Journal of Oil Palm Research Vol.30 (4) December 2018 p. 635-641
DOI: https://doi.org/10.21894/jopr.2018.0051

OXIDATIVE CHANGES IN REPEATEDLY HEATED VEGETABLE OILS

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ABSTRACT

Thermal oxidation of vegetable oils due to repeat heating is known to exert adverse effects on human health. The oxidative stability of vegetable oils is one of the important factors in determining suitable types of cooking oils and cooking methods. Therefore, the oxidative changes in several types of commonly used vegetable oils, namely: palm olein (POo), soyabean oil (SBO) and corn oil (CNO) were studied. The oil samples were heated repeatedly at 150°C for five rounds. The oxidation level of the oils was assessed by determining their respective changes in fatty acid composition, peroxide value (PV), free fatty acid (FFA) value and p*-anisidine value (AV). SBO and CNO contained significantly higher amount of unsaturated fatty acids (84.1% and 86.2%, respectively) compared to POo (56.8%). Consequently, PV, FFA and AV value of the oil samples increased according to their unsaturation level: POo < SBO < CNO. Therefore, POo was shown to have the greatest oxidative stability against thermal oxidation, followed by CNO and SBO.*

Keywords: thermal oxidation; vegetable oils.

Date received: 1 February 2018**; Sent for revision:** 5 February 2018**; Received in final form:** 10 April 2018**; Accepted:** 25 July 2018.

INTRODUCTION

It has been a regular practice to use the same cooking oil repeatedly as a cost saving measure. This practice is widely employed throughout Malaysia, ranging from household to the roadside food stalls and reputable food outlets in the cities. It has been reported that repeated heating of cooking oil at a high temperature in the presence of moisture and air can cause the oil to undergo several chemical reactions, such as thermal oxidation, hydrolysis, polymerisation and fission (Andrikopoulos *et al.*, 2002; Choe and Min, 2007). Consequently, the oil will decompose, followed by the generation of volatile compounds and a number of monomers and polymers (Andrikopoulos *et al.*, 2002). According to Jaarin *et al.* (2011), thermal oxidation of oil will give rise to the alteration of fatty acid configuration from *cis* isomer to *trans* isomer.

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The consumption of repeatedly heated cooking oils is known to be harmful for health (Owu *et al.*, 1998). Studies have shown that heating cooking oils repeatedly can result in lipid oxidation which generates unfavourable products, such as reactive oxygen species, that are associated to inflammation, carcinogenesis and cardiovascular diseases (Chung *et al.*, 1996; Sawa *et al.*, 1998; Akoh and Min, 2008; Grootveld *et al.*, 2001). Lipid oxidation also affects the chemical, nutritional and sensory properties of the oil and its products (Zahir *et al.*, 2014). Therefore, the degree of lipid oxidation is commonly used in determining the uses, quality and shelf-life of cooking oils. The quality of repeatedly heated cooking oil can be influenced by several factors, such as temperature, type of oil, heating duration, ventilation, type of heating vessel, oil saturation level and existence of pro-oxidants or antioxidants (Andrikopoulos *et al.*, 2002).

There are a wide variety of commonly utilised cooking oils derived from plant sources such as oil palm, sunflower, soyabean, olive and corn. The oxidative stability of cooking oils can be affected by

their chemical constitution, as well as their physical and physico-chemical characteristics (Karakaya and Simsek, 2011). Particularly, vegetable oils rich in unsaturated fatty acids are known to reduce the risk of cardiovascular diseases (Halvorsen and Blomhoff, 2011). However, they are more susceptible to oxidation or thermal degradation during heating and possibly exert negative effects on human health (Marinova *et al.*, 2012). Hence, choosing a suitable cooking oil based on the cooking method is essentially important.

