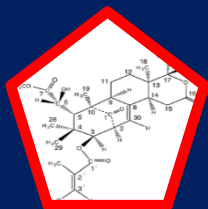
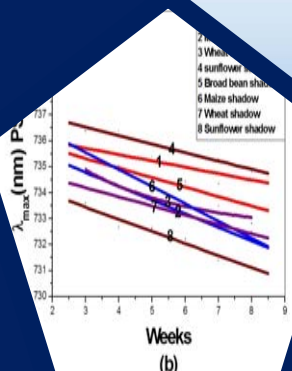




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Distribution of Fatty Acids in Fresh and Thermally Treated Oxidized Vegetable Cooking Oils

Azhari H. Nour^{1*}, Aini N. Mohamed², Abeer A. Idris¹, Awadalla B. Omer¹ and Mohammed B. Sulieman³

¹*Faculty of Pure and Applied Sciences, International University of Africa, 12223, Khartoum, Sudan*

²*Faculty of Industrial Sciences and Technology, University Malaysia Pahang, 26300, Malaysia*

³*Medicinal, Aromatic Plants, Research Institute, National Centre for Research, 2404, Khartoum, Sudan*

*Corresponding author: azharyhamid@yahoo.com

ABSTRACT

Deep fry cooking method is desirable to many consumers as it gives valuable sensory characteristic such as crispy texture, golden brown color and good aroma. The cooking oil is usually replenished and reused several times prior discarded for lower cost purpose by food manufacturer, fast food outlets and eateries operators. However, reusing and recycling the oil will degrade the nutritional and sensory quality of the oil as several processes occur such hydrolysis, polymerization, formation of conjugated dienes, formation and decomposition of hydroperoxides. The study aimed to investigate degradation level in cooking oil by the distribution of fatty acids and the presence of short chain fatty acids. The method involved converting fatty acids in oil to methyl ester prior analyzed by Gas Chromatography- Mass Spectroscopy (GC-MS). Then investigated the distribution of fatty acids and compared in fresh oil, waste oil collected from food outlets and the oil subjected to heating and frying in laboratory. Oil that were thermally treated and oxidized showed the decreasing in polyunsaturated fatty acids content and increasing in saturated fatty acids content. The ratio of linoleic acid to palmitic acid (18:2/16:0) decreased after oil was thermally treated and oxidized. The significant

difference between fresh and thermoxidized oils is the presence of octanoic acid (C8:0) in thermoxidized oil. An acceptable linear correlation ($r^2= 0.886$) was found between the changes in linoleic acid content and the amount of octanoic acid that was formed. The results obtained clearly support that octanoic acid could be as marker for oil that has is thermoxidized.

Keywords: Vegetables oil, Fatty acids, Thermoxidized, Degradation level, Octanoic acid

مستخلص

طريقة الطهي العميق هي أمر مرغوب فيه للعديد من المستهلكين لأنه يعطي خاصية حسية قيمة مثل الملمس المقرمش ، اللون البني الذهبي والرائحة الجيدة. وعادة ما يتم تجديد زيت الطهي وإعادة استخدامه عدة مرات قبل التخلص منه لأغراض أقل تكلفة من قبل الشركات المصنعة للأغذية و محلات الوجبات السريعة والمطاعم. ومع ذلك ، فإن إعادة استخدام الزيت وإعادة تدويره سيؤدي إلى تدهور الجودة الغذائية والحسية للزيوت ، حيث تحدث العديد من العمليات مثل التحلل ، والبلمرية ، وتشكيل داينات المترافقة ، وتشكيل وتحلل الهيدروبروكسيدات. هدفت الدراسة إلى دراسة مستوى التدهور أو الانحلال في زيت الطهي عن طريق توزيع الأحماض الدهنية ووجود الأحماض الدهنية قصيرة السلسلة. تضمنت الطريقة أولاً تحويل الأحماض الدهنية في الزيت الطازج إلى استرات الميثيل ومن ثم تحليلها بواسطة تحليل كروماتوغرافيا الغاز - مطياف الكتلة (GC-MS) ثم التحقيق في التوزيع الأحماض الدهنية ومقارنتها بالزيت الطازج ، وفضلات الزيوت التي تم جمعها من محلات الوجبات السريعة أو المطاعم والزيت الخاضع للتسخين والقلي في المختبر. فإن نتائج الدراسة أظهرت بان الزيت المعالج حرارياً والأكسدة أدى إلى انخفاض محتوى الأحماض الدهنية غير المشبعة المتعددة وزيادة محتوى الأحماض الدهنية المشبعة. انخفضت معدل نسبة حمض اللينوليك إلى حامض البالمتيك (18: 16/2: 0) بعد أن تم معالجة الزيت وأكسدته حرارياً. والفرق الكبير بين الزيوت الطازجة والمؤكسد حرارياً هو وجود حمض الأوكتانويك (C8:0) في الزيت المؤكسد حرارياً تم العثور على ارتباط خطي مقبول ($r^2 = 0.886$) بين التغيرات في محتوى حمض اللينوليك وكمية حمض الأوكتانويك التي تشكلت. النتائج التي تم الحصول عليها تدعم بوضوح أن حمض الأوكتانويك يمكن أن يكون علامة أو مقياس على أن الزيت قد تم أكسدتها حرارياً.

