

products, DSC can be a robust tool as it helps to fingerprint their thermal behavior, the characteristic melting and crystallization behavior. Moreover, several works have evaluated DSC application to detect adulteration of fats and oils. For instance, DSC was able to detect the presence of pig and buffalo body fat in cow and buffalo ghee (Lambelet & Ganguli, 1983); butter in tallow and margarine (Aktas & Kaya, 2001); lipase-catalyzed interesterified lard in refined-bleached-deodorised palm oil (Marikkar et al., 2001, 2002 a); animal fat in butter (Coni et al., 1994), canolla oil; (Marikkar et al., 2002 b); and palm olein (Marikkar et al., 2003).

On the other hand, the usage of chemometrics strategies, in fats and oils analysis has been reported. PCA and cluster analysis in particular, because of their capability to interpret large data sets have been used to define relationships that exist in fatty acid characterisation studies of food lipids (Kadegowda et al., 2008; Mates et al., 2007; Garcia-Lopez et al., 1996; and Martin-Carratala et al., 1998). Brodnjak-Voncina et al., (2005) applied PCA for the discrimination of various vegetable oils. In addition, many works have been done using PLS coupled with physical methods such as spectrometric to evaluate the quality of edible oils for example (Al-Alawi et al., 2004; Inon et al., 2003); and Maggio et al., 2010). Recently, Cerretani et al., (2011) applied DSC coupled with PLS to study the quantitation of fatty acids in olive oil.

The coupling of DSC and chemometrics techniques particularly Principal Components Analysis (PCA) and k mean clustering, to the authors best knowledge, there are no works on this application present in literature. Thus, this work's aim was to differentiate lard from other animal fats (chicken, beef fats) as well as their admixtures in different concentrations (lard-beef fat and lard-chicken fat) based on DSC data in combination with the Chemometrics technique of principal component analysis and k mean cluster.

2.0 Materials And Methods

We discussed below, the materials and methods of the Application of Principal Components Analysis (PCA) and k Mean Cluster for Differentiation of Lard from Other Edible Fats by using Differential Scanning Calorimetry (DSC).

2.1 Samples Preparation:

The extraction of fats from the samples was done according to the Bligh-Dyer method (1959) with some modifications. 300 to 400 mg of sample were approximately weighed; then, 4 ml methanol, 2 ml chloroform and 0.4 ml water were added; homogenized and vortexed for 30 sec. After that, 2 ml of chloroform and 2 ml of water were added; vortexed for 30 sec and centrifuged at $5000 \times g$ for 10 min, at room temperature. Using a Pasteur pipette, the upper layer (methanol/water) was then removed, and the lower layer (methanol/chloroform) was transferred into a clear tube. Finally, the organic solvents were removed using a rotary evaporator under reduced pressure, at 60°C . The extracted animal fat, lard, beef tallow (BT), chicken fat (CF) and a series of 14 samples containing (0.5 - 10 % w/w) of lard in BT, and CF were analyzed. Samples containing lard were assigned as adulterated; while pure beef fat, pure chicken fat and pure lard were marked as beef tallow (BT), chicken fat (CF) and lard (LD).

2.2 Thermal Analysis Of Samples By DSC

A Mettler Toledo DSC was used for analyzing the thermal characteristics of the oil samples. Nitrogen (99.999% purity) was the purge gas used and flowed at 20 ml/min. The instrument was calibrated with indium and dodecane. Samples of 8–10 mg were weighed into aluminium pans and covers were crimped into place. An empty, hermetically sealed aluminium pan was used as a reference. Prior to sample analysis, the baseline was obtained with an empty, hermetically sealed aluminium pan. All samples were subjected to the following temperature program: -80°C isotherms hold for 5 min, heated from -80 to 80°C at a rate of $5^{\circ}\text{C}/\text{min}$ (Marikkar et al., 2002). The manufacturer's software (7 Series/UNIX DSC software library) program was used to analyze and plot the thermal data. The melting and crystallization characteristics of each sample in a DSC scan were obtained using the DSC Data Analysis software. All determinations were carried out in triplicate and average of three measurements was used for data analysis.

2.3 Statistical Analysis

For differentiation and detection of lard from other animal fats, the onset temperature, enthalpy (the heat energy required for melting or released upon crystallization), and end set temperature measured for all samples were transformed to a multivariate data set. Then, the data was standardized using the Microsoft Excel software version 2007. Principal component analysis (PCA) and K-mean cluster were performed by using an Unscrambler software (X10) from Camo, USA. All analysis was done in triplicate; and the adulterated samples were used especially in the chemometrics analysis.

