OXIDATIVE STABILITY AND PHYSICOCHEMICAL PROPERTIES OF PALM OLEIN

SOEK SIN TEH*; SIAU HUI MAH** and RAZNIM ARNI ABD RAZAK*

ABSTRACT
Oxidised oil has been emerged as a root for many chronic diseases. The study was aimed to evaluate the effects of heating on oxidative stability and physicochemical properties of palm olein. Palm olein was heated to 80°C (P80) and 120°C (P120) up to 12 hr. The positional distribution of fatty acids, fatty acid composition (FAC) and iodine value (IV) were analysed using proton-Nuclear Magnetic Resonance (^1H-NMR) and carbon-Nuclear Magnetic Resonance (^13C-NMR). The slip melting point (SMP), peroxide value (PV), cloud point (CP) and free fatty acid (FFA) contents of the oil were determined according to MPOB Test Methods. Results implied that the regiospecificity of fatty acids in palm olein, FAC, IV and SMP remained unchanged (p>0.05) after both heating treatments for 12 hr. The FFA of P80 and P120 were gradually increased, even so, their FFA were within the acceptance level under prolonged heating for ≤ 10 hr. The CP of P80 and P120 were gradually increased due to an increase in the amount of FFA in the oils. In general, palm olein exhibited good oxidative stability after being heated for up to 2 hr at 80°C and 120°C beyond which the oil deteriorated. Overall, this study revealed that palm olein - as a cooking oil - was thermally and oxidatively stable under normal cooking environment.

Keywords: cloud point, fatty acid composition, free fatty acid, peroxide value, slip melting point.

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INTRODUCTION
Edible oils can be obtained from plant or animal sources. Common edible oils available in the market for cooking are soyabean oil, corn oil, safflower oil, rice bran oil, grape seed oil, olive oil, sunflower oil, peanut oil, palm oil, fish oil and canola oil. The cooking oils exist in different physical states, including solid, semi-solid or liquid, which depend on the percentage of saturated fatty acids (SFA) in the oil. Each country has its own preferable edible oils to be used for either cooking or culinary purposes. It is indeed depending very much on the cultivated plants in the countries, and the weather conditions. Heat is applied while cooking dishes at a temperature ranging from 60°C-180°C, which relies heavily on food preparation. Thus, some types of oils such as poly-unsaturated oils, which are susceptible to oxidation, will subsequently produce certain reactive chemicals or even toxic compounds that are hazardous to health (Grootveld et al., 2001). Therefore, prolonged cooking of such oils may be deemed undesirable as it makes the oils unsafe for consumption. Many well-established laboratory methods are available to assess oxidative stabilities of oils relating to peroxide value (PV), iodine value (IV), cloud point (CP) and free fatty acid (FFA) content (Hoekman et al., 2012).

* Malaysian Palm Oil Board, 6 Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia. E-mail: sssteh@mpob.gov.my

** School of Biosciences, Taylor’s University, Lakeside Campus, 47500 Subang Jaya, Selangor, Malaysia.
Having mentioned all the beneficial effects of the oil, it is important to maintain its quality from external heat and free radicals. Oxidative stability tests have been developed to ensure the quality, shelf life (Wambura et al., 2008), nutritional quality, palatability and toxicity (Choe and Min, 2006) of edible oils in order to be compliant with legislation and demands of consumers. Different degrees of oxidation of oils generate different hazardous constituents, including primary (1°), secondary (2°) and tertiary (3°) oxidative products such as lipid hydroperoxides, dienes and FFA (1° oxidation products) (Choe and Min, 2006); alcohols, aldehydes, ketones, hydrocarbons, epoxides and products of polymerisation or copolymerisation (2° oxidation products); peroxyacids and volatile fatty acids (3° oxidation products) (Dabrowska et al., 2015). The adverse effects of oxidised oils causing severe diseases have been widely discussed (Burenjargal and Totani, 2009; Totani and Burenjargal, 2008; Ogino et al., 2015; Shafaeizadeh et al., 2011).

