

ASSOCIATION OF FATTY ACIDS AND POLAR COMPOUND FRACTIONS WITH ACRYLAMIDE FORMATION DURING INTERMITTENT FRYING

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ABSTRACT

This study aimed to investigate the relationship of fatty acids composition (FAC) and total polar compounds (TPC) on acrylamide formation during intermittent frying of French fries. Four vegetable oils namely palm olein, red palm olein, soybean and sunflower oil were subjected to 80 frying cycles over five consecutive days. The FAC was analysed in the fresh oil (before frying) and 80th frying cycles while TPC was quantified for every 16th frying cycle. The saturated fatty acids (SFA) significantly increased over 80 frying cycles; however, polyunsaturated fatty acids (PUFA) demonstrated a significant reduction. A strong positive correlation ($r=0.864$, $p\leq 0.05$) was observed between acrylamide content and PUFA whereas a weak positive but significant correlation for SFA ($r=0.284$, $p\leq 0.05$) and monounsaturated fatty acids (MUFA) ($r=0.358$, $p\leq 0.05$). Polymerised triacylglycerols (PTAG), oxidised triacylglycerols (OxTAG), and diacylglycerols (DAG) were significantly increased over frying cycles. A weak but significant negative correlation ($r=-0.269$, $p\leq 0.05$) was observed between PTAG in oils and acrylamide in French fries. FAC and PTAG significantly contributed to the formation of acrylamide in French fries. This indicates that lipid oxidation, particularly tertiary oxidation, exhibits a significant contribution to acrylamide development during frying.

Keywords: acrylamide, fatty acids composition, intermittent frying, total polar compounds, vegetable oils.

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INTRODUCTION

Frying is one of the food preparation methods involving immersion of food in edible oil above the boiling point of water that cause changes in

the characteristics of fried products such as colour, texture and flavour of the fried products (Hubbard and Farkas, 2007). The dehydration process at excessive temperatures is reflected by a rapid heat and mass transfer between oil and food (Ahmad Tarmizi and Niranjana, 2013). Albeit considerable oil content in fried food, frying is still widely employed in domestic industrial sectors (Ahmad Tarmizi *et al.*, 2016). Intermittent frying refers to cooking at discrete batches when the oil is heated and kept after use for subsequent frying operation (Seppanen and Csallany, 2006). In the presence of oxygen, moisture, trace elements and free radicals, frying leads to oil degradation through myriad chemical reactions such as thermal oxidation, hydrolysis, and polymerisation (Andrikopoulos *et al.*, 2002).

As fried food absorbs oil, deterioration of oil used for frying not only yields undesired sensory characteristics of the fried product (Gertz and Behmer, 2014) but also generate new compounds that

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could be harmful to human health (Kushairi *et al.*, 2019). The presence of acrylamide is one of the major concerns associated with fried products. Acrylamide is classified by the International Agency for Research on Cancer (IARC) as a probably carcinogenic (group 2A) to humans (IARC, 1994). Potato-based products such as French fries are reported to have notable levels of acrylamide (Luning and Sanny, 2016; Sanny *et al.*, 2012). The European Commission has set a threshold for French fries at 500 $\mu\text{g kg}^{-1}$ (European-Commission, 2017). It is well-established that the Maillard reaction is the major pathway for the formation of acrylamide (Stadler *et al.*, 2002). Meanwhile, lipid oxidation is reported as the minor pathway for acrylamide manifestation when its precursor, which is acrylic acid, is developed via the degraded product namely acrolein (Daniali *et al.*, 2016; Gertz and Klostermann, 2002; Yasuhara *et al.*, 2003).

Fatty acid composition (FAC) is often used to determine the authenticity and stability of oils for frying (Ahmad Tarmizi *et al.*, 2016). Their thermal resistance is associated with the degree of saturation. Oils with higher saturated fatty acids are more stable than conventional liquids oils such as canola and sunflower oils (Ahmad Tarmizi *et al.*, 2016). The amount of double bonds in fatty acids determines the severity of oil oxidation. A higher degree of oil unsaturation is more prone to thermal oxidation (Ahmad Tarmizi *et al.*, 2016). Several publications have correlated the degree of unsaturation and thermal oxidation with acrylamide formation in the fried products (Lim *et al.*, 2014; Wang *et al.*, 2019). Factors such as frying conditions (Abdul Hamid *et al.*, 2019; Kamarudin *et al.*, 2018; Sanny *et al.*, 2010) and frying media (Ahmad *et al.*, 2021; Daniali *et al.*, 2018; Lim *et al.*, 2014; Zhang *et al.*, 2015) have shown to onset the formation of acrylamide in fried products.

