Effect of Fractional Crystallization on Fatty Acid and Triacylglycerol Compositions of Selected Native Lipids: An Overview

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Abstract- *Fractional crystallization has been recognized as a technique commonly used for modifying animal and plant lipids. When applied for semi-solid fats, fractional crystallization could yield a solid fraction called stearin and a liquid fraction known as olein. These derived lipid fractions were found to show remarkable differences from their parent lipids with regard to physico-chemical characteristics, fatty acids, and triacylglycerol compositions. Investigations on changes of chemical composition and the subsequent impact on physical characteristics are necessary for novel fat formulations in the oils and fats industry. In this overview, we tried to analyze the compositional changes caused by the fractional crystallization of avocado (Persea Americana) fat, engkabang (Shorea macrophylla) fat, lard, and mee fat (Madhuca longifolia). It is hoped that a critical discussion on this topic could provide some insight and future directions for the fractionation of several other unexploited native lipids.*

Keywords: Fractional crystallization, lipid stearin, lipid olein, tropical fats

I. INTRODUCTION

Fractional crystallization is a lipid modification process which is applied to several native plant and animal lipids to isolate novel fat products (Timms, 2005). The process of fractionation is generally divided into two major categories: dry fractionation and solvent fractionation. When compared to dry fractionation, solvent fractionation has been known to yield better recoveries of liquid olein and solid stearins (Marikkar and Ghazali, 2011). In the solvent crystallization approach, the lipid sample is generally mixed with a solvent medium and left at low temperature to crystallize. The precipitated fat at the bottom of the container will be filtered off to recover the high-melting fraction (HMF). After several rounds of recrystallization and filtration, the mother-liquor left in the process is evaporated to produce liquid called low-melting fraction (LMF) (Yanty, *et al.*, 2011a; Yanty, *et al.*, 2011b; Yanty, *et al.*, 2013; Marikkar, *et al.*, 2010). This process would normally cause tremendous changes in the composition and properties of the parent lipid. The triacylglycerol (TAG) molecules of the parent lipids will have different physico-chemical properties than those of the fat derivative obtained. The natural characteristics of the lipids would allow TAG molecules with lower melting point (MP) to remain in the liquid phase while those with higher MP tend to crystallize at certain temperature. After the phase separation become stabilized, it is possible to isolate the low-melting component known as olein (high in oleic acid) and high-melting component called stearin (high in stearic acid) (Yanty, *et al.*, 2011a; Yanty, *et al.*, 2011b; Yanty, *et al.*, 2013; Marikkar, *et al.*, 2010).

The information on lipid derivatives obtained from the fractionation of lipids could help in product development activities. For instance, palm stearin (used in shortening and margarine), and palm mid-fraction (used in cocoa butter substitutes) are examples of useful products produced generated from fractional crystallization of palm oil. Some confectionary fats and oils having high oxidative stability are also produced by fractional crystallization (Jin, *et al.*, 2018). Besides this, the fractional crystallization has helped to identify the changes in fatty acid and TAG compositions of the modified forms of lard such as lard stearin and lard olein (Marikkar and Yanty, 2014). This knowledge could help food control authorities to detect occurrence of the modified forms of lard in food systems (Yanty, *et al.*, 2011b). Fractionational crystallization would become the way forward in the future as it has the several advantages over both hydrogenation and interesterification. The foremost advantage is the fact that it is entirely a physical process with a low environmental impact (Timms, 2005). This overview intends to compare the varying nature of fractional crystallization behavior of four different native lipids, which has led to several novel fat products.

II. EXPERIMENTAL METHODOLOGY

A. *Sampling and fat extraction*

Mee fat was extracted from seeds of *Madhuca longifolia* collected from the North Central Province of Sri Lanka. Mee fat extraction from finely ground samples of dried *Madhuca longifolia* was carried out via the soxhlet extraction method using petroleum ether (at 40-60 °C) for 8 h (Marikkar, et al., 2010). Enkabang fat was extracted from seeds of *Shorea macrophylla* collected from Sarawak region of Malaysia. Enkabang fat extraction from finely ground samples of dried Engkabang seeds was carried out via the soxhlet extraction method using petroleum ether (at 40-60 °C) for 8 h (Nur Illyin, et al., 2013). Avocado fat was extracted from the flesh of mature avocado fruits collected from the Peninsular region of Malaysia. Fat extraction from finely ground samples of dried avocado fruits was carried out by soxhlet extraction method using petroleum ether (40–60°C) (Yanty, et al., 2011a). Lard was extracted from the adipose tissues of swine collected from the Peninsular region of Malaysia. After rendering the adipose tissues in an oven at 60°C for few hours, lard was squeezed out using a piece of muslin cloth as described previously by Marikkar et al (2001).

