



## Determination of types of fat ingredient in some commercial biscuit formulations

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### Abstract

A study was carried out to compare the composition and thermal profiles of the fat component of six brands of commercial biscuits (BA, BB, BC, BD, BE & BF) with those of lard and palm oil. Extraction of fat from biscuit samples was done using petroleum ether according to the soxhlet extraction procedure. The isolated fat samples along with lard and palm oil were analyzed using gas liquid chromatography (GLC), reversed-phase high performance liquid chromatography (RP-HPLC), and differential scanning calorimetry (DSC). According to GLC analysis, palm oil, lard and all six biscuit brands had either palmitic or oleic acid as major fatty acids. *Sn-2* positional analysis of fatty acids showed that oleic (> 60%) as the most dominant fatty acid of palm oil and biscuit brands BA, BB, BC, and BD while palmitic (> 60%) as the most dominant fatty acid of lard and biscuit brands BE and BF. RP-HPLC analysis showed that the triacylglycerol (TAG) profiles of lard and biscuit brands BE and BF were closely similar while those of brands BA, BB, BC, and BD and palm oil were similar. DSC analysis showed that the cooling and heating profiles of lard and brands BE and BF were similar, while those of palm oil and brands BA, BB, BC, and BD were similar. Hence, this study concluded that biscuit brands BE and BF are not suitable for consumers whose religious restriction prohibit the use of lard as food ingredient.

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### Introduction

Biscuit is one of the most popular bakery products eaten by consumers all around the world. They are made up of a combination of ingredients, which includes wheat-flour, shortening, sugar, millet, milk, and egg. The inclusion of shortening usually aids in the development of the structure of the batter of dough during the mixing process, by helping to produce tiny pockets of air bubbles that serve as the nuclei for leavening gasses (Stauffer, 2005; Cauvian and Young, 2006). As shortenings play an important role in the formulation of the structure of the batter or dough, it influences heavily on the volume, grain, and texture of the finished products (Ghotra *et al.*, 2002; Cauvian and Young, 2006). Shortening's contribution to the eating quality of biscuits is also related to its function of producing softness and tenderness (Ghotra *et al.*, 2002). Shortenings of different kinds are used in biscuit formulations depending on the type of finished product quality requirements. For instance, use of butter fat in biscuit formulations has been known for a long time in many countries (Cauvian and Young, 2006). Two important benefits of butter in biscuit formulation are its flavor and mouth feel. Use of plant based shortenings of palm oil in biscuit

formulation has become increasingly popular due to their functional properties as well as price advantages. Although vegetable-based shortenings are preferred in bakery products by consumers, there is a possibility for using animal fats as cheaper substitutes. For instance, lard has been well-known for its use as a fat ingredient in certain types of biscuits (Yanty *et al.*, 2011), cakes (Hartnett and Thalheimer, 1979), and piecrust (Lawson, 1995). The plastic behavior of lard is said to contribute much to its functional properties in many baking applications. Particularly, the semi-solid nature, wider plastic range and flow characteristics of lard were found to incorporate innumerable air cell during the preparation of dough (Hoffman, 1989; Kamel, 1992). However, use of lard as ingredient in biscuits is a serious concern to followers of Islamic and Orthodox Jewish religions as these religions prohibit the consumption of products containing lard. Hence, a considerable amount of effort has been devoted to the development of methodologies to detect lard in food products (Rohman and Che Man, 2012). Although there are methods for detection of lard in various oil products, it has now become necessary to demonstrate their usefulness in finished products such as biscuits. In this study, the use of fatty acid and TAG compositional data as well as

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thermal profiles has been investigated to detect lard containing biscuits in the market.

## Materials and Methods

### Materials

Four different local brands of biscuits (BA, BB, BC, & BD) and two different imported brands (BE & BF) of biscuits were purchased in three replicates from local superstores in Malaysia. Lard was extracted using three batches of adipose tissues of swine collected from local slaughter houses according to the method reported previously by Marikkar *et al.* (2001). 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.

