

## Differentiation of Fractionated Components of Lard from Other Animal Fats Using Different Analytical Techniques

(Perbezaan Komponen Berperingkat Lemak Babi daripada Lemak Haiwan Lain Menggunakan Teknik Analitik Berbeza)

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### ABSTRACT

*A study was conducted to differentiate fractionated components of lard namely lard olein (LO) and lard stearin (LS) from other common animal fats. Lard fractions and animal fats were analyzed using differential scanning calorimetry (DSC), elemental analyzer–isotope ratio mass spectrometry (EA-IRMS), gas chromatography mass spectrometry (GC-MS) and Fourier transform infrared spectroscopy (FTIR). Overlay of FTIR spectra did not help to pinpoint any characteristic feature to distinguish either LO or LS from other animal fats, but overlay of DSC cooling curves helped a successful discrimination. The determination of  $\delta^{13}\text{C}$  from EA-IRMS showed that the values corresponding to the fractionated components of lard were significantly ( $p < 0.05$ ) different from those of the other common animal fats. GC-MS analysis showed that direct comparison of overall fatty acid data was not able to discriminate LO and LS from other animal fats, but the application of principal component analysis (PCA) to fatty acid data helped a successful discrimination.*

*Keywords: EA-IRMS; FTIR; GC-MS; halal; lard; lard olein; lard stearin*

### ABSTRAK

*Satu kajian telah dijalankan untuk membezakan komponen berperingkat lemak babi iaitu lemak babi olein (LO) dan lemak babi stearin (LS) daripada lemak haiwan biasa yang lain. Pecahan lemak babi dan lemak haiwan dianalisis menggunakan kalori pengimbasan kebezaan (DSC), unsur penganalisis-isotop nisbah jisim spektrometri (EA-IRMS), kromatografi gas spektrometri jisim (GC-MS) dan spektroskopi transformasi Fourier inframerah (FTIR). Lengkung FTIR spektrum tidak membantu untuk menentukan apa-apa ciri-ciri untuk membezakan sama ada LO atau LS daripada lemak haiwan lain, tetapi lapisan lengkung penyejukan DSC telah berjaya membantu diskriminasi. Penentuan  $\delta^{13}\text{C}$  daripada EA-IRMS menunjukkan bahawa nilai yang sepadan dengan komponen berperingkat lemak babi secara ketara ( $p < 0.05$ ) berbeza daripada lemak haiwan biasa lain. GC-MS menunjukkan bahawa perbandingan langsung asid lemak keseluruhan data adalah tidak dapat membezakan LO dan LS dari lemak haiwan yang lain, tetapi penggunaan analisis komponen utama (PCA) kepada data asid lemak membantu diskriminasi yang berjaya.*

*Kata kunci: EA-IRMS; FTIR; GC-MS; halal; lemak babi; lemak babi olein; lemak babi stearin*

### INTRODUCTION

Determining the authenticity of foods is an ever increasing challenge to food analysts. As adulteration practices are becoming more subtle and complicated, the possibility for detection becomes extremely difficult. Despite this, the efforts taken on food authentication are still helpful to some extent to reduce false description/incorrect labeling, substitution of cheaper ingredients and other modes of adulteration. As speculative reports of food adulterations are on the rise in the social media, consumers around the world have become increasingly cautious about the quality of the food they purchase. Food manufactures are at times compelled to confirm the authenticity of their finished products or ascertain the source of origin of the ingredients used in formulations. This increased demand for authenticity also comes amid various legislative and

regulatory pushes that increase the level of regulation imposed on the food supply worldwide.

