

Physicochemical Properties and Thermal Behavior of Binary Blends of *Madhuca longifolia* Seed Fat and Palm Oil as a Lard Substitute

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Abstract—Fat extracted from pork is prohibited under halal and kosher food regulations. A study was carried out on *Madhuca longifolia* seed fat and palm oil to compare their physicochemical, solidification and melting characteristics to formulate halal alternative lipid substitutes. Various blends of *Madhuca longifolia* (ML) and palm oil (PO) was formulated in order to become similar to lard (LD). A total of three binary blends were prepared: ML:PO (97:3; w/w), ML:PO (95:5), ML:PO (93:7), and identified by the mass ratio of ML to PO. The fat blends were compared with LD in terms of the fatty acid and triacylglycerol compositions using gas chromatography and high performance liquid chromatography, respectively. In addition, the fat blends also being studied for thermal properties using differential scanning calorimetry and solid fat content using *p*-nuclear magnetic resonance. Although there were considerable differences between LD and the fat blends with regard to fatty acid and triacylglycerol compositions, some similarities were seen regarding to thermal properties and solid fat content profiles. The blend of ML:PO (97:3) displayed closer similarity to LD with respect to melting transition at -3.59 °C and its solid fat content profile showed the least difference to that of LD throughout the temperature range measured.

Index Terms—*Madhuca longifolia*, palm oil, fatty acid, triacylglycerol, thermal behavior, solid fat content

I. INTRODUCTION

Lard (LD) or pork fat is usually used as a shortening in food products such as cookies, cakes, pastries and bread. It showed that LD shortening has a good plasticity and palatability to the bakery products [1]. However, any food products containing LD are prohibited to scholars for both religions of Islamic and Orthodox [2], [3]. The consumption of food products rich in LD are known to associate with certain health risk such as coronary heart disease and hypercholesterolemia [1]. Therefore, formulating alternative halal fats are required for

Muslim and Jew scholars. The fruit seed of *Madhuca longifolia* could be one of the alternative halal fat and it is still among under-utilized material for oil production. To date, there is little information on the composition and utilization of *Madhuca longifolia* seed fat (ML). In India, the seed fat of *Madhuca longifolia* is often used as a cocoa butter substitute [4], [5].

There are still very few studies on alternative halal fats as a lard substitute [6]-[8]. Most studies even focuses only on physical properties of the fats rather than their chemical properties. One of the most important physical properties is solid fat content (SFC) at which its profile can be used to evaluate the special applications of fats [9], [10]. Therefore, SFC profile is very important criterion in mimicking or obtaining a specific fat-based product, in this case as a lard substitute. Chemical properties, on the other hand, are also an important quality attribute that should be taken into account during formulation especially in terms of nutritional value of the fat-based products.

The previous study showed that ML could be a replacement for LD after blending it with palm oil fraction [palm stearin (PS)] at different ratios [7]. It was demonstrated that addition of 1% of PS to ML gave the best blend as a LD substitute in terms of SFC profile and melting property as shown in the DSC thermogram. Afterwards, shortenings made of ML:PS binary blends was compared to LD shortening in term of microstructures, polymorphic forms and textural properties [11]. They found that ML:PS (99:1) shortening did not show significant ($p > 0.05$) difference compared to that of LD shortening in terms of consistency, hardness, adhesiveness and compression values. In addition, both LD and ML:PS (99:1) shortenings contained a mixture of β' and β polymorphs of which the β' form was the most predominant polymorph.

Malaysia is currently the world's largest producer and exporter of palm oil. Palm Oil (PO) has been used in many food products such as cooking oil, shortening,

margarine and others [12]. However, there is no report on utilization of PO for LD substitute. Based on Solid Fat Content (SFC) profile, both LD and PO have similar solidification behavior within the 25-40 °C temperature range, whereas ML is found to be compatible to that of LD, within a 0-25 °C temperature range [13]. It is assumed that the addition of PO into ML could give a better compatibility of solidification behavior to LD. Hence, this study is to investigate the potential of adding PO into ML to produce LD substitute.