B. *Fractionation procedure*

The procedures for obtaining high melting and low-melting components from all the four fats were roughly similar, but with slight variations. Briefly, a portion of the melted sample of individual fat was mixed with acetone in 1:2 (w/v) ratio. The solution was boiled at 60°C until it became uniformly dissolved, and then it was cooled and left at 5±1°C for 2 hours to crystallize. The precipitated fat was filtered off to give a high melting fat fraction called stearin. After removing the precipitate, the mother-liquor was evaporated under reduced-pressure to yield a liquid fraction called olein (Yanty, et al., 2011a; Yanty, et al., 2011b; Yanty, et al., 2013; Marikkar, et al., 2010).

C. *Analytical work*

Cloud point (CP), slip melting point (SMP) and iodine value (IV) of the fat samples were determined according to AOCS method Cc.6.25, AOCS method Cc.3.25, and AOCS method Cd Id-92, respectively (AOCS, 1999). The TAG compositional analyses were carried out on a Waters Model 510 liquid chromatography equipped with a differential refractometer Model 410 as a detector (Waters Associates, Milford, MA) using Merck Lichrosphere RP-18 column (5 µm) (12.5 cm × 4 mm i.d.; Merck, Darmstadt, Germany). The mobile phase was a mixture of acetone: acetonitrile (63.5:36.5 v/v) and the flow rate was 1.5 mL/min (Marikkar and Ghazali, 2011; Marikkar, et al., 2013; Yanty, et al., 2013a; Yanty, et al., 2013b; Marikkar, et al., 2010). Fatty acid methyl esters analyses were performed on a gas chromatograph (Agillent Technologies, Singapore) fitted with a FID detector using the polar capillary column RTX-5 (0.32 mm internal diameter, 30 m length and 0.25 µm film thickness; Restex Corp., Bellefonte, PA) (Marikkar and Ghazali, 2011; Marikkar, et al., 2013; Yanty, et al., 2013a; Yanty, et al., 2013b; Marikkar, et al., 2010).

III. RESULTS AND DISCUSSION

A. Impact of fractionation on physico-chemical parameters

The physical properties of food lipids are of primary determining factors to fulfill the processing requirements of novel product formulations. The data presented in Table 1 shows the data related to cloud point (CP), slip melting point (SMP) and iodine value (IV) of the selected food lipids and their fractions (Yanty, et al., 2011a; Yanty, et al., 2011b; Yanty, et al., 2013; Marikkar, et al., 2010). These are, in fact, some of the important analytical parameters that would be related to the TAG composition and fatty acid distribution in them. The four native lipids selected for this study were found to display considerable differences with regard to these parameters (Table 1). Hence, their fractionated components were also expected to show remarkable differences. One of the thermal characteristics of olein fractions is described by CP since they exist in the liquid form at room temperature. By definition, CP is the temperature at which seeds of crystals are found to emerge leading to a cloudy appearance (AOCS, 1999). The CP value of mee fat olein (MFO) (10.5 °C) and lard olein (LO) (3.25 °C) were within the range found in most of the commercially available palm olein samples. The oleins are generally expected to display some resistance to clouding phenomenon, and therefore

would be advantageous to use them as cooking oil (Marikkar, et al., 2010).

The IV of lipids are generally used to determine the degree of unsaturation in fatty acids. Higher the IV, the more were C=C bonds present in the lipid systems (Yanty, et al., 2011b). Among the lipids selected for the discussion, avocado fat (AB) had the highest IV (84.3), followed by lard (LD) (73.8), mee fat (MF) (61.1) and engkabang fat (EF) (30.5). This indicated that a high amount of unsaturated fatty acids were found in avocado fat when compared to any other lipids. After fractionation, the IV of low-melting components (olein) of the lipids were found to increase remarkably, while the IV of high-melting components (stearin) were decreased with respect to the corresponding native lipid samples. This *happened* to be the direct consequence of the migration of more unsaturated fatty acids into the liquid olein phase and more saturated fatty acids into the solid stearin fraction.