### Oil extraction

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

### 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 (Agilent Technologies, Singapore) fitted with a FID detector. All the instrumental conditions were as described in the previous report of Yanty *et al.* (2012). The identification of the peaks of the samples was done 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 peaks area.

### Analysis of fatty acids at the *sn*-2 position

The distribution of fatty acids at the *sn*-2 position of the TAG molecules was determined according to the modified method of Luddy *et al.* (1964). Neutral TAG of oil samples were isolated using column chromatography and hydrolyzed with hog pancreatic lipase (Fluka Chemie, Buchs, Switzerland). The resulting 2-monoacylglycerols (2-MAG) were isolated by using thin layer chromatographic plates placed on tank containing mixture of hexane/diethyl ether/acetic acid (50:50:1, vol/vol/vol). FAME of

isolated samples of 2-MAG were then determined according to the method described previously by Marikkar *et al.* (2003).

### Determination of 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). All the instrumental conditions including the type of column were as described in the previous report of Yanty *et al.* (2012). The identification of the peaks of the samples was done using a set of TAG standards purchased from Sigma-Aldrich (Deisehofen, Germany) as well as the TAG profiles of lard (Yanty *et al.*, 2012), and palm oil (Marikkar *et al.*, 2005) reported previously.

### Thermal analysis by differential scanning calorimetry

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). All the instrumental conditions including the sampling procedure were as described in the previous report of Yanty *et al.* (2012).

### Statistical analysis

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

## Results and Discussion

### Overall fatty acid composition

Fatty acid compositions of the lipids extracted from commercial brands of biscuits are compared with those of lard and palm oil as shown in Table 1. All local brands (BA, BB, BC, & BD) were found to contain palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) as major fatty acids. All of them had palmitic (41.02 - 45.35%) as the most dominant fatty acid followed by oleic (36.48 - 39.54%), and linoleic (10.25 - 12.32%) acids. According to previous studies, the major fatty acids of palm oil are also palmitic (48.7%), oleic (37.1%), and linoleic (8.1%) acids (Tan and Che Man, 2000; Marikkar *et al.*, 2002). Hence, the proportional distributions of the fatty acids of palm oil and those of the local brands of biscuits (BA, BB, BC, & BD) were found to be closely similar (Table 1). Although the two imported brands (BE & BF) were also found to contain palmitic, and oleic as major fatty acids, the

Table 1. Overall fatty acid composition of palm oil, lard and oils from six coded samples of commercial biscuits\*

	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	Others
Palm oil	0.33±0.01 <sup>b</sup>	1.10±0.00 <sup>b</sup>	43.99±0.06 <sup>a</sup>	0.18±0.01 <sup>b</sup>	4.36±0.06 <sup>d</sup>	39.14±0.20 <sup>b</sup>	10.25±0.06 <sup>d</sup>	0.67±0.01
BA	0.45±0.06 <sup>b</sup>	1.33±0.08 <sup>a,b</sup>	45.35±0.06 <sup>a</sup>	0.09±0.12 <sup>b</sup>	4.93±0.09 <sup>c</sup>	36.48±0.01 <sup>c</sup>	10.53±0.03 <sup>d</sup>	0.86±0.04
BB	0.24±0.00 <sup>b</sup>	1.06±0.00 <sup>b</sup>	43.23±0.01 <sup>a,b</sup>	0.16±0.00 <sup>b</sup>	4.32±0.00 <sup>d</sup>	38.49±0.01 <sup>b,c</sup>	11.55±0.02 <sup>b,d</sup>	0.96±0.01
BC	1.76±0.50 <sup>a</sup>	1.77±0.37 <sup>a</sup>	41.02±0.33 <sup>b</sup>	0.17±0.00 <sup>b</sup>	4.24±0.06 <sup>d</sup>	38.57±1.58 <sup>b</sup>	11.10±0.45 <sup>b,d</sup>	1.38±0.90
BD	0.32±0.11 <sup>b</sup>	1.06±0.1 <sup>b</sup>	41.10±0.22 <sup>b</sup>	0.16±0.00 <sup>b</sup>	4.14±0.16 <sup>d</sup>	39.54±0.76 <sup>b</sup>	12.32±0.40 <sup>b,c,d</sup>	1.38±0.09
BE	0.18±0.01 <sup>b</sup>	0.89±0.01 <sup>b</sup>	20.04±0.05 <sup>c</sup>	1.33±0.03 <sup>a</sup>	18.30±0.14 <sup>a</sup>	42.60±0.28 <sup>a</sup>	13.50±0.14 <sup>b,c</sup>	3.17±0.06
BF	0.18±0.00 <sup>b</sup>	0.89±0.02 <sup>b</sup>	21.27±1.56 <sup>c</sup>	1.32±0.01 <sup>a</sup>	18.23±0.07 <sup>a</sup>	42.23±0.33 <sup>a</sup>	12.80±1.13 <sup>b</sup>	3.09±0.17
Lard	0.09±0.01 <sup>b</sup>	1.24±0.01 <sup>a,b</sup>	22.68±0.48 <sup>c</sup>	1.42±0.05 <sup>a</sup>	12.70±0.28 <sup>b</sup>	38.24±0.13 <sup>b</sup>	20.39±0.04 <sup>a</sup>	3.25±0.01