The oxidation level of cooking oil can be evaluated by examining the chemical changes taking place during oil heating. The present study involved intermittent heating of palm olein (POo), soyabean oil (SBO) and corn oil (CNO) for five rounds at 150˚C and determining the fatty acid composition, peroxide value (PV), free fatty acid (FFA) and *p*-anisidine value (AV) of the fresh and heated oils. This study was conducted to prepare oxidised cooking oils which will be incorporated into the animal food pellets that will be used in breast cancer study. The results from this study are particularly useful when designing experimental studies or intervention trials to investigate the possible adverse effects of oxidised vegetable oils on human health.

MATERIALS AND METHODS

Sources of Vegetable Oils

POo (AVENA, Pacific Oils and Fats Industries Sdn Bhd, SBO [Soya Lite, SOCMA Trading (M) Sdn Bhd] and CNO (Vecorn, Yee Lee Edible Oils Sdn Bhd, were purchased from local supermarket in Malaysia.

Heating of Oil

The heating of oils was modified from the method of Owu *et al.* (1998). POo, SBO and CNO were heated at 150˚C in a stainless steel saucepot intermittently for five rounds of 20 min in each round. The oils were allowed to cool for at least 5 hr after each round. No fresh oil was added between the heating processes. The heating processes were repeated for two times using the same conditions, except that 2 kg of oils were tested in the first trial while 5 kg of oils were used in the second trial. Oil samples were obtained before and after each round of heating during the first trial and analysed for PV, AV and FFA value. After we have obtained the preliminary readings from the first trial, oil samples were collected before and after fifth round of heating during the second heating trial for fatty acid composition and the aforementioned analyses. The samples were flushed with nitrogen to prevent oxidation and stored at -20°C until analysis.

Preparation of Fatty Acid Methyl Ester

The oil samples from second trial were transesterified to fatty acid methyl ester (FAME) using modified method of Ichihara and Fukubayashi (2010). Oil sample of 100 µl was added into a test tube containing 1 ml of toluene and 2 ml of acidified methanol (2% v/v H_2SO_4 in methanol) for refluxing at 80˚C. After 2 hr, the reaction was stopped by adding 5 ml of NaCl. After that, 5 ml of hexane was added and centrifuged for 5 min at 2500 rpm. The hexane layer was transferred to a new test tube and the extraction process was repeated by using the same amount of hexane. The hexane extracts were then combined and added with 10 ml of $KHCO_3$ to maximise capacity. The mixture was centrifuged for 5 min at 2500 rpm. Hexane upper layer was removed into the vial and put under nitrogen blanket to evaporate off hexane, leaving the oil extract only which was then reconstituted with 70-100 µl of hexane. The sample was then analysed by gas chromatography (GC).

Fatty Acid Composition Analysis

Fatty acid composition of the oil samples from the second trial were measured by using GC on a SP-2560 column (100 m x 0.23 mm x 0.2 mm) (Agilent Technologies, USA). The detector used was a flame ionisation detector (FID) on an Autosystem (Perkin Elmer, USA). The carrier gas was helium at a flow rate of 1.0 ml min^{-1} and set as 40 psi. The injector temperature was set at 250°C. Hydrogen and oxygen were used for ignition. Oven temperature was set at 240°C for 58 min before ramping. A 37-fatty acid methyl ester mixture (Sigma-Aldrich, US) was utilised as external standard. The fatty acid composition was expressed as the percentage of total fatty acids (wt/wt) (*Table 1*).

Determination of PV

PVvalue was analysed based on MPOB Test Method p2.3: 2004 (Ainie *et al.*, 2005). Oil sample (5.0 g) was added into a conical flask and added with 50 ml of acetic-isooctane solution (3:2). After swirling until the sample has dissolved, 0.5 ml of saturated potassium iodide (KI) was added and the sample was allowed to stand for 1 min and followed with thorough shaking for at least three times. Then, 30 ml of distilled water was added immediately, followed by addition of 0.5 ml starch solution (5 g $litre^{-1}$) which acts as indicator. The sample was titrated against 0.01 M sodium thiosulphate ($Na_2S_2O_3.5H_2O$) until blue colour just disappeared. Blank titration was performed by repeating the same procedure without oil sample. The test was conducted in

