Effect of Heating on Compositional Characteristics and Oxidative Stability of Crude and Refined Rice Bran Oil

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Abstract: The compositional characteristics and oxidative stability of rice bran oil were determined by observing the formation of oxidative products and alteration in chemical composition of oils during microwave or oven heating. The values of oxidative indicators such as free acidity, peroxide, \textit{p}-anisidine, total oxidation, thiobarbituric acid and color values, increased faster in refined oils compared to crude ones during heating. In gas chromatography analysis, the percentages of total saturated, monounsaturated and polyunsaturated fatty acids in the studied oils such as lab extracted crude rice bran oil, lab extracted and refined rice bran oil, crude rice bran oil from commercial mill and refined rice bran oil from commercial mill were: 23.07 to 23.56, 41.15 to 42.38 and 34.38 to 35.88, respectively. The heating caused the reduction of polyunsaturated fatty acids content with increasing saturated fatty acids content, and these changes were greater in refined rice bran oil indicating extensive lipid oxidation occurred in refined oil. The change in triacylglycerol species content as determined by High-performance liquid chromatography, was lower in crude oil; the higher stability of these species in crude oil could have contribution to reduce oxidation. During thermal treatment, the generation of hydroperoxides, their degradation and formation of secondary oxidative products evaluated by Fourier-transform infrared spectroscopy, were lower in crude oils. However, the rate of formation of oxidative products in lab prepared samples was lower compared to that in the samples collected from commercial mill. Under extreme thermal condition, the order of oxidative stability: lab extracted crude rice bran oil > crude rice bran oil from commercial mill>lab extracted and refined rice bran oil > refined rice bran oil from commercial mill. The present results will be useful to oil seed processing mills in refining of rice bran oil for economic feasibility and better marketability.

Key words: rice bran oil, oxidation stability, fatty acids, triacylglycerol

1 Introduction

The nature has given rice bran oil (RBO) as a healthy gift to mankind. The RBO is obtained from rice bran, a byproduct of rice milling industry. It is gaining commercial importance as cooking oil especially for frying applications in the world. It contains balanced fatty acid profile and appreciable quantities of bioactive components with high antioxidative properties, and reduces cholesterol in blood\textsuperscript{1}. In general, the nonglyceride materials present in vegetable oils, are removed or reduced them to an optimum level, by the refining process. As a result, their deleterious effects on oil quality are reduced; the oil becomes suitable for human consumption. Now a days, consumers don’t like to use crude oil as it contains considerable amount of unacceptable materials that produce color and odor\textsuperscript{2}. Vegetable oils are refined by chemical or physical process to preserve the quality of oil by removing or reducing free fatty acids, phospholipids, and coloring materials that might have adverse effects on chemical composition, sensory characteristics, and stability of the oil\textsuperscript{3}. Simultaneously, the refining process also reduces or removes some useful bioactive phytochemicals present in the unsaponifiable fraction of
crude RBO and other vegetable oils$^{4,5}$. As a result, the crude oil differs from refined ones in terms of quality or oxidative stability, because of the presence of such phytochemicals in unsaponifiable fraction of crude oil which are removed by refining$^6$. But the oxidative status of the starting oil is critical for the shelf-life of the refined product. Farhoosh et al.$^6$ concluded that the oxidative stability of soybean and canola oils significantly decreased after the neutralization step and no considerable changes during the further refining steps. Mezouari et al.$^4$ stated that although refined rice bran oil showed good oxidative stability, when compared to crude oil its stability was decreased to some extent. Crude chufa oil subjected to refining process showed less oxidative stability than crude oils$^7$.

The modern way of life obliges individuals to reduce the time dedicated to prepare meals to the minimum possible. For preparing food, microwave heating is one of the most attractive cooking methods, commonly employed in households and especially in restaurants for its high speed, convenience and efficiency compared to conventional heating method. Heating of food in a microwave oven is caused by interaction of an electromagnetic field with the chemical constituents of food$^8$. During thermal treatment, the oils undergo oxidation and, thermolytic or oxidative reactions take place with the generation of volatile and nonvolatile oxidative products. The physico-chemical characteristics of the oil are altered by these reactions$^9$. The oxidative stability of an oil depends on its fatty acids and triacylglycerol composition, as well as the concentration of bioactive phytochemicals with antioxidant activities$^{10}$. Of course, each oil has a characteristic pattern of TAGs, and the properties of a particular oil depend on the abundance of different TAG molecular species$^{11}$. Several papers highlight the effect of refining process on the oxidation tendency, concentration of bioactive compounds and properties of RBO$^{12,13}$ and of some other seed oils$^{5,7,14,15}$ without applying any thermal treatment. However, Mezouari and Eichner$^{16}$ investigated the influence of stirring on the thermal degradation of refined RBO throughout the heating period at 180°C and found a significant loss of natural antioxidants with increasing the formation of polymeric TAG. Mezouari et al.$^4$ observed the effect of refining on the minor compounds of RBO and its thermal stability, and revealed the heating process enhanced the concentration in polymers and reduced the concentration in sterol in crude or refined RBO. To the best of our knowledge, no or very little information was reported on the influence of microwave or oven heating on the compositional characteristics and oxidative stability of crude and refined RBOs. Against this background, the present study was designed to explore the effect of microwave or conventional oven heating on the oxidative stability, fatty acid composition, and triacylglycerol species of the crude and refined RBO prepared in the laboratory, and to compare these data with those of the RBO samples collected from commercial oil mill.

