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Detection of the changes in fatty acid composition and physicochemical properties in selected edible oils heated at different degrees of time

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ABSTRACT

The study investigated the changes in chemical and fatty acid composition in selected edible oils due to continuous heating. Peroxide value of all the oil except rice bran oil went beyond the permissible limit of 10 $mEqO_2/kg$ after 90 min of heating. At first degree of heating, the saponification number of all the oils increased. But the values declined after second and third time of heating, indicating a high degree of saturation. Rice bran oil showed the most slower decreasing trend in case of iodine value. Acid value of all the oils increased with time of heating indicating the presence of free fatty acid. Fatty acid composition of all the oils seemed to be affected due to continuous heating when analyzed with GC–MS. Presence of essential fatty acids was affected and fully absent in some oil. Some foreign chemicals like glycidyl fatty acid esters, fumaric acid, succinic acid, cyclohexane, etc. were detected due to the heating of oils. Glycidyl fatty acid esters were discovered in the degradation of each oil. Cyclohexane is produced during the breakdown of palm and soybean oil. Conferring to the results, it is recommended that oils should not be used after the third degree of heating.

1. Introduction

Oils used in cooking are essential to both human health and the food processing industry (Kumar et al., 2016). Oils and fats contain more than twice as many calories as carbs or proteins of equal weight. In addition to carrying fat-soluble vitamins, they also include particular fatty acids that play a vital function in nutrition. The market and value of edible oils have increased due to the health advantages of essential fatty acids such as unsaturated fatty acids (FAO, 2010). Fatty acids are structurally of two types. Saturated fatty acids (such as myristic, stearic, and palmitic acids) have no double bonds, whereas unsaturated fatty acids have more than eight percent double bonds (for example, linoleic, oleic, and linolenic acids).

The most prevalent vegetable oils are palm, olive, soybean, sunflower, coconut, maize, cottonseed, and peanut. Vegetable oils like palm oil contain 50 % saturated fatty acids, 40 % monounsaturated fatty acids and 10 % polyunsaturated fatty acids (Wattanapenpaiboon & Wahlqvist, 2003). Whereas, soybean oils are rich in polyunsaturated fatty acids (PUFA) such as linoleic (18:2) and alpha-linolenic (18:3) acids (Sarkar et al., 2015). On the contrary, rice bran oil (RBO) has lower saturated fatty acid content (20 %) when compared to soybean and palm oil (Shakib et al., 2014). These three vegetable oils are most consumed oils among the Bangladeshi as well as South Asian people. According to Ates and Bukowski (2023), to meet demand, world vegetable oil inventories are expected to increase by 540,000 metric tons to 30.6 million metric tons in 2023/24, with soybean oil and rapeseed oil stocks leading the way.

Due to the double bonds present in fatty acids, this vegetable oils are prone to autoxidation and thus the fatty acid composition changes frequently due to heating. The majority of food for deep frying is fried at approximately 180 °C by dipping it in extremely hot oil (Godswill et al., 2018). Commonly, food vendors increase their profits by reusing edible oil for food preparation, particularly deep-frying. During repeated heating, the oxidative degradation of oil lipids accelerates, hazardous reactive oxygen species are produced, and the natural antioxidant content of the edible oil decreases (Leong et al., 2015). When cooking oil is

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subjected to a very high temperature during continuous heating, a series of complicated chemical reactions ensue, resulting in the loss of both the quality and nutritional worth of the cooking oil and the certain production of free radicals (Gogus & Smith, 2010). Cooking oils may cause harm by oxidizing our good cholesterol into bad cholesterol (Goswami et al., 2016). In the heating process, the chemical nature of the oil deteriorates, especially if there are unsaturation, due to the formation of compounds that could be toxic when its consumption is moderate, and very harmful to the health of man when it is chronic, added to the generation of other volatile compounds when the oil is in contact with food at high temperatures (Liu et al., 2021; Yang et al., 2021).