The SMP is one of the basic physical characteristics that refer to conventional melting point of both food lipids and waxy solid (Marikkar et al., 2010). The SMP of AB was the lowest (30 °C) among plant-lipids such as EF (37.25 °C) and MF (35.5 °C), but comparably similar to LD (27.5 °C). The SMP value being below the physiological temperature would normally indicate its suitability for edible applications such as fat substitute in confectionery industry (Marikkar et al., 2010). After fractionation, the stearin isolated from the native lipids were found to possess higher SMP value than the parent lipid. Owing to higher SMP value and harder nature, the lipids stearins may be useful as raw materials for preparation of sticks, hard margarine, commercial shortening and other solid formulations (O_Brien, 1998).

B. Impact of fractionation on fatty acid composition

The fatty acid distributions of AB, EF, LD, MF and their fractions have been presented in Table 1. Among the native lipid samples, higher amounts of unsaturated fatty acids were found with AB, MF and LD. In contrast, EF was found to have more saturated fatty acids than unsaturated fatty acids. Upon fractionation, the lipid stearins were found to possess increased amounts of saturated fatty acids with respect to their corresponding native samples. As shown Table 1, the nature of fatty acid distributions in lipid oleins were the other way around when compared to lipid stearins (Yanty, et al., 2011a; Yanty, et al., 2011b; Yanty, et al., 2013; Marikkar, et al., 2010). The oleins in general experienced increases in unsaturated fatty acids. During crystallization, TAG molecules with more saturated fatty acids would undergo precipitation easily; as such considerable amount of saturated fatty acids would migrate into the solid phase, while leaving behind more unsaturated fatty acids in the liquid phase (Yanty, et al., 2011a; Yanty, et al., 2011b; Yanty, et al., 2013; Marikkar, et al., 2010). Owing to this situation, the thermal stability of lipids stearins and oleins would be changed in accordance with the changes in the degree of unsaturation .

In this study, oleic acid (44.65%) was the major fatty acid of AB, followed by palmitic acid (30.37%). The fatty acid profile of AB of this study has been in agreement with the foundlings published in several other reports (Azizi and Najafedh, 2008). Percentage increase of palmitic acid (59.63%) was remarkable in avocado stearin (AS) while fatty acid such as oleic (29.28 %) and linoleic (5.62%) were declined considerably (Table 1). In fact, the changes in fatty acid distribution tended to decrease the iodine value while causing an increase in slip melting point of AS. In avocado olein (AO), oleic is the major fatty acid (60.51%) which has increased with respect to sample AB (Table 1). While palmitic acid (26.2 %) got reduced indicating the migration of more palmitic acid into the solid phase, the liquid phase would become more enriched with oleic acid. This was very much similar to the fatty acid changes happened during the fractional crystallization of MF. For instance, the distributions and changes of fatty acids in mee fat stearin (MFS) and mee fat olein (MFO) are similar to those of AS and AO except the increases noticed in linolenic acid percentage of MFO (Table 1). The distribution of fatty acids in EF was remarkably different from those of AB and MF. This could be due to the fact that it was a fat hard and brittle in nature, which was extracted from a tree species of different subclass. Stearic acid was the most dominant in EF (47.83%) while oleic acid was the major fatty acid in both AB (43.65%) and MF (44.02%). This contrasting feature would have strong influence on its fractionation behavior. When fractionated under similar conditions described before, the yield recoveries of the solid and liquid components were not quite comparable to those of AB and MF. Upon fractionation, the differences in fatty acids were only slight as for instance in engkabang fat stearin

(EFS), palmitic acid (16.92%) and stearic acid (49.12%) increased only slightly. In enkabang fat olein (EFO), on the other hand, the increases in the percentage of *unsaturated* fatty acids caused oleic acid to become the major fatty acid (44.29 %) (Table 1).

Table 1: Basic physico-chemical characteristics and fatty acid compositions (%) of some native lipids and their olein and stearin fractions[1].