2 Experimental

2.1 Sample and Reagent

Freshly milled rice bran (5 kg) was obtained directly from the milling system of a local automatic rice mill. The sample was packed in a polyethylene microwave-safe bag, adjusting moisture content 21%$^{17}$ and microwaved using a microwave oven (2450 MHz, output power 850 W) for 3 min to inactivate endogenous lipase. The bran was removed from the oven, cooled to room temperature, and stored in polyethylene bags at −15°C in a refrigerator. After microwave heating, the moisture content was 8.03%. Fresh samples of RBO (crude and fully chemically refined) were collected from local commercial oil mill. Thiobisurbitic acid was purchased from Himedia Laboratories (Mumbai, India), while acetic acid and standards were from Sigma-Aldrich Co. (St. Louis, MO, USA). Analytical-grade and HPLC-grade solvents and other chemicals were products of Merck (Darmstadt, Germany or Mumbai, India).

2.2 Extraction and refining of oil

The oil was extracted from rice bran using n-hexane as extracting solvent with a solid to solvent ratio of 1:10 (w/v) for 3 h in a magnetic stirrer at room temperature. The extract was filtered through Whatman No. 4 filter paper and the residue was further extracted twice with the same solvent under the identical conditions. The resultant extracts were combined and dried under reduced pressure at 45°C. The chemical refining process of crude RBO was conducted by conventional process$^{3,12}$. The crude oil (500 mL) was heated to 72°C and degummed by adding water with slow agitation for 30 min. Then the oil was settled for 30 min and the water with dissolved gum was drained out. The degummed oil was heated to 80°C and the required amount of NaOH (1%, w/w) was added. Then, it was stirred and left to react for 30 min at room temperature. The soap stock was separated from the neutralized oil by decantation and centrifugation. The oil was washed three times with water. The neutralized oil was brought to 90°C under vacuum and 1% (w/w) of bleaching earth was added. Once this temperature was reached, the oil was stirred for 30 min, cooled to room temperature and filtered through filter paper. The oil was then cooled gradually at 12°C for 4 h for crystallization of wax and filtered to remove the wax. The deodorization was carried out under vacuum at 245°C for 2 h using nitrogen as stripping gas. After deodorization, oil was cooled to 45°C, and stored at −15°C in a refrigerator until analysis. The sample codes were assigned as lab extracted crude RBO (LCO), lab extracted and refined RBO (LRO), crude RBO from commercial mill (MCO) and refined RBO from commercial mill (MRO).
2.3 Thermostability

For microwave heating, the oil samples (80 mL) were taken into 100 mL open glass beakers which were placed at equal distances on the turntable plate of a domestic microwave oven (MS 3042G, LG, China). The oil samples were microwaved for 0, 5, 10, 15 min at a frequency of 2450 MHz and output power 850 W. A calibrated thermocouple (Model HI 9043, Hanna Instruments Ltd Bedfordshire, UK) was used to determine the final oil temperature at various heating times. The final temperature of the oil samples was 211, 246, and 264°C for 5, 10, and 15 min, respectively. For conventional oven heating, the oil samples (80 mL) were poured into 100 mL open glass beakers, without stirring, and held in an electric oven at 180 ± 2°C in order to accelerate the oxidative degradation. Oil samples were withdrawn at intervals of 0, 3, 6 and 9 h. Finally, the oil samples were stored into capped glass bottles at −15°C for further studies.

2.4 Oxidative indices

American Oil Chemists’ Society official methods were followed for determining free fatty acid content (method Ca 5a-40), peroxide value (method Cd 8-53), and thiobarbituric acid value (method Cd 19-90). Determination for p-anisidine value (method p2.4) was conducted using a spectrophotometer according to PORIM test methods.

Oxidation value was calculated from Holm’s equation: TOTOX = 2PV + p-AV. For color value of the sample, the absorbance at 420 nm of 5.0% (w/v) solution of oil in chloroform was recorded using a spectrophotometer.

2.5 Fatty acid composition (FAC)

Fatty acid composition was evaluated by a gas chromatograph (7890A, Agilent Technologies, USA) after transesterification of fatty acids to their corresponding methyl esters following the PORIM method p3.4. The chromatographic system was composed of a polar SP-2250 (Supelco, Bellefonte, PA) capillary column (0.25 mm i.d. × 60 m × 0.2 μm) and a flame ionization detector. Nitrogen was used as carrier gas with a flow rate of 20 mL/min. The oven temperature was set as follows: initial temperature of 100°C, and programmed to increase to 250°C at 10°C/min. Fatty acids in the chromatogram were identified or quantified by comparing the retention times and peak areas of the unknowns with those of standard fatty acid methyl esters.

2.6 Molecular species of triacylglycerol (TAG)

The composition of molecular species of TAG in oil sample was evaluated by using a HPLC system (Agilent 1260 Infinity, USA) composed of a packed Poroshell 120 EC-C18 (Agilent, USA) column (50 mm × 4.6 mm i.d. × 2.7 μm) and evaporative Light Scattering Detector (ELSD). The mobile phase was acetone/acetonitrile (65:35, v/v) at a flow rate of 1 mL/min. Quantification of TAG was carried out by using standards (Sigma-Aldrich Co., St. Louis, MO, USA).

2.7 FT-IR spectroscopy

FTIR spectra of oil samples were recorded on a Fourier Transform Infrared (FTIR) Spectroscopy (IRAffinity-1S, Shimadzu Corporation, Kyoto, Japan) coupled with a high sensitivity pyroelectric detector (deuterated L-alanine doped triglycine sulphate). The spectra were obtained with a spectral resolution of 4 cm⁻¹ and frequency range from 4000 to 400 cm⁻¹.