\*Each fatty acid value in the table represents the mean ± standard deviation of three replicates. Means within column with different superscripts are significantly ( $p < 0.05$ ) different.

Table 2. *Sn-2* fatty acid composition of palm oil, lard and oils from six coded samples of commercial biscuits\*

	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	Others
Palm oil	0.33 ± 0.03 <sup>a</sup>	0.69 ± 0.08 <sup>a</sup>	16.43 ± 0.04 <sup>a</sup>	0.13 ± 0.06 <sup>a</sup>	1.18 ± 0.08 <sup>a</sup>	62.65 ± 0.2 <sup>c</sup>	17.33 ± 0.19 <sup>b</sup>	0.62 ± 0.22
BA	0.4 ± 0.07 <sup>a</sup>	0.72 ± 0.06 <sup>a</sup>	13.65 ± 0.21 <sup>b</sup>	0.19 ± 0.04 <sup>a</sup>	1.43 ± 0.11 <sup>a</sup>	63.75 ± 0.28 <sup>b</sup>	18.05 ± 0.06 <sup>a</sup>	0.55 ± 0.05
BB	0.46 ± 0.03 <sup>a</sup>	0.68 ± 0.07 <sup>a</sup>	15.45 ± 0.5 <sup>a</sup>	0.14 ± 0.05 <sup>a</sup>	1.76 ± 0.2 <sup>b</sup>	62.45 ± 0.07 <sup>c</sup>	17.72 ± 0.33 <sup>b</sup>	0.57 ± 0.09
BC	0.27 ± 0.06 <sup>a</sup>	0.61 ± 0.07 <sup>a</sup>	12.35 ± 0.18 <sup>c</sup>	0.22 ± 0.09 <sup>a</sup>	1.15 ± 0.08 <sup>a</sup>	65.87 ± 0.13 <sup>a</sup>	18.07 ± 0.07 <sup>a</sup>	0.82 ± 0.18
BD	0.41 ± 0.04 <sup>a</sup>	0.81 ± 0.04 <sup>a</sup>	12.01 ± 0.01 <sup>c</sup>	0.29 ± 0.11 <sup>a</sup>	1.78 ± 0.08 <sup>b</sup>	66.38 ± 0.14 <sup>a</sup>	16.81 ± 0.06 <sup>c</sup>	0.91 ± 0.36
BE	-	2.64 ± 0.06 <sup>b</sup>	66.53 ± 0.36 <sup>d</sup>	1.18 ± 0.46 <sup>b</sup>	8.23 ± 0.15 <sup>c</sup>	10.30 ± 0.42 <sup>d</sup>	7.32 ± 0.047 <sup>d</sup>	3.80 ± 0.22
BF	-	2.69 ± 0.27 <sup>b</sup>	66.79 ± 0.49 <sup>d</sup>	1.04 ± 0.37 <sup>b</sup>	7.83 ± 1.85 <sup>c</sup>	10.67 ± 0.95 <sup>d</sup>	7.51 ± 0.21 <sup>d</sup>	3.47 ± 0.24
Lard	-	2.72 ± 0.20 <sup>b</sup>	67.54 ± 1.80 <sup>d</sup>	1.16 ± 1.63 <sup>b</sup>	8.26 ± 0.71 <sup>c</sup>	9.88 ± 0.70 <sup>d</sup>	7.41 ± 5.30 <sup>d</sup>	3.06 ± 0.48