Therefore, oxidation stability of oils should be monitored well in order to minimise any possible hidden risks of chronic diseases. The US Department of Health and Human Services (HHS) recommended that the safe minimum internal temperature for cooking meat and poultry is 165°F (74°C) to ensure food safety whereas the preferred oven temperature for whole meat roasts is 250°F (121°C). Therefore, two cooking temperatures, 80°C and 120°C were chosen in this study. Our study aimed to determine the effects of the cooking temperatures on the oxidative stability of palm olein. Its quality indicators such as positional distribution of fatty acids, fatty acid composition (FAC), IV, slip melting point (SMP), PV, CP and FFA on palm olein were determined using established test methods.

MATERIALS AND METHODS

Materials

Palm olein was purchased from local supermarket in Selangor, Malaysia. Deuterated chloroform (CDCl₃) was purchased from Sigma Aldrich, Switzerland. All other analytical grade chemicals were purchased from R&M Chemicals, Malaysia.

Sample Preparation

Palm olein was heated at 80 ± 3°C (sample denoted as P80) and aliquot oil samples were collected at 2-hour interval for 12 hr and labelled as T0, T2, T4, T6, T8, T10 and T12 for analyses. The steps above were repeated by heating palm olein at 120 ± 3°C (sample denoted as P120).
Positional Distribution of Fatty Acids, FAC and IV of Samples

The fatty acid positional distribution in samples and their saturation levels were determined using a Nuclear Magnetic Resonance (NMR) spectrometer (JEOL ECZ-600 MHz) (Teh et al., 2016). About 100 mg of the samples were dissolved in 0.5 ml of CDCl₃ and analysed via quantitative ¹³C-NMR and ¹H-NMR. The fatty acid positional distribution in the triglycerides of the samples were acquired with specific parameters: 15.0 s of relaxation delay, 8192 of data points, 90º of pulse angle and spectral width of 1500 Hz at which the acyl chain carbonyl carbons resonate. The FAC of the samples were detected according to Miyake (1998) with slight modifications as follows: 1500 Hz of spectral width, 16 384 of data points and 1.82 s of acquisition time. The analyses were performed in triplicate for each sample. The IV of the samples was calculated using data from ¹H-NMR according to AOCS Cd 1c-85:

\[
IV = C_{18:1} (0.860) + C_{18:2} (1.732) + C_{18:3} (2.616)
\]

Free Fatty Acids (FFA) Content

Twenty grams (20 g) of sample was dissolved in 50 ml of neutralised ethanol. The mixture was heated at 40°C. The mixture was shaken gently and titrated with 0.02 M potassium hydroxide (KOH) solution until permanent pink colour was observed. A blank determination was carried out simultaneously with the sample. The analysis was performed in triplicate for each sample. The FFA content (as wt% of palmitic acid) was calculated according to AOCS Official Method Ca 5a-40: FFA (2009) as below:

\[
FFA \text{ as palmitic acid, wt\%} = \left(\frac{25.6 \times M \times V}{m}\right)
\]

where \( M \) = Molarity of standardised KOH (mol litre⁻¹),
\( V \) = Volume of KOH used (ml) and
\( m \) = Mass of the sample (g)

Slip Melting Point (SMP)

The sample was melted and filled into a capillary tube for approximately 1 cm from the open end of the capillary tube and kept at -5°C for 24 hr. The capillary was then tied to the lowest part of the thermometer and immersed in a temperature-controlled water bath. The SMP of the sample was recorded once the oil began to rise in the capillary tube. The analysis was performed in triplicate for each sample according to MPOB Test Method p4.2:2004: Determination of SMP (2004).

Cloud Point (CP)

CP of the samples were conducted using MPOB Test Method p4.3:2004: Determination of CP (2004). In brief, the sample was heated at 130°C for 5 min, then immediately immersed and cooled in a temperature-controlled water bath. A thermometer was immersed in the sample. The CP of the sample was recorded once the lowest part of the immersed thermometer was no longer visible. The analysis was performed in triplicate for each sample.