Sample	Cloud point (°C)	Iodine value (g I_2/ 100 g)	Slip melting point (°C)	Fatty acid (%)								
				C 14:0	C 16:0	C 16:1	C 18:0	C 18:1	C 18:2	C 20:0	SFA	USFA
AB	-	84.30 ± 0.14	30.00 ± 0.71	-	30.37 ± 0.06	5.20 ± 0.02	1.30 ± 0.01	43.65 ± 0.04	17.45 ± 0.04	-	31.67 ± 0.07	68.33 ± 0.07
AS	-	42.80 ± 0.20	42.50 ± 0.71	-	59.63 ± 0.05	2.26 ± 0.01	2.88 ± 0.00	29.08 ± 0.04	5.62 ± 0.00	-	62.51 ± 0.05	37.50 ± 0.05
AO	-	103.70 ± 0.15	-	-	26.20 ± 0.04	6.08 ± 0.03	1.10 ± 0.02	60.51 ± 0.06	5.90 ± 0.03	-	27.30 ± 0.06	72.70 ± 0.06
EF	-	30.50 ± 0.71	37.25 ± 0.45	-	16.58 ± 0.35	-	47.83 ± 0.13	32.49 ± 0.06	1.00 ± 0.02	2.10 ± 0.01	66.51	33.49
EFS	-	28.10 ± 0.57	38.50 ± 0.35	-	16.92 ± 0.22	-	49.12 ± 0.04	30.92 ± 0.04	1.00 ± 0.01	2.04 ± 0.01	68.08	31.92
EFO	-	44.75 ± 0.64	25.50 ± 0.71	-	21.48 ± 0.13	-	24.87 ± 0.01	44.29 ± 0.03	8.26 ± 0.01	1.10 ± 0.00	47.45	52.55
LD	-	73.80 ± 0.34	27.50 ± 0.71	1.24 ± 0.01	22.68 ± 0.48	1.42 ± 0.05	12.70 ± 0.28	38.24 ± 0.13	20.39 ± 0.04	-	-	-
LS	-	45.98 ± 0.02	45.75 ± 0.35	1.23 ± 0.15	31.68 ± 0.81	0.72 ± 0.05	25.15 ± 0.11	24.97 ± 1.00	14.04 ± 0.06	-	-	-
LO	3.25 ± 0.35	103.00 ± 0.06	-	1.46 ± 0.15	21.76 ± 0.01	2.30 ± 0.01	6.38 ± 0.03	42.76 ± 0.18	23.62 ± 0.03	-	-	-
MF	-	61.10 ± 0.35	35.5 ± 0.50	-	20.88 ± 1.51	-	22.05 ± 0.90	44.02 ± 1.10	7.85 ± 0.77	-	43.40 ± 2.15	51.85 ± 2.55
MFS	-	47.05 ± 0.05	46.5 ± 0.70	-	25.28 ± 1.33	-	29.01 ± 0.84	38.38 ± 1.60	5.72 ± 0.56	-	54.28 ± 2.46	44.10 ± 2.86
MFO	10.50 ± 0.50	64.40 ± 0.45	-	-	19.28 ± 1.20	-	16.20 ± 0.67	53.12 ± 0.95	9.61 ± 0.88	-	35.48 ± 2.60	62.73 ± 2.58

Each value in the table represents the mean of two replicates. Abbreviations: AB, avocado butter; AS, avocado butter stearin; AO, avocado butter olein; EF, engkabang fat; EFS, engkabang fat stearin; EFO, engkabang fat olein; L, lard; LS, lard stearin; LO, lard olein; MF, mee fat; MFS, mee fat stearin; MFO, mee fat olein; SFA, saturated fatty acid; USFA, unsaturated fatty acid

This clearly *demonstrated* the fractional crystallization behavior of EF was somewhat different from those of AB and MF.

LD being an animal fat did not display a contrast to the fractional behaviors of three plant fats discussed earlier. Under similar fractionation conditions described before, the *yield* recoveries of the solid and liquid components were quite comparable to those of AB and MF. The fatty acid profile of LD used in this study was roughly similar to those reported by other researchers previously (Marikkar, et al., 2021). In LD, oleic acid (38.24%) was the dominant fatty acid, followed by palmitic acid (22.68%) and linoleic acid (20.39%). However, palmitic acid became the most dominant fatty acid in lard stearin (LS) (76.57%) followed by stearic acid (25.15 %). Meanwhile, in LO, there was increased in the proportions of the unsaturated fatty acids such as oleic (42.76 %) and linoleic (23.62%).