The thermal oxidation mechanism mainly involves initiation, propagation, and termination (Choe and Min, 2009). Peroxide value (PV), which measures hydroperoxides in the oil, is used to evaluate the initial stages of oxidation. The *p*-anisidine value (AnV), which is denoted as secondary oxidation, quantifies the level of non-volatile aldehydes and ketones in oil during frying (Ahmad Tarmizi *et al.*, 2016). The advancement of secondary oxidation or known as tertiary oxidation is mainly responsible for the formation of compounds with relatively higher molecular weight. The measurement of total polar compounds (TPC) is perceived as one of the most objective methods to determine the cumulative oil degradation from primary, secondary, and tertiary oxidation. TPC can be further cascade down to several fractions namely polymerised triacylglycerols (PTAG), diacylglycerols (DAG), monoacylglycerols (MAG), oxidised triacylglycerols (OxTAG) and free fatty

acids (FFA) (Santos *et al.*, 2013). Many European countries have set the TPC maximum limit between 25% and 30% in used oils (Berger, 2005). A considerable amount of TPC in the oil as a result of high OxTAG and PTAG build-up could possibly affect human health (Petersen *et al.*, 2013). Animal studies have shown that PTAG and OxTAG are potent for higher risk of skin diseases (Cohn, 2002) and gastrointestinal irritants (Saguy and Dana, 2003). Realising the amount of frying oil imparts the fried products is significant, oil characteristics are highly concern in ensuring oil deterioration is minimal (Ahmad Tarmizi *et al.*, 2016).

Most authors reported the insignificant association between PV and acrylamide content in fried food (Abd Razak *et al.*, 2021; Ahmad *et al.*, 2021; Kuek *et al.*, 2020; Williams, 2005; Zhang *et al.*, 2015). Despite our current study did not observe any significant correlation between AnV and acrylamide content in fried beef nuggets, the relationship was reported to be significant in French fries (Abd Razak *et al.*, 2021; Kuek *et al.*, 2020). In fact, Abd Razak *et al.* (2021) has recently observed a significant association between TPC and acrylamide content in French fries. As most publications highlighted a significant correlation between the FAC and acrylamide content (Lim *et al.*, 2014; Wang *et al.*, 2019), limited studies have been carried out to explore the relationship between TPC and acrylamide. Thus, our study aimed to establish a relationship between TPC and FAC of different vegetable oils on acrylamide formation during intermittent frying of French fries. The acrylamide data from our published article (Kuek *et al.*, 2020) was used in the present study to determine the strength of the relationship between FAC and TPC with acrylamide concentration.

MATERIALS AND METHODS

Raw Materials

Palm olein, soybean oil and sunflower oil of the same batch were purchased from local supermarkets. The red palm olein (industrial grade) was procured from a palm oil refinery that is located in Selangor, Malaysia. Shoestring pre-fried French fries ($0.5 \times 0.4 \text{ cm}^2$ in cross-section and 6 to 7 cm long) of the same batch were obtained from a local frozen food distributor. The potato product was stored at -20°C upon purchase and thawed at 4°C overnight before the frying experiment.

Frying Experiment

The frying setup was executed with reference to Ahmad Tarmizi *et al.* (2019a) with some slight modification. Approximately 5 kg of oil was firstly

weighed, placed into two split pots of fryer DFT-6000 (MSM, Malaysia) and heated to 180°C. A batch of 150 g of par-fried potato strips was fried for every cycle for 3.5 min with the interval of 30 min for 8 hr daily for five consecutive days. The frying protocol was designed to simulate conditions in the food service sector (Ismail, 2005). Upon completion of each frying cycle, the finished product was collected and stored in a zip-lock bag at -20°C. Approximately 450 g of used oil was sampled in a dark amber bottle and cooled to room temperature before flushing with nitrogen and stored at -20°C for subsequent analyses. Fresh oil was added to the fryer on the next day of operation to replenish oil sampling. Each type of oil underwent frying experiments in duplicate.

Chemicals and Reagents

The n-hexane, tetrahydrofuran, sodium sulphate, and silica gel 60 (0.063 to 0.200 mm, 70 to 230 mesh ASTM) were purchased from Merck (Darmstadt, Germany). Petroleum ether (PE), diethyl ether (DE), and sea sand (acid wash) were purchased from Systerm (Selangor, Malaysia).

Analysis of Oil

Fatty acid composition (FAC). The quantification of FAC was carried out following AOCS Official Method Ce 1i-07 (AOCS, 2011) using Hewlett-Packard 6890 Series gas chromatography (Agilent Technologies, Palo Alto, CA, USA). The GC was equipped with a BPX70 capillary column (60 m × 0.25 mm, i.d. 0.25 µm film thickness), flame ionisation detector (FID), electronic integrator, and data processor (J&W Scientific, Folsom, USA). The sample injection volume was set at 1 µL while helium gas was used as the carrier gas with the flow rate of 0.8 mL min⁻¹ and a pre-column split ratio of 100:1. The temperature for the injection port and FID detector was set at 240°C while the column temperature remained at 185°C. Fatty acid methyl esters (FAME) were quantified by comparing their retention times and peak areas. Reference mix comprising of RM-5 and RM-6 methyl ester standards (Supelco, Dorset, United Kingdom) was used considering the variation of fatty acids in the studied oils. The percentage of mass fractions is used to express the level of each fatty acid.

