# SEED FAT FROM *Madhuca longifolia* AS RAW MATERIAL FOR HALAL ALTERNATIVE FATS

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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 oil to compare their solidification and melting characteristics to formulate halal alternative lipid substitutes. Initially, a direct comparison of pork fat and Madhuca longifolia seed fat was done with respect to fatty acid and triacylglycerol compositions using chromatographic techniques and thermal properties by differential scanning calorimetry (DSC) and pulse NMR spectroscopy. By subjecting these two fats to fractional crystallization under controlled temperature in acetone, their solid and liquid components were isolated separately. The thermal properties of the solid and liquid components from pork fat were also compared to those of lipid derivatives from Madhuca longifolia seed fat using DSC and NMR techniques. As the analytical data obtained from DSC and pulse NMR techniques showed that the thermal properties of these two fats and their components were compatible, Madhuca longifolia seed fat could be a useful raw material for formulation as halal alternative fats.*

**KEYWORDS.** Animal fats, halal alternatives, lard, *Madhuca longifolia*, seed fats

## INTRODUCTION

Animal body fats such as pork fat, beef tallow, mutton tallow and chicken fat have long been used as ingredients in food preparation. Pork fat adulteration in food ingredients an unresolved outstanding issue, which has caused uneasiness amongst the consumers from certain religious groups (Riaz & Chaudry, 2004). According to past studies, pork fat has been used as an ingredient in certain types of biscuits, snacks, rice and moon cakes (Wikipedia, 2012). Owing to the growing public concern about halal status of food in many parts of the world, producing safe and high quality halal food is desired to ensure consumer health and successful domestic and international trade. However, research into the development of alternative plant-based ingredients for halal applications is currently limited.

*Madhuca longifolia*, which belongs to the family Sapotaceae, is a large woody tree distributed in the North-Central part of Sri Lanka. It is reported to originate from South-east Asia. Several parts of the tree are known for uses in traditional medicine. Likewise, its uses in Indian folk medicine are described elsewhere in the literature (Ramadan *et al*., 2006). *Madhuca longifolia* flowers seasonally and produces green fleshy fruits containing three to four ellipsoidal seeds. According to past reports, the fruit seeds may compose around 50 % oil (w/w, db) (Ramadan *et al*., 2006). The crude oil extracted from the seeds is locally known as mee fat, which is pale yellow and remains as a semi-solid in the tropical temperatures. A few reports have already appeared in literature highlighting the compositional characteristics of Indian Mahua seed fat and indicating its potential uses as confectionery fat (Ramadan *et al*., 2006; Ramadan & Moersel, 2006). Marikkar *et al.* (2010) also reported the composition and thermal properties of *Madhuca longifolia* seed fat and its fractions. However, these studies did not consider exploring the compatibility of mee fat to pork fat as an ingredient in food products. Hence, the objective of this study is to compare the properties of mee fat, and pork fat as well as to compare their high-and low melting fractions.

## MATERIALS AND METHODS

### *Materials*

Pork fat was extracted using three batches of adipose tissue of pork collected from local slaughter houses according to the method reported previously by Marikkar *et al.* 2001. Dried fruit seeds of *Madhuca longifolia* were collected from three different locations in the North Central Province of Sri Lanka. All chemicals used in this experiment were of analytical or HPLC grade.

### *Methods*

#### *Oil Extraction*

Oil extracted from finely ground samples of dried *Madhuca longifolia* seeds by the soxhlet extraction method using petroleum ether (40–60°C) (AOAC 2007). The extracted oils were kept in an oven at 60°C for 1 hr to expel solvent before storing at -20°C. Before analysis, the oil samples were removed from frozen storage, left static at room temperature for 1 hr and then warmed at 60°C until they became completely molten.

#### *Fractional Crystallization of Pork Fat*

Pork fat was melted at 60°C and mixed with acetone in 1:2 (w/v) ratio. The solution was boiled at 60°C until uniformly dissolved and left at 5 ± 1°C for 24 hr to crystallize. The precipitated fat was filtered off to give a high melting fat fraction (LS). After removing the precipitate, the liquor was evaporated under reduced pressure to yield a liquid called low-melting fraction (LO).