Occurrence of fat extracted from swine in food is an important concern for followers of certain religious faiths. *Halal* and *kosher* food regulations implemented in a number of countries prohibit the use of either lard or its fractionated products such as LS and LO in any food system (Regenstein et al. 2003). Hence, several studies have been undertaken previously to evaluate the use of different analytical approaches to discriminate lard from other animal fats. Marikkar et al. (2001) demonstrated the use of DSC analysis to distinguish lard from chicken fat, beef fat and mutton fat. Later on, Che Man et al. (2011) used FTIR spectroscopy to investigate the possibility of discriminating admixtures of animal fats containing lard. Subsequently, Chin et al. (2009) followed by Dias

et al. (2010) employed GC×GC-Time of Flight (ToF) MS to differentiate lard from other animal fats using branched fatty acids containing carbon atoms of more than 18. In this regard, chemometric methods such as principal component analysis were also employed to solve problems related to discrimination of identity (Christy & Egeberg 2006). In any of these studies, discriminating the fractionated lard components namely, LO and LS from other animal fats was not investigated. However, lard as an edible fat could be utilized in diversified forms in food systems. LS has been used either as shortening or fat component to improve the firmness of lard, while LO was suitable as a frying medium or bread pan lubricating agent. Since the use of LS and LO are also prohibitive under *Halal* and *kosher* food regulations, there is a need to develop methods based on various analytical techniques to discriminate them from other permissible food items. Hence, the objective of this study was to evaluate gas chromatography mass spectrometry (GC-MS), elemental analyzer-Isotope ratio mass spectrometry (EA-IRMS), differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR) techniques for discrimination of LO and LS from other animal fats such as chicken fat, beef fat and mutton fat.

## MATERIALS AND METHODS

### MATERIALS

The samples of animal fats namely lard, beef fat (BF), mutton fat (MF) and chicken fat (CF) were extracted from adipose tissues of animals collected from local slaughter houses located at three different places in Sri Serdang, Malaysia. Adipose tissues were rendered at an oven temperature of 100°C for 6 h and animal fats were filtered separately through double-folded muslin cloth to remove impurities. After adding the samples with small proportions of anhydrous sodium sulphate to remove residual moisture, they were filtered through Whatman No. 2 filter paper and stored at 4°C (Marikkar et al. 2001). All the reagents and chemicals used in this study were either analytical or HPLC grade unless otherwise specified (acetone, hexane, sodium hydroxide). A standard mixture containing 37 fatty acid methyl esters (FAME) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

### FRACTIONAL CRYSTALLIZATION OF LARD

Fractional crystallization was carried out using acetone as solvent medium. Lard was melted at 60°C and mixed with acetone in 1:2 (w/v) ratios. The solution was boiled at 60°C until it became uniformly dissolved and left at 5±1°C for 24 h to crystallize. The precipitated fat was filtered off to give a high melting fat fraction known as lard stearin (LS). After removing the precipitate, the mother-liquor was evaporated under reduced pressure to yield a liquid called lard olein (LO) (Yanty et al. 2013).

### FATTY ACID ANALYSIS

FAMES were prepared by dissolving 50 mg portion of oil in 0.8 mL of hexane and adding 0.2 mL portion of 1 M solution of sodium methoxide (PORIM 1995). The clear supernatant of the solution was transferred into a 2 mL auto-sampler vial. The top hexane layer FAME solution (1 µL) was injected on an Agilent 6890N GC (Agilent Technologies, Singapore) equipped with a polar capillary column DB-23 (0.15 mm internal diameter, 60 m length and 0.25 µm film thickness; Agilent Technologies, Singapore) and an Agilent 5973 MS. Split injection was conducted with a split ratio of 100:1, using helium as a carrier gas at a flow rate of 1.00 mL/min. The temperature of the column was 50°C (for 1 min) and programmed to increase to 175°C at the rate of 25°C/min. Then, it was increased at the rate of 4°C/min to 230°C for 5 min. The temperatures of the injector and detector were maintained at 250°C. The identification of individual peak of FAMES was done by comparing their retention time with those of 37 FAME standards (Supelco Bellefonte, PA). The percentage of individual fatty acid of samples was calculated as the ratio of the partial area to the total area (Yanty et al. 2013).