\*Each fatty acid value in the table represents the mean ± standard deviation of three replicates. Means within column with different superscripts are significantly ( $p < 0.05$ ) different.

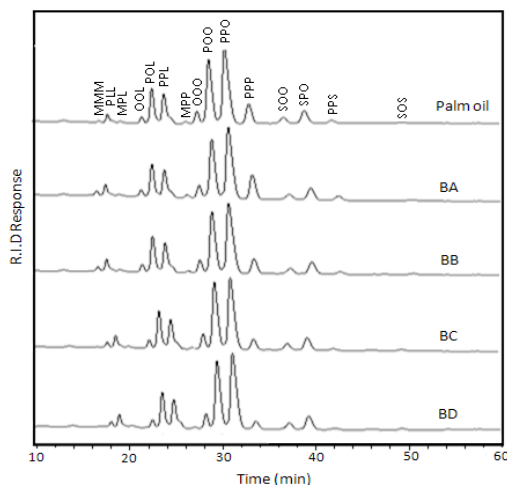


Figure 1. TAG profiles of palm oil and oils of four coded local commercial biscuits

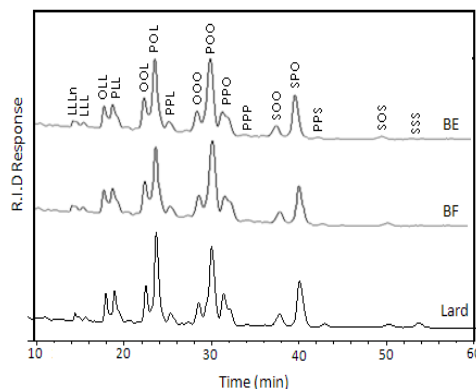


Figure 2. TAG profiles of lard and oils of two coded imported commercial biscuits

third most dominating fatty acid in them was stearic acid. Both of them were found to have oleic (42.23 - 42.60%) as the most dominant fatty acid followed by palmitic (20.04 - 21.27%), and stearic (18.23 - 18.30%) acids. The differences in the proportions of stearic and linoleic acids would make the imported brands to be distinctly different from the local brands. However, the proportional distributions of fatty acids in the imported brands (BE & BF) were somewhat comparable to that of lard (Table 1).

### *Sn-2 fatty acid composition*

*Sn-2* fatty acid compositions of the lipids extracted from commercial brands of biscuits are compared with those of palm oil and lard as shown in Table 2. Most of the local brands (BA, BB, BC, & BD) were found to contain oleic (C18:1), linoleic (C18:2), and palmitic (C16:0) as major fatty acids. However, the distributional proportions of these fatty acids differed considerably from those of the overall fatty acid compositions of these biscuit brands as shown

in Table 1. All of them had oleic (62.45 - 66.38%) as the most dominant fatty acid followed by linoleic (16.81 - 18.07%), and palmitic (12.01 - 15.45%) acids. According to Table 2, palm oil also had oleic (C18:1), linoleic (C18:2), and palmitic (C16:0) as major fatty acids in its *sn-2* fatty acid composition and the relative distributions of these fatty acids are somewhat comparable to those of the four local brands of biscuits. By contrast, the two imported brands (BE & BF) and lard were found to display a higher proportion of palmitic (66.53 - 67.54%) and lower contents of oleic and linoleic acids (17.29 - 17.62%) at the *sn-2* positional fatty acid composition. In fact, lard having a higher proportion of palmitic and lower contents of oleic and linoleic acid at the *sn-2* position of the glycerol backbone is a unique feature, which has been established in many previous studies (Marikkar *et al.*, 2003; Yanty *et al.*, 2011). Hence, it can be deduced that the fat ingredient from BE and BF are quite similar to lard.