The technique of gas chromatography-mass spectrometry (GC–MS) is frequently used to identify and quantify specific components in edible oils (Seppänen-Laakso et al., 2002). It is commonly used to identify short-chain low-molecular-weight fatty acid methyl ester due to its speed, dependability, and user friendliness (Fang et al., 2013; Barriuso et al., 2013; Dayrit et al., 2011). Food vendors and restaurant owners inquired about the repeated use of edible oils since they have been penalized for using the same oil many times. This study intends to determine the extent to which frequent heating of edible oils (soybean oil, palm oil, and rice bran oil) poses no health concerns. Again, repeated heating causes many modifications to the fatty acid profile of oils. Consequently, some beneficial fatty acids may not be present after cooking. This study aims to determine the changes in the fatty acid composition of consumed edible oils to determine if there are any significant changes in the nutritional value of the oils, to educate the general public about the harmful effects of consuming foods prepared with repeatedly heated edible oil, and to provide experimental data for future use by the appropriate legal authorities.

2. Material and methods

2.1. Heating of oil sample

The refined vegetable cooking oil (Soybean oil, palm oil and rice bran oil) (5 L) of a standard food grade was purchased from the local market of Jashore, Bangladesh. An aliquot of oil (1 L) was separated and labeled as unheated cooking oil (UHCO). Another aliquot of each oil (4 L) was heated in a pan on a gas stove of 30″ × 27″ × 36″ dimension (at 180 °C) for 30 min and then was cooled to room temperature. The ratio of oxygen contact area (oil surface) to its volume was 0.378 cm^{-1}. The temperature of the heated oil was controlled by checking it with a digital thermometer (Precision Plus, accuracy ±0.05 °C). From this, a sample of 1 L will be separated and labeled as single heated cooking oil (SHCO). Then the single heated was heated again for 30 min to obtain doubly heated cooking oil (DHCO). The same process was repeated to obtain triple heated cooking oil (THCO). This process of heating and cooling of the oil was performed without addition of fresh oil and the whole process took 1 h 30 min. The viscous dark brown oil sample (approximately 3 L) thus obtained was then stored in amber color bottles to prevent photodegradation of PAHs (Perumalla Venkata & Subramanyam, 2016).

2.2. Chemicals and reagents

Fatty acid methyl ester (FAME) standards of C14:0, C16:0, C18:0, C18:1, C18:2 were purchased from Sigma-Aldrich (Steinheim, Germany). FAMEs of C18:1 isomer mixture, C18:2 isomer mixture (No. 47791) and C18:3 isomer mixture (No. 47792) were purchased from Supelco Inc. (Bellefonte, PA, USA). All other chemicals and solvents used were of the highest quality AR grade commercially available.

2.2.1. Analysis of constituents of oil

The quality of oils to be used in this study for treatment was investigated by the determination of their peroxide value (PV), saponification number (SN), iodine value (IV), acid value (AV) according to official methods described by AOAC (2023).

2.2.2. Peroxide value

The peroxide value determines the concentration of hydroperoxide, the primary oxidation products.

0.5 g of the oil was weighed and taken into a conical flask. 25 ml of the solvent was added and air above the liquid was displaced with CO_2. 1 ml of the potassium iodide solution was added, the flask was stoppered and it was allowed to stand for 1 min (with shaking). Then 35 ml of distilled water was added and the liberated iodine was titrated with 0.1 N sodium thiosulphate solution using starch as indicator. The conical flask was vigorously shaken at the end to remove the last traces of iodine from the chloroform layer. A blank titration was carried out simultaneously (The thiosulphate consumption should be negligible). Peroxide value of the oil was determined using the following formula:

$$\text{Peroxide value (mill equivalents or mill moles) per 1000 g of oil} = \frac{(\text{Sample titre} - \text{Blank titre}) \times \text{Normality of sodium thiosulphate solution} \times 1000}{\text{Weight of oil taken}}$$

2.2.3. Saponification number

The oil was filtered. It was ensured that the sample was free of impurities and moisture. 1 g of the sample was weighed in a 250 ml conical flask having a ground glass join. 25 ml of alcoholic KOH solution was pipetted and a few glass beads or pieces of pumice were added. The flask was connected to an air condenser of at least 65 cm (or preferably 202 cm) long. The sample was reflasked on a water bath or a hot plate for not more than 1 h. Gently but steadily the sample was boiled until it was saponified as indicated by the absence of oily matter and presence of clear solution was appeared. The flask and the condenser were allowed to cool. The inside of the condenser was washed with about 10 ml of hot ethanol neutral to phenolphthalein. 1 ml of phenolphthalein indicator was added and titrated with standard HCl. A blank determination was conducted alongside. Then the saponification number was calculated using the following formula:

$$\text{Saponification number of the oil} = \frac{(\text{Blank titre} - \text{Sample titre}) \times \text{Normality of KOH solution} \times 56.01}{\text{Weight of sample (g)}}$$

Table 1
Physicochemical properties of selected vegetable oils heated in different degrees.