### FTIR MEASUREMENTS

FTIR spectra of all fat samples (lard, CF, BF, MF, LO and LS) were recorded using an FTIR Nicolet 6700 spectrometer (Thermo Nicolet, Madison, WI) equipped with deuterated triglycine sulphate (DTGS) detector and KBr beam splitter. The samples were placed in contact with the horizontal attenuated total reflectance (HATR) element (ZnSe crystal) at controlled ambient temperature (25°C) using a Pasteur pipette. FTIR spectra were collected in the mid-infrared region of 4000–650 cm<sup>-1</sup> by co-adding 32 scans and at a resolution of 4 cm<sup>-1</sup>. All spectra were ratioed against a background of air spectrum. After each scan, a new reference air background spectrum was taken. Spectra were recorded as absorbance values at each data point in triplicate. The manufacturer's software (OMNIC operating system, version 7.0 Thermo Nicolet) program was used to analyze spectral data (Che Man et al. 2011).

### THERMAL ANALYSIS

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, Schwyzbeanch, Switzerland). Nitrogen (99.999% purity) was used as the purge gas at a rate of ~20 mL/min. Approximately, 4–8 mg of melted sample was placed in a standard DSC aluminium pan and sealed hermetically. An empty, hermetically sealed DSC aluminium pan was used as the reference. The oil samples were subjected to the following temperature program: 70°C isotherm for 1 min, cooled at 5°C/min to -70°C (Yanty et al. 2013).

#### DETERMINATION OF $\delta^{13}\text{C}$ VALUES

About 0.2  $\mu\text{g}$  of each animal fat was weighed and loaded into a clean tin capsule to determine their  $\delta^{13}\text{C}$  values. The capsules containing samples were placed in auto-sampler system of elemental analyzer (Europa Scientific, UK) to be burnt in an  $\text{O}_2$  atmosphere of the combustion  $\text{CuO}$  tube with its temperature set at  $960^\circ\text{C}$ . Combustion gases were eluted through a reduction column by a stream of He gas and passed into the gas chromatograph where  $\text{CO}_2$ , still in the He stream, was separated from the other gases. The gas stream was then entered into the IRMS system (Sercon Ltd., Crewe, U.K.) where the  $\text{CO}_2$  gas was analyzed by comparison with NBS-22 reference material (with a  $\delta^{13}\text{C}$  value of  $-30.03\%$ ). During every batch of analyses, an empty tin capsule was analyzed as the blank to check the background (Liu et al. 2007). The results were referenced to Vienna Pee Dee Belemnite (V-PDB). The isotopic values were calculated against the international isotope reference standards: NBS-22 (International Atomic Energy Agency, Vienna, Austria) for  $^{13}\text{C}/^{12}\text{C}$  measurements (Osorio et al. 2011).

#### STATISTICAL ANALYSIS

Data were statistically analyzed by one-way analysis of variance (ANOVA) using MINITAB (version 14) statistical package at 0.05 probability level. For grouping and classification models, PCA was carried out using Unscrambler 9.7 (Camo, USA) software.

#### RESULTS AND DISCUSSION

##### FATTY ACID COMPOSITION OF FATS

The data presented in Table 1 compares the fatty acid distribution of LS and LO with those of lard, CF, BF and MF. Some of the data on fatty acid compositions of animal fats obtained in this study were in accordance with the findings reported previously (Marikkar 2015; Rohman & Che Man 2010). As a common feature, LO, CF and lard were found to possess higher percentage of unsaturated fatty acids than saturated fatty acids. Similar to CF and lard, oleic (C18:1) was the major fatty acid of LO followed by linoleic (C18:2) and palmitic (C16:0) acids. However, LO and lard showed differences with respect to stearic acid (C18:0). This was mainly due to the fact that a portion of stearic acid (C18:0) would have migrated in to the solid phase during crystallization of lard. According to statistical analysis, there were no significant ( $p>0.05$ ) differences between LO and CF with respect to oleic (C18:1), myristic (C14:0) and lauric acids (C12:0), but significant ( $p<0.05$ ) differences were noted with regard to the rest of the fatty acids. On the other hand, LO differed significantly ( $p<0.05$ ) from BF and MF with respect to all fatty acids except lauric (C12:0). This was due to the fact that both BF and MF had higher percentage of saturated fatty acids than unsaturated fatty acids when compared to LO.