Total polar compound (TPC) fractions. The TPC was performed according to IUPAC 2.507 with minor modification (Dobarganes *et al.*, 2000). About 2.5 g of the sample was measured in a 50 mL volumetric flask. PE and DE solution (87:13, respectively) was then added up to the mark. The empty round bottom flask dried in an oven at 103°C overnight and allowed to cool in a desiccator until

constant weight. A 45 cm glass column was packed with 25 g of silica gel, suspended in PE-DE mixture, and layered with 4 g of sea sand.

The nonpolar fraction was eluted using a 150 mL PE-DE mixture for approximately 60 to 65 min and collected in a round bottom flask. A similar procedure was repeated for polar fractions using DE. The round bottom flask was then dried using a rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland) at 60°C, rotation at 130 rpm for 15 min, and vacuum level at 380 mbar, and rotation at 130 rpm for 15 min. After drying, nitrogen gas was then flushed slowly into the flask for another 15 min and weighed. About 0.5 g of residue in the round bottom flask was taken out and re-dissolved using 10 mL of tetrahydrofuran. The solution was then filtered using a 0.45 µm nylon membrane filter and collected in a vial before injection into high performance liquid chromatography (HPLC).

Partitioning of polar compound fractions. Polar compound fractions namely OxTAG, PTAG, DAG, MAG, and FFA were quantified using HPLC equipped with an evaporative light scattering detector (ELSD) (Agilent 1260 Infinity, Santa Clara, USA) and three PLgel 500 Å columns consist of 5 µm particle size (7.5 mm × 300 mm) in series (Agilent 1260 Infinity, Santa Clara, USA). Tetrahydrofuran was used as a mobile phase used at the flow rate of 1 mL min⁻¹. Temperature columns were set at 40°C while the evaporator and nebuliser of the ELSD were fixed at 40°C and 30°C, respectively. The nitrogen flow rate of the ELSD was established at 1.7 standard L min⁻¹ (SLM). Analysis time for each sample required 30 min to complete. The principle of quantifying the polar compounds fractions was based on the molecule size. Fractions with larger molecule size will appear as the earliest peak on the chromatogram: PTAG > OxTAG > DAG > MAG > FFA. It is important to note that partitioning of polar fractions is made from the basis of polarity reflecting specific routes of a chemical reaction during frying.

Statistical Analysis

General linear model analysis of variance (ANOVA) was performed using Minitab Statistical Software 17 (Minitab Inc., Pa, USA). The means of analysis were evaluated following Tukey's multiple comparison test. Pearson's correlation was used to determine the strength of the relationship between quality parameters in the oil and acrylamide content in the fried product. The correlation was confirmed for possible violations from the model assumptions during the analysis. *p*-value, which was less or equal to 0.05, indicates the significance of the relationship between variables.

RESULTS AND DISCUSSION

Table 1 shows the significant variation ($p \leq 0.05$) occurring in FAC in all oils across 80 frying cycles. Alteration of fatty acids is associated with the levels of unsaturated fatty acids, heat treatment, and number of frying cycles (Alireza *et al.*, 2010). It is also noted that myristic acid (C14:0), stearic acid (C18:0) and oleic acid (C18:1) were significantly increased over 80 frying cycles regardless of oil types. This observation was in agreement with the findings by Ahmad Tarmizi *et al.* (2019a) and Li *et al.* (2017). An increase in SFA was mainly contributed by the conversion of unsaturated fatty acids into saturated form as a result of oxidation (Alireza *et al.*, 2010). Indeed, the elevation of palmitic acid (C16:0), C18:0 and C18:1 was likely of oxidation of linoleic acid (C18:2) and linolenic acid (C18:3) during frying (Li *et al.*, 2017). The C18:1 significantly increased in sunflower and soybean oils as a function of frying

cycles (Table 1). In the case of sunflower oil, an increase of C18:1 showed in this study contradicted with Romero *et al.* (1998) but consistent with the observation made by Ahmad Tarmizi *et al.* (2019a). However, C18:1 was significantly lowered in palm olein, and this coincides with Li *et al.* (2017). Interestingly, C18:1 of red palm olein did not show any significant changes even after 80 frying cycles compared to palm olein (Table 1). The C18:2 and C18:3 demonstrated a significant reduction across frying cycles in all oils (Table 1), which was found similar with Li *et al.* (2017). In the light of soybean oil, Sebedio *et al.* (1990) observed an enhancement of C18:1, C18:2 and C18:3 in soybean oil due to degradation of C18:2 and C18:3. Reduction of C18:2 and C18:3 can be explained by higher tendency of the oil towards oxidation (Alireza *et al.*, 2010). Oxidation of unsaturated fatty acids lowered the unsaturated fatty acid content (Li *et al.*, 2017).