Name of the oil sample	Time of heating*	Peroxide value ($mEqO_2$/kg)	Saponification number (mg KOH/g oil)	Iodine value (g I_2/100 g of oil)	Acid value (mg KOH/g oil)
Palm oil	Unheated (P_0)	4.23 ± 0.90^a	192.00 ± 0.89^a	49.54 ± 0.60^a	10.14 ± 0.63^a
	Single heated (P_1)	7.67 ± 0.23^b	191.76 ± 0.31^a	47.27 ± 0.44^b	12.87 ± 0.43^b
	Doubly heated (P_2)	10.25 ± 0.87^c	184.24 ± 0.44^b	47.41 ± 0.18^b	14.24 ± 0.98^c
	Triple heated (P_3)	13.21 ± 0.88^d	180.35 ± 0.60^c	44.34 ± 0.28^c	15.34 ± 0.74^c
Soybean oil	Unheated (S_0)	4.21 ± 0.23^a	187.23 ± 0.23^a	189.55 ± 0.25^a	0.59 ± 0.54^a
	Single heated (S_1)	5.39 ± 0.43^a	186.62 ± 0.54^a	187.19 ± 0.29^b	0.62 ± 0.23^b
	Doubly heated (S_2)	6.76 ± 0.22^b	183.60 ± 0.32^b	186.67 ± 0.65^b	0.67 ± 0.32^c
	Triple heated (S_3)	13.97 ± 0.32^c	181.56 ± 0.25^c	185.97 ± 0.56^c	0.71 ± 0.56^d
Rice bran oil	Unheated (R_0)	2.33 ± 0.23^a	184.87 ± 0.56^a	100.12 ± 0.45^a	1.23 ± 0.45^a
	Single heated (R_1)	5.23 ± 0.33^b	183.98 ± 0.87^b	99.09 ± 0.76^a	1.51 ± 0.16^b
	Doubly heated (R_2)	6.78 ± 0.45^c	183.87 ± 0.56^b	96.87 ± 0.56^b	1.55 ± 0.56^b
	Triple heated (R_3)	9.13 ± 0.52^d	181.34 ± 0.77^c	91.34 ± 0.87^c	1.59 ± 0.37^c

* Each oil was heated 3 times with 30 min intervals. All the values are presented in mean ± SD. Values with different superscripts within each oil at different heating stages indicates significant difference at $p < 0.05$.

2.2.4. Iodine value

0.6 g oil was weighed accurately into a clean dry 250 ml glass stoppered conical flask, and 10 ml of carbon tetrachloride was added. 25 ml of Wij's solution (solution of iodine and iodine monochloride) was added, mixed and stored in a dark cupboard for 30 min. 15 ml of 10 % potassium iodide solution and 100 ml of distilled water were added. The solution was titrated with 0.1 N $Na_2S_2O_3$ solution using starch as an indicator near the end point. A blank determination was carried out alongside without the sample and iodine value was calculated using the following formula:

$$\text{Iodine value of the oil} = \frac{(\text{Blank determination} - \text{Sample determination}) \times \text{N of Sodium thiosulphate} \times 12.69}{\text{Weight of sample (g)}}$$

2.2.5. Acid value

10 g of oil was weighed. The sample was dissolved in hot 100 ml of neutralized ethanol and was titrated using 0.01 or 0.1 N alkali using phenolphthalein as indicator. The conical flask was shaken vigorously during titration and the solution was kept warm. The following formula was used to calculate the acid value of oil:

$$\text{Acid value} = \frac{\text{ml of alkali} \times \text{N of alkali} \times 56.01}{\text{Weight of sample (g)}}$$

