeocha, Comparative analysis on the bleaching of crude palm oil using activated groundnut hull, snail shell and rice husk, Heliyon 7 (2021) e07747.
17. N. Zhang, Y. Li, S. Wen, Y. Sun, J. Chen, Y. Gao, A. Sagymbek, X. Yu, Analytical methods for determining the peroxide value of edible oils: A mini-review, Food Chem 358 (2021) 129834.
18. W. Zeng, Y. Endo, Lipid Characteristics of Camellia Seed Oil, J Oleo Sci 68 (2019) 649–658.
19. C.E.W. Malti, M. Boussaïd, L. Belyagoubi, M. Paoli, M. Gibernau, F. Tomi, F. Atik Bekkara, C. Bekhechi, Chemical Variability of the Essential Oil of Pituranthos scoparius from Algeria., Chem Biodivers 15 (2018).
20. H. Zhao, W. Li, X. Zhao, X. Li, D. Yang, H. Ren, Y. Zhou, Cu/Zn superoxide dismutase (SOD) and catalase (CAT) response to crude oil exposure in the polychaete Perinereis aibuhitensis, Environmental Science and Pollution Research 24 (2017) 616–627.
21. R. Pratap, K. Pillai, R. Khanam, F. Islam, Shibli Jameel Ahmad, M. Akhtar, Protective effect of irbesartan, an angiotensin II receptor antagonist, alone and in combination with aspirin on middle cerebral artery occlusion model of focal cerebral ischemia in rats, Hum Exp Toxicol 30 (2011) 354–362.
22. O. Temiz, In vivo neurotoxic effects of emamectin benzoate in male mice: evaluation with enzymatic and biomolecular multi-biomarkers, Environmental Science and Pollution Research 29 (2022) 8921–8932.
23. M.A. Ali, M.A. Islam, N.H. Othman, A.M. Noor, M. Ibrahim, Effect of rice bran oil addition on the oxidative degradation and fatty acid composition of soybean oil during heating, Acta Sci Pol Technol Aliment 18 (2019) 427–438.
24. M. Sochacka, J. Giebułtowicz, M. Remiszewska, P. Suchocki, P. Wroczyński, Effects of Selol 5% supplementation on tissue antioxidant enzyme levels and peroxidation marker in healthy mice, Pharmacological Reports 70 (2018) 1073–1078.
25. G. Hageman, H. Verhagen, B. Schutte, J. Kleinjans, Biological effects of short-term feeding to rats of repeatedly used deep-frying fats in relation to fat mutagen content, Food and Chemical Toxicology 29 (1991) 689–698.
26. S. Bhardwaj, S.J. Passi, A. Misra, K.K. Pant, K. Anwar, R.M. Pandey, V. Kardam, Effect of heating/reheating of fats/oils, as used by Asian Indians, on trans fatty acid formation, Food Chem 212 (2016) 663–670.