3. Results And Discussion

3.1 DSC Cooling Thermograms For The Pure Samples

DSC is a suitable technique to characterise such phase transitions as crystallisation and melting of vegetable oils that require the intake or release of thermal enthalpy. All DSC thermograms, obtained upon cooling of fat samples, are reported in Figure 6 which indicates typical DSC cooling curves of the pure samples BT, CF, and LD. In general, all samples showed two main well-defined exothermic events. In the lard sample (LD), the first peak (minor) was appeared at low temperature $\sim (-45^{\circ}\text{C})$ and the major and sharp peak with a shoulder was at higher temperature $\sim (-2^{\circ}\text{C})$. Chicken fat (CF) and beef tallow (BT) also showed almost similar cooling profiles; in CF the major exothermic peak appeared at $\sim (-41^{\circ}\text{C})$, and the second one was at $\sim 5^{\circ}\text{C}$. Meanwhile in BT sample, a clear shifting in exothermic event was observed where the first peak was at $\sim (-40^{\circ}\text{C})$, and the second peak was shown at $\sim (-2^{\circ}\text{C})$.

This cooling behavior of the pure samples was due to the amount of saturated and unsaturated triacylglycerols presented in the samples. It means the sample that contains more unsaturated TAGs; its cooling thermograms will shift more to the lower temperature side. The results of the previous chapter emphasises this; where the percentages of unsaturated TAGs of the lard (LD), chicken fat (CF), and beef tallow (BT) were 89, 85, and 74 % respectively. According to Che Man & Tan (2002) and Tan & Che Man, (2002), the cooling curves are more interpretable than those obtained from

the melting ones. This study also followed similar trends, where cooling thermograms provides better definition of different animal profiles.

3.2 DSC Melting (Heating) Thermograms For The Pure Samples

Heating thermograms profiles of the samples in Figure 7 helped to distinguish two major peaks in all samples. In lard sample, the first major peak was started to appear at low temperature $\sim (-50^{\circ}\text{C})$ and the other one with shoulders was at higher temperature $\sim 2^{\circ}\text{C}$. In CF sample, exhibited two small endothermic events where the first melting peak showed at $\sim (-46^{\circ}\text{C})$, and the end peak was at $\sim (-8^{\circ}\text{C})$. BT also showed different melting profiles; the first major endothermic peak with a shoulder appeared at $\sim (-44^{\circ}\text{C})$, and the second one was at higher temperature $\sim 4^{\circ}\text{C}$. This melting behaviour of samples was due to the amount of saturated and triacylglycerols presented in the samples. Meaning that, the sample that contains more saturated TAGs; its melting thermograms will shift more to the higher temperature region. From the previous chapter it was observed that the percentage of saturated TAGs of beef tallow (BT) was the highest followed by chicken fat (CF), and ended by lard (LD) (23, 13, and 9 % respectively).