II. MATERIALS AND METHODS

A. Materials

Dried fruit seeds of *Madhuca longifolia* were collected from different locations in the North Central Province of Sri Lanka. A sample of palm oil was obtained as a generous gift from the Malaysian Palm Oil Board (MPOB). All chemicals used in this experiment were of analytical or HPLC grade.

B. Oil Extraction

Oil extraction from finely ground samples of dried *Madhuca longifolia* seeds was carried out using the soxhlet extraction method with petroleum ether (40-60 °C) [14]. Extracted oil was kept in an oven at 60 °C for 1 h to expel the solvent and immediately transferred into a blue-capped bottle before storing at -20 °C. Before analysis, oil sample was taken out from freezer, left to stand at room temperature for 1 h, and then warmed at 60 °C until completely molten.

C. Determination of Fatty Acid Composition

Fatty Acid Methyl Ester (FAME) was prepared by dissolving a 50 mg portion of oil in 0.8 mL of hexane and adding a 0.2 mL portion of a 1 M solution of sodium methoxide [15]. The analysis was done using a gas chromatograph (Agilent Technologies, Singapore) fitted with an FID detector. The polar capillary column RTX-5 (0.32 mm internal diameter, 30 m length and 0.25 mm film thickness; Restex Corp., Bellefonte, PA) was used. The oven temperature was programmed as follows: initial temperature of 50 °C (for 1 min), programmed to increase to 200 °C at 8 °C per min⁻¹. Both injector and detector temperatures were maintained at 200 °C throughout the analysis. The split ratio was 58:1 and the carrier gas (helium) flow rate was 1.0 mL min⁻¹. Individual peak of FAMEs was identified by comparing its retention time with a reference mixture of FAME standards (Supelco, Bellefonte, PA).

D. Determination of the Triacylglycerol (TAG) Composition

The TAG composition was determined using a Waters Model 510 liquid chromatography equipped with a differential refractometer Model 410 as the detector (Waters Associates, Milford, MA). The analysis of TAG was performed on a Merck Lichrosphere RP-18 column (5 µm) (12.5 cm x 4 mm internal diameter; Merck, Darmstadt, Germany). The mobile phase was a mixture of acetone: acetonitrile (63.5:36.5) and the flow rate was

1.5 mL min⁻¹. The oven temperature was maintained at 30 °C. The injector volume was 10 µL of 5% (w/w) oil in chloroform. Individual peak of TAGs was identified by comparing its retention time with a set of TAG standards purchased from Sigma-Aldrich (Deisehofen, Germany) as well as TAG profiles of lard, *Madhuca longifolia* fat and palm oil [13].

E. Determination of Thermal Behavior

Thermal analysis was carried out on a Mettler Toledo differential scanning calorimeter (DSC 823 Model) equipped with a thermal analysis data station (STARe software, Version 9.0x, Schwerzenbach, Switzerland). Nitrogen (99.99% purity) was used as the purge gas at a rate of ~20 mL min⁻¹. Approximately 4-8 mg of molten sample were placed in a standard DSC aluminum pan and then hermetically sealed. An empty, hermetically sealed, DSC aluminum pan was used as the reference. The sample was preheated at 60 °C to destroy any thermal history of the oil, then cooled at 5 °C min⁻¹ to -60 °C, and held for 1 min. The sample was again heated from -60 °C to 60 °C at the same rate [16].

F. Determination of Solid Fat Content

The determination of SFC was carried out using a Bruker Minispec (Model Mq 20) pulse Nuclear Magnetic Resonance (pNMR) spectrometer (Karlsruhe, Germany) [17]. The sample in the NMR tube was melted at 90 °C for 15 min, followed by chilling at 0 °C for 60 min, and then held at each measuring temperature (0, 5, 10, 15, 20, 25, 30, 35, 40, 45 °C) for 30 min prior to measurement. Melting, chilling and holding of the samples were done in pre-equilibrated thermostatted glycol containing baths with 0.1 °C precision.