### *TAG composition*

TAG profiles of lipids extracted from the four local brands of biscuits (BA, BB, BC, & BD) are

Table 3. Triacylglycerol (TAG) composition of palm oil, lard and oils from six coded samples of commercial biscuits\*

TAG	Palm oil	BA	BB	BC	BD	BE	BF	Lard
LLLn	-	-	-	-	-	1.32±0.02 <sup>a,b</sup>	1.17±0.01 <sup>b</sup>	1.54±0.21 <sup>a</sup>
LLL	-	-	-	-	-	0.47±0.00 <sup>a,b</sup>	0.68±0.02 <sup>a</sup>	0.68±0.21 <sup>a,b</sup>
MMM	0.21±0.13 <sup>b</sup>	0.90±0.01 <sup>b</sup>	0.88±0.02 <sup>b</sup>	1.11±0.01 <sup>a</sup>	1.09±0.02 <sup>a</sup>	-	-	-
OLL	-	-	-	-	-	5.07±0.04 <sup>a</sup>	4.76±0.04 <sup>b</sup>	4.68±0.08 <sup>b</sup>
PLL	2.08±0.05 <sup>c</sup>	2.67±0.02 <sup>d</sup>	2.99±0.01 <sup>c,d</sup>	2.96±0.02 <sup>c</sup>	3.01±0.04 <sup>c,d</sup>	7.05±0.02 <sup>a</sup>	6.31±0.04 <sup>b</sup>	7.05±0.06 <sup>a</sup>
MPL	0.54±0.19 <sup>a</sup>	0.21±0.01 <sup>c,d</sup>	0.29±0.02 <sup>b</sup>	0.20±0.03 <sup>d</sup>	0.28±0.04 <sup>c,b</sup>	-	-	-
OOL	1.62±0.06 <sup>f</sup>	1.54±0.00 <sup>f</sup>	1.77±0.01 <sup>e</sup>	2.11±0.02 <sup>d</sup>	1.79±0.03 <sup>e</sup>	7.55±0.08 <sup>a</sup>	7.21±0.01 <sup>b</sup>	6.93±0.04 <sup>c</sup>
POL	9.96±0.06 <sup>c,d</sup>	9.59±0.01 <sup>d</sup>	10.68±0.01 <sup>c</sup>	10.96±0.03 <sup>c</sup>	10.26±0.03 <sup>c</sup>	21.45±0.01 <sup>a</sup>	20.05±0.03 <sup>b</sup>	20.00±0.27 <sup>b</sup>
PPL	10.19±0.11 <sup>b</sup>	9.46±0.01 <sup>d</sup>	10.45±0.07 <sup>a</sup>	10.01±0.01 <sup>e</sup>	9.99±0.02 <sup>c</sup>	2.86±0.01 <sup>e</sup>	1.82±0.03 <sup>g</sup>	2.62±0.02 <sup>f</sup>
MPP	0.64±0.13 <sup>a,b</sup>	0.82±0.02 <sup>a</sup>	0.52±0.01 <sup>b,c</sup>	0.41±0.03 <sup>c</sup>	0.34±0.01 <sup>c</sup>	-	-	-