TABLE 1. FATTY ACID COMPOSITION OF VEGETABLE OILS ACROSS FRYING CYCLES

Fatty acid (%)	Frying cycle	Palm olein	Sunflower oil	Red palm olein	Soybean oil
C12:0	Fresh	0.41 ± 0.00 ^{ab}	0.00 ± 0.00 ^{ca}	0.31 ± 0.01 ^{bb}	0.00 ± 0.00 ^{cb}
	80 th	0.44 ± 0.01 ^{aa}	0.00 ± 0.00 ^{da}	0.35 ± 0.02 ^{ba}	0.05 ± 0.00 ^{ca}
C14:0	Fresh	1.04 ± 0.00 ^{ab}	0.08 ± 0.01 ^{db}	0.65 ± 0.07 ^{ba}	0.09 ± 0.00 ^{cb}
	80 th	1.16 ± 0.03 ^{aa}	0.25 ± 0.06 ^{ca}	0.57 ± 0.07 ^{bb}	0.22 ± 0.01 ^{ca}
C16:0	Fresh	37.96 ± 0.00 ^{ab}	6.17 ± 0.10 ^{db}	35.08 ± 0.38 ^{bb}	10.73 ± 0.02 ^{cb}
	80 th	43.46 ± 0.20 ^{aa}	11.71 ± 0.04 ^{da}	40.16 ± 0.31 ^{ba}	16.50 ± 0.02 ^{ca}
C16:1	Fresh	0.26 ± 0.02 ^{aa}	0.10 ± 0.00 ^{cb}	0.20 ± 0.00 ^{ba}	0.08 ± 0.00 ^{db}
	80 th	0.20 ± 0.01 ^{ab}	0.13 ± 0.01 ^{ba}	0.21 ± 0.01 ^{aa}	0.10 ± 0.00 ^{ca}
C18:0	Fresh	3.97 ± 0.00 ^{bb}	3.28 ± 0.02 ^{db}	3.78 ± 0.05 ^{cb}	4.36 ± 0.01 ^{ab}
	80 th	4.57 ± 0.10 ^{ba}	3.96 ± 0.08 ^{ca}	4.44 ± 0.22 ^{ba}	5.01 ± 0.01 ^{aa}
C18:1	Fresh	43.05 ± 0.62 ^{ba}	31.05 ± 0.05 ^{cb}	46.38 ± 0.47 ^{aa}	23.28 ± 0.01 ^{db}
	80 th	42.58 ± 0.38 ^{bb}	35.40 ± 0.08 ^{ca}	44.97 ± 0.53 ^{ba}	28.05 ± 0.02 ^{da}
C18:2	Fresh	11.74 ± 0.01 ^{da}	57.49 ± 0.07 ^{aa}	12.10 ± 0.12 ^{ca}	52.83 ± 0.03 ^{ba}
	80 th	6.90 ± 0.12 ^{db}	46.75 ± 0.06 ^{ab}	8.11 ± 0.04 ^{cb}	43.75 ± 0.02 ^{bb}
C18:3	Fresh	0.34 ± 0.01 ^{ba}	0.32 ± 0.05 ^{ca}	0.32 ± 0.01 ^{ba}	7.47 ± 0.00 ^{aa}
	80 th	0.13 ± 0.02 ^{cb}	0.24 ± 0.04 ^{bb}	0.12 ± 0.01 ^{cb}	4.57 ± 0.00 ^{ab}
C20:0	Fresh	0.38 ± 0.00 ^{ab}	0.23 ± 0.01 ^{ba}	0.38 ± 0.0 ^{ab}	0.38 ± 0.01 ^{ab}
	80 th	0.50 ± 0.06 ^{aa}	0.27 ± 0.01 ^{ca}	0.45 ± 0.02 ^{aba}	0.42 ± 0.01 ^{ba}
C20:1	Fresh	0.00 ± 0.00 ^{ca}	0.21 ± 0.04 ^{aba}	0.16 ± 0.00 ^{ba}	0.20 ± 0.00 ^{aa}
	80 th	0.00 ± 0.00 ^{ca}	0.16 ± 0.02 ^{ba}	0.17 ± 0.01 ^{ba}	0.19 ± 0.00 ^{ab}
C22:0	Fresh	0.00 ± 0.00 ^{ca}	0.71 ± 0.01 ^{aa}	0.00 ± 0.00 ^{cb}	0.42 ± 0.01 ^{bb}
	80 th	0.00 ± 0.00 ^{da}	0.70 ± 0.03 ^{aa}	0.05 ± 0.00 ^{ca}	0.54 ± 0.01 ^{ba}
C24:0	Fresh	0.00 ± 0.00 ^{aa}	0.00 ± 0.00 ^{aa}	0.00 ± 0.00 ^{aa}	0.00 ± 0.00 ^{aa}
	80 th	0.00 ± 0.00 ^{aa}	0.00 ± 0.00 ^{aa}	0.00 ± 0.00 ^{aa}	0.00 ± 0.00 ^{aa}
Others	Fresh	0.85 ± 0.00 ^{aa}	0.07 ± 0.01 ^{db}	0.35 ± 0.01 ^{bb}	0.15 ± 0.00 ^{cb}
	80 th	0.26 ± 0.05 ^{bb}	0.44 ± 0.01 ^{aa}	0.45 ± 0.01 ^{aa}	0.21 ± 0.00 ^{ba}
SFA	Fresh	43.76 ± 0.00 ^{ab}	10.47 ± 0.10 ^{db}	40.38 ± 0.29 ^{bb}	15.98 ± 0.03 ^{cb}
	80 th	50.14 ± 0.22 ^{aa}	16.88 ± 0.08 ^{da}	46.31 ± 0.32 ^{ba}	23.04 ± 0.03 ^{ca}
MUFA	Fresh	43.31 ± 0.63 ^{ba}	31.25 ± 0.15 ^{cb}	46.64 ± 0.48 ^{aa}	23.43 ± 0.11 ^{db}
	80 th	42.78 ± 0.38 ^{bb}	35.58 ± 0.11 ^{ca}	45.43 ± 0.54 ^{bb}	28.21 ± 0.11 ^{da}
PUFA	Fresh	12.08 ± 0.01 ^{da}	57.81 ± 0.08 ^{ba}	12.42 ± 0.12 ^{ca}	60.30 ± 0.03 ^{aa}
	80 th	7.04 ± 0.13 ^{db}	46.99 ± 0.09 ^{bb}	8.23 ± 0.04 ^{cb}	48.31 ± 0.03 ^{ab}
C18:2/C16:0	Fresh	0.31	9.32	0.34	4.93
	80 th	0.16	3.99	0.20	2.65