كلمات مفتاحية: الزيوت النباتية ، الأحماض الدهنية ، الأوكسدة الحرارية ، مستوى التدهور أو الانحلال ، حمض الأوكتانويك ،

1. INTRODUCTION

Deep fry cooking method is desirable to many consumers as it gives valuable sensory characteristic such as crispy texture, golden brown color and good aroma. The cooking oil is usually replenished and reused several times prior discarded for lower cost purpose by food manufacturer, fast food outlets and eateries operators. However, reusing and recycling the oil will degrade the nutritional and sensory quality of the oil as several processes occur such hydrolysis, polymerization, (Bhattacharya et al., 2008; Ramadan., 2010), formation of conjugated dienes, formation and decomposition of hydroperoxides (Yoon et al., 2000), especially at high temperature between 160°C to 180°C (Hageman et al., 1988; Alvis et al., 2009; Ramadan, 2010) with the presence of moisture and oxygen. The processes lead to the formation of decomposition product which consists of volatile and non volatile components and the later can accumulate in the degraded oil, absorbed into food, ingested, thus impose threat to health. The effect of thermoxidized oil to health has been extensively reported. Leong et al. (2008) reported that feeding heated oil to rats resulted in increase of blood pressure and organ damage. Oxidized oil also is said to be genotoxic (Hageman et al., 1988), mutagenic (Bautista et al., 2001), promote intestinal tumor (Aladedunye and Przybylski 2009), organoleptic failure (Hein et al., 1998), carcinogenic (Yildiz et al., 2006), liver damage, promote deleterious effect in the

endothelial function (Williams et al., 1999; Rueda-Clausen et al., 2007) and growth retardation (Al-Harbi, 1993).

Due to the hazardous impact of thermoxidized oil to health, food production process especially by the food manufacturer, fast food outlet, eateries, need to be monitored especially by regulatory authorities. But the question remain is what is the best, simplest method that can be applied as routine analytical approach to determine quality of oil. Usually, sensory evaluation such as the odor of the oil is conducted; however this evaluation depends heavily on the perception of the evaluator. Other physical methods involve smoke points (Augustin et al., 1987), viscosity, color of the oil, refractive index, dielectric constant. Chemical methods involve free fatty acids value, iodine value, peroxide value; p-anisidine values content of carbonyls or conjugated double bounds, non-urea adduct forming esters, and polymer contents (Al-Harbi, 1993; Gil et al., 2004; Naz et al., 2005; Abdulkarim et al., 2007; Bansal et al., 2010). All of the methods involve measuring non specific compounds and depends on the type of food, oil and the condition during frying operation and only can roughly determine the quality of oil (Hein et al., 1998). Different factors during frying such as frying temperature, emulsifiers, trace metals, food scraps, free fatty acids and alkaline-reacting materials (Bhattacharya et al., 2008) in the frying oil will cause different type of degradation. For example, oil with high free fatty acids numbers are oil that has been exposed to food moisture as free fatty acids are caused by hydrolysis. In one operation, the rate of hydrolysis may be twice

that of the rate of oxidation, whereas in another operation, the reverse may occur. Hence, a specific method may be ideal for one operation but completely useless in another (Fritsch, 1981). Moreover, oil consists of triglycerides with a variety in chain length and degree of saturation, so different oil will react different way towards degradation factor. According to Bhattacharya et al., (2008), degradation of polyunsaturated oils, such as soybean or sunflower oil is relatively more rapid.

Determination of total polar material is recognized as the most reliable method to measure oil degradation (Fritsch, 1981; Marquez-Ruiz et al., 1995; Gil et al., 2004; Bansal et al., 2010), however, these values were adopted for polyunsaturated oils and might not be applicable to more saturated oils, as these oils are less susceptible to oxidation during frying (Al-Harbi, 1993). Moreover, this method is time consuming and the use of large volume of solvents is considered potential environmental problem (Innawong et al., 2004; Ramadan, 2010).