OOO	3.97±0.32 <sup>c,d</sup>	3.94±0.02 <sup>d</sup>	3.70±0.13 <sup>d</sup>	4.36±0.01 <sup>b</sup>	4.27±0.04 <sup>b,c</sup>	5.10±0.01 <sup>a</sup>	5.19±0.03 <sup>a</sup>	4.33±0.21 <sup>b</sup>
POO	24.76±1.18 <sup>a</sup>	21.89±0.01 <sup>c</sup>	23.34±0.02 <sup>b</sup>	24.89±0.01 <sup>a</sup>	24.79±0.04 <sup>a</sup>	22.34±0.04 <sup>c</sup>	24.91±0.02 <sup>a</sup>	20.67±0.11 <sup>d</sup>
PPO	31.61±0.17 <sup>b</sup>	30.27±0.02 <sup>d</sup>	31.15±0.08 <sup>c</sup>	30.19±0.01 <sup>d</sup>	32.16±0.02 <sup>a</sup>	7.49±0.01 <sup>g</sup>	9.70±0.01 <sup>f</sup>	10.63±0.01 <sup>c</sup>
PPP	4.77±1.77 <sup>c</sup>	9.27±0.05 <sup>a</sup>	5.48±0.01 <sup>b</sup>	3.84±0.02 <sup>d</sup>	2.88±0.03 <sup>e</sup>	0.35±0.02 <sup>f</sup>	0.40±0.01 <sup>f</sup>	0.38±0.00 <sup>f</sup>
SOO	2.72±0.29 <sup>d</sup>	2.02±0.02 <sup>g</sup>	2.21±0.01 <sup>f</sup>	2.51±0.01 <sup>e</sup>	2.50±0.02 <sup>e</sup>	3.28±0.02 <sup>c</sup>	3.80±0.01 <sup>a</sup>	3.62±0.04 <sup>b</sup>
SPO	5.65±0.01 <sup>d</sup>	5.19±0.02 <sup>e</sup>	5.32±0.03 <sup>e</sup>	5.31±0.01 <sup>e</sup>	5.72±0.02 <sup>d</sup>	13.18±0.01 <sup>a</sup>	11.67±0.03 <sup>c</sup>	12.52±0.12 <sup>b</sup>
PPS	0.92±0.01 <sup>b</sup>	1.78±0.01 <sup>a</sup>	0.95±0.01 <sup>b</sup>	0.74±0.03 <sup>c</sup>	0.53±0.03 <sup>d</sup>	0.55±0.0 <sup>d</sup>	0.49±0.01 <sup>d</sup>	0.81±0.00 <sup>c</sup>
SSO	0.42±0.13 <sup>c</sup>	0.51±0.01 <sup>c</sup>	0.32±0.02 <sup>c</sup>	0.43±0.03 <sup>c</sup>	0.43±0.01 <sup>c</sup>	0.92±0.02 <sup>a,b</sup>	1.07±0.05 <sup>a</sup>	0.83±0.01 <sup>b</sup>
SSS	-	-	-	-	-	0.36±0.03 <sup>b</sup>	0.36±0.01 <sup>b</sup>	1.31±0.01 <sup>a</sup>
Others	-	-	-	-	-	0.71±0.01	0.45±0.01	1.43±0.13