2.3. Analysis of the fatty acid composition by GC–MS

Gas chromatography-mass spectrometry analysis was carried out with Clarus® 690 gas chromatograph (PerkinElmer, CA, USA) using a column (Elite-35, 30 m length, 0.25 mm diametre, 0.25 μm thickness of film) and it was equipped with Clarus® SQ 8 C mass spectrophotometre (PerkinElmer, CA, USA). 1 μL sample was injected (splitless mode) and pure Helium (99.999 %) was used as a carrier gas at a constant flow rate (1 mL/min) of 40 min run time. The sample was analyzed in EI (electron ionization) mode at high energy (70 eV). Though inlet temperature was constant at 280 °C, column oven temperature was set at 60 °C (for 0 min), raised at 5 °C per minute to 240 °C and hold for 4 min. The sample compounds were identified comparing to the National Institute of Standards and Technology (NIST) database. Trans fatty acids in all the edible oils heated in different degrees were detected using AOCS (2005). The absolute amounts of trans FAs in the oil were calculated based on the peak intensity of the internal standard.

2.4. Statistical analysis

The sample analysis was carried out in triplicate and the heating process of each oil was carried out with three repetitions. A statistical study was performed using one-way analysis of variance (ANOVA) and SPSS for Windows version 25 to compare the changes in chemical properties of each oil sample. Individual mean differences were evaluated as the least significant difference (LSD), with the significance threshold set at $p < 0.05$.

3. Results and discussion

3.1. Analysis of constituents of oil

3.1.1. Peroxide value

The peroxide value found for selected edible oils is presented in the Table 1. Peroxide Value (PV) determines the autoxidation of oils, i.e. rancidity of the oil. It also reveals the amounts of free radicals forming in the oil when the oil is subjected to different degrees of heating. The standard value of different edible oils may vary, but it is suggested by the Codex 210 standard for fats and oils that the peroxide value of the fresh edible oil should not exceed 10 $mEqO_2$/kg (Woodbury et al., 1998). We obtained two sorts of information regarding edible oils from Table 1. First, the PV of all three oils is within the acceptable range during unheated stage. With the exception of rice bran oil, all PV values surpassed the standard PV after triple heating. The increase in PV indicates generation of lipid peroxide by products (Jaarin & Kamisah, 2012). Similar finding was reported by Khaled et al. (2023).

Again, with the exception of rice bran oil, the other two heating oils surpassed the standard value at the double (palm oil) or triple (soybean oil) heating. Slow rate of increase in PV of rice bran oil may be attributed due to the protective effect of oryzanol present (Latha & Nasirullah, 2014). It implies that rice bran oil is less susceptible to oxidation than the other two oils. To conclude from PV analysis, the study recommends that rice bran oil may be used for cooking four times, however palm and soybean oils should not be used for cooking more than three times.

3.1.2. Saponification number

Khaled et al. (2023) discovered a heating influence on the saponification number of palm, soybean, and rice bran oil. At the first degree of heating, the saponification number increased considerably in all cases. Saponification numbers increased considerably ($p < 0.05$) in our study, following the same pattern. The saponification number began to decline after the second and third heating, and this decrease was most noticeable in palm and soybean oil. A comparable research of Adejumo and Alakowe (2013) revealed a steady decline in saponification number. Odewole and Oyeniyi (2016) reported the same first time decrease in saponification number of groundnut oil and increase in further heating. After repeated heating cycles, the unsaturated fatty acids start to degrade faster and shortens the average chain length which eventually drops the saponification number (Valantina et al., 2015). Table 1 shows that both soybean and rice bran oil met the criteria established by FAO/WHO (2009), which was 181.60 mg KOH/g of oil after third degree of heating. However, in the case of palm oil, it exceeded the standard value, indicating that it followed a high degree of saturation as heating time increased.

3.1.3. Iodine value

The results shown in Table 1 showed that the iodine value of all three oils declined as heating time increased. However, in the case of rice bran oil, it was more significant. At the unheating stage, the IV of rice bran oil was 100.12 ± 0.45 g I_2/100 g of oil. It began to decline, and the rate of decline was faster (approximately 77 percent) than the other two oils. This might be owing to the presence of double bonds in rice bran oil. Similar findings were reported by Yoon et al. (1987). Gharby et al. (2014) reported the decrease in iodine value due to the continuous heating of palm, sunflower and rapeseed oil respectively. They found a drop in iodine value of palm oil of at least 2 units after 30 h of heating. Our study also found a drop of almost 4.22 units in triply heated samples. Cuesta et al. (1991) found that heating attributed to the destruction of the double bonds of unsaturated fatty acids of oil and thus alters the iodine value.