#### *Fractional Crystallization of Mee Fat*

Mee fat was melted at 60°C and mixed with acetone in 1:2 (w/v) ratio. The solution was boiled at 60°C until uniformly dissolved and left at 5 ± 1°C for 2 hr to crystallize. The precipitated fat was filtered off to give a high-melting fat fraction. The filtrated liquor was re-crystallized in the same condition for another 2 hr and a second fat fraction was collected. It was combined with the previous one and labelled high melting fraction (MS). The filtrated liquor was again left at 5 ± 1°C for 24 hr to allow the remaining solid to precipitate. After removing the precipitate, the liquor was evaporated under reduced pressure to yield a liquid called low-melting fraction (MO).

#### *Determination of Cloud Point (CP), Slip Melting Point (SMP) and Iodine Value (IV)*

CP, SMP and 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).

#### *Determination of Fatty Acid Composition*

Fatty acid methyl esters (FAME) were prepared by dissolving 50 mg portion of oil in 0.8 ml of hexane and adding 0.2 ml portion of 1M solution of sodium methoxide (PORIM 1995), then analyzed on a gas chromatograph (Agillent Technologies, Singapore) fitted with a FID detector. The polar capillary column RTX-5 (0.32 mm internal diameter, 30 m length and 0.25 µm film thickness; Restex Corp., Bellefonte, PA) was used. The oven temperature was programmed as follows: initial temperature of 50°C (for 1 min), increased to 200°C at 8°C/min. Both injector and detector temperatures were maintained at 200°C throughout the analysis. The carrier gas (helium) flow rate was 1.0 mL/min and the split ratio was 58:1. The peaks of the samples were identified with reference to a chromatographic profile containing FAME standards (Supelco, Bellefonte, PA). The percentage of fatty acid was calculated as the ratio of the partial area to the total peak area (Marikkar *et al.,* 2005).

***Determination of Triacylglycerol (TAG) Composition***

The TAG composition was determined using 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 × 4 mm i.d.; 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. The oven temperature was maintained at 30°C. The injector volume was 10 μL of 5% (w/w) oil in chloroform. Each sample was chromatographed two times, and the data were reported as area percentage (Marikkar *et al.*, 2003). The peaks of the samples were identified using a set of TAG standards purchased from Sigma-Aldrich (Deisehofen, Germany) as well as the TAG profiles of lard (Rashood *et al.*, 1996) reported previously.

***Thermal Analysis by Differential Scanning Calorimetry (DSC)***

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. Approximately 4-8 mg of molten sample was placed in a standard DSC aluminum pan and then hermetically sealed. An empty, hermetically-sealed DSC aluminum pan was used as the reference. The oil/fat samples were subjected to the following temperature program: The sample was held at 70°C isotherm for 1 min to destroy the thermal history, cooled at 5°C /min to -70°C and held for 1 min. The sample was then heated from -70°C to 70°C at the same rate (Marikkar *et al.*, 2003).

***Determination of Solid Fat Content***

Solid fat content (SFC) was measured using a Bruker Minispec (Model Mq 20) pulse Nuclear Magnetic Resonance (pNMR) spectrometer (Karlsruhe, Germany), according to AOCS 1999. 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 for 30 min prior to measurement. Samples were melting, chilled and held in pre-equilibrated thermostatted glycol baths, accurate to 0.1°C. SFC measurements were taken at 5°C intervals over the range of 0-60°C.

***Statistical Analysis***

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

## RESULTS AND DISCUSSION

***Basic Physico-chemical Parameters***

The slip melting point (SMP), cloud point (CP) and iodine value (IV) of pork fat, mee fat and their fractions are shown in Table 1. The SMP of pork fat is found to be 27.50°C, while mee fat is found to have higher SMP value (35.50°C). As a result of fractionation, the solid component of the untreated samples (pork and mee fats) are mainly consists of higher melting TAG. Hence, the SMP of stearin fractions were found to be higher than that of native samples. According to Table 1, the SMP of pork fat stearin (45.75°C) was lower than mee fat stearin (46.50°C). Being liquid at room temperature, the thermal characteristics of olein fractions were described by CP. CP of pork fat olein is found to be lower, by about 7 units than mee fat olein. The IV of pork fat was 73.80, while mee fat was 61.10. The IV of pork fat stearin was found to be lower than mee fat stearin. On the other hand, the IV of pork fat olein was found to be higher, by about 39 units than mee fat olein. As IV represents the degree of unsaturation of fatty matter, the values recorded for the fractions shows a reverse relationship in SMP.