\*Each value in the table represents the mean ± standard deviation of two replicates. Means within row with different superscripts are significantly ( $p < 0.05$ ) different. LLLn dilinoleoyl-3-linolenoleoyl glycerol, LLL trilinoleoyl glycerol, MMM trimyristoyl glycerol, OLL dilinoleoyl-1-oleoyl glycerol, PLL dilinoleoyl-1-palmitoyl glycerol, MPL myristoyl-palmitoyl-linoleoyl glycerol, OOL dioleoyl-3-linoleoyl glycerol, POL palmitoyl-oleoyl-linoleoyl glycerol, PPL dipalmitoyl-1-linoleoyl glycerol, MPP dipalmitoyl-1-myristoyl glycerol, OOO trioleoyl glycerol, POO dioleoyl-1-palmitoyl glycerol, PPO dipalmitoyl-3-oleoyl glycerol, PPP tripalmitoyl glycerol, SOO dioleoyl-1-stearoyl glycerol, PSO palmitoyl-stearoyl-oleoyl glycerol, PPS dipalmitoyl-3-stearoyl glycerol, SSS tristearoyl glycerol

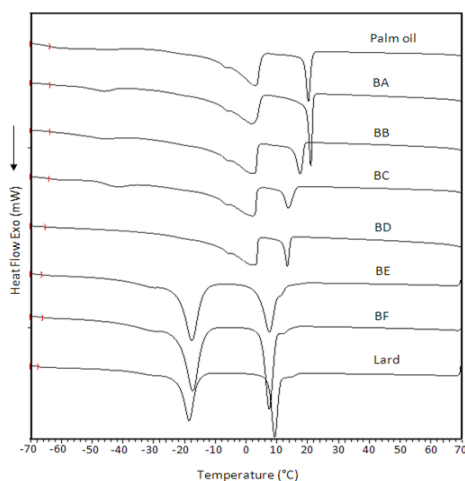


Figure 3. DSC cooling curves of palm oil, lard and oils of six coded samples of commercial biscuits

compared with that of palm oil as shown in Figure 1. As the TAG distributional profile of palm oil has already been established in many previous studies, it can be used for comparison purposes (Marikkar *et al.*, 2005; Yanty *et al.*, 2012). As such, the TAG peaks of palm oil were identified as 1:MMM, 2: PLL, 3: MPL, 4: OOL, 5: POL, 6: PPL, 7: MPP, 8:OOO, 9: POO, 10: PPO, 11: PPP, 12: SOO, 13: SPO, 14: PPS, and 15: SOS (M, myristic; L, linoleic; P, palmitic; O, oleic; S, stearic) based on the retention times of a set of TAG standards as well as the previous reports (Marikkar *et al.*, 2005; Yanty *et al.*, 2012). Most of the local brands of biscuits were found to have TAG profiles similar to that of palm oil as they were also found to have PPO, POO, PPL and POL as major TAG species. The quantitative data presented in Table 3 showed a comparison between palm oil and biscuit

brands with regard to the proportional distribution of individual TAG molecular species. The local brands BB, BC, and BD displayed minimal differences from palm oil with regard to the proportions of the dominant TAG molecular species, namely POO, PPO, and POL (Table 3). However, sample BA showed some remarkable deviations with respect to the proportions of PPO and PPP.

An overlay of chromatograms shown in Figure 2 was to compare the TAG profiles of lipids extracted from the two imported brands (BE and BF) with that of lard. Lard was found to possess POO, POL, SPO, and PPO as the major TAG molecular species, which is in agreement with the findings reported previously (Marikkar *et al.*, 2005; Yanty *et al.*, 2012). According to Table 3, these were also the dominant TAG molecular species present in biscuit brands BE and BF. Meanwhile, other TAG molecular species such as OOO, OOL, PPL, and SOO were found to occur in lesser amounts in lard as well as brands BE and BF. According to Table 3, the noted differences in the proportions of POL and PPO would make the local brands to be distinctly different from the imported brands. Hence, these two TAG molecular species could be taken as effective discriminating parameters to distinguish the local and imported brands of biscuits.

#### Thermal characteristics by DSC cooling curves

The DSC cooling curves of lipids extracted from different brands of biscuits are compared to those of lard and palm oil as shown in Figure 3. The DSC cooling curve of palm oil used in this study was found

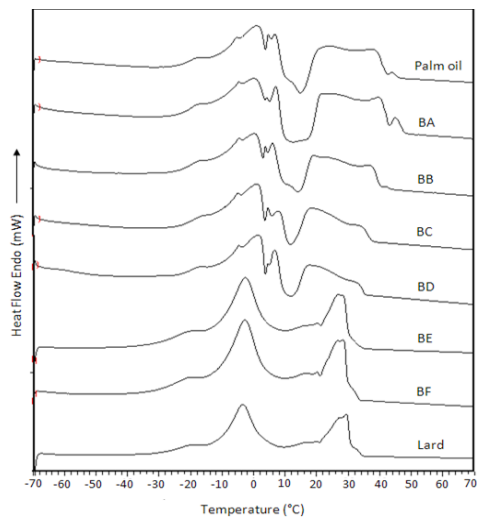


Figure 4. DSC heating curves of palm oil, lard and oils of six coded samples of commercial biscuits