3.1.4. Acid value

The acid value of all three oils in Table 1 rose considerably ($p < 0.05$), indicating that the amount of free fatty acids rises as heating time increases. The more the amount of unsaturated fatty acids present in an oil, the longer it takes heating to destroy their double bonds and release free fatty acids, which is the reason of rancid odor in fats and oils. In our case, the acid value of each oil started to increase soon after the heating started. Similar findings were reported by Habarakada et al. (2021). But when comparing within the selected oils in our study, it had been observed that, palm oil had the highest change in acid value (about 45 %). It may be due to less amount of unsaturated fatty acid chain present when compared to soybean and rice bran oil. Again, acid value and saponification number of the edible oils has got certain relation in this study. Saponification number represents the average molecular weight

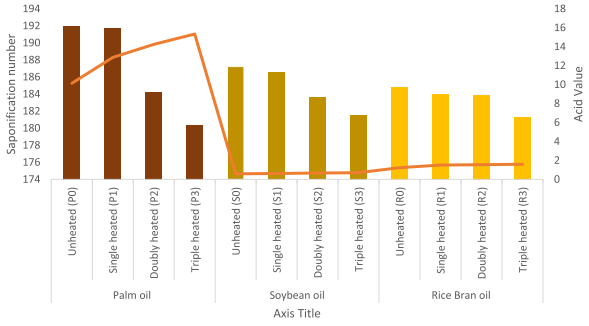

Fig. 1. Relationship between Saponification number and acid value of selected vegetable oils at different heating time.

Table 2
Fatty acid concentration changes (w/w%) in selected vegetable oils in different degrees of heating.

Name of the oil	Name of the fatty acid	Unheated	Single heated	Doubly heated	Triply heated
Palm oil	Myristic acid (C14:0)	1.02 ± 0.23^{a}	0.96 ± 0.23^{a}	0.65 ± 0.23^{b}	ND
	Palmitic acid (C16:0)	39.16 ± 0.21^{a}	37.87 ± 0.29^{b}	31.32 ± 0.23^{c}	28.52 ± 0.33^{d}
	Stearic acid (C18:0)	6.63 ± 0.23^{a}	ND	ND	ND
	Oleic acid (C18:1)	36.03 ± 0.34^{a}	35.54 ± 0.26^{a}	29.22 ± 0.45^{b}	29.01 ± 0.14^{b}
	Linoleic acid (C18:2)	10.05 ± 0.45^{a}	8.33 ± 0.43^{b}	7.34 ± 0.59^{c}	6.89 ± 0.42^{c}
Soybean oil	Myristic acid (C14:0)	1.28 ± 0.98^{a}	1.03 ± 0.43^{b}	0.87 ± 0.45^{c}	0.22 ± 0.25^{d}
	Palmitic acid (C16:0)	40.13 ± 0.34^{a}	39.36 ± 0.24^{a}	32.23 ± 0.05^{b}	29.23 ± 0.23^{c}
	Stearic acid (C18:0)	6.87 ± 0.23^{a}	6.29 ± 0.45^{b}	5.98 ± 0.23^{b}	4.09 ± 0.12^{c}
	Oleic acid (C18:1)	22.72 ± ± 0.45^{a}	20.33 ± 0.23^{b}	18.09 ± 0.14^{c}	18.00 ± 0.46^{c}
	Linoleic acid (C18:2)	8.59 ± 0.54^{a}	8.24 ± 0.35^{b}	6.85 ± 0.12^{c}	5.76 ± 0.35^{d}
Rice bran oil	Myristic acid (C14:0)	3.28 ± 0.34^{a}	2.75 ± 0.21^{b}	1.87 ± 0.43^{b}	1.07 ± 0.23^{c}
	Palmitic acid (C16:0)	12.17 ± 0.56^{a}	10.75 ± 0.23^{b}	5.07 ± 0.45^{c}	4.54 ± 0.45^{c}
	Stearic acid (C18:0)	3.87 ± 0.23^{a}	2.89 ± 0.67^{b}	2.58 ± 0.53^{bc}	2.29 ± 0.23^{c}
	Oleic acid (C18:1)	32.22 ± 0.34^{a}	25.35 ± 0.55^{b}	24.34 ± 0.37^{b}	21.54 ± 0.67^{c}
	Linoleic acid (C18:2)	25.32 ± 0.31^{a}	24.22 ± 0.24^{b}	22.22 ± 0.22^{cd}	21.02 ± 0.34^{d}