TABLE 1. FATTY ACID COMPOSITION OF THE FRESH AND FIVE ROUNDS HEATED OIL SAMPLES

Fatty acid % (wt/wt)	POo		SBO		CNO	
	Fresh	5th	Fresh	5th	Fresh	5th
	Heating		Heating		Heating	
Σ Saturated fatty acids	43.2	44.0	15.9	16.7	13.9	14.0
Lauric acid ($C_{12:0}$)	0.2	0.2	N.D.	N.D.	N.D.	N.D.
Myristic acid ($C_{14:0}$)	0.9	0.9	N.D.	N.D.	N.D	N.D
Palmitic acid ($C_{16:0}$)	37.8	38.6	10.9	11.7	11.0	11.1
Stearic acid ($C_{18:0}$)	4.0	4.0	4.1	4.2	1.9	1.9
Arachidic acid ($C_{20:0}$)	0.3	0.3	0.9	0.8	1.0	1.0
Σ Monounsaturated fatty acids	45.6	45.3	23.4	23.0	32.3	32.5
Palmitoleic acid ($C_{16:1}$)	0.1	0.1	0.1	0.1	0.1	0.1
Oleic acid ($C_{18:1}$)	45.5	45.2	23.3	22.9	32.2	32.4
Σ Polyunsaturated fatty acids	11.2	10.7	60.7	60.3	53.9	53.6
Linoleic acid ($C_{18:2}$ *n6-C*)	11.0	10.5	54.2	54.1	53.1	52.8
Linolenic acid ($C_{18:3}$)	0.2	0.2	6.5	6.2	0.8	0.8

Note: Values are expressed as mean of duplicates.
N.D. – not detected.
POo – palm olein; SBO – soyabean oil; CNO – corn oil.

triplicates for all samples. PV was calculated using the following formula:

PV (meq kg^{-1}) = [1000 x (V_1-V_0) x concentration of sodium thiosulphate]/sample weight, g
where, V_1 = titration volume of the sample and
V_0 = titration volume of the blank

Determination of Free Fatty Acid Value

The procedure was conducted based on MPOB Test Method p2.5: 2004 (Ainie *et al.*, 2005). Neutralised isopropanol was prepared by heating 50 ml of isopropanol to 40˚C, followed by addition of 0.5 ml of phenolphthalein solution. Then, the mixture was neutralised by adding 0.1 M NaOH solution dropwise until permanent pink colour appeared. The oil sample (5.0 g) was mixed with 50 ml of neutralised isopropanol in a conical flask by shaking gently and placed on a hot plate (40˚C). After adding one drop of phenolphthalein solution, the sample was titrated against standard sodium hydroxide (NaOH) solution (0.005 M) using phenolphthalein as an indicator. The test was conducted in triplicates for all samples. FFA was calculated using the following formula:

FFA (%) = [(Molecular weight of fatty acid x volume of KOH used x molarity of sodium hydroxide x) / (sample weight, g)]

Determination of *p*-AV

The AV of oil samples were determined according to MPOB Test Method p2.4: 2004 (Ainie *et al.*, 2005). A 1.5 g of oil sample was weighed in a 25 ml volumetric flask and mixed with 25 ml of *iso*-octane. The absorbance (Ab) of the mixture at 350 nm was measured with *iso*-octane as blank using UV/VIS Spectrometer (Lamda 12, Perkin Elmer, USA). The 5 ml of the mixture was then pipetted into a test tube and 5 ml of *iso*-octane into another test tube. The *p*-anisidine solution of 1 ml in acetic acid (25 g $litre^{-1}$) was added to both test tubes and allowed to react for 10 min. The absorbance (As) of the sample solution was measured with the test tube containing isooctane and *p*-anisidine solutions as a blank. The AV was calculated using the following equation: AV = 25×(As-Ab)/m, where *m* is the mass of the sample. The test was conducted in triplicates for all samples.