***Fatty Acid Composition***

The fatty acid distributions of pork fat, mee fat and their fractions are compared in Table 1. The major fatty acids of pork fat were oleic acid (38.24%), followed by palmitic (22.68%) and linoleic (20.39%) acids. According to most of the previous studies, pork fat is generally found to have more unsaturated fatty acids (USFA) than saturated fatty acids (SFA) (Nurjuliana *et al*., 2010; Rashood *et al*., 1996). On the other hand, the predominant fatty acids of mee fat were oleic (44.02%), stearic (22.05%) and palmitic (20.88%) acids, but very little linoleic acid (7.85%). Because of its fatty acid composition, the SFA of mee fat (42.93%) was found to be higher than that of pork fat (36.62%). When compared to native mee fat, stearin fractions may contain more SFA than USFA. With respect to native samples, tremendous increases in the proportions of stearic and palmitic could be noticed with concurrent decrease of oleic and linoleic acids. In olein fractions, the proportion of oleic and linoleic acids were increased, with concurrent decrease of stearic and palmitic acids. According to Table 1, the proportion of oleic and linoleic acids of pork fat olein were found to be lower, by about 10 and 14 units, respectively than mee fat olein.

***Triaclyglycerol Composition***

The TAG distributional profiles of pork fat, mee fat and their fractions are compared as shown in Table 2. The major TAG molecular species of pork fat are LPO, OPO, StPO and PPO comprising 61.5% of the total. This is agreement with the previous findings of Rashood *et al.* (1996). According to Table 2, pork fat is found to contain 51.34% of UUS and 26.60% of USS as the predominant TAG molecular groups. In the meantime, mee fat consisted of OPO, StPO, PPO and OOSt as the predominant TAG molecular species, but very little amount of LPO (4.26%). Presence of OLL, LLL, and LLLn in significant proportions would make pork fat have higher amount of triunsaturated (UUU) in comparison to mee fat. Mee fat is also found to have diunsaturated (UUS) (44.98%) and disaturated (USS) (42.19%) as the dominant TAG molecular groups. Stearins of both fats were found to have SPO and PPO as the major TAG molecular species, but the second most abundant TAG molecular species in mee fat stearin was StStO (23.78%). The most dominant TAG molecular groups in both stearins were USS and UUS. On the other hand, the most predominant TAG molecular species of pork fat olein was OPO (26.11%), followed by LPO (24.52%) and PLL (9.33%). Pork fat olein is having UUS (64.26%) and UUU (23.82%) as the predominant TAG molecular groups, but very little amount of USS (7.84%) TAG molecules. Unlike pork fat olein, mee fat olein consists of UUS (55.29%), USS (30.20%) and UUU (15.16%), as its TAG molecular groups, with OPO (28.65%), OOSt (24.05%) and StPO (14.05%) as the predominant TAG molecules.

**Table 1. Basic physico-chemical characteristics and fatty acid compositions (%) of pork fat, mee fat and their fractions.**

| | Iodine value (g $I_2$/100 g) | Slip melting point (°C) | Cloud point (°C) | C 14:0 | C 16:0 | C 16:1 | C 18:0 | C 18:1 | C 18:2 | Others |
|---|---|---|---|---|---|---|---|---|---|---|
| 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 | 3.33±0.12 |
| MF | 61.1±0.35 | 35.5±0.5 | - | - | 20.88±1.51 | - | 22.05±0.9 | 44.02±1.1 | 7.85±0.77 | - |
| 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 | 2.21±0.02 |
| MS | 47.05±0.05 | 46.5±0.7 | - | | 25.28±1.33 | - | 29.01±0.84 | 38.38±1.6 | 5.72±0.56 | - |
| LO | 103±0.06 | - | 3.25±0.35 | 1.46±0.15 | 21.76±0.01 | 2.30±0.01 | 6.38±0.03 | 42.76±0.18 | 23.62±0.03 | 1.72±0.2 |
| MO | 64.4±0.45 | - | 10.5±0.5 | - | 19.28±1.2 | - | 16.2±0.67 | 53.12±0.95 | 9.61±0.88 | - |