LS being the higher melting fraction of lard displayed a fatty acid composition which was markedly different from that of either lard or LO. Similar to BF and MF, LS was also found to possess higher percentage of saturated fatty acids than unsaturated fatty acids. The major fatty acids of LS were palmitic (C16:0) followed by oleic (C18:1) and stearic (C18:0), comprising about 83% of the total FA distribution. These fatty acids were also the most abundant in BF and MF as reported in this study as well as previous reports (Marikkar et al. 2005). Although no significant ( $p>0.05$ ) differences were noticed among LS, BF and MF with respect to lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids, there were significant ( $p<0.05$ ) differences among them with regard to stearic (C18:0) and linoleic (C18:2) acids.

##### DSC COOLING CURVES OF FATS

The DSC cooling curves of LS and LO are compared to those of BF, CF and MF as shown in Figure 1. When compared to lard and other animal fats, both LS and LO displayed significant differences on the cooling curves that can easily distinguish them from the rest of the samples. For instance, the cooling curve of LO as shown in Figure 1 displayed its major exothermic transition at  $-36.1^\circ\text{C}$  and the minor peaks at  $-2.5^\circ\text{C}$ . In comparison to either lard or any other animal fat, it did not display any high-melting thermal transition in the temperature region above  $0^\circ\text{C}$ . On the other hand, other animal fats displayed cooling profiles that were more complex in nature involving multiple thermal transitions both in the high-and low-melting regions. Unlike LO, both BF and MF showed sharp thermal transitions at  $18.2$  and  $29.0^\circ\text{C}$ , respectively. As discussed previously in other reports (Marikkar 2015; Rohman & Che Man 2010), this difference could be due to the dominance of low-melting TAG molecules largely esterified with highly-unsaturated fatty acids in LO.

The high melting fraction, LS, displayed major and minor transitions at  $18.4^\circ\text{C}$  and  $21.8^\circ\text{C}$ , respectively, but no significant thermal transition was seen in the temperature range below  $15.0^\circ\text{C}$ . On the other hand, other animal fats displayed broad thermal transitions below  $15.0^\circ\text{C}$ . For instance, other animal fats namely BF, MF and CF displayed their low-melting transitions in the negative temperature region. The reason for these thermal property differences shown by LS and other animal fats could be most probably related to their TAG compositional differences. After fractionation from lard, LS has lost larger proportion of the unsaturated TAG molecules from its compositions. As more and more saturated TAG molecules get accumulated in LS during fractional crystallization, it was found to become enriched with more saturated fatty acids (Table 1). These extreme changes in the molecular composition would have strong impact on the thermal profiles displayed by lipids as discussed in several previous studies (Marikkar 2015). As a consequence, the thermal transition corresponding to the unsaturated TAG molecules in the negative temperature region was seemed to disappear in LS.

TABLE 1. Comparison of the fatty acid composition (peak area %) of lard olein and lard stearin with those of other animal fats<sup>1</sup>

Fatty acid	Lard olein	Lard stearin	Lard	Chicken fat	Beef fat	Mutton fat
C12:0	0.22±0.00 <sup>a</sup>	0.1±0.00 <sup>a</sup>	0.58±0.56 <sup>a</sup>	0.64±0.93 <sup>a</sup>	0.11±0.05 <sup>a</sup>	0.46±0.06 <sup>a</sup>
C14:0	1.26±0.40 <sup>b</sup>	1.00±0.17 <sup>b</sup>	1.27±0.22 <sup>b</sup>	1.62±0.65 <sup>b</sup>	6.15±0.31 <sup>a</sup>	6.40±0.49 <sup>a</sup>
C16:0	21.16±0.60 <sup>c</sup>	29.15±1.06 <sup>a</sup>	22.77±0.96 <sup>bc</sup>	25.39±1.01 <sup>ab</sup>	31.07±0.78 <sup>a</sup>	27.38±1.22 <sup>a</sup>
C16:1	1.60±0.19 <sup>c</sup>	0.78±0.05 <sup>cd</sup>	1.46±0.07 <sup>bcd</sup>	5.32±0.48 <sup>a</sup>	2.56±0.07 <sup>b</sup>	0.52±0.18 <sup>d</sup>
C18:0	7.13±0.68 <sup>c</sup>	26.33±0.77 <sup>b</sup>	11.88±0.86 <sup>d</sup>	4.84±0.18 <sup>f</sup>	16.53±1.25 <sup>c</sup>	30.90±0.50 <sup>a</sup>
C18:1	43.57±0.54 <sup>a</sup>	27.69±1.54 <sup>d</sup>	40.49±2.18 <sup>ab</sup>	43.94±1.77 <sup>a</sup>	35.70±1.71 <sup>bc</sup>	29.82±1.04 <sup>cd</sup>
C18:2	21.76±2.66 <sup>a</sup>	12.02±0.10 <sup>b</sup>	18.23±3.45 <sup>a</sup>	18.26±1.64 <sup>ab</sup>	6.59±0.61 <sup>c</sup>	1.61±0.06 <sup>c</sup>
Others	3.31	2.92	3.18	N.d.	1.28	2.9
ΣSFA	30.69	58.40	37.54	32.48	55.50	68.84
ΣUSFA	66.93	40.49	60.18	67.52	44.85	31.95