Statistics

All results were expressed as means ± standard deviation. Significant differences among the groups were determined by using analysis of variance (ANOVA), followed by Turkey's Multiple Comparison Test using IBM SPSS Statistics for Windows version 21.0 (Armonk, New York, USA). All results were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Heat is one of the significant factors that increase the rate of oxidation processes of oils (Choe and Min, 2006). In this study, three types of vegetable oils, *i.e.* POo, SBO and CNO were heated at 150°C, which is the temperature commonly used during food preparation, for five rounds intermittently. Fatty acid composition of oil has a substantial effect on its quality and oxidative stability. The fatty acid composition of fresh and five rounds-heated POo, SBO and CNO from the second trial were analysed using GC. Their respective

fatty acid composition in percentage (%), and the mean of the total saturated fatty acids (SFA), monosaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are recorded in *Table 1*.

From *Table 1*, all the three types of oils consist of nine fatty acids. Three to five types of SFA of chain length ranging from C_{12} to C_{20}, and four types of unsaturated fatty acids of chain length ranging from C_{16} to C_{18} were found in all the oil samples. The most abundant fatty acid in POo was monounsaturated oleic acid ($C_{18:1}$), accounted for approximately 45.5%. This finding was in line with the data reported by Chowdhury *et al.* (2007). On the other hand, bi-unsaturated linoleic acid ($C_{18:2\ n\text{-}6C}$) was the most abundant fatty acids found in the SBO and CNO, accounted for approximately 54.2% and 54.1% respectively.

The total SFA was the highest in fresh POo (43.2%), which was about three-fold higher than in fresh SBO (15.9%) and fresh CNO (13.9%) (*Table 1*). Palmitic acid ($C_{16:0}$) was the major SFA for all the three types of oils, which was comparable to the findings of Kostik *et al*. (2013) and Zambiazi *et al.* (2007). Before heating, the total unsaturated fatty acid content in SBO (84.1%) and CNO (86.2%) were significantly higher than in POo (56.8%). All the oil samples consist of two types of MUFA, namely: palmitoleic acid ($C_{16:1}$) and oleic acid ($C_{18:1}$). POo contained the highest percentage of MUFA (45.6%), followed by CNO (32.3%) and SBO (23.4%). PUFA content in SBO (60.7%) was higher than in CNO (53.9%), which was in accordance with findings reported by Zambiazi *et al.* (2007).

As reported by Kushairi *et al*. (2018), POo had a relatively balanced proportion of SFA to unsaturated fatty acids, while SBO and CNO contained more than 80% of unsaturated fatty acids. These findings indicate that POo has greater stability against oxidation compared to other vegetable oils with high unsaturated fatty acids. There were no significant changes in the fatty acid composition of the oil samples after five rounds of heating (*Table 1*). However, according to Moreno *et al.* (1999), intense heating of oils may reduce the unsaturation level of fatty acids. Therefore, this indicates that higher temperature or prolonged heating duration are required to alter the fatty acid composition of the oil samples.

Tables 2 and *3* depict the extent of changes in the PV, AV and FFA values of POo, SBO and CNO after subjected to five rounds of heating in the first and second trials respectively.

PV was used as an indicator for the primary oxidation of oils, with hydroperoxides as the main primary products of lipid oxidation (Karakaya and Simsek, 2011). In the present study, the lowest PV value for fresh oil samples was found in POo, followed by CNO and SBO (*Tables 2* and *3*). This might be due to the high SFA level found in POo which is shown to provide greater storage stability

TABLE 2. PEROXIDE, FREE FATTY ACID AND ANISIDINE VALUES OF PALM OLEIN, SOYABEAN OIL AND CORN OIL AFTER FIVE ROUNDS OF HEATING (first trial)