C. Impact of fractionation on triacylglycerol composition

A comparison of the TAG distribution profiles of AB, EF, LD, MF and their fractions is shown in Table 2. When these lipids were subjected to fractional crystallization, the majority of the tri-saturated and di-saturated TAG molecules would migrate into their respective solid phases, leaving behind most of the mono-saturated and tri-unsaturated TAG molecules in the solvent phase. Nevertheless, the way of migration of different TAG species present in the individual fat might differ drastically. Hence, the solid and liquid fractions would display drastic differences in the TAG compositions when compared to the parent native lipids. In the original form of AB, the most dominant TAG molecule was POO (22.76 %), followed by POL (19.29 %) and PPO (12.43 %). After fractionation, in the solid fraction AS, the amounts of TAG molecules such as tri-palmitin (PPP) (38.31%) and oleo-dipalmitin (PPO) (30.08%) were increased with concurrent reductions in linoloyle-diolene (OOL) (1.1%), linoloyle- palmitoyle-olene (POL) (1.64%), and tri-olene (OOO) (5.28) (Table 2). This resulted in the increase of SMP with a decrease in iodine value of AVS. On the other hand, liquid fraction AO contained POO, OOO, OOL, and POL as major TAG molecules. The percentage increases of TAG moleules such as OOO (22.40%) and OOL (16.99%) in AO were relatively higher than the same TAG molecules found in either AB or AS (Table 2).

MF is pale yellow in color and remains as a semi-solid under the tropical temperature conditions. As shown in Table 2, the TAG profile of MF is compatible to that of crude palm oil. It can be reduced that the combination of TAG molecules formed by palmitic, oleic and steric acids could be responsible for the semi-solid nature of MF. According to the data presented in Table 2, OOP was the most dominant TAG molecule of MF followed by POS and OOS. Other TAG molecules such as POP, POS, and SOS were also present in MF in considerable amounts (Table 2). Upon fractionation, in the liquid fraction mee olein (MFO), the TAG molecular species such as OOO, OOP and OOS experienced increments while TAG species such as POS and SOS undergone decreases. In the solid fraction mee stearin (MFS), TAG molecular species such as POP, POS, and SOS experienced increments while there were considerable decreases in the proportion of OOS, OOP, OOO, and PLO.

Among the food lipids selected in this overview, EF is considered to be the hardest in its physical nature. Although the composition and thermo-physical properties of EF have been extensively discussed in a previous report (Nur Illiyin et al., 2013), its fractional crystallization behavior was not received adequate attention. In the composition (96.8 %) of EF, the disaturated TAG molecular species becoming predominant was a noteworthy feature. Upon fractionation, this type of TAG molecules increased slightly in the stearin fraction (EFS) (97.12 %) with respect to the native sample. Meanwhile in the olein fraction (EFO), diunsaturated TAG molecules (49.38 %) increased by replacing the disaturated TAG molecules. This might be due to the significant increases in the proportion of di-oleo stearin (OOSt) in the composition (29.66 %) of EFO as opposed to EF (1.38 %).

LD was the only animal fat selected for the discussion in this overview. According to the data presented in Table 2, LPO, OPO, PPO and SPO were the major TAG molecular species comprising 61.5% of the total. As shown in Table 2, these four were also the predominant TAG molecular species of LS, but the amount of PPO and SPO have increased remarkably with concurrent reductions in the amounts of LPO and OPO. Some remarkable differences were also seen in the distribution of OOS, SPO, and PPS in LS. With respect to native LD, the TAG composition of LO was found to deviate considerably as it was

Table 2: Triacylglycerol (TAG) compositions (%) of some native lipids and their olein and stearin fractions