G. Statistical Analysis

The results were expressed as mean value ± standard deviation of three replicates. Data were statistically analyzed by one-way analysis of variance (ANOVA), using Tukey's Test of MINITAB (version 15) statistical package at 0.05 probability level.

III. RESULTS AND DISCUSSION

A. Fatty Acid Composition

Fatty acids are essential components of edible fats and oils in which they can be found in ester form with a glycerol backbone as a triacylglycerol. Fatty acid (FA) composition is important for its nutritional value content. They can be divided into two categories namely saturated and unsaturated fatty acids. Fatty acid composition of ML:PO blends and LD is compared in Table I.

The major FA of LD were oleic (38.24%), palmitic (22.68%), linoleic (20.39%) and stearic (12.70%) acids. Lard is generally found to have more unsaturated fatty acids (USFA) than saturated fatty acids (SFA) [1], [18]. The major FA of ML were oleic (47.58%), palmitic (22.38%), stearic (21.53%) and linoleic (8.51%) acids [7]. Meanwhile, the major FA of PO were palmitic (43.99%), oleic (39.24%) and linoleic (10.25%) acids [13]. As shown in Table I, the overall predominant FA of ML:PO

blends were oleic (ranging from 47.01 to 47.33%), followed by palmitic (ranging from 23.03 to 23.79%) and stearic (ranging from 20.33 to 21.03%) acids, but very little linoleic acid (ranging from 8.56 to 8.63%). The increment amount of PO into ML caused slight increment in the proportion of palmitic (from 23.03 to 23.79%), stearic (from 21.03 to 20.33%), linoleic (from 8.56 to 8.63%) margaric (from 0.03 to 0.08%) and arachidic (from 0.01 to 0.03%) acids with concurrent decreases in the amounts of stearic (from 21.03 to 20.33%) and oleic (from 47.33 to 47.01%) acids.

TABLE I. FATTY ACID COMPOSITION (%) OF ML:PO BLENDS AND LD

	ML:PO (97:3)	ML:PO (95:5)	ML:PO (93:7)	LD
FA				
C12:0	0.01±0.01 ^b	0.02±0.01 ^b	0.02±0.01 ^b	0.09±0.01 ^a
C14:0	0.03±0.01 ^c	0.06±0.01 ^b	0.08±0.02 ^{b,c}	1.24±0.01 ^a
C16:0	23.03±0.07 ^c	23.46±0.01 ^b	23.79±0.03 ^a	22.68±0.48 ^d
C16:1	trace	0.01±0.01 ^b	0.01±0.01 ^b	1.42±0.05 ^a
C18:0	21.03±0.04 ^a	20.66±0.02 ^b	20.33±0.01 ^c	12.70±0.28 ^d
C18:1	47.33±0.01 ^{a,b}	47.16±0.07 ^{b,c}	47.01±0.01 ^c	38.24±0.13 ^d
C18:2	8.56±0.05 ^c	8.60±0.10 ^{b,c}	8.63±0.09 ^b	20.39±0.04 ^a
C18:3	trace	0.01±0.01 ^b	0.01±0.02 ^b	0.98±0.01 ^a
C20:0	0.01±0.00 ^d	0.02±0.01 ^c	0.03±0.01 ^b	0.67±0.01 ^a
Others	n.d	n.d	n.d	1.59
USFA	55.89	55.78	55.66	61.03
SFA	44.11	44.22	44.34	38.97

Each value in the table represents the mean of two determinations. Means within each row bearing different superscripts are significantly ($p<0.05$) different.