<sup>1</sup>Each fatty acid value in the table represents the mean ± standard deviation of three replicates. Means within each row with different superscripts are significantly (p<0.05) different. Abbreviations: SFA, saturated fatty acid; USFA, unsaturated fatty acid.

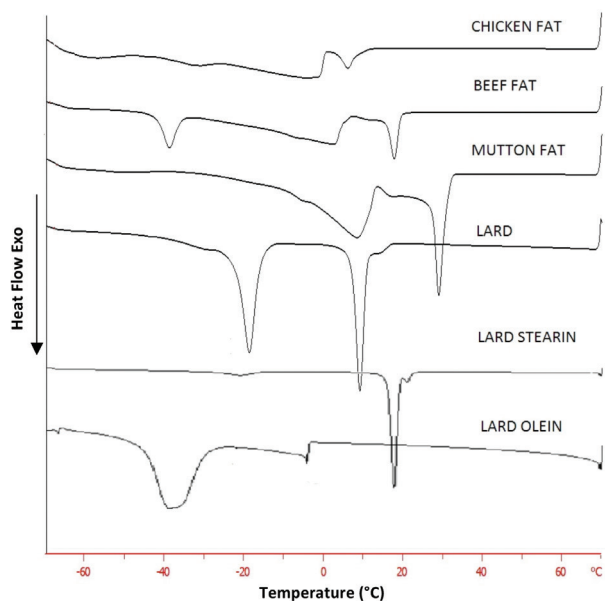


FIGURE 1. DSC cooling curves for chicken fat, beef fat, mutton fat, lard, lard stearin and lard olein

#### FTIR SPECTRA OF FATS

The overlay of FTIR spectra shown in Figure 2 compares the spectral features of LO and LS with those of lard, CF, BF and MF. Previous reports on the characterization of the spectral features of LO and LS were inadequate in the literature. Although the overall appearances of the spectra of LO and LS were roughly similar, differences were seen mainly in the intensity of their bands in certain regions and the exact frequency where maximum absorbance was produced. According to previous reports (Guillen et al. 2003; Safar et al. 1994; Van de Voort & Sedman 2000), the differences in the spectral patterns of edible oils and fats were attributed to molecular and structural differences such as degree of unsaturation and chain length, monounsaturated to polyunsaturated acyl group ratio, variations in *trans* fatty acid content and presence

of naturally occurring minor components in oils. In mid IR spectroscopy of oils and fats, a medium absorption band at approximately  $3008\text{ cm}^{-1}$  was due to the *cis* olefinic stretching vibration and its intensity and the value of exact frequency might vary depending on the degree of unsaturation of the oil sample. As shown in Figure 2, LO and chicken fat showed high intensity and high frequency value for this band while the low intensity and lower frequency values were recorded for LS, BF and MF. It was because LO and chicken fat has higher degree of unsaturation while LS, BF and MF displayed lower degree of unsaturation (Table 1). The two strong absorption bands occurred at  $2918$  and  $2853\text{ cm}^{-1}$  were due to the methylene asymmetrical stretching and methylene symmetrical stretching vibrations, respectively (Guillen et al. 2003; Safar et al. 1994; Van de Voort & Sedman 2000). These two bands, however, did not show any particular characteristic feature which could be used to differentiate LO and LS from lard and other animal fats.