Note: ^{a-d} values within the same row with different lowercase letters are significantly different ($p \leq 0.05$).

^{A-B} values within the same fatty acid chain column with different lowercase letters are significantly different ($p \leq 0.05$).

Value are the means ± SD of duplicate experiments with triplicate determinations.

An increase in SFA for all oils was found significant while polyunsaturated fatty acids (PUFA) exhibited a significant decline over frying cycles. A higher degree of unsaturation reflects the tendency of oil towards oxidation (Ahmad Tarmizi *et al.*, 2016). Oxidation of C18:3 causes an increase in SFA but reduces the PUFA content (Li *et al.*, 2017). Unlike PUFA and SFA, the trend for monounsaturated fatty acids (MUFA) as depicted in *Table 1* is very much dependent on the oil types. For sunflower and soybean oils, MUFA increased significantly over 80 frying cycles. However, palm olein displayed a significant drop in MUFA with the increase of frying cycles. In the case of red palm olein, MUFA did not show any significant changes over frying cycles due to the absence of alteration in C18:1. The ratio of C18:2/C16:0 is calculated to distinguish the degree of oxidative deterioration in used oils (Alireza *et al.*, 2010). *Table 1* also shows the reduction of C18:2/C16:0 for all vegetable oils throughout the frying cycles. Such observation was somewhat similar to Zribi *et al.* (2014), who emphasised on the C18:2/C18:1 decrement over frying time indicates the occurrence of lipid deterioration under excessive heating. Sunflower oil exhibited the highest C18:2/C16:0 as its relatively higher C18:2 content (Alireza *et al.*, 2010). Interestingly, palm olein demonstrated the lowest ratio of C18:2/C16:0 when compared to other oils which reflect its stability and resistance towards heat even after five days of frying. This study also revealed that C18:2/C16:0 for red palm olein and palm olein was apparently identical (*Table 1*). This further demonstrated that both palm olein and red palm olein were found stable and thermally resistant against high temperature under long frying operation.

Table 2 shows the Pearson's correlation between the degree of saturation and acrylamide content upon extended frying. The results showed a strong positive correlation ($r=0.864$, $p\leq 0.05$) between acrylamide with PUFA while a weak positive but significant correlation was pronounced in SFA ($r=0.284$, $p\leq 0.05$) and MUFA ($r=0.358$, $p\leq 0.05$). Comparison of results with published literature could not be made due to the absence of research works that associate FAC with acrylamide content fried products. Nevertheless, Ehling and Shibamoto (2005) reported on the heating of asparagine with C16:0 could generate a relatively high acrylamide content. The presence of SFA could onset the acrylamide formation albeit the correlation was comparatively weak. A similar study by Ehling and Shibamoto (2005) also concluded that heating asparagine in lipid with higher unsaturated fatty acids induces acrylamide formation of which the C18:1 and C18:2 generated 10 folds higher in acrylamide in comparison to C16:0 when heating was undertaken at 180°C. *Table 2* also concluded that the correlation between PUFA and acrylamide

was stronger than MUFA and SFA considering PUFA is highly prone to oxidation. L alas (2009) reported that C18:2 is more susceptible to oxidation when compared to C18:1 considering the presence of conjugated diene. The results obtained agree with the outcome from Capuano *et al.* (2010) where an increase in unsaturation is highly prone to lipid oxidation and thus, promotes acrylamide formation. Lipid oxidation generates more carbonyl groups such as aldehydes and ketones that can further react with asparagine to form acrylamide even in the absence of reducing sugar (Ehling and Shibamoto, 2005). Yasuhara *et al.* (2003) also opined that acrylamide could be developed through the formation of acrolein. Acrolein, which is one of the oil breakdowns, can further oxidise to acrylic acid and subsequently reacts with ammonia to form acrylamide.