Abbreviations: FA, fatty acid; ML, *Madhuca longifolia* seed fat; PO, palm oil; LD, lard; n.d, not detected; USFA, unsaturated fatty acid; SFA, saturated fatty acid

When compared to LD, the formulated blends had tremendous proportions of oleic and stearic acids. Oleic acid is known as monounsaturated oil, which plays important role in many health benefits [19], [20]. Even though stearic acid is a saturated FA, it has been shown to have a neutral effect on blood total and low density lipoprotein (LDL) cholesterol levels [21]-[25]. Because of its FA composition, the USFA of ML:PO blends was found to be lower (ranging from 55.66 to 55.89%) than that of LD (61.03%). Therefore, the formulated blends may have more nutritional characteristics such as less saturated fat and no cholesterol.

Even so, based on a quantitative basis, none of FA in ML:PO blends was found to become compatible to those of LD. It is suggested to add soybean, canola or sunflower oils in order to increase the amount of linoleic acid in the formulated blends. Furthermore, the fatty acid distribution in ML:PO blends could also be improved by enzymatic interesterification using 1,3 specific lipases. It is not recommended to use non-specific lipases because these enzymes do not usually bring significant changes in the fatty acid distribution of interesterified fat blends [26], [27].

B. Triacylglycerol Composition

The TAG compositions of ML:PO blends are compared to that of LD as shown in Table II. The predominant triacylglycerol (TAG) of LD were POO (20.67%), followed by POL (20%), SPO (12.52%) and

PPO (10.63%). The major TAG of ML were POO (25.64%), SPO (14.86%), SOO (12.05%), OOO (11.06%) and PPO (10.39%) [7]. PO was composed of PPO (31.68%), POO (24.76%) and PPL (10.19%) as dominant TAG molecules [13].

TABLE II. TRIACYLGLYCEROL COMPOSITION (%) OF ML:PO BLENDS AND LD

TAG	ML:PO (97:3)	ML:PO (95:5)	ML:PO (93:7)	LD
LLL _n	n.d	n.d	n.d	1.54±0.21 ^a
LLL	n.d	n.d	n.d	0.68±0.21 ^a
OLL	0.85±0.01 ^b	0.83±0.00 ^c	0.80±0.01 ^d	4.68±0.08 ^a
MMM	0.01±0.01 ^a	0.01±0.00 ^a	0.02±0.01 ^a	n.d
PLL	0.87±0.04 ^c	0.89±0.03 ^{b,c}	0.92±0.01 ^b	7.05±0.06 ^a
MPL	0.02±0.01 ^c	0.03±0.00 ^b	0.04±0.00 ^a	n.d
OOL	4.89±0.00 ^b	4.83±0.01 ^c	4.75±0.01 ^d	6.93±0.04 ^a
POL	6.81±0.02 ^d	6.88±0.01 ^{b,c}	6.95±0.11 ^b	20.00±0.27 ^a
PPL	2.32±0.01 ^d	2.49±0.01 ^c	2.65±0.01 ^a	2.62±0.01 ^b
OOO	10.85±0.00 ^a	10.71±0.01 ^b	10.57±0.01 ^c	4.33±0.21 ^d
POO	25.62±0.04 ^a	25.60±0.07 ^{a,b}	25.58±0.04 ^b	20.67±0.11 ^c
PPO	11.01±0.20 ^c	11.46±0.05 ^b	11.88±0.02 ^a	10.63±0.01 ^d
PPP	0.46±0.04 ^c	0.55±0.02 ^b	0.64±0.01 ^a	0.38±0.00 ^d
SOO	11.77±0.01 ^a	11.59±0.01 ^b	11.40±0.01 ^c	3.62±0.04 ^d
SPO	14.58±0.02 ^a	14.41±0.01 ^b	14.21±0.01 ^c	12.52±0.12 ^d
PPS	0.35±0.01 ^c	0.36±0.00 ^c	0.37±0.00 ^b	0.81±0.00 ^a
SOS	6.19±0.01 ^a	6.07±0.01 ^b	5.95±0.00 ^c	0.83±0.01 ^d
SSS	0.22±0.00 ^b	0.22±0.00 ^b	0.21±0.00 ^c	1.31±0.01 ^a
Others	3.15	3.10	3.06	1.41
UUU	16.59	16.37	16.12	18.16
UUS _t	45.07	44.96	44.85	51.34
US _t St	34.12	34.46	34.73	26.60
StStSt	1.04	1.14	1.24	2.50

Each value in the table represents the mean of two determinations. Means within each row bearing different superscripts are significantly ($p<0.05$) different.