Toward the central part of the spectra, all six fats showed strong absorption band at  $1748.5\text{ cm}^{-1}$ , which is usually assigned to stretching vibration of carbonyl functional group. The carboxylic acid absorption occurring at about  $1710\text{ cm}^{-1}$  would normally be not resolved from this peak and partly explains the broadening effect (Van de Voort & Sedman 2000). All six fats were found to exhibit a band near  $1457\text{ cm}^{-1}$ , which was the bending mode of methylene group. Further down the frequency, all six fats exhibited another band near at  $1378\text{ cm}^{-1}$  which is usually assigned to symmetrical bending vibration of  $-\text{CH}(\text{CH}_3)$  bond (Safar et al. 1994). Both bands near  $1220\text{ cm}^{-1}$  as well as  $1163\text{ cm}^{-1}$  are associated with the vibration of the C-O ester groups and the bending vibration of the  $\text{CH}_2$  group (Guillen et al. 2003). Below this region, all six fats showed a dual-band at  $1119.2$  and  $1093\text{ cm}^{-1}$ , where the magnitude of the bands were inversely related to the proportion of saturated acyl groups and oleic acyl group, respectively (Guillen et al. 2003; Safar et al. 1994; Van de Voort & Sedman 2000). All these showed that the possibility to

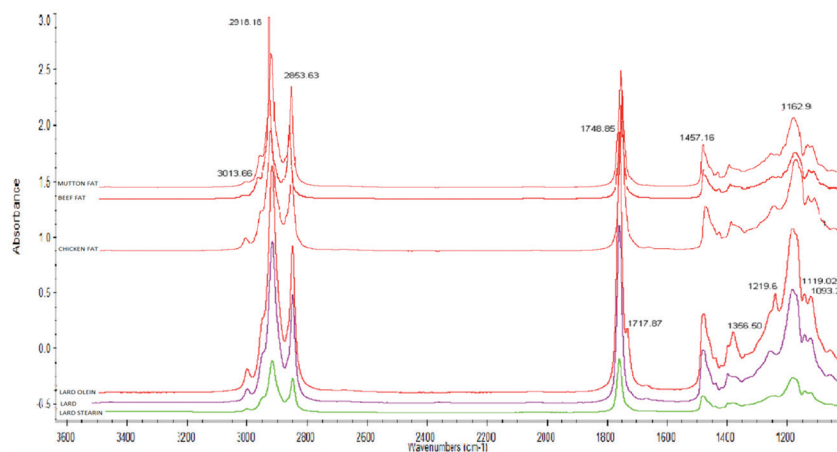


FIGURE 2. FTIR spectra for chicken fat, beef fat, mutton fat, lard, lard stearin and lard olein in the mid infrared region ( $4000\text{--}650\text{ cm}^{-1}$ )



pinpoint any characteristic feature in FTIR to distinguish either LO or LS from other animal fats was little.

#### $\delta^{13}\text{C}$ VALUES OF FATS

The data presented in Table 2 was to compare the  $\delta^{13}\text{C}$  values of LO and LS with those of lard and other animal fats. There was hardly any report giving details of  $\delta^{13}\text{C}$  values of LO and LS in the literature. According to Table 2, the highest  $\delta^{13}\text{C}$  value of  $-20.3\%$  was found for CF while the lowest value of  $-33.2\%$  was found for MF. As reported previously by Nina et al. (2013), significant ( $p < 0.05$ ) differences were noticed between the  $\delta^{13}\text{C}$  values of lard and other common animal fats namely CF, BF, and MF. According to Gonzalez et al. (1999), the  $\delta^{13}\text{C}$  value of swine's adipose tissue was found to range from  $-22.14$  to  $-23.87\%$  and the value was similar to  $\delta^{13}\text{C}$  value of lard extracted from the adipose tissues. However, no significant differences were noticed between the  $\delta^{13}\text{C}$  values of lard and its fractionated components (LO and LS) indicating that EA-IRMS technique cannot differentiate lard and its fractionated components. This should be expected as LO and LS were fat components derived from the same lard sample, whose  $^{13}\text{C}/^{12}\text{C}$  ratio would remain a constant even after fractionation.