Peroxide Value (PV)

One gram (1 g) of the sample was dissolved in 50 ml of 3:2 acetic acid:iso-octane solution. Next, 0.5 ml of saturated potassium iodide solution was added into the mixture and shaken gently for 1 min, followed by 30 ml of distilled water. The mixture was then titrated with 0.01 M sodium thiosulphate (Na₂S₂O₃) solution with constant vigorous agitation. When the yellow colour of the solution was almost faded, 0.5 ml of 5 g litre⁻¹ starch solution was added and the titration continued until the blue colour faded. A blank determination was carried out simultaneously with the sample. The analysis was performed in triplicate for each sample. The PV was calculated according to MPOB Test Method p2.3:2004 as below:

\[
Peroxide \text{ value, PV (meq O}_2 \text{ kg}^{-1}) = \left(\frac{(V_a - V_b) \times 1000}{m}\right)
\]

where \( V_a \) = Volume of Na₂S₂O₃ solution (ml) used,
\( V_b \) = Volume of Na₂S₂O₃ solution (ml) used for the blank,
\( M \) = Molarity of standardised Na₂S₂O₃ (mol litre⁻¹) and
\( m \) = Mass of the sample (g)

Statistical Analysis

All data obtained were performed using statistical analysis software, GraphPad Prism 7 and presented as mean ± standard deviations. The data were analysed by one-way analysis of variance (ANOVA), followed by Tukey’s Multiple Comparison Test. Results were considered to be statistically significant when the \( p \) value was < 0.05.

RESULTS AND DISCUSSION

Oxidative stability and physico-chemical properties of palm olein were examined in this study under two different heating conditions, i.e. 80°C (P80) and 120°C (P120). The positional distribution of fatty acids in the resulting oils were determined using quantitative ¹³C-NMR spectroscopy. The results (Tables 1a and 1b) indicated that the SFA at the sn-1,3 positions of P80 ranged from 64.1%-67.4% while
Nevertheless, P120 exhibited 65.8%-68.0% of SFA at sn-1,3 positions and 92.1%-94.0% of UFA at sn-2 position. The positional distribution of fatty acid profiles amongst aliquot samples of P80 namely T0, T2, T4, T6, T8, T10 and T12 were comparable based on the statistical analysis. Similar trend was observed for P120 after heating at the same time interval. No significant difference (p>0.05) was found between the two sets of palm olein (P80 and P120) and control (without heating treatment). The findings demonstrated that heating palm olein at 80°C and 120°C did not give any significant effect on the regiospecific distribution of fatty acids as compared to control as no rearrangement of fatty acids was observed. Previous studies indicated that the sn-2 position of fatty acids in oils plays an important role in regulating cholesterol metabolism (Redgrave et al., 1988) while the sn-1,3 positions in regulating fat absorption (Small, 1991). These results showed that increasing reaction time and heating temperature did not alter the structure of palm olein, hence indicating no significant effect on the serum cholesterol level and fat deposition.

The FAC of P80 and P120 (Table 2) were determined by 1H-NMR spectroscopic method. All of the oils tested showed similar percentages of SFA (45.3%-48.3%), oleic acid (39.4%-43.8%), linoleic acid (10.7%-11.9%) and linolenic acid (0.2%-0.4%). Both P80 and P120 possessed comparable FAC profiles which were consistent with those via quantitative 13C-NMR analysis (p>0.05) as compared to the fatty acids at the sn-1,2,3 positions of the oils. These identical FAC profiles might be attributable to a slower oxidation rate of palm olein due to its high content of SFA, which would have assisted in counterbalancing the detrimental effects of UFA. This finding was consistent with literature data which revealed that autoxidation and thermal oxidation are highly associated with the degree of unsaturation levels of oils (Brodnitz, 1968; Tian and Dasgupta, 1999); higher amount of UFA will cause a greater rate of oxidation. Therefore, IV is important to evaluate the quality as well as predict the oxidising tendency of oil. The changes in IV of P80 and P120 were insignificant after prolonged heating at 80°C and 120°C, as compared to control (Table 3). The data postulated an equitable balanced...
saturation/unsaturation fat in palm olein which helped decelerating oil deterioration.