Each fatty acid value in the table represents the mean ± standard deviation of two replicates.
Abbreviations: LD, pork fat; MF, mee fat; LS, pork fat stearin; MS, mee fat stearin; LO, pork fat olein; MO, mee fat olein

**Table 2. Triacylglycerol (TAG) compositions (%) of pork fat, mee fat and their fractions.**

| TAG | LD | MF | LS | MS | LO | MO |
|---|---|---|---|---|---|---|
| LLLn | 1.54±0.21 | ND | 0.22±0.00 | ND | 2.29±0.01 | ND |
| LLL | 0.68±0.21 | ND | 0.23±0.00 | ND | 1.43±0.01 | ND |
| OLL | 4.68±0.08 | ND | 2.11±0.01 | ND | 6.01±0.01 | ND |
| PLL | 7.05±0.06 | ND | 3.26±0.01 | ND | 9.33±0.04 | ND |
| OOL | 6.93±0.04 | 3.0±0.26 | 3.40±0.02 | 1.45±0.00 | 8.48±0.01 | 2.60±0.1 |
| LPO | 20.00±0.30 | 4.26±0.37 | 9.32±0.00 | 1.77±0.00 | 24.52±0.11 | 2.59±0.15 |
| PPL | 2.62±0.04 | 1.19±0.04 | 3.96±0.01 | 0.35±0.03 | 2.63±0.03 | Tr. |
| OOO | 4.33±0.21 | 9.85±0.22 | 2.46±0.04 | 3.47±0.04 | 5.61±0.07 | 12.56±0.05 |
| OPO | 20.67±0.11 | 22.92±0.88 | 9.48±0.03 | 9.00±0.01 | 26.11±0.01 | 28.65±0.06 |
| PPO | 10.63±0.01 | 11.92±0.66 | 22.87±0.03 | 13.95±0.06 | 3.05±0.05 | 12.07±0.02 |
| PPP | ND | Tr. | ND | 0.2±0.1 | ND | Tr. |
| OOSt | 3.62±0.04 | 17.80±00 | 1.79±0.01 | 7.42±0.02 | 4.30±0.02 | 24.05±0.07 |
| StPO | 12.52±0.12 | 19.34±0.44 | 30.19±0.01 | 35.09±0.04 | 2.16±0.00 | 14.05±0.00 |
| PPSt | 0.81±0.00 | Tr. | 2.53±0.04 | 1.76±0.04 | ND | Tr. |
| StStO | 0.83±0.01 | 9.74±0.58 | 2.29±0.01 | 23.78±0.04 | ND | 3.45±0.2 |
| StStSt | 1.31±0.01 | ND | 4.14±0.01 | ND | ND | ND |
| Unknown | 1.84±0.09 | - | 1.78±0.02 | 1.86±0.1 | 4.12±0.35 | - |
| UUU | 18.16 | 12.85 | 8.42 | 4.92 | 23.82 | 15.16 |
| UUS | 51.34 | 44.98 | 23.85 | 18.19 | 64.26 | 55.29 |
| USS | 26.60 | 42.19 | 59.31 | 73.17 | 7.84 | 30.20 |
| SSS | 2.12 | - | 6.67 | 1.96 | - | - |

Each value in the table represents the mean ± standard deviation of two replicates.
Abbreviations: LD, pork fat; MF, mee fat; LS, pork fat stearin; MS, mee fat stearin; LO, pork fat olein; MO, mee fat olein; O, oleic; P, palmitic; L, linoleic; Ln, linolenic; St, stearic; Tr., trace; ND, not detected; UUU, triunsaturated; UUS, diunsaturated; USS, disaturated; SSS, triunsaturated.