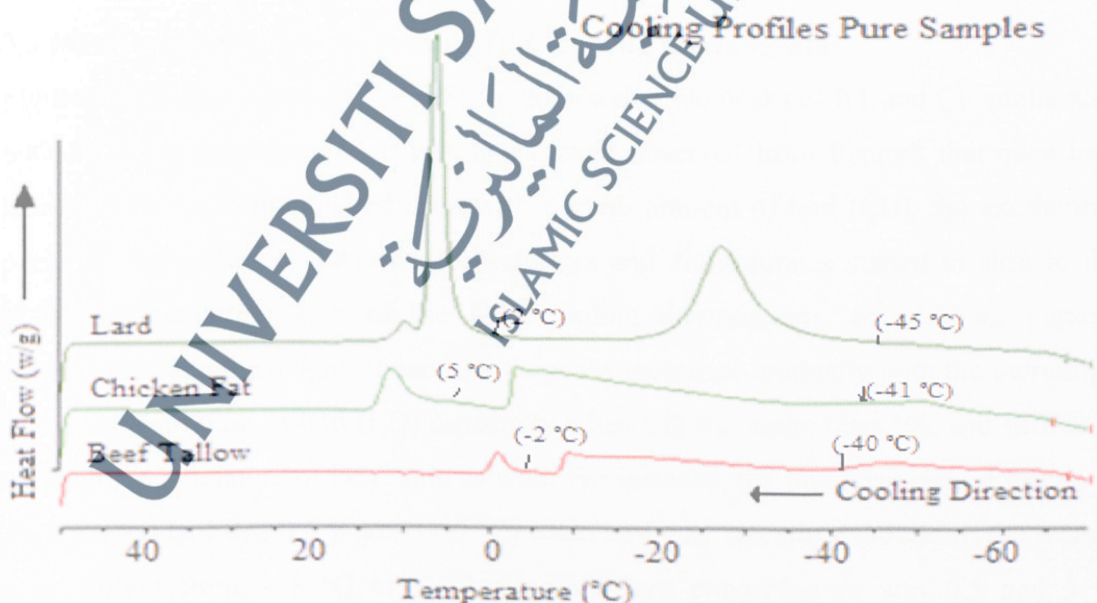


Fig.6 DSC cooling thermograms of the pure sample: (a) LD, (b) BT and (c) CF

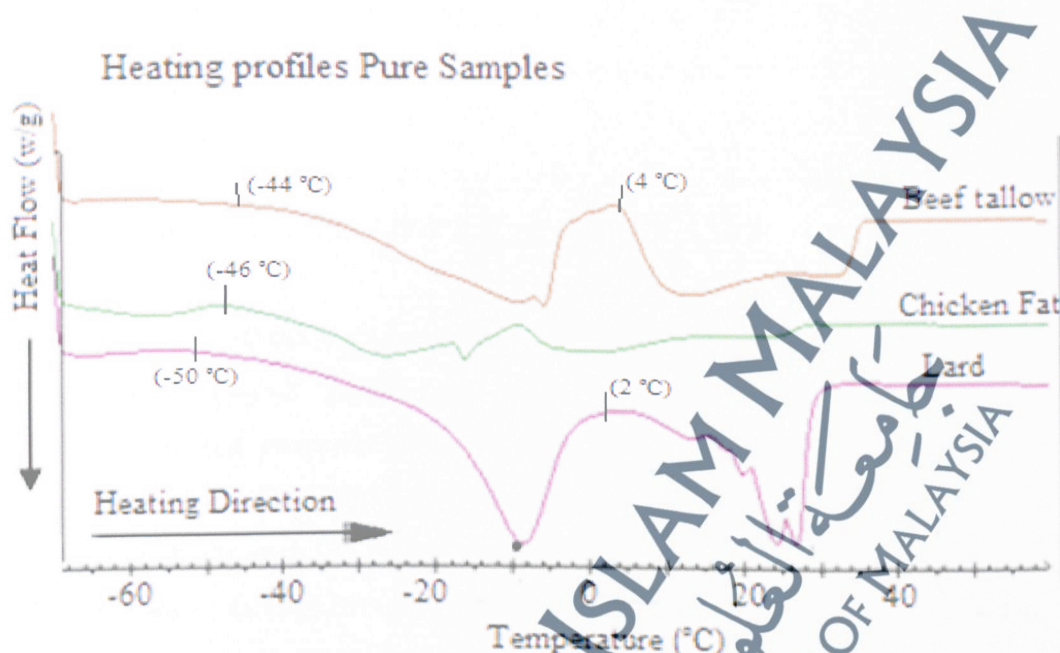


Fig. 7 DSC melting thermograms of the pure samples: (a) BT, (b) CF and (c) LD

3.3 Monitoring of adulterants using DSC cooling thermograms

Figure 8 and figure 9 showed the DSC major exothermic peaks of BT and CF adulterated with different concentration of LD. It was well observed from Figure 8 that once beef tallow (BT) was contaminated even with a small amount of lard (LD), the exothermic peaks corresponding to TAG of unsaturates and diunsaturates started to shift to the higher temperature region of the DSC cooling thermograms. In addition, Figure 8 showed also that the height of exothermic peaks increased gradually with the increasing of the concentration of lard (LD) especially when LD was more than 1%, and shifted to the higher temperature of DSC side as well. For instance, the first peak moved from $\sim (-40^{\circ}\text{C})$ when lard was 0.5 % to $\sim (-37^{\circ}\text{C})$ when lard was 5%, while the end of the second peak shifted from $\sim 8^{\circ}\text{C}$ to $\sim 12^{\circ}\text{C}$ when lard concentration was 0.5 and 5 % respectively. This peaks movement can be a very strong indicator for BT contamination by LD.

On the other side, DSC cooling thermograms of CF contaminated with lard in Figure 9 has shown an opposite shifting in the exothermic events to the lower temperature side of the DSC cooling thermograms, and this was observed only when the concentration of LD was more than 2 %. For instance, the first peak was at $\sim (-41^{\circ}\text{C})$ and the end of the second peak was $\sim 3^{\circ}\text{C}$ when LD percentage was 0.5 %, but they shifted to $\sim (-42^{\circ}\text{C})$ and $\sim 1^{\circ}\text{C}$ respectively when the concentration of LD increased to 5 %. This peaks shifting was due to the composition of TAG in CF and LD.

Moreover, the exothermic peaks differ in the liberated energy (enthalpy) for crystal formation (aggregation) (Saadi et al., 2012). Thus, in the mixture of BT and LD, the enthalpy decreased proportionally with the increasing of LD percentage in BT. It (enthalpy) changed from 36.87 (J/g) (0.5 % CF fat and LD; where the enthalpy was increased gradually with the increment of LD concentration. It changed from 20.9 (J/g) (0.5 % lard) to 25.45 (J/g) (5 % LD). These crystallisation behavior results correspond to the nature and percentage of triacylglycerols (TAGs) of the studied fats and oils (Tan & Che Man, 2002). In addition, based on the report of Saadi et al. (2012) the crystallisation rate is directly proportional to high melting TAGs such as PPO, PPS, and SOS content.