	Heating	Sample		
		POo	SBO	CNO
PV	0	0.638±0.200^{a}	1.768±0.836^{a}	1.206±0.551^{a}
	1	8.023±1.142^{b}	15.719±1.437^{b}	5.426±2.433^{b}
	2	15.459±0.324^{c}	16.462±0.992^{b}	18.523±0.436^{c}
	3	21.620±0.273^{d}	23.410±2.020^{c}	24.055±0.273^{d}
	4	27.791±0.469^{e}	28.556±1.485^{d}	28.472±1.276^{e}
	5	28.442±1.421^{e}	29.543±2.291^{d}	34.592±2.221^{f}
FFA	0	0.044±0.009^{a}	0.047±0.001^{a}	0.051±0.003^{a}
	1	0.046±0.036^{a}	0.081±0.013^{a}	0.076±0.005ab^{c}
	2	0.050±0.038^{a}	0.098±0.003^{a}	0.098±0.010abc^{d}
	3	0.060±0.045^{a}	0.228±0.023^{b}	0.137±0.015bc^{d}
	4	0.071±0.054^{a}	0.244±0.014^{b}	0.156±0.009c^{d}
	5	0.080±0.060^{a}	0.263±0.025^{b}	0.177±0.016^{d}
AV	0	2.057±0.096^{a}	2.657±0.508^{a}	5.603±0.463^{a}
	1	3.825±0.284^{a}	11.797±0.196^{b}	6.511±0.642a
	2	8.604±0.192^{b}	35.422±0.813^{c}	12.367±0.760^{b}
	3	13.508±1.166^{c}	45.454±1.995^{d}	28.540±3.956^{c}
	4	21.169±0.566^{d}	57.463±2.321^{e}	38.235±3.217^{d}
	5	28.169±0.975^{e}	59.528±0.587^{e}	48.384±1.192^{e}

Note: Values are expressed as means ± standard deviation. Values bearing different superscripts within the same column for each oil sample are significantly different ($P < 0.05$).
POo – palm olein. SBO – soyabean oil; CNO – corn oil.
PV – peroxide value. FFA – free fatty acid. AV – *p*-anisidine value.

TABLE 3. PEROXIDE, FREE FATTY ACID AND ANISIDINE VALUES OF PALM OLEIN, SOYABEAN OIL AND CORN OIL AFTER FIVE ROUNDS OF HEATING (second trial)

Sample	Heating	Sample		
		POo	SBO	CNO
PV	0	0.963±0.057[a]	3.921±0.116[a]	3.126±0.118[a]
	5	21.861±0.245[b]	29.504±0.928[b]	25.464±0.237[b]
FFA	0	0.090±0.007[a]	0.113±0.016[a]	0.110±0.013[a]
	5	0.112±0.014[a]	0.290±0.010[b]	0.169±0.003[b]
AV	0	2.744±0.498[a]	3.641±0.458[a]	4.862±0.525[a]
	5	25.376±0.199[b]	49.788±0.510[b]	48.461±0.200[b]

Note: Values are expressed as means ± standard deviation. Values bearing different superscripts within the same column for each oil sample are significantly different ($P < 0.05$).
POo – palm olein. SBO – soyabean oil; CNO – corn oil.
PV – peroxide value. FFA – free fatty acid. AV – *p*-anisidine value.

as compared to the more unsaturated fatty acid-rich oils (Ferrari *et al.*, 2005).

PV in all types of oils were increased as the frequency of heating increased. The increased PV indicate greater formation of primary oxidation products in the oil during the heating process (Jaarin and Kamisah, 2012). In both trials, SBO and CNO that consist of mainly unsaturated fatty acids were shown to have higher PV compared to POo which consists of mainly saturated fatty acids, as reported by Kaleem *et al.* (2015). It can be seen that the peroxide formation in POo and SBO increased in a similar trend. Significant increase in PV can be observed after four rounds of heating followed by a small increment (2.15% and 3.15% for POo and SBO, respectively) after five rounds of heating. A decline in the rate of PV formation after five rounds of heating might be due to the increasing rate of formation of secondary products of oxidation from peroxide.