***Melting Profile by DSC***

The DSC melting profile of pork fat, mee fat and their fractions are compared in Fig.1 (untreated samples), Fig. 2 (solid fractions) and Fig. 3 (liquid fractions). In Fig. 1, the melting profiles of pork fat and mee fat are represented by curves (A) and (B), respectively. Both curves showed two well-separated endothermic regions, namely the low and high melting regions. The region below 10°C is due to low-melting TAG group, while the region above 10°C is designated as high-melting TAG group. Mee fat is found to display its $T_{endset}$ at 38.86°C, which is somewhat closer to that of pork fat (35.70°C). In Fig. 2, the melting profiles corresponding to the stearins of pork fat and mee are represented by curves (E) and (F), respectively. In both of these curves, there is hardly any significant thermal transition in the temperature region below 10°C. While the $T_{endset}$ of mee fat stearin was found to be at 59.87°C, the corresponding value of pork fat stearin was 51.36°C.This difference in $T_{endset}$ values could be attributed to the enrichment of disaturated (USS) TAG molecular groups in mee fat stearin rather than pork fat stearin during the fractional crystallization (Table 2). In Fig. 3, the melting profiles of the oleins of pork fat and mee fat are represented by curves (I) and (J), respectively. In comparison with the untreated samples, oleins from both fats are found to display wide and broad low-melting transitions. In addition, the shifting peak maxima of thermal transitions of both olein fractions toward the low-temperature region indicated the changing proportional distribution of different TAG molecules. The reason for the shift in thermal transitions could be attributable to the increasing proportion of diunsaturated (UUS) and triunsaturated (UUU) TAG groups in the liquid fractions. However, $T_{endset}$ value of pork fat olein (14.08°C) is found to be somewhat lower than that of mee fat olein (29.28°C). This feature might be due

to higher proportions of UUS (64.26%) and UUU (23.82%) in pork fat olein than those of mee fat olein [UUS (55.29%) and UUU (15.16%)].

***Cooling Profile by DSC***

The DSC melting profile of pork and mee fats are compared in Fig.1, their solid fractions in Fig. 2 and their liquid fractions in Fig. 3. In Fig. 1, the cooling profiles of pork and mee fat are represented by curves (C) and (D), respectively. Similar to melting profile, the DSC crystallization profile of pork fat and mee fat also had two well-separated transitions. In curves (C) and (D), two distinguished regions could be identified by taking 0°C as the point of reference. The region below 0°C represents a low-melting TAG group, while the region above 0°C is designated as high-melting TAG group. In fact, the more widely separated high and low-melting transitions could be an indication of a better fractionation in both of these fats. The $T_{onset}$ of mee fat is found to be higher (26.86°C) than that of pork fat (18.25°C). In Fig. 2, the cooling profiles of the stearins of pork fat and mee are represented by the curves (G) and (H), respectively. It is clear that cooling profiles of both solid fractions are completely different from those of untreated fats. The absence of any significant thermal transition below 20°C was an indicative feature, which could be used to establish their identity as the solid fractions. It could be assumed that the drastic reductions in both diunsaturated (UUS) and triunsaturated (UUU) TAG molecular groups (Table 2) would lead to the disappearance of the low-melting transitions in stearins of both of these fats. However, mee fat stearin is found to display somewhat higher $T_{onset}$ (52.17°C) than that of pork fat stearin (33.53°C).