*ND = Not Detected. All the values are presented in mean ± SD. Values of each row with different superscripts for each fatty acids in each oil at different heating stages indicates significant difference at $p < 0.05$.

of the fatty acids present in the edible oils studied. As heat was applied to the oil samples, long chain fatty acids broke down into shorter ones and as a result, the saponification number of all the edible oils went on decreasing. As the saponification value decreased and the amount of short chain fatty acid or free fatty acids went on increasing, the acid value of the oils at different heating time went on increasing. This relation is clearly visible in Fig. 1.

3.2. Analysis of the fatty acid composition, foreign compounds and trans-fatty acids present in the oils by GC–MS

3.2.1. Analysis of fatty acid composition in palm oil

According to the Codex Standard for Named Vegetable Oils CSX 210 - 1999 (CODEX, 1999), fresh palm oil should contain at least 0.5–2.0 percent of myristic acid as well as palmitic acid, stearic acid, oleic acid, and linoleic acid should be 39.3–47.5 percent, 3.5–6.0 percent, 36.0–44.0 percent, and 9–12 percent of total fatty acid (w/w%) present in the oil respectively. Table 2 displays the data received from this investigation.

The results of the research revealed that there had been considerable alterations in fatty acid percentage as a result of continual heating. As the heat duration increased, the quantity of myristic and oleic acid decreased. Palmitic acid in our sample continued to decrease as a result of the extended heat. Linoleic acid has undergone slow decreasing with the duration of prolonged heat. It is undeniable that prolonged heating has a negative impact on the fatty acid content of palm oil. From table 3, it can be seen that the researchers tried to establish the relation of iodine value and the changes in fatty acid composition of palm oil. Iodine value measures the amount of unsaturation in oils. Iodine value goes on decreasing as the applied heat attempted to break the double bounds of C18:1 and C18:2 unsaturated fatty acids present on the studied palm oil.

Table 3
Relationship between fatty acid concentration changes and iodine value of selected edible oils in different degrees of heating.

Name of the oil	Heating time	C14:0	C16:0	C18:0	C18:1	C18:2	Iodine value (g I_2/100 g of oil)
Palm oil	P_0	1.02 ± 0.23^{a}	39.16 ± 0.21^{a}	6.63 ± 0.23^{a}	36.03 ± 0.34^{a}	10.05 ± 0.45^{a}	49.54 ± 0.60^{a}
	P_1	0.96 ± 0.23^{a}	37.87 ± 0.29^{b}	ND	35.54 ± 0.26^{a}	8.33 ± 0.43^{b}	47.27 ± 0.44^{b}
	P_2	0.65 ± 0.23^{b}	31.32 ± 0.23^{c}	ND	29.22 ± 0.45^{b}	7.34 ± 0.59^{c}	47.41 ± 0.18^{b}
	P_3	ND	28.52 ± 0.33^{d}	ND	29.01 ± 0.14^{b}	6.89 ± 0.42^{c}	44.34 ± 0.28^{c}
Soybean oil	S_0	1.28 ± 0.98^{a}	40.13 ± 0.34^{a}	6.87 ± 0.23^{a}	22.72 ± 0.45^{a}	8.59 ± 0.54^{a}	189.55 ± 0.25^{a}
	S_1	1.03 ± 0.43^{b}	39.36 ± 0.24^{a}	6.29 ± 0.45^{b}	20.33 ± 0.23^{b}	8.24 ± 0.35^{a}	187.19 ± 0.29^{b}
	S_2	0.87 ± 0.45^{c}	32.23 ± 0.05^{b}	5.98 ± 0.23^{bc}	18.09 ± 0.14^{c}	6.85 ± 0.12^{b}	186.67 ± 0.65^{bc}
	S_3	0.22 ± 0.25^{d}	29.23 ± 0.23^{c}	4.09 ± 0.12^{c}	18.00 ± 0.46^{c}	5.76 ± 0.35^{c}	185.97 ± 0.56^{c}
Rice bran oil	R_0	3.28 ± 0.34^{a}	12.17 ± 0.56^{a}	3.87 ± 0.23^{a}	32.22 ± 0.34^{a}	25.32 ± 0.31^{a}	100.12 ± 0.45^{a}
	R_1	2.75 ± 0.21^{b}	10.75 ± 0.23^{b}	2.89 ± 0.67^{b}	25.35 ± 0.55^{b}	24.22 ± 0.24^{b}	99.09 ± 0.76^{a}
	R_2	1.87 ± 0.43^{bc}	5.07 ± 0.45^{c}	2.58 ± 0.53^{bc}	24.34 ± 0.37^{b}	22.22 ± 0.22^{c}	96.87 ± 0.56^{b}
	R_3	1.07 ± 0.23^{c}	4.54 ± 0.45^{c}	2.29 ± 0.23^{c}	21.54 ± 0.67^{c}	21.02 ± 0.34^{d}	91.34 ± 0.87^{c}