TAG	AB	AS	AO	EF	EFS	EFO	LD	LS	LO	MF	MFS	MFO
LLLn	1.87±0.00	2.67±0.13		-	-	-	1.54±0.21	0.22±0.00	2.29±0.01	ND	ND	ND
LLL	0.85±0.07	0.47±0.03	1.17±0.08	-	-	-	0.68±0.21	0.23±0.00	1.43±0.01	ND	ND	ND
OLL	3.23±0.02	0.47±0.03	6.00±0.15	-	-	-	4.68±0.08	2.11±0.01	6.01±0.01	ND	ND	ND
OOA	-	-	-	-	-	1.70 ±0.08	-	-	-	-	-	-
OOL	9.00±0.03	1.10±0.00	16.99±0.24	-	-	0.44±0.01	6.93±0.04	3.40±0.02	8.48±0.01	3.00±0.26	1.45±0.00	2.60±0.10
OOO	11.42±0.01	5.28±0.01	22.40±0.43	0.12±0.01	0.36±0.01	3.27±0.01	4.33±0.21	2.46±0.04	5.61±0.07	9.85±0.22	3.47±0.04	12.56±0.05
OOS	0.52±0.01	0.64±0.11	1.14±0.13	-	-	-	-	-	-	-	-	-
OOSt	-	-	-	1.38±0.01	1.28±0.01	29.66±0.05	3.62±0.04	1.79±0.01	4.30±0.02	17.80±00	7.42±0.02	24.05±0.07
OPO	22.76±0.02	12.29±0.08	8.83±0.17	0.68±0.01	0.41±0.01	15.99±0.01	20.67±0.11	9.48±0.03	26.11±0.01	22.92±0.88	9.00±0.01	28.65±0.06
Others	6.83±0.04	2.25±0.88	21.01±1.66	-	-	-	-	-	-	-	-	-
PLL	5.63±0.11	0.18±0.04	5.63±0.11	-	-	0.29±0.12	7.05±0.06	3.26±0.01	9.33±0.04	ND	ND	ND
POL	13.16±0.20	1.64±0.01	13.16±0.20	-	-	1.74±0.22	20.00±0.30	9.32±0.00	24.52±0.11	4.26±0.37	1.77±0.00	2.59±0.15
POSt	-	-	-	35.58±0.07	38.20±0.08	12.28±0.03	-	-	-	-	-	-
PPL	4.03±0.06	1.65±0.05	0.17±0.01	0.15±0.00	0.14±0.01	3.12±0.12	2.62±0.04	3.96±0.01	2.63±0.03	1.19±0.04	0.35±0.03	Tr.
PPO	12.43±0.00	30.08±0.38	0.22±0.01	7.27±0.04	7.33±0.03	16.53±0.04	10.63±0.01	22.87±0.03	3.05±0.05	11.92±0.66	13.95±0.06	12.07±0.02
PPP	2.88±0.06	38.31±0.56	0.61±0.01	-	-	-	ND	ND	ND	Tr.	0.20±0.10	Tr.
PPS	0.11±0.01	3.97±0.07	-	-	-	-	-	-	-	-	-	-
PPSt	-	-	-	0.28±0.01	0.23±0.02	0.98±0.04	0.81±0.00	2.53±0.04	ND	Tr.	1.76±0.04	Tr.
SPO	0.57±0.02b	1.71±0.02	-	-	-	-	-	-	-	-	-	-
StOA	-	-	-	3.82±0.12	3.69±0.11	-	-	-	-	-	-	-
St PO	-	-	-	-	-	-	12.52±0.12	30.19±0.01	2.16±0.00	19.34±0.44	35.09±0.04	14.05±0.00
StStO	-	-	-	49.98±0.13	47.76±0.05	2.65±0.01	0.83±0.01	2.29±0.01	ND	9.74±0.58	23.78±0.04	3.45±0.20

StStSt	-	-	-	0.33±0.02	0.33±0.02	-	1.31±0.01	4.14±0.01	ND	ND	ND	ND
Unknown	-	-	-	0.41	0.24	11.35	1.84±0.09	1.78±0.02	4.12±0.35	-	1.86±0.1	-
SSS	-	-	-	0.59	0.61	0.98	2.12	6.67	-	-	1.96	-
USS	-	-	-	96.8	97.12	34.58	26.6	59.31	7.84	42.19	73.17	30.20
UUS	-	-	-	2.06	1.69	49.38	51.34	23.85	64.26	44.98	18.19	55.29
UUU	-	-	-	0.12	0.36	3.71	18.16	8.42	23.82	12.85	4.92	15.16

Each value in the table represents the mean ± standard deviation of two replicates. Abbreviations: O, oleic; P, palmitic; L, linoleic; Ln, linolenic; St, stearic; Tr., trace; ND, not detected; UUU, triunsaturated; UUS, diunsaturated; USS, disaturated; SSS, triunsaturated. For other abbreviatios see Table

found to possess OOL, OPO, LPO, and PLL as dominant TAG molecules, but almost negligible amounts of the TAG molecules di-palmito-stearin (PPS), oleo-distearin (SSO), and tri-stearin (SSS).