2.8 Statistical analysis

All the determinations were run in triplicate unless otherwise stated and the data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed and significant differences between means ascertained by Duncan’s multiple range tests using IBM SPSS 22 statistics. The statistical significance was declared at p < 0.05.

3 Results and Discussion

3.1 Oxidation indices

Free fatty acids (FFA) are often used as an indicator for lipid hydrolysis by lipase and hydrolysis may be promoted by the reaction of oil with moisture. The accumulation of FFA in the oils were significantly increased (p < 0.05) with heating time progressed and this hydrolytic degradation was higher in refined oils compared to crude ones (Table 1). The rate of increment ranged from 192–230 and 267–370% for microwaved crude and refined oils, respectively whereas, oven heated crude and refined oils showed increments 223–245 and 286–395%, respectively. A similar trend was followed by Mezouari and Eichner during storage of crude or refined RBO. The higher amount of FFA accumulated in refined oils indicated more hydrolysis of triacylglycerols to form fatty acids occurred in refined oils during heating. It also revealed that the high initial FFA of crude oil did not influence the hydrolytic degradation of RBO during heating. The peroxide value (PV) is used as a measure of oxidation of oil, fat and fatty food. The PV were found to be increased in all oil samples during thermal treatment (Table 1). At all corresponding heating times, the refined oil displayed significantly (p < 0.05) higher PV than that of crude ones, indicating a higher extent of hydroperoxides in refined ones. The highest concentrations of hydroperoxides were determined at 10 min or 6 h of heating after which the rate slowed down. This decrease could be explained by the formation of secondary oxidation products from very unstable primary oxidation products. Bester et al. reported that PV decreased as oxidation proceeds due to rapid decomposition of hydroperoxides.
The present findings are consistent with previous results found by Abbas et al.\textsuperscript{21} when groundnut oil was submitted to heating at 170°C. The sample MRO showed highest increment (675% for microwave and 800% for oven) in PV with the lowest detected in LCO (181% for microwave and 193% for oven). Such results revealed the refined RBO is more thermolabile and produces higher quantities of peroxides under extreme heating condition. The p-Anisidine value (p-AV) is a measure of secondary products, mainly non-volatile carbonyls, of fat and oil oxidation, which results from the breakdown of hydroperoxides\textsuperscript{21}. As like PV, the p-AV increased with heating time and the increment was significantly higher\textsuperscript{(p<0.05)} in refined samples during thermal treatment (Table 1). Moreover, the increment in p-AV was higher at earlier stage and lower at later stage of heating period. The p-AVs in LCO, LRO, MCO and MRO significantly\textsuperscript{(p<0.05)} increased from 5.31, 4.17, 6.19 and 4.54 in untreated control to 26.31, 33.32, 32.04 and 39.46 for 15 min microwaved samples, and to 30.60, 38.03, 36.90 and 43.76 for 9 h oven heated samples, respectively. During the heat treatment, the lower rate of increment of secondary oxidation products in crude oil as measured by p-AV, revealed the considerably extended shelf-life of crude oils. The total oxidation (TOTOX) value measures both hydroperoxides and their breakdown products, and provides a better estimation of the progressive oxidative deterioration of fats and oils. At the end of 15 min or 9 h heating, the percent of increment in TOTOX value was the