TABLE 2. PEARSON'S CORRELATION BETWEEN ACRYLAMIDE CONCENTRATION AND SFA, MUFA AND PUFA OF VEGETABLE OILS, RESPECTIVELY

Parameters	Pearson's correlation	
	<i>r</i>	<i>p</i> -value
SFA	0.284	$p\leq 0.05$
MUFA	0.358	$p\leq 0.05$
PUFA	0.864	$p\leq 0.05$

A significant increase ($p\leq 0.05$) in TPC of palm olein, red palm olein, sunflower oil and soybean oil was also evidenced as a function of frying cycle (*Table 3*) as frying oil was continuously exposed to elevated temperature, oxidation of oil increased and thus, the TPC (Aniolowska and Kita, 2016). The TPC development was likely contributed to the increase of OxTAG and hydrolytic breakdown products such as FFA and DAG. The rate of TPC development over frying time is much dependent on the oil composition (Ahmad Tarmizi *et al.*, 2016). Many European countries tolerate an upper TPC limit of 24% to 27% due to health concerns of used oil (Li *et al.*, 2016). By taking 24% as a benchmark, frying in palm olein and soybean oil surpassed the limit after the 48th frying cycle, whereas sunflower oil gave a shorter frying time (32nd cycle). The results in *Table 3* clearly indicate that red palm olein was the most stable as its TPC content was still acceptable even after the 60th frying cycle.

With respect to polar compound fractions, PTAG, OTAG, and DAG of all vegetable oils were significantly increased ($p\leq 0.05$) over frying cycles (*Table 3*). PTAG is developed when oils undergo tertiary oxidation and thermal alteration (Ahmad Tarmizi *et al.*, 2019b). The PTAG of sunflower and soybean oils were found to be higher than other oils. This result can be explained by the higher C18:2 content in oils. Oils rich in C18:2 are more easily polymerised during frying than those oils containing

higher C18:1 (Tompkins and Perkins, 2000). It is also reported that the formation rate of cyclic monomers and polymers was higher when the oils contain considerable level of C18:3 (Tompkins and Perkins, 2000).

Results in Table 3 showed that the OxTAG for all oils significantly increased ($p \leq 0.05$) across frying cycles. The trends of OxTAG in palm olein and soybean oil exhibited a rapid increase until the 48th cycle and did not show any notable increase thereafter. The results were consistent with the observation made by Ahmad Tarmizi *et al.* (2019b). For sunflower oil, OxTAG rapidly increased until the 32nd frying cycle and stabilised throughout the remaining frying cycle. Such occurrence is due to lipid oxidation where aldehydes and ketones were converted into compounds with high-molecular-

weight (Tsaknis *et al.*, 2002). However, a similar profile was not observed in red palm olein as OxTAG continued to build up consistently throughout 80 frying cycles. The increase in OxTAG was possibly caused by higher concentration of beta-carotenes that could be responsible as pro-oxidant during frying (Palozza *et al.*, 2003). Sunflower and soybean oils gave higher OxTAG when compared to palm olein and red palm olein even before frying. The result was similar to those observed by Li *et al.* (2017), who concluded that the presence of OxTAG was prominent in sunflower oil. However, after 80 frying cycles, all oils yielded comparable OxTAG contents due to prolonged oxidation during frying. According to Li *et al.* (2017), OxTAG swiftly increases at the beginning of frying and reaches a plateau as the frying progresses.