TABLE 2. Comparison of  $\delta^{13}\text{C}$  values of lard stearin and lard olein with those of other animal fats<sup>1</sup>

Animal Fat	<i>n</i>	$\delta^{13}\text{C}$ (‰)	
		Mean	<i>Sd</i>
Chicken fat	3	$-20.3^d$	0.93
Lard	3	$-23.2^c$	0.97
Lard olein	3	$-23.4^c$	0.41
Lard stearin	3	$-22.6^c$	0.89
Beef fat	3	$-29.5^b$	0.87
Mutton fat	3	$-33.2^a$	0.32

<sup>1</sup>Means within each column with different superscripts are significantly ( $p < 0.05$ ) different. Abbreviation: *Sd*, standard deviation.

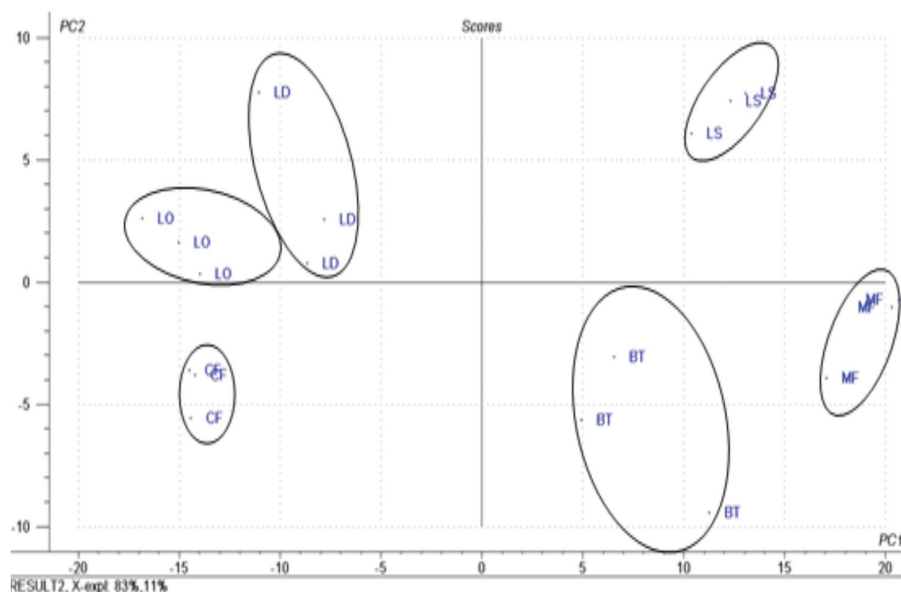
#### PCA OF FATTY ACID DATA

According to the fatty acid distributional data presented in Table 1, lard, LO and CF were found to contain palmitic, oleic and linoleic acid as the major fatty acids while LS, BF and MF had a common characteristic feature by having palmitic, stearic and oleic acids as their major fatty acids. In such situations, it might be difficult to find differentiation of LO and LS from other animal fats using mere comparison of the overall fatty acid data. However, application of multivariate data handling techniques such as PCA would enable researchers to extract more subtle information that could lead to the differentiation among them. PCA has already been recognized for its capability to identify patterns in data and expressing the data in such a way as to emphasize their similarities and differences (Brodnjak-Voncina et al. 2005). For instance, this approach has been studied for authentication of commercial edible oils (Lee et al. 1998), classification of