### Table 1  Change in FFA, PV, and p-AV values of crude and refined rice bran oil during heating.

| Parameter | Heat treatment | Heating time (min/h) | LCO | LRO | MCO | MRO |
|-----------|----------------|----------------------|-----|-----|-----|-----|
| FFA (%)  | Microwave      | 0                    | 3.25 ± 0.04\textsuperscript{bc} | 0.15 ± 0.01\textsuperscript{aA} | 5.60 ± 0.08\textsuperscript{aB} | 0.20 ± 0.02\textsuperscript{aB} |
|          |                | 5                    | 5.08 ± 0.08\textsuperscript{bC} | 0.29 ± 0.01\textsuperscript{aA} | 9.17 ± 0.06\textsuperscript{aB} | 0.45 ± 0.02\textsuperscript{aB} |
|          | Oven           | 0                    | 3.25 ± 0.04\textsuperscript{bc} | 0.15 ± 0.01\textsuperscript{aA} | 5.60 ± 0.08\textsuperscript{aB} | 0.20 ± 0.02\textsuperscript{aB} |
|          |                | 3                    | 6.09 ± 0.05\textsuperscript{bC} | 0.34 ± 0.01\textsuperscript{aA} | 10.42 ± 0.17\textsuperscript{aB} | 0.56 ± 0.02\textsuperscript{aB} |
|          |                | 6                    | 8.46 ± 0.15\textsuperscript{bC} | 0.45 ± 0.02\textsuperscript{aA} | 15.00 ± 0.05\textsuperscript{aC} | 0.82 ± 0.03\textsuperscript{aB} |
|          |                | 9                    | 10.51 ± 0.12\textsuperscript{bC} | 0.58 ± 0.03\textsuperscript{aA} | 19.31 ± 0.20\textsuperscript{aB} | 0.99 ± 0.02\textsuperscript{aB} |
| PV (meq O\textsubscript{2}/kg oil) | Microwave      | 0                    | 3.77 ± 0.02\textsuperscript{bc} | 1.59 ± 0.02\textsuperscript{aA} | 3.94 ± 0.02\textsuperscript{aB} | 1.78 ± 0.02\textsuperscript{aB} |
|          |                | 5                    | 5.91 ± 0.06\textsuperscript{bA} | 7.43 ± 0.06\textsuperscript{bB} | 8.73 ± 0.06\textsuperscript{bB} | 9.05 ± 0.04\textsuperscript{aB} |
|          | Oven           | 0                    | 3.77 ± 0.02\textsuperscript{bc} | 1.59 ± 0.02\textsuperscript{aA} | 3.94 ± 0.02\textsuperscript{aB} | 1.78 ± 0.02\textsuperscript{aB} |
|          |                | 3                    | 6.62 ± 0.05\textsuperscript{bA} | 8.94 ± 0.03\textsuperscript{bB} | 8.92 ± 0.06\textsuperscript{bB} | 11.00 ± 0.06\textsuperscript{aC} |
|          |                | 6                    | 11.04 ± 0.15\textsuperscript{bA} | 11.35 ± 0.07\textsuperscript{aA} | 13.04 ± 0.04\textsuperscript{aB} | 16.02 ± 0.15\textsuperscript{aC} |
|          |                | 9                    | 10.15 ± 0.03\textsuperscript{aA} | 10.93 ± 0.12\textsuperscript{bB} | 11.00 ± 0.06\textsuperscript{aB} | 12.73 ± 0.06\textsuperscript{aC} |
| p-AV     | Microwave      | 0                    | 5.31 ± 0.04\textsuperscript{ab} | 4.17 ± 0.04\textsuperscript{aA} | 6.19 ± 0.18\textsuperscript{aC} | 4.54 ± 0.49\textsuperscript{aA} |
|          |                | 5                    | 11.46 ± 0.47\textsuperscript{bA} | 13.08 ± 0.50\textsuperscript{bB} | 13.26 ± 0.31\textsuperscript{bB} | 17.28 ± 0.09\textsuperscript{aC} |
|          | Oven           | 0                    | 5.31 ± 0.04\textsuperscript{ab} | 4.17 ± 0.04\textsuperscript{aA} | 6.19 ± 0.18\textsuperscript{aC} | 4.54 ± 0.49\textsuperscript{aA} |
|          |                | 3                    | 13.28 ± 0.57\textsuperscript{bA} | 18.39 ± 0.08\textsuperscript{bC} | 16.40 ± 0.95\textsuperscript{aB} | 20.29 ± 0.02\textsuperscript{aD} |
|          |                | 6                    | 26.15 ± 0.05\textsuperscript{aA} | 35.30 ± 0.22\textsuperscript{aA} | 32.20 ± 0.15\textsuperscript{aB} | 40.17 ± 0.15\textsuperscript{aD} |
|          |                | 9                    | 30.60 ± 0.05\textsuperscript{aA} | 38.03 ± 0.02\textsuperscript{aA} | 36.90 ± 0.60\textsuperscript{bB} | 43.76 ± 0.14\textsuperscript{aD} |

Each value in the table represents the mean of three replicates ± SD.

Values within a row with the same uppercase letters are not significantly different at \( p < 0.05 \).

Values within a column with the same lowercase letters are not significantly different at \( p < 0.05 \).

LCO- lab extracted crude RBO, LRO- lab extracted and refined RBO, MCO- crude RBO from commercial oil mill, and MRO-refined RBO from commercial oil mill.
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Table 2 Change in TOTOX, TBA and color values of crude and refined rice bran oil during heating.

| Parameter | Heat treatment | Heating time (min/h) | Sample       |
|-----------|----------------|----------------------|--------------|
|           |                |                      |  LCO | LRO | MCO | MRO |
| TOTOX     | Microwave      | 0                    | 12.86±0.08<sup>ac</sup> | 7.35±0.07<sup>aa</sup> | 14.07±0.15<sup>b</sup> | 8.10±0.46<sup>ab</sup> |
|           |                | 5                    | 23.28±0.47<sup>ab</sup> | 27.93±0.53<sup>bb</sup> | 30.71±0.30<sup>c</sup> | 35.38±0.09<sup>cd</sup> |
|           |                | 10                   | 41.57±0.27<sup>ab</sup> | 53.94±0.33<sup>bc</sup> | 54.61±0.16<sup>c</sup> | 62.91±0.24<sup>cd</sup> |
|           |                | 15                   | 46.02±0.23<sup>ab</sup> | 52.84±0.14<sup>bc</sup> | 53.48±0.11<sup>c</sup> | 62.78±0.07<sup>cd</sup> |
|           | Oven           | 0                    | 12.86±0.08<sup>bc</sup> | 7.35±0.07<sup>aa</sup> | 14.07±0.15<sup>bd</sup> | 8.10±0.46<sup>ab</sup> |
|           |                | 3                    | 26.51±0.64<sup>ab</sup> | 36.26±0.03<sup>bc</sup> | 34.24±0.85<sup>bc</sup> | 42.29±0.11<sup>b</sup> |
|           |                | 6                    | 48.24±0.36<sup>ab</sup> | 58.00±0.19<sup>bc</sup> | 58.28±0.22<sup>b</sup> | 72.21±0.17<sup>bc</sup> |
|           |                | 9                    | 50.90±0.03<sup>bc</sup> | 59.90±0.24<sup>bc</sup> | 58.90±0.79<sup>bc</sup> | 69.22±0.12<sup>cd</sup> |