Alternately, the oxidative stability of oils can be examined by measuring their FFA contents. Literature data revealed that FFA is more prone to oxidation (Molteberg et al., 1996; Mahesar et al., 2014) compared to that of esterified fatty acids (Kinsella et al., 1978). The FFA contents of P80 and P120 are presented in Figure 1. The results between P80 and control (i.e., 0.051 ± 0.001) were insignificant except for T10 (0.055 ± 0.001) and T12 (0.058 ± 0.003) which were heated for ≥ 10 hr. The FFA content (calculated as wt% of palmitic acid) of P80 fell within the acceptable level (0.1 wt%) for refined, bleached, deodourised palm olein as stipulated in Palm Oil Refiners Association of Malaysia Standard Specifications for processed palm oil. In contrast, FFA content of P120 was highly associated with the reaction time. Although the FFA content of palm olein heated at 120°C for 10 hr was within the acceptance level, the percentage increased to 53.0% after 10 hr (i.e., 0.101 ± 0.002) which slightly exceeded the maximum limit (0.1 wt%). The results implied that prolonged heating of palm olein for more than 10 hr at high temperature (120°C) should be avoided even though palm olein is generally regarded as thermally stable.

The SMP and CP are important parameters for oil quality monitoring in food and cosmetic industries. In addition, CP can also determine storage stability of an oil. The SMP of palm olein is 16.3°C, which is consistent with literature data (Siew, 1990). The SMP of both P80 and P120 did not differ significantly even with increasing reaction time and heating temperature compared to the control (Figure 2). Nonetheless, their CP increased to different extents gradually, with P120 showing more intense changes throughout the treatment period. Also, significant difference was observed between the two sets. A gradual increase of FFA content at 120°C in the oils resulted in higher CP (Figure 3). This was probably due to the higher melting point of the increased FFA present and/or changes of the types of SFA, which in turn increased the crystallisation rate of the oils. For instance, the melting points of lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) are ranging from 43.0°C-70.0°C.

### Table 1b. Positional Fatty Acid Compositions (mol 100 mol⁻¹ of total fatty acids) of Palm Olein Heated at 120°C (P120) from 0 to 12 hr (T0, T2, T4, T6, T8, T10 and T12)