*N.D = Not Detected. Here, P, S, R represent the Palm, Soybean and Rice bran oil respectively. Superscripts 0, 1, 2, 3 in the oil name represent the single, doubly and triple heated oils. All the values are presented in mean ± SD. Values with different superscripts for each fatty acids in each oil at different heating stages indicates significant difference at $p < 0.05$.

Due to their structural nature, unsaturated fatty acids are prone to oxidation and this oxidation process is higher when heat is applied. It is notable in the study that, palm oil unsaturated fatty acids concentration changed with a huge difference compared to the other two oils.

3.2.2. Analysis of fatty acid composition in soybean oil

According to Firestone (2013), a fresh sample of soybean oil should have 39.3–47.5 percent palmitic acid. Stearic acid, oleic acid, and linoleic acid should be 3.5–6.0 percent, 22–36 percent, and 9–12 percent (w/w%) of total fatty acids respectively. Table 2 displays the results received from this investigation.

According to the table's findings, all of the fatty acids found in soybean oil see variations in their concentration. Linoleic acid, for example, one of the necessary fatty acids, dropped as heating time increased. It is sometimes lower than the normal range for that fatty acid. Only myristic acid defied the trend here. In the GC–MS analysis, lauric acid was not found in either the unheated or heated samples.

From Table 3, it can be seen that unsaturated fatty acid composition was changed simultaneously with the change in iodine value. But in case of soybean oil, the change was little compared to palm oil. This was because the smoke point of the soybean oil which was higher than the palm oil.

3.2.3. Analysis of fatty acid composition in rice bran oil

According to Firestone (2013), a fresh sample of rice bran oil should include palmitic acid, stearic acid, oleic acid, and linoleic acid in the proportions of 12–28 percent, 1–4 percent, 31.9–50.0 percent, and 16–43.6 percent (w/w%) of total fatty acids. Table 2 displays the results received from this investigation. According to the table, every single fatty acid in the oil undergoes noteworthy alterations. Palmitic acid and linoleic acid are two of the most affected.

From Table 3 in the case of rice bran oil, iodine value decreases also affected the fatty acid composition changes. The unsaturated fatty acids here undergone decreasing due to oxidation associated with heat applied to the samples. The C18:2 fatty due to their double C—C bond, has undergone slower degradation and slowed down the iodine value changes for the rice bran oil studied here.

3.2.4. Analysis of foreign compounds present in edibles oils by GC–MS

Several foreign chemicals were discovered to be formed along with fatty acid modifications as a result of the continuous heating of edible oils in this investigation. When such substances are eaten, they are very harmful to human health. For example, glycidyl fatty acid esters, fumaric acid, succinic acid, cyclohexane, and so on. In this investigation, glycidyl fatty acid esters (GEs) were discovered in the degradation of each oil caused by prolonged heating. Cyclohexane is produced during the breakdown of palm and soybean oil. Palm oil was discovered to contain fumaric acid and succinic acid.

Table 4
Change in trans fatty acid content present in edible oils due to different degree of heating.