All of the method above will give results which depend heavily on the type of oil, frying condition, type of food. Moreover, all the methods above are tedious, some use a large amount of solvent and it will give quantitative results. Hence, it is better to have simple method, independent of factors involve during frying process and will give conclusive results. Specific component need to be detected, regardless of its amount, and will give clear cut difference between unused oil and degraded oil, and can act as marker for oil degradation. Research done by Berdeux et al. (1999; 2002); Velasco et al.

(2005), can be proposed as conclusive method to differentiate between unused and recycled oil. The analytical approach is based on converting fatty acid to simplest fatty acids derivatives which is the methyl ester, prior analyzed by gas chromatography. They discovered the presence of short chain fatty acid which is the octanoic (C8:0) in thermally treated and oxidized oil. Hence, C8:0 can be proposed as component that can give clear cut difference between fresh oil and thermoxidized oil. This fatty acid is the results of decomposition of an unsaturated fatty acid which is linoleic acid (18:2) during thermal treatment. With the presence of air and high temperature, linoleic acids decomposed to hydroperoxides. Due the instability of hydroperoxides, it decompose further through homolytic β -scission of the alkoxy radicals coming from allylic hydroperoxides to form ultimately volatile and non-volatile oxidation compounds, the latter including short-chain glycerol-bound compound. (Kamal Eldin et al., 1997; Berdeux et al., 1999).

However, their research was conducted on highly unsaturated oil such as corn oil, sun flower oil, and palm olein and in model systems of triacylglycerols and fatty acid methyl esters. It is not known whether the result can be applied to palm oil, which is highly saturated and more resistant to oxidation and the sample in their research was applied only to samples heated in the lab, no sampling of waste oil was conducted, to signify the real frying condition and difference variables involve in frying process. So this study was focused to detect the presence of octanoic acid in thermoxidized and degraded

palm oil. Palm oil was chosen as sample in this study owing to their common use as cooking medium by Malaysian. Waste oil samples were collected several types of food outlets. Fresh oil samples were subjected to heating and frying in laboratory, to act as control sample.

2. MATERIALS AND METHODS

2.1 Oil samples

Two brands of high oleic acid palm oil (denoted as Sample A, Sample B), one brand of corn oil (denoted as sample C) were purchased from local hypermarket on 13, April 2010, Kuantan. Sample A, B, and C acted as control sample in this present study. Chicken nuggets were selected as food medium, bought from local hypermarket. Sample of waste oil were collected from food outlets which are eateries, caterers, banana fritters food stall, and fast food outlet. These samples were denoted as Sample D, Sample E, Sample F and Sample G respectively. The samples were collected at different type food outlet to signify different frying condition such as the type of and the brands of oil that is used. The outlet operators were asked to follow their normal frying procedure and save about 1 liter of the oil sample. The fresh counterparts of the oil samples were purchased from local outlet. No fresh counterpart for sample G because the origin of the oil was not revealed by the fast food outlet operators. All the samples were kept in 20°C until further use.

2.2 The frying protocol

One kilograms of sample A, B, and C were fried in 3L stainless steel domestic deep fryer at 185°C – 200°C with total frying time of 6 hours. This condition was chosen as it resembles the actual frying practices. Chicken nuggets which acted as food medium were fried in 200 g batches, 10 minutes apart for 6 hours. Heating conditions was applied by keeping all the process variables the same as those in frying except there was no food in the oil. No replenishment of the oil during frying and heating to avoid dilution of decomposed and oxidized product.

2.3 Esterification procedure

Exactly, 200 mg of oil sample were dissolved in 10 mL of hexane in test tube, then 4 mL of 2M methanolic KOH was added to the tube. Then the tube was vortex occasionally. After 15 minutes, the hexane phase was collected and washed twice with 4 mL water to get rid of remaining catalyst and methanol. The solution then was dried over anhydrous sodium sulfate and filtered using filter paper. All solvents and chemicals used are of analytical grade.