| Absorbance at 420 nm | Microwave | 0 | 0.56±0.01<sup>ac</sup> | 0.22±0.02<sup>aa</sup> | 0.49±0.01<sup>ab</sup> | 0.20±0.01<sup>ab</sup> |
|                       | 5         | 0.59±0.03<sup>ab</sup> | 0.35±0.02<sup>aa</sup> | 0.60±0.01<sup>ab</sup> | 0.37±0.02<sup>ab</sup> |
|                       | 10        | 0.78±0.02<sup>ab</sup> | 0.55±0.02<sup>ab</sup> | 0.73±0.01<sup>ab</sup> | 0.57±0.05<sup>ab</sup> |
|                       | 15        | 0.99±0.02<sup>ab</sup> | 0.79±0.03<sup>ab</sup> | 0.89±0.03<sup>ab</sup> | 0.73±0.03<sup>ab</sup> |
|                       | Oven      | 0 | 0.56±0.01<sup>ac</sup> | 0.22±0.02<sup>aa</sup> | 0.49±0.01<sup>ab</sup> | 0.20±0.01<sup>ab</sup> |
|                       |           | 3 | 0.62±0.01<sup>ab</sup> | 0.36±0.04<sup>ab</sup> | 0.61±0.01<sup>bc</sup> | 0.39±0.04<sup>ab</sup> |
|                       |           | 6 | 0.79±0.01<sup>ac</sup> | 0.56±0.02<sup>ab</sup> | 0.75±0.04<sup>bc</sup> | 0.64±0.03<sup>ab</sup> |
|                       |           | 9 | 0.95±0.03<sup>ab</sup> | 0.80±0.03<sup>ab</sup> | 0.99±0.07<sup>bc</sup> | 0.75±0.03<sup>ab</sup> |

Each value in the table represents the mean of three replicates ± SD.
Values within a row with the same uppercase letters are not significantly different at p < 0.05.
Values within a column with the same lowercase letters are not significantly different at p < 0.05.
LCO- lab extracted crude RBO, LRO- lab extracted and refined RBO, MCO- crude RBO from commercial oil mill, and MRO-refined RBO from commercial oil mill.

highest in MRO (675% for microwave and 755% for oven) and lowest in LCO (258% for microwave and 296% for oven) (Table 2). The lower TOTOX values revealed the refining process of crude oil reduced the heat stability of rice bran oil. The thiobarbituric acid (TBA) value is used to measure secondary lipid peroxidation products. During heating, the concentration of thiobarbituric acid was observed increasing tendency for all samples; but no regular pattern of increment was recoded as like AV and p-AV (Table 2). The TBA values were increased sharply for the oils at earlier phase of heating followed by a decrease. This probably resulted from the volatilization of secondary oxidation products or their break down. As indicated by TBA, the increase in concentration of oxidative products in refined oil was higher in comparison with crude sample during heating. The data from PV and p-AV also confirmed the above statement that showed the refined oil to be more susceptible to oxidation compared to crude one at extreme thermal condition. The similar changes were observed in RBO<sup>3</sup> and in peanut oil<sup>4</sup> during chemical refining process. This might be attributed to decreased amount of tocols and other natural antioxidants in refined oil. This implies the negative impact of the refining process on the oxidative stability of RBO. However, the refining process decreases the amounts of some oxidative products which was expected, indicating quality improvement of the crude oil. During heating the rate of formation of oxidative products in laboratory extracted oils (LCO and LRO) was lower compared
to the samples (MCO and MRO) collected from commercial mill for this study. However, the initial amount of oxidation products in commercial oil sample was found to be higher than laboratory extracted oil. This might be due to the precise control of process parameters and small-scale operations in the laboratory extraction/refinery unit. Sairam et al. observed that the defatted rice bran prepared in the laboratory, possessed better nutrient profile and antioxidant capacity than those the samples purchased from commercial rice mill. As heating continued up to 15 min or 9 h, the oil became darker because of the generation of primary and secondary oxidation products from the splitting of unsaturated fatty acid. As a consequence, the colour absorbance value of crude or refined RBO measured at 420 nm, were significantly (p < 0.05) increased (Table 2). Before heating, the absorbance value of refined oil (0.20–0.22) was lower than that of crude oil (0.49–0.56); this resultant from the removal of coloring pigments by the bleaching of crude oil. A sharp increase in absorbance value of refined oil was noticed during heating and highest increment was measured in MRO (265–275); this indicates the impact of refining of crude RBO. This confirms the results of other oxidative parameters as discussed earlier that showed the refined RBO to be more susceptible to oxidation at elevated temperature compared to crude ones.

### 3.2 Fatty acid composition (FAC)

Some food-processing techniques can affect fatty acid composition of oils when hardly subjected to successive heating. The FAC of oil can be an indicator of its stability, physical properties and nutritional value. Rice bran oil consisted of mainly C18:1, C18:2 and C16:0 with the concentration of 41.88–42.18, 32.42–34.22, and 19.61–20.51%, respectively while, C14:0, C16:1, C18:0, C18:3, C20:0 and C22:0 make up less than 2% (Table 3). Saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids accounted 23.07–23.56, 41.15–42.38, and 34.38–35.88% of the total fatty acids, respectively. The present data of FAC are in accordance with those reported elsewhere. The present results revealed the refining process did not significantly influence the FAC in RBO with exception of slight decrease in the concentration of C18:2 for the lab extracted sample. These results agreed with Pestana et al. who confirmed no significant effect of refining process of crude oil on the composition of fatty acids. As depicted in Table 4, the relative content of PUFA was decreased, while that of SFA or MUFA increased in RBO oils under oxidative stress. A similar trend was found by Abbas et al. during thermo-oxidative degradation of groundnut oil. But the change in relative content of fatty acids was low in crude samples compared to refined ones during thermal treatment. The highest reduction of PUFA was estimated in MRO (12.39% for microwave and 15.87% for oven) and lowest in LCO (4.46% for microwave and 6.10% for oven). The present data revealed the change in FAC in the sample LCO during microwave or oven heating was lower compared to the rest samples, indicating its less tendency to the formation of oil oxidation products and polymeric substances by oxidative degradation of PUFAs. Slow rate of formation of such products in MCO during