Abbreviations: TAG, triacylglycerol; ML, *Madhuca longifolia* seed fat; PO, palm oil; LD, lard; L, linoleic, Ln, linolenic; O, oleic, M, margaric; P, palmitic; S, stearic; U, unsaturated; St, saturated; n.d, not detected

Table II in general indicated that the overall major TAG of ML:PO blends were POO (ranging from 25.58 to 25.62%), SPO (ranging from 14.21 to 14.58%), SOO (ranging from 11.4 to 11.77%) and PPO (ranging from 11.01 to 11.88%). After addition of PO into ML, the disaturated (US_tSt) (ranging from 34.12 to 34.73%) and trisaturated (StStSt) (ranging from 1.04 to 1.24%) were found to increase steadily with concurrent decreases in diunsaturated (UUSt) (ranging from 44.85 to 45.07%) and triunsaturated (UUU) (ranging from 16.12 to 16.59%). When compared to LD, the formulated blends were known to be composed higher amount of US_tSt but lower amount of UUSt, UUU and StStSt. The increments of US_tSt TAG molecules in the ML:PO blends could be due to the presence of higher proportions of PPO (31.61%), POO (24.76%) and PPL (10.19%) in PO [13]. The amount of saturated TAG molecules is important in the shortening production as it can maintain the stability of products [28] without hydrogenation that may lead to the production of *trans* fats.

The addition of PO in ML generally increased the amount of other palmitic based TAG molecules in the formulated blends. On the contrary, the addition of PS in ML was found to decrease the amount of palmitic based TAG molecules such as POL and POO [7] with concurrent increase the amount of other TAG molecules. Based on TAG composition, none of TAG in ML:PO

blends was found to become compatible to those of LD. As mentioned earlier, the changes of TAG distribution could be done through enzymatic interesterification. It was found that there were increases of UUS_t and US_t in engkabang fat: canola oil (EF:CaO) blends after reacted with non-specific *Candida antarctica* lipase [26].

C. Thermal Behavior

1) Cooling thermogram

The crystallization behaviors of ML:PO blends and LD are shown in Fig. 1. Lard had cooling transitions at two widely different temperature regions: two major transitions at low temperature region [-18.7 °C (a3)] and high temperature region [10.3 °C (a2) with a shoulder peak at 16.8 °C (a1)]. The onset of crystallization (T_{onset}) of LD was 18.25 °C. As a comparison, mee fat (*Madhuca longifolia* seed fat) consisted of sharp peak at 26 °C, a minor broad peak at -4.5 °C and a minor sharp peak at -24.3 °C [7].