2.4 Fatty acids analysis

About, 1 μ L of the methyl ester solution was injected into an Agilent 6890 GC equipped with flame ionisation detector (FID), HP Innowax capillary column (30 m X 0.25 mm). Helium was used as carrier gas at 1.5 mL/min. Both injection port and detector temperatures were 250°C. The column temperature was programmed from 50°C for 1 minute, increased to

70°C at 5°C min⁻¹, held for 9 minutes, and further programmed to 200°C at 15°C min⁻¹, held for 10 minutes. Final temperature was 230°C, held for 4 minutes. Split ratio of 50:1 was used. Fatty acids were identified by comparison of retention time with authentic standards (C4-C24, Supelco 37 component FAME Mix). Results of fatty acids are given as percentage area of the sum of all identified peaks

3. RESULTS AND DISCUSSION

Fatty acids composition

The fatty acids composition of control samples and collected samples at food outlets are presented in Table 1 and Table 2, respectively. Results are given based on the average of three replications with standard deviation. Sample A and B, which were the control samples of palm oils, gave fatty acids composition that is characteristic of palm oil, where the amount of palmitic acid and oleic acid were high. Saturated and polyunsaturated fatty acids composition is almost equal. Sample C, which was the control sample of corn oil, gave fatty acids composition that is characteristic of corn oil, where content of linoleic acid is prominent. As expected, sample D, E and F showed characteristic of palm oil, while sample G, which the origin of the oil was not revealed, also showed characteristic of palm oil.

A total of 10 fatty acids were detected in fresh oil and 11 in heated, fried, and waste oil. The carbon range for fresh oil is from C10 to C20 and C8 to C20 in waste oil. Major fatty acids in all samples were stearic acid, palmitic

acid, oleic acid and linoleic acids. The number of double bonds in unsaturated fatty acids is from one to three double bonds.

Figure 1 shows fatty acids saturation degree in the oil sample. After thermo oxidation, the content of polyunsaturated fatty acids in degraded oil of all samples decreased, while the saturated acid content increased. The content of saturated fatty acids is not really increase in direct sense, but more to the indirect effect of the polyunsaturated content decrease. Sample A, B which were control sample of palm oil, were measured as a group, Sample D, E and F which were palm oil sample were measured as a group. The decrease in unsaturation is evidence of the transformation occurring in the polyunsaturated fatty acids which the oil initially contains. The higher the temperature during frying is the higher the decrease of fatty acids unsaturation (Moya Moreno et al., 1999).

There was decrease in the amount of linoleic acid in waste oil compared to fresh oil. The deterioration of linoleic acid was more pronounced in Sample C which was corn oil. This is because the higher the unsaturation degree of fatty acid, the higher the susceptibility towards oxidation. This decrease can be attributed to the destruction of double bonds by oxidation, scission, and polymerization (Cowan, 1954). According to Berdeux et al. (2002), linoleic and oleic acids are the most representative fatty acids undergoing degradation in the oils. However, in this study, there is no decrease in the content of oleic acid. Probably this is because oleic acid is more resistant towards oxidation. Bansal et al. (2010) discovered the same

finding in their research of deep fried and heated palm olein. However, Cuesta et al. (1991) found that oleic acids showed a tendency to decrease in frying of olive oil. Rossi et al. (2009), observed higher degradation rate of sunflower oil, compared to other types of vegetable oil, due to the high content of linoleic acid. Aladedunye and Przybylski (2009) observed progressive decrease in linoleic acid of canola oil throughout frying period. Takeoka et al. (1997) found that of oils heated at 190 °C corn oil had the fastest rate of deterioration reaching the limit. The ratio of linoleic acid to palmitic acid (C18.2/C16:0) has been suggested as a valid indicator of the level of PUFA deterioration (Aladedunye and Przybylski, 2009). The lower the value of C18.2/C16:0 ratios, (increase in palmitic acid and a decrease in linoleic acid), the higher the degradation level. Table 1 and Table 2 show the value of C18.2/ C16:0 for each sample. As can be seen from those Tables, thermally treated oils show lower value of C18.2/ C16:0. Bansal et al. (2010) also discovered the same result with their study on repeated frying of palm olein.

The most significant thing about the result is octanoic acid (C8:0) only detected in thermoxidized oils, which were the heated, fried and waste oil. Figure 2 presents the chromatogram of fresh, heated, fried and waste oil. The presence of octanoic acid is represented by a peak eluted at 13.256 minutes retention times and the peak could only be detected in chromatogram of waste, heated and fried oil. The result is similar with studies conducted by Berdeux et al. (1999, 2002); Velasco et al. (2005) in analyzing fatty acids in fresh and thermoxidized oil. C8:0 is the result of linoleic acid oxidation. This

was supported by an acceptable correlation ($r^2=0.8806$) between the changes in linoleic acid level to the amount of octanoic acid formed in this study. Figure 3 shows the correlation graph. As can be seen from Table 1, the amount of C8:0 formed was high in Sample C (fried corn oil), and this is in correlation with its higher content of linoleic acid in corn oil. Table 2, showed the amount of C8:0 in Sample G was higher compared to other waste oil. This result is expected as Sample G, being waste oil collected from a well known fast food outlet, appeared very degraded with high viscosity and very dark in color. This also showed that, fast food outlet operators tend to reuse cooking oil until it is really degraded before being disposed, probably for lower cost purpose. Other samples showed almost equal amount of C8:0. However, regardless the amount of C8:0 in the degraded oil, it can be proposed as marker for used oil as it is not detected in fresh oil.