V. CONCLUSION

This overview shows that fractional crystallization behavior of individual native lipid is tightly-dependent on the nature of distribution of their TAG composition. Because of this reason, EF was found to exhibit a completely different fractionation behavior when compared to the other three native lipids. During crystallization, TAG molecular species with low-degree of unsaturation will go into the solid phase leaving behind the TAG molecules with high-degree of unsaturation. Hence, determining the composition of food lipids subjected for fractional crystallization is important to have a clear picture of fractionation behavior. Owing to the migration of different TAG molecules between the solid and liquid phases, the degree of unsaturation or unsaturated to saturated fatty acid ratio were greatly affected. Therefore, it shows that there were distinguishable differences among the four native lipids and their solid and liquid fractions based on basic physico-chemical characteristics, fatty acid composition and tri-acylglyceride profile. As a result, in all four lipids, stearin component obtained were found to display high melting point and low iodine value while olein components displayed low melting point and high iodine value. This may have important implications on the oxidative stability, nutritional values, and product applications of the derived fractions.

REFERENCES

AOCS. 1999. Official methods and recommended practices of the American Oil Chemists' Society. 5th ed. Champaign, IL: American Oil Chemists' Society.

Azizi S.N., Najafzadeh S. (2008) Fatty acids and volatile compounds in avocado cultivated in North of Iran. World Appl Sci J 5: 1–4.

Jun Jin, Liang Jie, Liyou Zheng, Min Cheng, Dan Xie, Qingzhe Jin, Xingguo Wang (2018) Characteristics of palm mid-fractions produced from different fractionation paths and their potential usages. *International Journal of Food Properties* 21:1, 58-69.

Marikkar, J. M. N., Yanty, N. A. M. (2014). Effect of chemical and enzymatic modifications on the identity characteristics of lard: A review. *International Journal of Food Properties*, *17*(2), 321–330.

Marikkar, J. M. N., Yanty, N. A. M. (2012). Seed fat from *Madhuca longifolia* as raw material for halal alternative fats. *Borneo Science* 31, 84–94.

Marikkar JMN, Lai OM, Ghazali HM, Che Man YB (2001) Detection of lard and randomized lard as adulterants in refined-bleached-deodorized palm oil by differential scanning calorimetry. *J Am Oil Chem Soc* 78: 1113–1119.

Marikkar, J. M. N., Ghazali, H. M. (2011). Effect of *Moringa oleifera* oil blending on fractional crystallization behavior of palm oil. *International Journal of Food Properties*, *14*(5), 1049–1059.

Marikkar, J.M.N., Ghazali, H.M., Long, K. (2010). Composition and thermal characteristics of *Madhuca longifolia* seed fat and its solid and liquid fractions. *Journal of Oleo Science*, *59*(1), 7–14.

Nur Illiyin, M.R., Marikkar, J.M.N., Shuhaimi, M., Mahiran, B., Miskandar, M.S. (2013). A comparison of the thermo physical behaviour of engkabang (*Shorea macrophylla*) seed fat - canola oil blends and lard. *J. Am. Oil Chem. Soc.* 90, 1485–1493.

O_Brien, R. D. (1998). Baking shortening. In: Fats and oil: formulating and processing for applications (pp. 343–382). USA: Technomic Publishing Co. Inc.

Timms, R. E. (2005). Fractional crystallization - the fat modification process for the 21st century. *European Journal of Lipid Science and Technology* 107:48-57.

Yanty, N. A. M., Marikkar, J. M. N., Che Man, Y. B. (2011a). Effect of fractional crystallization on composition and thermal characteristics of avocado (*Persea americana*) butter. *Journal of Thermal Analysis and Calorimetry*, *111*(3), 2203–2209.

Yanty, N. A. M., Marikkar, J. M. N., Shuhaimi, M. (2013). Effect of fractional crystallization on the composition and thermal properties of engkabang (*Shorea macrophylla*) seed fat and cocoa butter. *Grasas Y Aceites* 64: 546–553.

Yanty, N. A. M., Marikkar, J. M. N., Man, Y. B. C., & Long, K. (2011b). Composition and thermal analysis of lard stearin and lard olein. *Journal of Oleo Science*, *60*(7), 333–338.