| Sample Types of fatty acids | sn-1,3 | sn-2 | sn-1,2,3 |
|----------------------------|--------|------|---------|
| Palm olein (control)       |        |      |         |
| SFA                        | 67.1 ± 1.2 | 6.7 ± 0.3 | 46.8 ± 0.8 |
| MUFA                       | 28.1 ± 1.4 | 66.3 ± 0.4 | 41.0 ± 1.0 |
| PUFA                       | 4.8 ± 0.2  | 27.0 ± 0.2 | 12.2 ± 0.1 |
| T0                         |        |      |         |
| SFA                        | 67.3 ± 0.7 | 7.9 ± 0.9 | 47.8 ± 0.8 |
| MUFA                       | 29.3 ± 0.8 | 70.4 ± 3.5 | 42.8 ± 0.7 |
| PUFA                       | 3.4 ± 0.6  | 21.7 ± 2.6 | 9.4 ± 0.7  |
| T2                         |        |      |         |
| SFA                        | 66.6 ± 0.5 | 7.6 ± 0.5 | 47.4 ± 0.5 |
| MUFA                       | 29.3 ± 0.7 | 71.8 ± 3.6 | 43.1 ± 1.9 |
| PUFA                       | 4.1 ± 1.1  | 20.6 ± 4.1 | 9.5 ± 1.7  |
| T4                         |        |      |         |
| SFA                        | 66.8 ± 1.4 | 6.9 ± 1.4 | 46.6 ± 0.3 |
| MUFA                       | 27.9 ± 0.6 | 70.6 ± 2.6 | 42.3 ± 0.4 |
| PUFA                       | 5.3 ± 0.8  | 22.5 ± 1.2 | 11.1 ± 0.2 |
| T6                         |        |      |         |
| SFA                        | 66.6 ± 0.7 | 7.6 ± 2.0 | 46.9 ± 0.2 |
| MUFA                       | 29.2 ± 0.6 | 66.1 ± 1.2 | 41.5 ± 0.6 |
| PUFA                       | 4.2 ± 1.3  | 26.3 ± 0.8 | 11.6 ± 0.7 |
| T8                         |        |      |         |
| SFA                        | 65.8 ± 1.9 | 6.0 ± 0.4 | 46.2 ± 0.5 |
| MUFA                       | 28.8 ± 2.0 | 66.8 ± 4.0 | 41.2 ± 2.0 |
| PUFA                       | 5.4 ± 0.2  | 27.2 ± 4.0 | 12.6 ± 1.6 |
| T10                        |        |      |         |
| SFA                        | 66.7 ± 0.7 | 6.1 ± 0.5 | 46.7 ± 0.4 |
| MUFA                       | 28.0 ± 1.1 | 67.8 ± 2.6 | 41.1 ± 0.5 |
| PUFA                       | 5.3 ± 0.9  | 26.1 ± 2.2 | 12.2 ± 0.2 |
| T12                        |        |      |         |
| SFA                        | 68.0 ± 0.1 | 7.3 ± 0.3 | 47.8 ± 0.3 |
| MUFA                       | 27.6 ± 1.1 | 66.3 ± 1.6 | 40.5 ± 1.4 |
| PUFA                       | 4.4 ± 1.1  | 26.4 ± 1.7 | 11.7 ± 1.2 |

Note: SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. All data shown p > 0.05 compared to control.
The rancidity of the oils, as depicted via PV, serves as an indicator for early oxidation stages of oils. Lipids hydroperoxides are 1° oxidation products, which are very stable in the absence of metals at ambient temperature. However, the hydroperoxides concentration increases when oxidation takes place and continues rising until it reaches the advanced oxidation stages (Choe and Min, 2006). Low PV is generally an indicator of good quality oil. In this study, both P80 and P120 showed PV < 10 meq O₂ kg⁻¹ under heating up to reaction time of 2 hr. Palm olein heated at 120°C showed more severe PV changes. Significant changes were observed between P80 and P120 from T2 to T12 compared to control; and the highest PV achieved were 29.11 ± 0.85 and 57.88 ± 1.74, respectively (Figure 4). The maximum acceptance level of the PV for edible oils is 10 milliequivalents of active oxygen per kg of oil (meq O₂ kg⁻¹) according to CODEX STAN 210-1999. The results revealed that thermal oxidation occurred in palm olein under heating at 80°C and 120°C and became more severe and undesirable under prolonged heating.

### TABLE 2. FATTY ACID COMPOSITION (FAC) OF PALM OLEIN HEATED AT 80°C AND 120°C FROM 0 TO 12 HR (T0, T2, T4, T6, T8, T10 and T12)