Table 1: Heating and cooking effect on individual fatty acid contents (% of total fatty acids) of control sample

Fatty Acids	Control Sample					
	Sample A		Sample B		Sample C	
	Fresh	Heated	Fried	Fresh	Heated	Fried
Saturated FA						
C8:0	nd	0.02 ^a	0.09 ^a	nd	0.09 ^a	0.09 ^a
C10:0	0.015 ^a	0.016 ^a	0.020 ^a	0.02 ^a	0.08 ^a	0.02 ^a
C12:0	0.16 ^a	0.18 ^a	0.21 ^a	0.19 ^a	0.47 ^a	0.21 ^a
C14:0	1.04 ^a	1.06 ^a	1.07 ^a	0.98 ^a	1.10±0.07	1.05±0.04
C16:0	37.89±0.02	36.61±0.02	39.24±0.02	39.17 ^a	42.67±3.02	42.04±3.02
C18:0	4.23 ^a	4.17 ^a	4.31 ^a	4.14 ^a	4.46±0.27	4.48±0.11
C20:0	0.36 ^a	0.32 ^a	0.13 ^a	0.20 ^a	0.20 ^a	0.14 ^a
Total	43.70	42.38	45.07	44.70	49.07	48.03
Monounsaturated						
C16:1, n-9c	0.18 ^a	0.31 ^a	0.38 ^a	0.17 ^a	0.17 ^a	0.33±0.14
C18:1, n-9c	43.60±0.03	44.33±0.01	45.14±0.05	44.17 ^a	43.28±0.76	43.72±0.75
Total	43.63	44.64	45.52	44.34	44.04	44.05
Polymunsaturated						
C18:2, n-6c	12.31±0.02	12.40±0.02	8.60±0.06	10.61 ^a	7.25±2.91	7.35±1.55
C18:3, n-6c	0.36 ^a	0.32 ^a	0.38 ^a	0.38 ^a	0.38 ^a	0.38 ^a
Total	12.67	12.72	8.98	10.99	7.63	7.73
C18:2/C16:0	0.33	0.33	0.21	0.27	0.16	0.17

nd, not detected
^a, standard deviation <0.01

Table 2: Heating and cooking effect on individual fatty acid contents (% of total fatty acids) of collected oil sample

Fatty Acids	Sample D		Sample E		Sample F		Sample G
	Fresh	Waste	Fresh	Waste	Fresh	Waste	Waste
Saturated FA							
C8:0	nd	0.03 ^a	nd	0.02 ^a	nd	0.02 ^a	0.06 ^a
C10:0	0.02 ^a	0.02 ^a	0.02 ^a	0.02 ^a	nd	0.02 ^a	0.02 ^a
C12:0	0.23 ^a	0.20 ^a	0.18 ^a	0.23 ^a	0.18 ^a	0.18 ^a	0.28 ^a
C14:0	0.96 ^a	0.96 ^a	0.94 ^a	0.96 ^a	1.00 ^a	1.07 ^a	1.00 ^a
C16:0	37.38 ^a	38.39 ^a	38.52 ^a	38.82±0.09	36.94 ^a	36.67 ^a	39.82 ^a
C18:0	4.14 ^a	4.28 ^a	4.19 ^a	4.13 ^a	4.05 ^a	4.19 ^a	4.71 ^a
C20:0	0.15 ^a	0.14 ^a	0.15 ^a	0.19 ^a	0.21 ^a	0.31 ^a	0.32 ^a
Total	42.88	44.02	44.00	44.37	42.38	42.46	46.21
Monounsaturated							
C16:1, n-9c	0.18 ^a	0.18 ^a	0.16 ^a	0.18 ^a	0.18 ^a	0.31 ^a	0.23 ^a
C18:1, n-9c	45.23 ^a	44.60±0.02	44.48±0.02	44.23±0.04	45.57 ^a	44.30±0.04	43.08±0.04
Total	45.41	44.78	44.64	44.41	45.75	44.61	43.31
Polysaturated							
C18:2, n-6c	11.10 ^a	9.96 ^a	10.62 ^a	10.60±0.13	11.51 ^a	12.35±0.02	9.93 ^a
C18:3, n-6c	0.38 ^a	0.38 ^a	0.37 ^a	0.37 ^a	0.36 ^a	0.36 ^a	0.38 ^a
Total	11.48	10.34	10.99	10.97	11.87	12.71	10.31
C18:2/C16:0	0.30	0.26	0.27	0.27	0.31	0.33	0.24