TABLE 3. POLAR COMPOUND FRACTIONS OF VEGETABLE OILS ACROSS FRYING CYCLES

Parameters (%)	Frying cycle	Palm olein	Sunflower oil	Red palm olein	Soybean oil
PTAG	0 th	0.02 ± 0.00 ^{CE}	0.40 ± 0.03 ^{AF}	0.01 ± 0.00 ^{DF}	0.12 ± 0.03 ^{BE}
	16 th	2.68 ± 0.16 ^{CD}	8.12 ± 0.30 ^{AE}	2.51 ± 0.12 ^{CE}	7.16 ± 0.24 ^{BD}
	32 nd	6.12 ± 0.22 ^{CC}	14.04 ± 0.21 ^{AD}	6.36 ± 0.19 ^{CD}	11.87 ± 0.41 ^{BC}
	48 th	8.97 ± 0.17 ^{CB}	18.40 ± 0.27 ^{AC}	8.25 ± 0.54 ^{CC}	16.15 ± 1.02 ^{BB}
	60 th	10.08 ± 0.83 ^{CA}	21.26 ± 0.21 ^{AB}	10.55 ± 0.56 ^{CB}	16.55 ± 1.58 ^{BB}
	80 th	10.69 ± 2.05 ^{DA}	22.95 ± 0.32 ^{AA}	13.06 ± 0.36 ^{CA}	19.06 ± 0.39 ^{BA}
OxTAG	0 th	0.53 ± 0.17 ^{BD}	1.84 ± 0.02 ^{AE}	0.42 ± 0.11 ^{BF}	1.81 ± 0.42 ^{AD}
	16 th	4.73 ± 0.13 ^{CC}	6.34 ± 0.11 ^{BD}	3.56 ± 0.47 ^{DE}	7.07 ± 0.27 ^{AC}
	32 nd	8.42 ± 0.59 ^{AB}	8.75 ± 0.13 ^{AC}	8.33 ± 0.31 ^{AD}	8.67 ± 0.55 ^{AB}
	48 th	10.54 ± 0.43 ^{AA}	9.91 ± 0.43 ^{AB}	9.75 ± 0.71 ^{AC}	10.00 ± 0.76 ^{AA}
	60 th	11.15 ± 0.52 ^{AA}	11.37 ± 0.40 ^{AA}	11.00 ± 0.47 ^{AB}	10.86 ± 0.61 ^{AA}
	80 th	11.96 ± 1.54 ^{ABA}	11.61 ± 0.28 ^{BA}	13.20 ± 0.84 ^{AA}	11.76 ± 1.54 ^{ABA}
DAG	0 th	6.67 ± 0.03 ^{AB}	1.55 ± 0.05 ^{CD}	3.47 ± 1.03 ^{BD}	1.00 ± 0.11 ^{DD}
	16 th	8.05 ± 0.24 ^{AA}	1.25 ± 0.02 ^{DF}	3.83 ± 0.24 ^{BD}	1.47 ± 0.04 ^{CC}
	32 nd	7.72 ± 0.07 ^{AA}	1.38 ± 0.05 ^{DE}	5.07 ± 0.13 ^{BC}	1.60 ± 0.13 ^{CC}
	48 th	7.72 ± 0.17 ^{AA}	1.65 ± 0.04 ^{DC}	5.38 ± 0.34 ^{BC}	1.91 ± 0.16 ^{CB}
	60 th	7.78 ± 0.40 ^{AA}	1.97 ± 0.05 ^{DB}	6.28 ± 0.36 ^{BB}	2.37 ± 0.60 ^{CAB}
	80 th	8.19 ± 0.89 ^{AA}	2.37 ± 0.05 ^{DA}	7.54 ± 0.30 ^{BA}	2.64 ± 0.08 ^{CA}
MAG	0 th	0.00 ± 0.00 ^{BA}	0.00 ± 0.00 ^{BA}	0.01 ± 0.00 ^{AB}	0.00 ± 0.00 ^{BA}
	16 th	0.00 ± 0.00 ^{BA}	0.00 ± 0.00 ^{BA}	0.01 ± 0.00 ^{AB}	0.00 ± 0.00 ^{BA}
	32 nd	0.00 ± 0.00 ^{AA}	0.00 ± 0.00 ^{AA}	0.00 ± 0.00 ^{CC}	0.00 ± 0.00 ^{AA}
	48 th	0.00 ± 0.00 ^{AA}	0.00 ± 0.00 ^{AA}	0.00 ± 0.00 ^{CC}	0.00 ± 0.00 ^{AA}
	60 th	0.00 ± 0.00 ^{BA}	0.00 ± 0.00 ^{BA}	0.01 ± 0.00 ^{BB}	0.00 ± 0.00 ^{BA}
	80 th	0.00 ± 0.00 ^{BA}	0.00 ± 0.00 ^{BA}	0.03 ± 0.00 ^{AA}	0.00 ± 0.00 ^{BA}
FFA	0 th	0.02 ± 0.00 ^{DB}	0.26 ± 0.03 ^{AA}	0.05 ± 0.01 ^{BC}	0.13 ± 0.00 ^{BB}
	16 th	0.03 ± 0.01 ^{BA}	0.19 ± 0.02 ^{AB}	0.04 ± 0.01 ^{BC}	0.17 ± 0.03 ^{AA}
	32 nd	0.03 ± 0.02 ^{CA}	0.24 ± 0.01 ^{AA}	0.07 ± 0.02 ^{EB}	0.20 ± 0.02 ^{BA}
	48 th	0.05 ± 0.01 ^{BA}	0.29 ± 0.04 ^{AA}	0.09 ± 0.03 ^{BB}	0.29 ± 0.09 ^{AA}
	60 th	0.06 ± 0.02 ^{CA}	0.30 ± 0.07 ^{AA}	0.11 ± 0.02 ^{BAB}	0.21 ± 0.02 ^{AA}
	80 th	0.06 ± 0.03 ^{CA}	0.33 ± 0.08 ^{AA}	0.16 ± 0.03 ^{BA}	0.24 ± 0.04 ^{AA}
TPC	0 th	7.24 ± 0.24 ^{AD}	4.05 ± 0.11 ^{BF}	3.97 ± 1.42 ^{BF}	3.09 ± 0.59 ^{CE}
	16 th	15.50 ± 0.53 ^{AC}	15.90 ± 0.37 ^{AE}	9.94 ± 0.79 ^{BE}	15.88 ± 0.12 ^{AD}
	32 nd	22.38 ± 0.55 ^{BB}	24.40 ± 0.37 ^{AD}	19.90 ± 0.06 ^{CD}	22.37 ± 0.96 ^{BC}
	48 th	27.28 ± 0.23 ^{BA}	30.24 ± 0.66 ^{AC}	23.48 ± 1.29 ^{CC}	28.35 ± 1.95 ^{BB}
	60 th	28.96 ± 1.68 ^{CA}	35.00 ± 0.49 ^{AB}	27.95 ± 1.43 ^{CB}	30.90 ± 1.41 ^{BB}
	80 th	30.90 ± 4.82 ^{CA}	37.26 ± 0.28 ^{AA}	34.00 ± 0.98 ^{BCA}	33.70 ± 0.04 ^{BA}