### Table 3  Fatty acid composition (%) of rice bran oils before heating.

| Fatty acids          | Name of Sample | LCO   | LRO   | MCO   | MRO   |
|----------------------|----------------|-------|-------|-------|-------|
| Myristic acid (C14:0)|                | 0.29±0.01<sup>a</sup> | 0.80±0.31<sup>b</sup> | 0.32±0.01<sup>a</sup> | 0.33±0.02<sup>a</sup> |
| Palmitic acid (C16:0)|                | 20.49±0.12<sup>ab</sup> | 19.61±0.75<sup>a</sup> | 20.51±0.02<sup>ab</sup> | 19.83±0.27<sup>a</sup> |
| Palmitoleic acid (C16:1) |              | 0.11±0.0<sup>1</sup>   | 0.20±0.07<sup>b</sup>   | 0.19±0.01<sup>1</sup>   | 0.09±0.02<sup>1</sup>   |
| Stearic acid (C18:0) |                | 2.00±0.21<sup>1</sup>   | 1.79±0.13<sup>a</sup>   | 1.86±0.02<sup>1</sup>   | 1.97±0.06<sup>1</sup>   |
| Oleic acid (C18:1)  |                | 42.04±0.23<sup>a</sup> | 42.18±0.43<sup>a</sup> | 41.88±0.02<sup>a</sup> | 41.89±0.23<sup>a</sup> |
| Linoleic acid (C18:2) |               | 34.22±2.11<sup>b</sup> | 32.90±0.13<sup>a</sup> | 32.42±0.03<sup>b</sup> | 33.02±0.13<sup>a</sup> |
| Linolenic acid (C18:3) |                | 1.66±0.17<sup>a</sup>   | 1.65±0.16<sup>a</sup>   | 1.96±0.04<sup>b</sup>   | 1.84±0.07<sup>b</sup>   |
| Arachidic acid (C20:0) |               | 0.40±0.06<sup>b</sup>   | 0.66±0.06<sup>b</sup>   | 0.68±0.00<sup>b</sup>   | 0.74±0.20<sup>b</sup>   |
| Behenic acid (C22:0) |                | 0.13±0.04<sup>b</sup>   | 0.21±0.04<sup>b</sup>   | 0.19±0.01<sup>b</sup>   | 0.30±0.01<sup>b</sup>   |
| **ΣSaturated fatty acids** |            | 23.31 | 23.07 | 23.56 | 23.17 |
| **ΣMonounsaturated fatty acids** |    | 41.15 | 42.38 | 42.07 | 41.98 |
| **ΣPolyunsaturated fatty acids** |            | 35.88 | 34.55 | 34.38 | 34.86 |

Each value is the mean ± standard deviation of triplicate determinations. Values within a row with the same lowercase letters are not significantly different at p < 0.05.
Table 4  Changes in saturated, monounsaturated and polyunsaturated fatty acids in rice bran oils during microwave and oven heating.