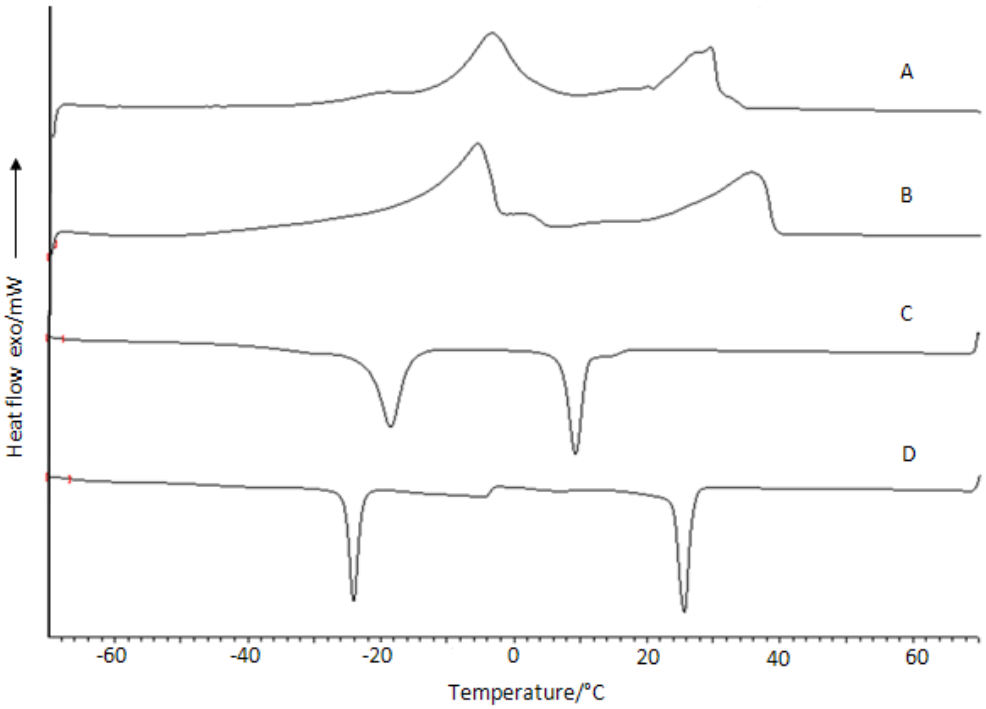


**Figure 1. DSC heating thermograms of (A) pork fat and (B) mee fat, and cooling thermograms of (C) pork fat and (D) mee fat.**

According to Table 2, mee fat stearin has a higher proportion of USS (73.17%) than pork fat stearin (59.31%). In Fig. 3, the cooling profiles of the oleins of pork fat and mee fat are represented by curves (K) and (L), respectively. Oleins from both fats are also found to display profiles completely different from those of untreated samples. In comparison to the untreated samples, the thermal transitions of both oleins were also found to shift to the lower temperature region due to increases in the proportions

of UUU and UUS TAG molecular groups as given in Table 2. Nevertheless, the $T_{onset}$ of mee fat olein (17.32°C) was found to be very much higher than that of pork fat olein (-1.72°C).