In addition to PV, FFA value is also commonly used to monitor the oil quality. FFA are one of the products of the hydrolysis of oils. During the heating of oil, di- and monoacylglycerols, glycerols and FFA were produced due to degradation of ester linkage of triacylglycerols (Choe and Min, 2007). In both trials, FFA values in all the three oil samples increased with increasing heating frequency (*Tables 2* and *3*), which were in accordance to the findings reported by Warner *et al.* (1997).

Hydrolysis occurs more readily in oil consisting predominantly of unsaturated fatty acids than oil consisting primarily of saturated fatty acids as the solubility of unsaturated fatty acids in water is higher than saturated fatty acids (Nawar, 1969). This was shown by the result in this study, where FFA values of the oils after five rounds of heating were increased with their unsaturation level: POo < CNO < SBO. This result was in accordance to the outcome reported by Tan *et al.* (2001), which revealed that FFA value of SBO was higher than CNO after heating for 20 min. Besides, Fekarurhobo *et al.* (2009) showed that FFA value of SBO was higher than palm oil, which is correlated to their level of unsaturation. Furthermore, di- and monoacylglycerols, glycerol and FFAs will promote further hydrolysis in oil (Choe and Min, 2007). Besides, glycerol which is relatively volatile at 150°C-200°C is partially evaporated from the oil and the glycerol remaining in the oil will stimulate the generation of FFA via hydrolysis (Min, 1989). Therefore, in this study, the amount of FFA in all types of oils increased with the frequency of heating, as reported by Chung and Choe (2001). An increment in FFA content indicates a higher level of thermal oxidation in oil (Yoshida *et al.*, 1992).

During lipid oxidation, the primary oxidation products decompose to form secondary oxidation products, including aliphatic aldehydes, ketones, alcohols, acids and hydrocarbons, that are more stable during the heating process (Poiana, 2012). AV is reliable and commonly used to measure secondary oxidation products (Halvorsen and Blomhoff, 2011; Tompkins and Perkins, 1999). The reaction between *p*-anisidine and secondary oxidation products in oils produces yellow-coloured products that are detected at 350 nm. In this study, AV increased with the increasing number of heating for all the three types of oils. Similar result was reported by Tompkins and Perkins (1999), where AV was positively correlated to the heating time. SBO had the highest AV, followed by CNO and lastly POo.

In addition, AV of SBO and CNO after heating for five rounds were significantly higher than POo, which were due to the high proportion of PUFA present in SBO and CNO. Besides, the percentage of $C_{1882}^{n6\text{-}C}$, $C_{18:1}$ and $C_{18:3}$ acids, the predominant types of unsaturated fatty acids that are susceptible to oxidation are significantly higher in SBO and CNO, compared to POo (Leyton *et al.*, 1987). Furthermore, during secondary oxidation,

$C_{18:3}$ and $C_{18:2}^{n6\text{-}C}$ acids are more readily oxidised than oleic acid (Yun and Surh, 2012). The content of linolenic and linoleic acids was the highest in SBO (60.27%), followed by CNO (53.75%) and lastly POo (11.26%). Therefore, the initial rate of increment in AV values corresponded to the content of linolenic and linoleic acids: SBO > CNO > POo.

CONCLUSION

Our findings showed an increased in oxidation levels of vegetable oils subjected to repeated heating. Vegetable oils with higher unsaturation levels are more susceptible to thermal oxidation compared to vegetable oils rich in saturated fatty acids. Therefore, it is not recommended to heat vegetable oils rich in unsaturated fatty acids more than once as they may exert negative effects on human health. Saturated fats such as POo which is more stable against thermal oxidation can be used if repeated heating is necessary for culinary purposes.

ACKNOWLEDGEMENT

The authors would like to thank the Director-General of MPOB for permission to publish this article. This research work was fully supported by MPOB and we appreciate the guidance from Tan Sri Dr Augustine Ong. We also would like to thank the laboratory staff in the Nutrition Unit, MPOB for the technical assistance.

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