| Sample | Heating time (min/h) | Fatty acid composition (%) |   |   |   |
|--------|----------------------|---------------------------|---|---|---|
|        |                      | Saturated fatty acids     | Monounsaturated fatty acids | Polyunsaturated fatty acids | P/S |
|        |                      |                           |                           |                            |     |
| LCO    | 0                    | 23.31 (100)               | 41.15 (100)               | 35.88 (100)                | 1.54 |
|        | 5                    | 23.69 (101.63)            | 41.28 (100.31)            | 35.04 (97.66)              | 1.48 |
|        | 10                   | 23.13 (99.23)             | 41.95 (101.94)            | 34.90 (97.27)              | 1.51 |
|        | 15                   | 23.35 (100.17)            | 42.39 (103.01)            | 34.28 (95.54)              | 1.47 |
|        | 0                    | 23.31 (100)               | 41.15 (100)               | 35.88 (100)                | 1.54 |
|        | 3                    | 23.39 (100.34)            | 42.39 (103.01)            | 34.21 (95.35)              | 1.46 |
|        | 6                    | 23.56 (101.07)            | 42.66 (103.67)            | 33.78 (94.15)              | 1.43 |
|        | 9                    | 23.75 (101.89)            | 42.57 (103.45)            | 33.69 (93.90)              | 1.42 |
|        | 0                    | 23.07 (100)               | 42.38 (100)               | 34.55 (100)                | 1.50 |
|        | 5                    | 23.34 (101.17)            | 41.99 (99.08)             | 33.00 (95.51)              | 1.41 |
|        | 10                   | 24.03 (104.16)            | 44.10 (104.06)            | 31.88 (92.27)              | 1.33 |
|        | 15                   | 23.80 (103.16)            | 44.43 (104.84)            | 31.77 (91.95)              | 1.33 |
| LRO    | 0                    | 23.76 (100)               | 42.07 (100)               | 34.38 (100)                | 1.46 |
|        | 3                    | 24.83 (107.63)            | 43.62 (102.93)            | 31.55 (91.32)              | 1.27 |
|        | 6                    | 25.74 (111.57)            | 43.88 (103.54)            | 30.91 (89.46)              | 1.20 |
|        | 9                    | 27.17 (117.77)            | 45.73 (107.90)            | 29.42 (85.15)              | 1.08 |
|        | 0                    | 23.56 (100)               | 42.07 (100)               | 34.38 (100)                | 1.46 |
|        | 5                    | 23.66 (100.42)            | 42.24 (100.40)            | 34.09 (99.15)              | 1.44 |
|        | 10                   | 23.53 (99.87)             | 42.55 (101.14)            | 33.91 (98.63)              | 1.47 |
|        | 15                   | 23.64 (100.34)            | 44.20 (105.06)            | 32.15 (93.51)              | 1.36 |
| MCO    | 0                    | 23.56 (100)               | 42.07 (100)               | 34.38 (100)                | 1.46 |
|        | 3                    | 23.72 (100.68)            | 43.08 (102.40)            | 33.19 (96.54)              | 1.40 |
|        | 6                    | 23.92 (101.52)            | 44.75 (106.37)            | 31.32 (91.10)              | 1.31 |
|        | 9                    | 24.54 (104.16)            | 45.40 (107.91)            | 30.06 (87.43)              | 1.22 |
|        | 0                    | 23.17 (100)               | 41.98 (100)               | 34.86 (100)                | 1.50 |
|        | 5                    | 23.67 (102.16)            | 42.15 (100.40)            | 34.17 (98.02)              | 1.44 |
|        | 10                   | 23.72 (102.37)            | 44.58 (106.19)            | 31.70 (90.93)              | 1.34 |
|        | 15                   | 25.68 (110.83)            | 43.79 (104.31)            | 30.54 (87.61)              | 1.19 |
| MRO    | 0                    | 23.17 (100)               | 41.98 (100)               | 34.86 (100)                | 1.50 |
|        | 3                    | 25.50 (110.05)            | 43.70 (104.09)            | 31.13 (98.30)              | 1.22 |
|        | 6                    | 25.15 (108.54)            | 44.75 (106.60)            | 30.10 (86.34)              | 1.20 |
|        | 9                    | 26.45 (114.15)            | 45.88 (109.29)            | 29.33 (84.13)              | 1.11 |

Number in parenthesis is relative % of saturated, monounsaturated and polyunsaturated fatty acids based on the initial saturated, monounsaturated and polyunsaturated fatty acids content before heating. P/S- ratio of polyunsaturated to saturated fatty acids. Each value is the mean of triplicate determinations.

LCO- lab extracted crude RBO, LRO- lab extracted and refined RBO, MCO- crude RBO from commercial oil mill, and MRO- refined RBO from commercial oil mill.
heating, was also found. This may be occurred due to the protective effect of bioactive constituents present in crude RBO. Moreover, the ratio of polyunsaturated to saturated fatty acids (P/S) of all samples decreased with oxidation time, which enables to evaluate the oil oxidation. The highest decreased amount was observed for the refined sample indicating the oxidation proceeded more rapidly in the refined oils than in the crude ones during thermal treatment.

3.3 Triacylglycerol (TAG) composition

The effect of heat treatment on the TAG molecular species (P, palmitic; M, myristic; O, oleic; L, linoleic) in RBO evaluated by HPLC are depicted in Figs. 1 and 2. The rice bran oil contained high amount of POL (23.21–24.19%) followed by OOL (15.02–16.02%), POO (12.57–13.18%), PLL–MOL (12.20–12.40%) and OOO (8.80–9.40%); the amounts of PLP, POP, SOO and POS at lesser concentration. Still, microwave or oven heating did not inflicted changes (with few exception) in TGA profile of crude oils, because of small amount of species containing more than four double bonds in TAGs. In this study, the percentage of POL, OOL, OLL and PLL-MOL decreased, whereas, in most case, the percentages of POO and OOO remained unchanged or slightly increased with increasing heating time, probably due to PUFA degradation. The change in TAG species as stated above was found to be higher in the refined samples than that of crude ones (Figs. 1 and 2; a, b, c, d). The present results concurred with the literature values for pumpkin seed oil. Of the above species, most significant reduction was found in OLL (Figs. 1a and 2a). This reduction was expected due to the reduction in linoleic acid concentration by oxidation. This is in good agreement with findings of Abbas et al. who reported that the highest reduction in OLL when groundnut oil heated at 170°C. The amounts of OLL decreased from 13.20, 13.10, and 12.95% in untreated samples to 12.47, 11.02, and 10.57% in 15 min microwaved samples and to 9.37, 7.13, and 6.13% in 9 h heated sample for LCO, MCO, and LRO, respectively. Yoshida and Takagi also reported that the influence of heating depends on the concentration of oleic and linoleic acids present in TGA molecule. From

![Changes of triacylglycerol composition of crude (LCO- lab extracted crude, MCO- crude from commercial oil mill) and refined (LRO- lab extracted and refined) rice bran oils during microwave heating.](image)

Fig. 1 Changes of triacylglycerol composition of crude (LCO- lab extracted crude, MCO- crude from commercial oil mill) and refined (LRO- lab extracted and refined) rice bran oils during microwave heating. (a) OLL, (b) PLL-MOL, (c) OOL, (d) POO, (e) OOO, (f) POO. Each value is the mean ± standard deviation of triplicate determinations. Values in each heating grouping with different letters on bar, are significantly different (p < 0.05).
the above discussion, it may be concluded that the change in TAG species was higher in refined oil compared to crude ones through the microwave or oven heating. The rate of fatty acid breakdown is related to the number of double bonds in the carbon chain of the molecule. As the number of double bonds increases, the rate of oxidation increases. Good agreement between the fatty acid and TAG composition was also found in this study.