nd: not detected

^a: standard deviation < 0.01

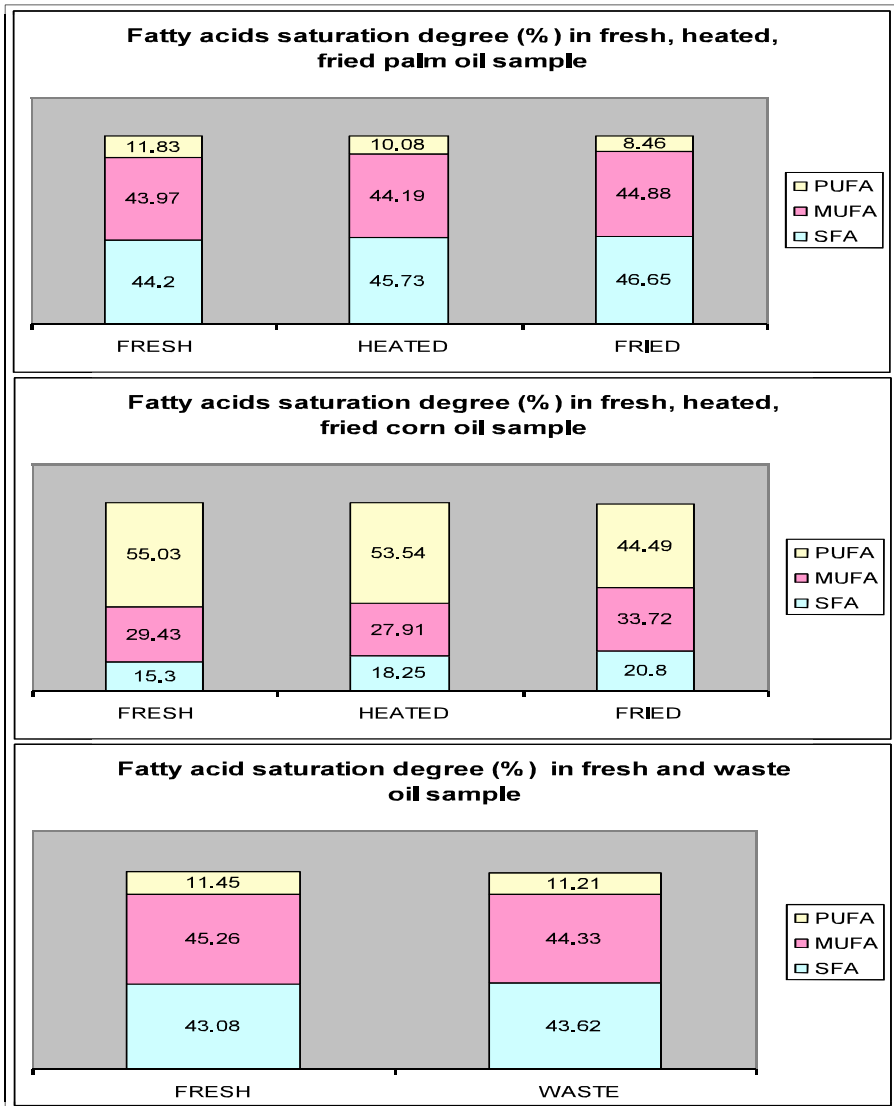
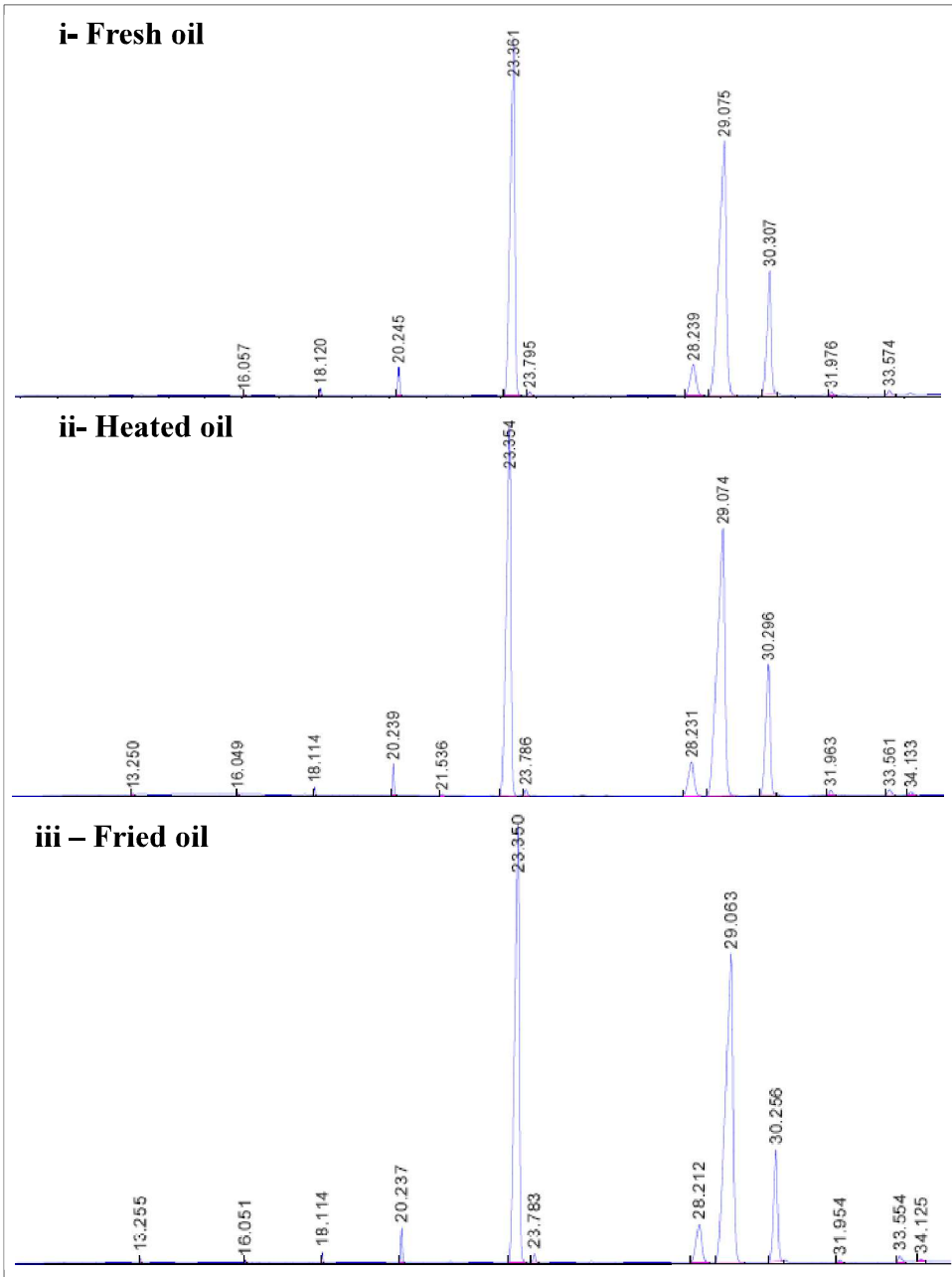