Note: ^{a-d} values within the same row with different letters are significantly different ($p < 0.05$).
^{A-F} values within the same column with different letters are significantly different ($p < 0.05$).
 Value are the means ± SD of duplicate experiments with triplicate determinations.

Irrespective of oil types, the DAG showed a significant increase ($p \leq 0.05$) as frying progressed (Table 3). Elevation in DAG is due to the hydrolytic reaction when moisture released from food interacts with oil during frying (Li *et al.*, 2017). In palm olein, DAG built-up coincides with frying cycles where short-chain fatty acids attached to TAG molecules are more susceptible to hydrolysis (Li *et al.*, 2017). It is a known fact that palm olein and red palm olein have naturally higher DAG content than other soft oils (Berger, 2005). For such circumstances, the measurement of TPC to solely distinguish the degree of oil deterioration seems unfair to palm oil products as the inherently higher DAG content could lead to misleading information on oil quality and stability (Ahmad Tarmizi *et al.*, 2019b). On that note, the focus should emphasise more on PTAG and OxTAG in judging the degree of oil deterioration.

The Pearson's correlation between polar compound fractions and acrylamide content upon extended frying is summarised in Table 4. A weak negative correlation ($r = -0.269$, $p \leq 0.05$) was observed between PTAG in oils and acrylamide content in the fried product. However, there was no correlation ($p > 0.05$) found between TPC, OxTAG, PTAG + OxTAG, and DAG on the presence of acrylamide in the fried product irrespective to frying media. Comparison of results with published literature was not possible due to the unavailability of such studies. Gertz *et al.* (2003) reported that TPC development enables to motivate heat conductivity of the oil over frying time. Enhancement of heat transfer would further allow more interaction between acrylamide precursor and hot oil, which in turn, increases the incidence of acrylamide formation. Our study, however, showed that the formation of acrylamide in fried products ceased with the PTAG built-up over frying time as evidenced by a weak negative correlation (Table 4). PTAG evolution would compete with glycerol molecules to form either acrylamide or skewed towards polymerisation to form PTAG.

TABLE 4. PEARSON'S CORRELATION BETWEEN ACRYLAMIDE CONCENTRATION AND PTAG, OTAG AND DAG OF VEGETABLE OILS, RESPECTIVELY

Parameters	Pearson's correlation	
	<i>r</i>	<i>p</i> -value
TPC	-0.152	0.181
PTAG	-0.269	$p \leq 0.05$
OxTAG	-0.023	0.838
PTAG + OxTAG	-0.209	0.065
DAG	0.192	0.091

This study showed that the alteration of FAC for all vegetable oils was significant across 80 frying cycles. The SFA gave a significant increase while PUFA exhibited a significant opposite trend

as a result of prolonged frying. A significant positive correlation was perceived among SFA, MUFA, and PUFA in vegetable oils and acrylamide content in the fried product. The results denoted that a higher degree of oil unsaturation could fasten lipid oxidation and thus, increase the manifestation of acrylamide formation. The amount of TPC developed in vegetable oils also portrayed a significant increase over frying cycles. Considerable elevation of PTAG, OxTAG, and DAG upon the increment of the frying cycle is due to the occurrence of oxidation aside from hydrolysis and polymerisation during frying. A significant negative correlation was observed between PTAG in vegetable oils and acrylamide content in French fries.

CONCLUSION

This study showed that the alteration of FAC for all vegetable oils was significant across 80 frying cycles. The SFA gave a significant increase while PUFA exhibited a significant opposite trend as a result of prolonged frying. A significant positive correlation was perceived among SFA, MUFA, and PUFA in vegetable oils and acrylamide content in the fried product. The results denoted that a higher degree of oil unsaturation could fasten lipid oxidation and thus, increase the manifestation of acrylamide formation. The amount of TPC developed in vegetable oils also portrayed a significant increase over frying cycles. Considerable elevation of PTAG, OxTAG, and DAG upon the increment of the frying cycle is due to the occurrence of oxidation aside from hydrolysis and polymerisation during frying. A significant negative correlation was observed between PTAG in vegetable oils and acrylamide content in French fries.

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