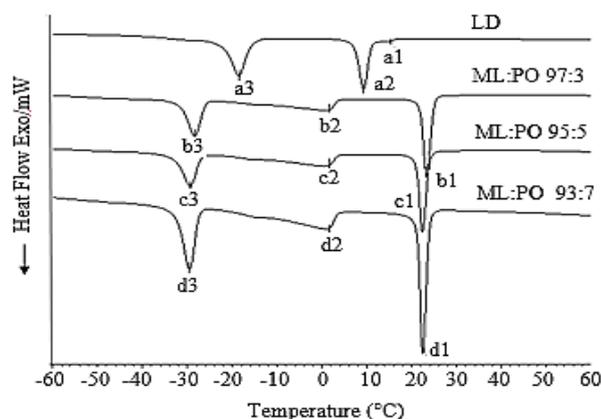


Figure 1. Cooling thermogram of ML:PO blends and LD

Fig. 1 also indicated that the ML:PO blends had three transitions; a major sharp peak at around 22 to 24 °C (b1, c1 and d1), a broad minor peak at around 0.5 to 1 °C (b2, c2 and d2) and a minor sharp peak at around -28 to -30 °C (b3, c3 and d3). When compared to original ML, the peaks of formulated blends were shifted to lower temperature region. This could be due to high melting TAG molecules in the formulated blends tend to crystallize at the higher temperature region [29]. The physical properties of fats and oils are strongly influence by the changes in their degree of unsaturation [30], [31]. In addition, the crystallization onset of ML:PO blends were higher (above 20 °C) than that of LD (18.25 °C). As mentioned earlier, these facts are due to higher amount of SFA (Table I) and US_tSt (Table II). Therefore, none of ML:PO blends displayed any similarity to LD with respect to the positions of thermal transitions in cooling thermogram.

On the other hand, the cooling profile of ML:PS blends was found to be compatible to the cooling profile of ML:PO blends as presented by the previous study [7]. Their peak temperature and peak area were though different. The major and minor peaks were also found to be shifted to higher temperature region after addition of

PS in ML. These changes could be due to the presence of higher disaturated TAG molecules in the ML:PS blends.

2) Heating thermogram

The melting behaviors of ML:PO blends and LD are shown in Fig. 1. Lard had heating transitions at two widely different temperature regions: two major transitions at -3.59 °C (a1) (low melting region) and 29.01 °C (a2) with a shoulder peak at 32.46 °C (a3) (high melting region). The endset of melting (T_{endset}) of LD was 35.7 °C. As indicated in Fig. 2, the melting transitions of ML:PO blends could also be divided into low melting region (below 10 °C) and high melting region (above 20 °C). Those blends had higher endset of melting transition (around 40 °C) compared to that of LD (35.7 °C). In addition, a minor peak of c4 and d4 (ML:PO (95:5) and ML:PO (93:7), respectively) at around 35 °C tend to form a shoulder peak (c3 and d3, respectively) at around 29 °C when the amount of PO increased in the fat blends. The shifting of endset melting transitions of all ML:PO blends and appearance of a new shoulder peak in ML:PO (95:5) and ML:PO (93:7) blends were in accordance with the decreasing degree of unsaturation noted in FA (Table I) and TAG composition (Table II).

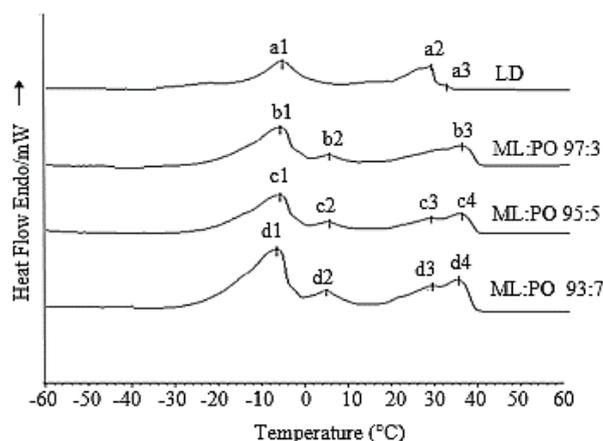


Figure 2. Heating thermogram of ML:PO blends and LD

Based on heating thermogram, a closer similarity between ML:PO (97:3) and LD was seen at the peak-maximum of (b1) at -3.59 °C. Similarly, the heating thermogram of ML:PS blends also had one major peak at low melting region (around -5 °C), one minor peak at middle melting region (around 5 °C) and one minor peak (around 37 °C) with a shoulder peak (around 30 °C) at high melting region. All melting peak maxima were found to be shifted to higher temperature region resulting in higher endset of melting transitions. The closest similarity of ML:PS (99:1, 40 °C) and LD (-3.59 °C) was found at a major peak of melting thermogram [7].