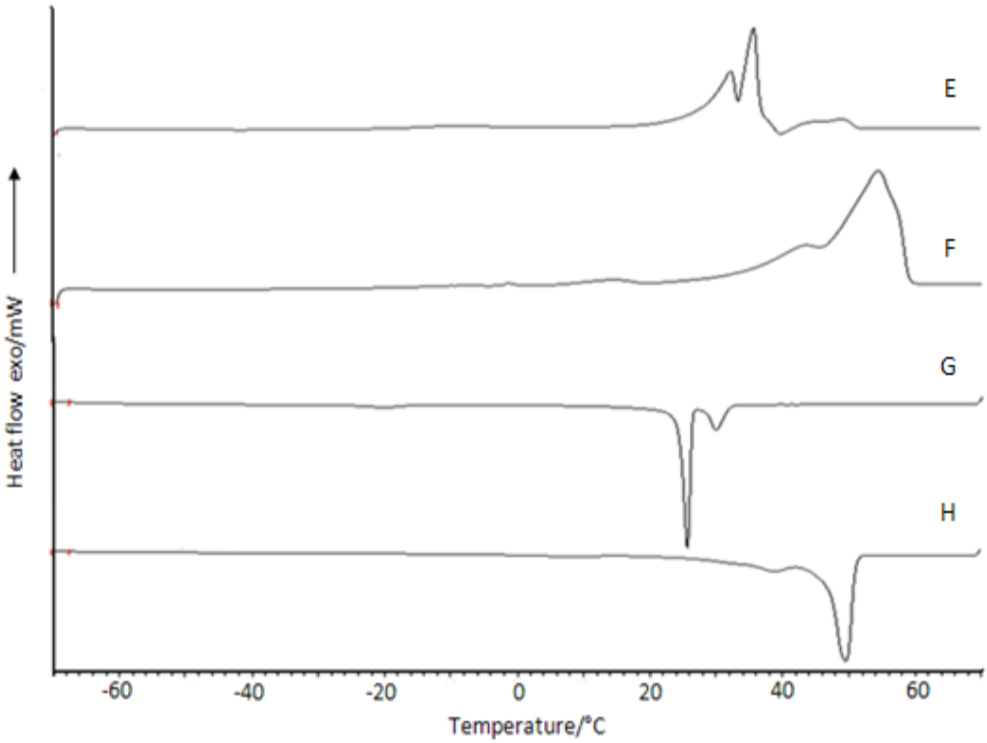


**Figure 2. DSC heating thermograms of (E) pork fat stearin and (F) mee fat stearin, and cooling thermograms of (G) pork fat stearin and (H) mee fat stearin.**

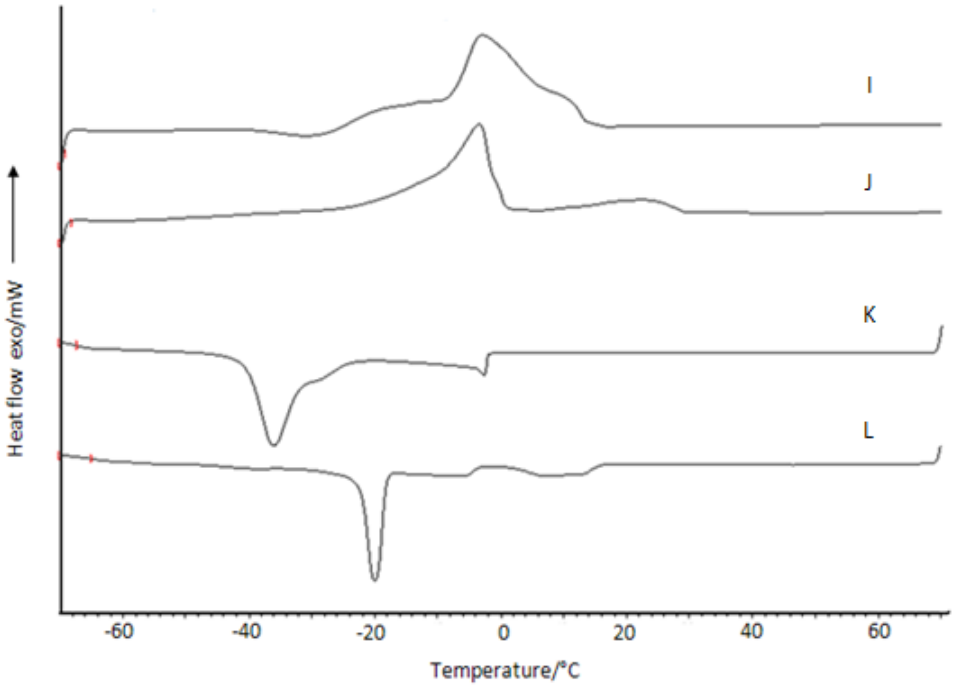


**Figure 3. DSC heating thermograms of (I) pork fat olein and (J) mee fat olein, and cooling thermograms of (K) pork fat olein and (L) mee fat olein.**