3.4 Evaluation by FT-IR

FTIR analysis provides a rapid means of evaluating the oxidative state of an oil or of monitoring changes in oil undergoing thermal stress. An increase or decrease in some of the wave number regions was observed in this study. However, only the regions which were generated for the certain oil oxidation products, were evaluated. Figures 3 and 4 illustrate the remarkable spectral changes under experimental conditions found in crude and refined RBO. The identified functional groups responsible for IR absorption peak are: 3008 (C–H stretching vibration of the cis-double bond); 2927 and 2854 (asymmetric and symmetric stretching vibration of CH\textsubscript{2}, resp.); 1745 (C=O stretching vibrations); 1465 (bending vibrations of the CH\textsubscript{2} and CH\textsubscript{3}); 1161 (C–O stretching vibration). The intensity of the cis-double bond near 3008 cm\textsuperscript{-1} (shoulder) suffers a slow shifting toward higher values during heating. This increase may be attributed to the generation of free radicals by the heating which initiate primary oxidation reaction of unsaturated fatty acids. This reaction resulted in primary oxidation products containing cis and conjugated double bonds as what happened in the case of the autoxidation of oleic and linoleic acid. The present results were in good agreement with those reported elsewhere. The intensities (absorbances) of the bands at 2927 and 2854 cm\textsuperscript{-1}, increased with increasing heating process due to surrounding chemical changes as a result of oxidation process. There is another major peak near 1745 cm\textsuperscript{-1} which corresponded to the carbonylic compounds formed by the degradation of hydroperoxide during heating; the intensity of it increased with thermal process. A very weak band at 1465 cm\textsuperscript{-1} tended to increase with the oxidation treatment. Another weak band at 1161 cm\textsuperscript{-1} related to the proportion in the sample of saturated acyl groups; the intensity of this band showed similar change during heating and increased its intensity. A similar trend was observed for the bands 2927, 2854, 1745, 1465, and 1161 cm\textsuperscript{-1} by Ali et al. in pumpkin seed oil during heat treatment and also by Valdés et al. in almonds.

![Fig. 2](image)

**Fig. 2** Changes of triacylglycerol composition of crude (LCO- lab extracted crude, MCO- crude from commercial oil mill) and refined (LRO- lab extracted and refined) rice bran oils during oven heating. (a) OLL, (b) PLL-MOL, (c) OOL, (d) POL, (e) OOO, (f) POO. Each value is the mean ± standard deviation of triplicate determinations. Values in each heating grouping with different letters on bar, are significantly different (p<0.05).

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during accelerated oxidation. In this work, the peak intensities of refined oils were shifted significantly in comparison with crude samples during heating. This indicates a clear effect of refining process on oxidative tendency of RBO. Under present experimental conditions, the significant change in IR peak intensities found in refined oil attributed the oxidation proceeds more rapidly in refined samples compared to crude ones. However, in most case, the intensities at different bands were found to be slightly higher in MCO and MRO than those in LCO and LRO at the end of heating. These results are in accordance with those found by the change in the oxidative indices, FAC, and TGA.

4 Conclusions

The refining process reduced some oxidative products from crude RBO and improved its basic quality parameters. The present results acquired from the compositional characteristics and oxidation indices revealed: (a) there was no significant difference in the fatty acids and TAG species contents in crude and refined RBOS; (b) exposing the oils to microwave or oven heating caused the formation of hydroperoxides and secondary oxidation products both in crude and refined RBOS; (c) when comparing the oxidative indices, fatty acids, TAG species and IR absorbances of crude and refined RBOS, it may be confirmed the greater tendency to form primary and secondary oxidative products, of refined oil compared to crude ones during heating; (d) in contrast to the samples MCO or MRO, the LCO or LRO had the slightly lower rate of oxidative deterioration.

Fig. 3 Changes of FT-IR spectra of crude and refined rice bran oil during microwave heating. a) 0 min heating b) 5 min heating c) 10 min heating d) 15 min heating. LCO- lab extracted crude RBO, LRO- lab extracted and refined RBO, MCO- crude RBO from commercial oil mill, and MRO- refined RBO from commercial oil mill.
Characteristics and Stability of Crude and Refined Rice Bran Oil

After 15 min microwave or 9 h oven heating the remaining of a large quantity of some bioactive compounds may be responsible for relatively good keeping quality of crude oil. Finally, the crude oil differs from the refined ones in quality or oxidative stability for the presence of minor bioactive compounds in unsaponifiable fraction of crude oil. These phytochemicals are removed during the refining which results in the reduction of thermal stability of refined RBO. These findings will suggest a new path-way to food and nutraceutical industries for high-quality rice bran oil production for human consumption.

Fig. 4 Changes of FT-IR spectra of crude and refined rice bran oil during oven heating. a) 0 h heating b) 3 h heating c) 6 h heating d) 9 h heating. LCO- lab extracted crude RBO, LRO- lab extracted and refined RBO, MCO- crude RBO from commercial oil mill, and MRO- refined RBO from commercial oil mill.

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