D. Solid Fat Content

Solid fat content of a fat is mainly responsible for many of its characteristics, including general appearance, oil exudation, functional and organoleptic properties. Its profile is important since it has a great influence on the suitability of fat or fat blend for a specific application

[17]. The SFC profile of LD and ML:PO blends are shown in Fig. 3. The SFC of LD at 0 °C was 30.8%. Throughout the temperature region between 10 to 20 °C, the SFC values of ML:PO blends have always been lower compared to that of LD. The largest decline in SFC of the formulated blends between 10 to 20 °C could be due to large amount of TAG that was liquefied within this temperature range. The lower SFC values in this temperature range could produce shortening that is specifically suitable in cool climate [32].

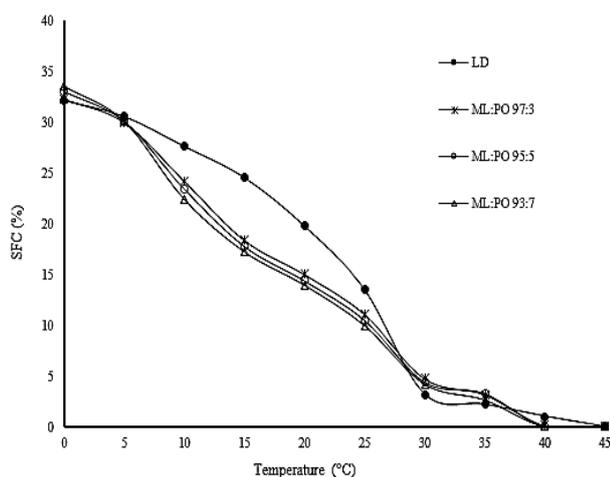


Figure 3. Solid fat content of ML:PO blends and LD

The SFC values of LD and ML:PO blends were also to be found closely similar to each other at 0, 5, 25, 30, 35 and 40 °C. While the SFC value of LD, ML:PO (97:3) and ML:PO (95:5) blends tend to become 0% at 40 °C, that of ML:PO (93:7) blend got 0% (completely melt) at higher temperature at 45 °C. This result could be due to higher proportion of UStSt and StStSt TAG molecules in ML:PO (93:7) blend compared to those of ML:PO (97:3) and ML:PO (95:5) blends.

In general, the difference in the SFC profiles of LD and ML:PO blends could be attributed to their differences in TAG molecular distributional patterns (Table II). Formulated blends with approximately 20-40% of SFC at 0 °C and 5-40% at room temperature are favorable for production of sausages with acceptable texture and consistency [33]. Among the three ML:PO blends, ML:PO (97:3) blend was showed better compatibility to LD at most of temperatures in the range (Fig. 3). Hence, the SFC profile of ML:PO (97:3) blend was the most suitable for fat-based ingredient in meat products. Ref. [34] revealed that the SFC profile of chemically interesterified vegetable oils found to be altered between 10 and 40 °C. In addition, the SFC of enzymatically interesterified EF:CaO blends became lower compared to original blend within the temperature range of 0-35 °C [26]. Therefore, the SFC profile of these formulated blends might be improved by chemical and enzymatic interesterification.

IV. CONCLUSION

This study was attempted to explore the possibility of producing a fat blend to mimic the thermal melting

behavior and SFC profiles of LD by blending ML and PO. Among formulated blends of ML:PO, ML:PO (97:3) blend was found to show the closest similarity to the properties of LD. Although there were differences in FA and TAG compositional data, ML:PO (97:3) blend and LD showed the closest similarity in terms of both melting transition at -3.59 °C and SFC values at 0, 5, 30, 35 and 40 °C. For future study, physical properties of these formulated blends should further be investigated for their polymorphic form, textural, rheological and microstructural properties. In addition, the physicochemical and thermal properties of the formulated blends can further be improved through chemical and enzymatic interesterification. Eventually, studies should also be done to evaluate the compatibility of formulated blends as food ingredients for meat products such as sausage, burger patties, salami, etc and bakery products such as cakes, cookies, etc.

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