to exhibit a sharp high-melting exo-thermic peak at 20.1°C and a broad low-melting exo-thermic peak at 3.05°C. These features of palm oil were comparably similar to those reported previously (Marikkar *et al.*, 2005; Yanty *et al.*, 2012). The overlay of the DSC cooling curves showed that the four local brands namely BA, BB, BC, and BD were found to possess thermal profiles somewhat similar to that of palm oil. Having a sharp high-melting transition (0 to -4°C) and a broad low-melting thermal transition with peak maxima in the range of -51.0 to -53.0°C was a common feature among them. However, the positions of individual DSC thermal transitions of these local brands were slightly different from those of palm oil due to slight differences in fatty acid and TAG compositions (Tables 1 and 3). These differences are nothing unusual since the effect of compositional differences on DSC thermal transitions of oils and fats has been discussed in many previous reports (Tan and Che Man, 2000; Marikkar *et al.*, 2001; Marikkar *et al.*, 2005). On the other hand, the DSC cooling curves displayed by the two imported brands namely, BE and BF were found to display profiles similar to the characteristic features of lard profile. For instance, both of these samples and lard displayed a sharp high-melting thermal transition appearing in the range of 8.0 to 10.0°C and a sharp low-melting thermal transition appearing in the range of -17.0 to 19.0°C. Lard possessing a sharp high and low-melting transition in wide separation was a common characteristic feature found in many previous studies (Marikkar *et al.*, 2001; Yanty *et al.*, 2012). As mentioned earlier, the DSC thermal transitions of BE and BF do not tally exactly with those of lard due to slight differences in their fatty acid and TAG compositions (Tables 1 and 3). According to Figure 3, the major differences in the low-melting transitions

of thermal profiles would make the two imported brands to be distinctly different from those of the four local brands. Hence, the nature of the shape and positions of the low-melting transition could be taken as effective discriminating parameter to distinguish the local and imported brands of biscuits.

#### *Thermal characteristics by DSC heating curves*

The DSC heating curves of lipids extracted from different brands of biscuits are compared to those of lard and palm oil as shown in Figure 4. For convenience, the heating curve of palm oil can be perceived as possessing a broad high melting group (HMG) of peaks representing the higher melting triacylglycerols and a broad low melting group (LMG) peak representing the lower melting triacylglycerols. The HMG peak appeared to be in the temperature range starting from 16.0 to 45.75°C while those belonging to LMG appeared in the temperature region extending from -21.5 to 15.0°C. These thermodynamic features of the heating curve of palm oil are in accordance with the previous reports (Tan and Che Man, 2000; Marikkar *et al.*, 2001). According to the overlay presented in Figure 4, lipids extracted from the four local brands displayed profiles somewhat similar to that of palm oil. As a common feature, all four local brands were found to display thermal curves with two broad distinguishable HMG and LMG regions though the positions of the thermal transitions of their profiles were not exactly similar to that of palm oil. As mentioned before, this could be attributed to the fatty acid and TAG compositional differences among them (Tables 1 and 3). On the other hand, the DSC profiles of the two imported brands BE and BF, and lard were found to be comparably similar in many features. Both of these brand possessed with two sharp HMG and LMG regions as similar to lard, whose thermal profile was also found to possess two major sharp endo-thermic peaks at -3.6 and 29.2°C, to represent its HMG and LMG regions (Yanty *et al.*, 2011; Yanty *et al.*, 2012). As seen before, the positions of the individual thermal transitions of these two brands were found to deviate slightly from those of lard due to compositional differences as elaborated before (Tables 1 and 3).

#### **Conclusions**

This study demonstrated the ways of identifying biscuits formulated with lard on the basis of fatty acid and TAG compositional data, as well as DSC cooling and heating profiles. Biscuit brands BE and BF, and lard were found to have relatively higher stearic acid contents than the four local brands used in this study.

The occurrence of excessive amounts of palmitic acid in the *sn*-2 position of these two imported brands (BE & BF) was a characteristic feature synonymous with lard. Overlay of TAG chromatograms showed that brands BE and BF had TAG profiles similar to that of lard while brands BA, BB, BC, & BD displayed TAG profiles similar to that of palm oil. Likewise, overlay of DSC cooling and heating curves showed that brands BE and BF had thermal profiles similar to that of lard while brands BA, BB, BC, & BD displayed thermal profiles similar to that of palm oil.

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