Figure 1: Fatty acids saturation degree in fresh, heated, fried and waste oil SFA; saturated fatty acids, MUFA; monounsaturated fatty acids, PUFA; polyunsaturated fatty acids.



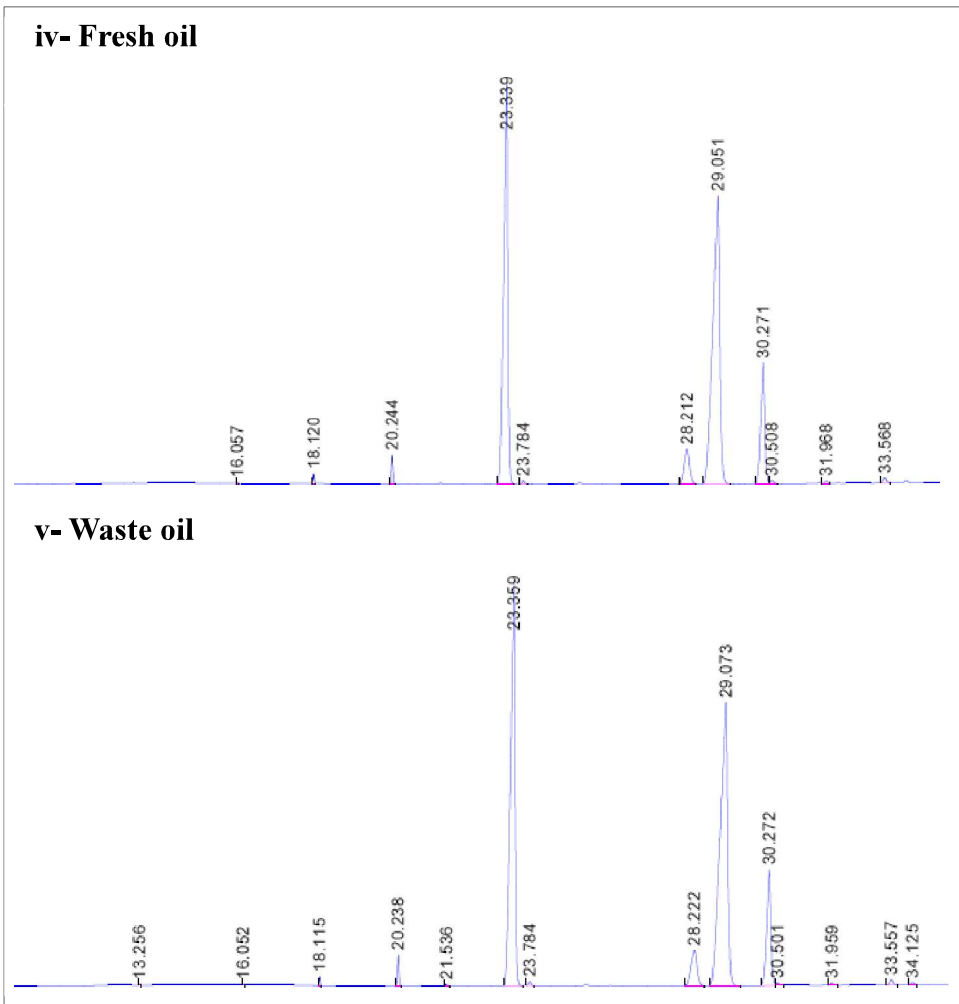


Figure 2: i- chromatogram of sample A, fresh; ii- chromatogram of sample A, heated; iii- chromatogram of sample A, fried; iv- chromatogram of sample D, fresh; v- chromatogram of sample D, waste

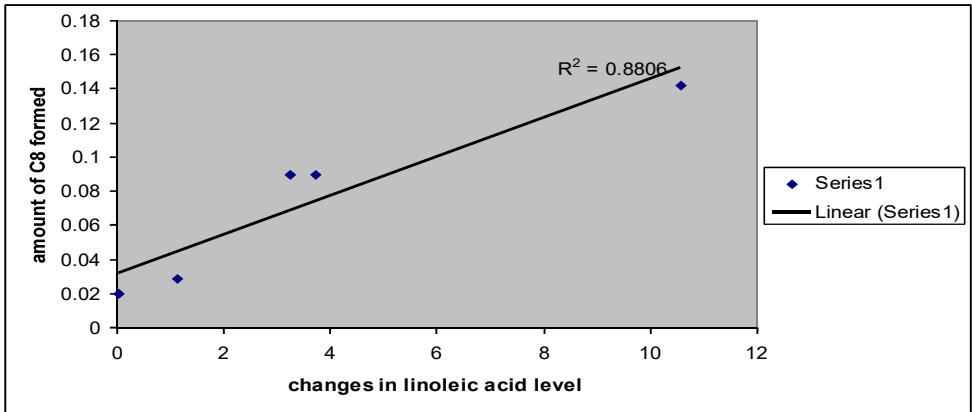


Figure 3: Correlation between changes of linoleic acids level and the amount of octanoic acid formed

4. CONCLUSION

As oil becomes thermoxidised, the level of polyunsaturated fatty acids is decreased, the level of saturated fatty acids increased. The ratio of linoleic acid to palmitic acid (18:2/16:0) decreased in thermally treated oil. Octanoic acid only was detected in degraded oils (heated, fried and waste oils). The higher the linoleic acid content in oil, the higher the level of octanoic acid formed. It shows that formation of C8 is the results of linoleic acid degradation and C8 could act as marker to determine whether the oil has been thermally degraded and oxidized. Moreover, the methodology to detect C8:0 is simple, thus can be applied as routine analysis by regulatory bodies such as food quality controller and health administer. However, there is limitation in this study. The numbers of samples collected are small, so care should be taken in extrapolating this finding.

Conflicts of interest

The authors declare no conflicts of interest.

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