Sample name	Unheated (mg/g)	Single heated (mg/g)	Doubly heated (mg/g)	Triple heated (mg/g)
Palm oil (18:1t)	ND	ND	ND	ND
Soybean oil (18:1t)	ND	ND	ND	ND
Rice bran oil (18:1t)	ND	ND	ND	ND
Palm oil (18:2t)	ND	1.53 ± 0.12^{a}	2.31 ± 0.32^{b}	2.67 ± 0.32^{b}
Soybean oil (18:2t)	ND	0.92 ± 0.39^{a}	1.12 ± 0.12^{b}	1.56 ± 0.002^{b}
Rice bran oil (18:2t)	ND	0.75 ± 0.032^{a}	0.89 ± 0.045^{b}	0.97 ± 0.022^{b}
Palm oil (18:3t)	ND	0.12 ± 0.022^{a}	0.23 ± 0.067^{b}	0.27 ± 0.012^{b}
Soybean oil (18:3t)	ND	0.87 ± 0.034^{a}	1.82 ± 0.34^{b}	1.89 ± 0.12^{b}
Rice bran oil (18:3t)	ND	0.18 ± 0.05^{a}	0.25 ± 0.03^{b}	0.32 ± 0.072^{c}

*N.D = Not Detected. All the values are presented in mean ± SD. Values with different superscripts in each oil at different heating stages indicates significant difference at $p < 0.05$.

Glycidyl fatty acid esters (GEs) are process pollutants. They are created during the manufacturing of edible oils through a deodorization process that takes place at high temperatures (Shimamura et al., 2021). The food's water vaporizes during cooking, starting the hydrolysis process. Glycerol, free fatty acids, monoacylglycerides, and diacylglycerides are produced when the ester bonds of the triacylglycerides in the vegetable oil are broken down by the water vapor (Jaarin et al., 2018) . Diacylglycerols (DAG) are the precursors of GEs in edible vegetable oils that turns into GEs when reacts with products of oil degradation (Destaillats et al., 2012). Glycidol has been linked to genotoxicity and carcinogenesis (Aasa et al., 2016). This chemical was found in all of the oils examined here. More specifically, the quantity of these molecules, such as glycidyl oleate and glycidyl palmitate, is observed to increase with the degree of heating.

Maleic acid and maleic anhydride in edible oils reacts with each other at elevated temperature (at about 130 °C) to produce fumaric acid and maleic acid is easily reduced to succinic acid during heating (Wojcieszak et al., 2015). There were growing concerns on the safety of fumaric acid esters (FAE) as several cases were reported of acute renal toxicity in patients treated with FAE (Balak, 2015). Other foreign substances, if taken in sufficient quantities, can potentially have significant health effects in humans.

3.2.5. Analysis of trans-fatty acids present in the sample edible oils

Prolonged heating of the sample edible oils shown the presence of trans fatty acids. Although C18:1t trans fatty acid was not detected in any of the oils at different heating stages. In case of C18:2t and C18:3t trans fatty acids all the oils shown an increasing trend (Table 4). Though none of the oil contained trans fatty acids that cross the levels of trans fatty acid suggested by AOCS (2005). Hence, it can be concluded that due to heating sample edible oils at 180 °C or such temperature has no significance in trans fatty acid development. Similar findings were reported by Yi et al. (2014) and Wakako et al. (2010).

4. Conclusion

Repeated heating of oil causes changes in fatty acid composition and physicochemical properties of edible oils which are detrimental to human health. Heating induces different reaction inside the oils and makes oil unsafe for human consumption. In this study, it was observed that oils like palm and soybean oil that contain higher amount of saturated fatty acid undergo different chemical reaction and worsens the oil quality. This study revealed that the selected vegetable oils should not be used after third degree of heating as the overall quality of the oils started to get worse for cooking as well as for human consumption. Fatty acids of the oils started to degrade due to continuous heating, releasing different foreign toxic chemicals. Heating caused many essential fatty acids to alter their structure. These changes were mostly observed in the following order: palm > soybean > rice bran oil. The findings of the study can be used by the legal authorities to lay down legislations against repeated heating of oil. Further research may be performed to find out if there is any effect on fatty acid composition due to the reactions occurring between different foodstuffs and oil under different heating temperatures and durations.

CRediT authorship contribution statement

B.M. Khaled: Writing – original draft, Resources, Investigation, Formal analysis, Conceptualization. **Mrityunjoy Biswas:** Writing – review & editing. **Adda Ann Sina:** Writing – review & editing, Visualization, Validation, Data curation. **Asraful Alam:** Software, Methodology, Formal analysis. **Md. Abdul Alim:** Supervision, Conceptualization. **Md. Emran Hossain:** Methodology, Formal analysis. **Md. Nazmus Saqib:** Writing – review & editing. **Md. Suman Rana:** Data curation. **Sumia Rahman Sweet:** Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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