***Solidification Behaviour***

The solid fat content (SFC) profiles of pork fat, mee and their fractions are compared as shown in Fig. 4. The SFC values of pork fat and mee fat at 0°C were 30.8% and 33.1%, respectively. In between the temperature range 0°C to 25°C, mee fat is found to display a SFC profile closely similar to that of pork fat. The apparent similarities in the SFC values of pork fat and mee fat in the range of 0 - 25°C are probably due to some similarities between them with regard to the distribution of TAG molecular species. According to Table 2, both mee fat and pork fat are found to possess a clear descending order with respect to the proportional distribution of diunsaturated (UUS), disaturated (USS), triunsaturated (UUU), and trisaturated (SSS) groups of TAG molecular species (i.e UUS > USS > UUU > SSS). Throughout the temperature region, the SFC values of pork fat stearin have always been higher than those of mee fat stearin, but the SFC profile of pork fat stearin suddenly dropped at around 35°C. Although the stearins of mee fat and pork fat are found to possess a high proportion of USS TAG molecular group, the proportion of SSS TAG molecular group in mee fat stearin (1.96%) was lower than that of pork fat stearin (6.67%). However, mee fat stearin is found to have SFC profile closely similar to that of pork fat stearin in the temperature range 35 - 45°C. In contrast, the SFC values of mee fat olein were always higher than those of pork fat olein as shown in Fig. 4. Even though UUS and UUU are the predominant TAG molecular groups in olein fractions, the excessive amount of USS (30.20%) in mee fat olein could contribute to this feature. Nevertheless, mee fat olein is found to have SFC profile closely similar to that of pork fat olein at temperatures above 10°C.

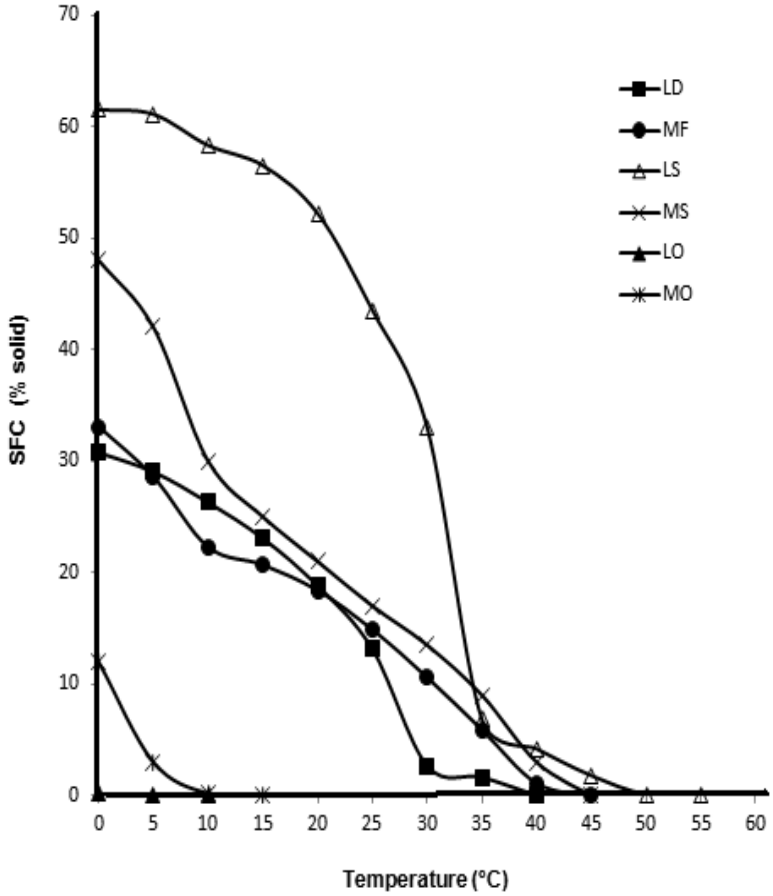


**Figure 4. Solid fat content profiles of pork fat (LD), mee fat (MF), pork fat stearin (LS), mee fat stearin (MS), pork fat olein (LO) and mee fat olein (MO).**

## CONCLUSION

This study demonstrates that mee fat has some common thermal characteristics with pork fat by having thermal transitions at low and high temperatures. Mee fat is found to display a SFC profile, closely similar to that of pork fat in the temperature range 0 - 25°C. During fractional crystallization, both of them are found to yield solid stearin and liquid olein. Pork fat stearin was found to have SFC values higher than that of mee fat stearin at temperatures below 35°C. However, the SFC values of both stearins are found to be somewhat similar to each other at temperatures above 35°C. On the other hand, the SFC values of pork fat olein are found to be lower than those of mee fat olein from 0 - 5°C, and at 10°C they tend to become 0. In conclusion, mee fat could be used as an alternative ingredient for halal fats.

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