coffee varieties (Alves et al. 2003) and peanut cultivars (Shin et al. 2011). For the application of PCA in this study, stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids were selected as independent variables since they were found to occur in higher proportions in all animal fats concurrently showing significant ( $p < 0.05$ ) differences among the animal fats. The score plot of fatty acids from the fractionated components of lard and other animal fats shown in Figure 3 represents the projection of samples defined by principal component 1 (PC1) describing 83% of the variation while PC2 accounted for 11% of the variation, making up 94% of variance explained in PC1 and PC2. Based on the group separation illustrated in Figure 3, lard and LO were located in upper left quadrant, while CF was situated in lower left quadrant. The close proximity between LO and lard was probably due to their close similarity in fatty acid distribution (Table 1). In Figure 3, LS was found in the upper right quadrant while MF and BF were found to be in lower right quadrant. Fatty acid variables giving higher influence on the group separation of the samples in the score plot could be traced by the analysis of the loading plot. According to the loading plot in Figure 4, stearic, oleic and linoleic acids were the three fatty acids, which showed greater influence in group separation into six different clusters. As explained by Cordella et al. (2003), a variable which was farther from the origin of axis contributed to the most variation in the statistical model generated by the PCA. Among these, stearic acid was the most discriminating variable that influences the separation of the sample series along PC1 axis, while linoleic and oleic acids were the most discriminating variables along PC2 axis.

#### CONCLUSION

This study investigated the use of GC-MS, EA-IRMS, DSC and FTIR techniques for discrimination of LO and LS from other animal fats namely CF, BF and MF. LO and LS successfully differentiated from other animal fats using EA-IRMS fats since there were significant differences with regard to their carbon isotope ratios ( $\delta^{13}\text{C}$ ). Analysis by FTIR spectroscopy did not show any characteristic feature peculiar to either LO or LS to discriminate them from other animal fats. The use of DSC for the same purpose was easier as the nature of the DSC profiles displayed by LO and LS were distinctly different from those of CF, BF and MF. According to GC-MS results, direct comparison of overall fatty acid data may not be useful to discriminate LO and LS from other animal fats. However, successful discrimination was achieved when the fatty acid data was subjected to PCA. According to the outcome of PCA, stearic, oleic and linoleic acids were found to be the most discriminating parameters in the clustering lard fractions away from other animal fats. Hence, the  $\delta^{13}\text{C}$  values and combination of fatty acid composition and PCA could be highly potential methods to differentiate fractionated components of lard from other animal fats.



Abbreviations: LO, Lard Olein; LS, Lard Stearin; LD, Lard; CF, Chicken fat; BF, Beef fat; MF, Mutton fat

FIGURE 3. Score plot of PCA of lard stearin, lard olein and other animal fats based on fatty acid composition

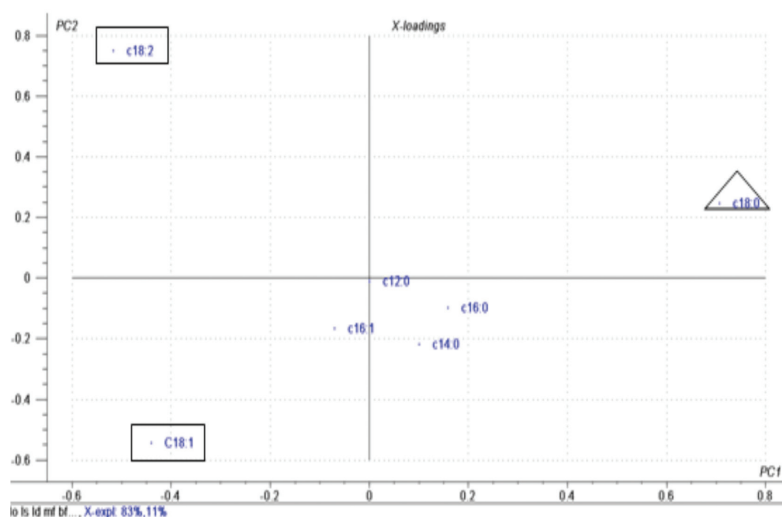


FIGURE 4. Loading plot of PCA of lard stearin, lard olein, and other animal fats based on fatty acid composition (PC1:  $\Delta$ , PC2:  $\square$ )

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