| Sample | Types of fatty acids | Total FAC of test oils (g 100 g⁻¹ total fatty acids) |
|--------|----------------------|------------------------------------------------------|
|        |                      | 80°C                                                 | 120°C                                               |
| Palm olein (control) | C18:1 | 41.3 ± 0.7 | 41.2 ± 0.8 |
|        | C18:2 | 11.9 ± 0.2 | 11.6 ± 0.5 |
|        | C18:3 | 0.3 ± 0.1 | 0.3 ± 0.0 |
|        | SFA   | 46.5 ± 1.0 | 46.9 ± 0.9 |
| T0     | C18:1 | 42.0 ± 1.4 | 40.8 ± 0.6 |
|        | C18:2 | 11.8 ± 1.2 | 11.7 ± 0.3 |
|        | C18:3 | 0.3 ± 0.1 | 0.3 ± 0.0 |
|        | SFA   | 45.9 ± 0.5 | 47.2 ± 1.0 |
| T2     | C18:1 | 39.4 ± 0.6 | 40.6 ± 0.8 |
|        | C18:2 | 11.8 ± 0.3 | 11.8 ± 0.4 |
|        | C18:3 | 0.4 ± 0.1 | 0.3 ± 0.1 |
|        | SFA   | 48.3 ± 1.1 | 47.3 ± 0.8 |
| T4     | C18:1 | 41.0 ± 0.7 | 40.2 ± 0.6 |
|        | C18:2 | 11.5 ± 0.6 | 11.5 ± 0.7 |
|        | C18:3 | 0.2 ± 0.1 | 0.3 ± 0.0 |
|        | SFA   | 47.4 ± 0.9 | 48.0 ± 0.8 |
| T6     | C18:1 | 41.4 ± 1.1 | 41.4 ± 0.4 |
|        | C18:2 | 11.3 ± 1.0 | 11.3 ± 0.8 |
|        | C18:3 | 0.3 ± 0.1 | 0.3 ± 0.0 |
|        | SFA   | 46.8 ± 0.3 | 47.0 ± 1.1 |
| T8     | C18:1 | 40.5 ± 0.8 | 41.3 ± 1.3 |
|        | C18:2 | 11.7 ± 0.3 | 11.3 ± 0.9 |
|        | C18:3 | 0.3 ± 0.1 | 0.4 ± 0.2 |
|        | SFA   | 47.5 ± 0.8 | 47.0 ± 0.9 |
| T10    | C18:1 | 40.8 ± 0.8 | 43.8 ± 1.4 |
|        | C18:2 | 11.8 ± 0.8 | 10.7 ± 1.8 |
|        | C18:3 | 0.3 ± 0.0 | 0.2 ± 0.1 |
|        | SFA   | 47.1 ± 0.9 | 45.3 ± 0.8 |

Note: C18:1 - oleic acid; C18:2 - linoleic acid; C18:3 - linolenic acid; SFA - saturated fatty acids. All data shown p > 0.05 compared to control.
Palm olein, a cooking oil was thermally stable under heating at 80°C and 120°C within the first 2 hr as was evidenced by the PV measurement. The FFA content of palm olein fell within the acceptance level under prolonged heating for ≤ 10 hr at 80°C and 120°C. Palm olein exhibited good oxidative stability property which was associated with its high SFA content. In short, the changes in the extent of oxidative stability and physicochemical properties of palm olein depend on the heating time and temperature.

| Reaction time (hr) | IV |
|-------------------|----|
|                   | 80°C | 120°C |
| Control           | 55.8 ± 0.2 | 56.3 ± 1.1 |
| 0                 | 56.8 ± 1.0 | 56.2 ± 0.8 |
| 2                 | 57.5 ± 1.3 | 56.1 ± 0.9 |
| 4                 | 55.5 ± 0.7 | 56.2 ± 1.2 |
| 6                 | 55.8 ± 0.8 | 55.2 ± 0.4 |
| 8                 | 56.0 ± 1.1 | 55.9 ± 0.8 |
| 10                | 55.8 ± 0.8 | 56.2 ± 0.8 |
| 12                | 56.3 ± 1.7 | 56.7 ± 1.5 |

Note: All data shown $p > 0.05$ compared to control.

CONCLUSION

Palm olein, a cooking oil was thermally stable under heating at 80°C and 120°C within the first 2 hr as was evidenced by the PV measurement. The FFA content of palm olein fell within the acceptance level under prolonged heating for ≤ 10 hr at 80°C and 120°C. Palm olein exhibited good oxidative stability property which was associated with its high SFA content. In short, the changes in the extent of oxidative stability and physicochemical properties of palm olein depend on the heating time and temperature.

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