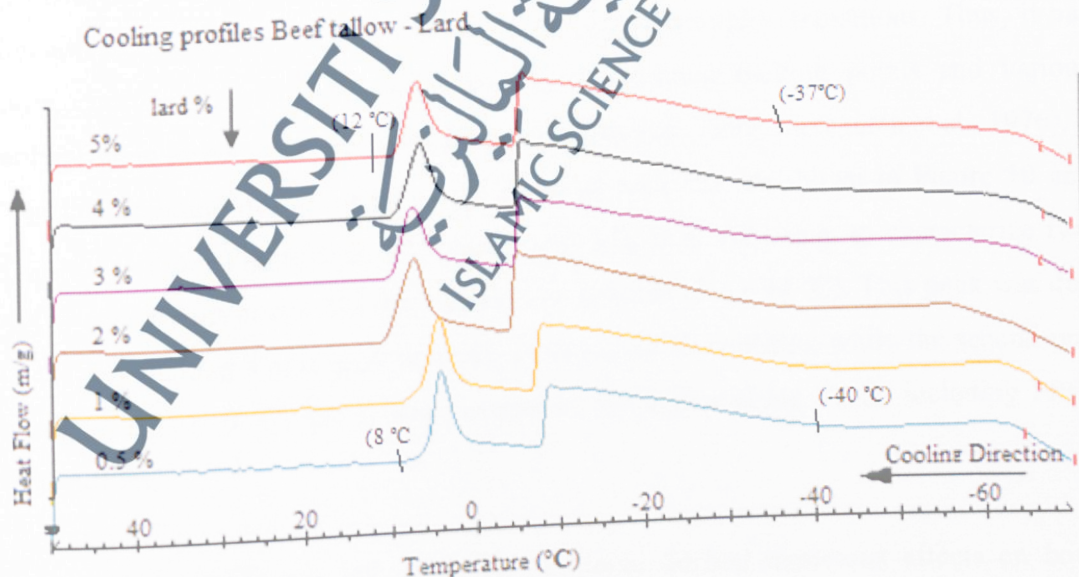


Fig. 8 DSC cooling thermograms of beef tallow adulterated with different concentrations of lard

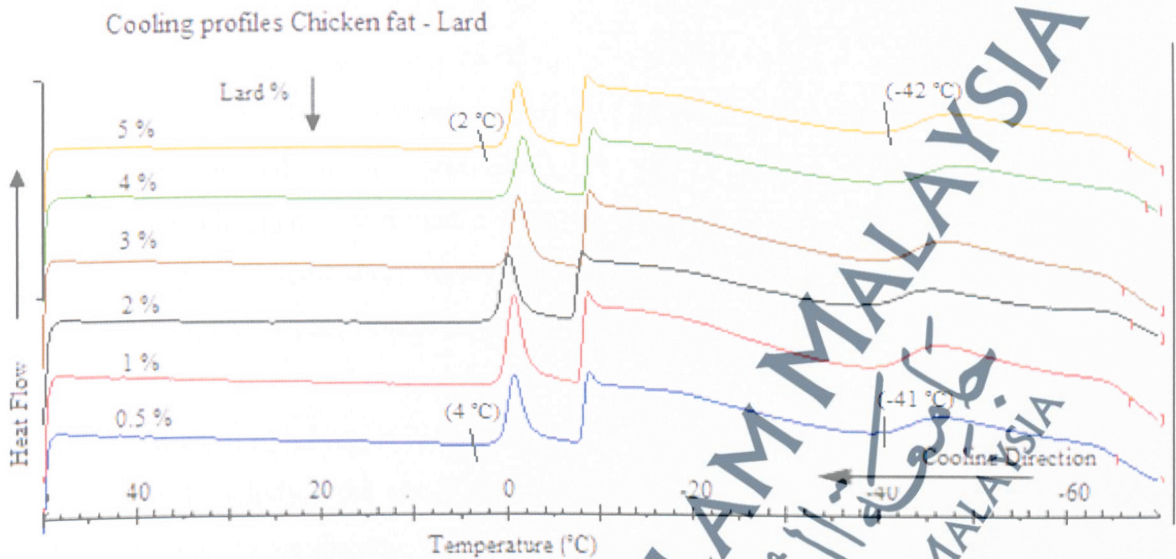


Fig. 9 DSC cooling thermograms of chicken fat adulterated with different concentrations of lard.

3.4 Monitoring Of Adulterants Using DSC Heating Thermograms

According to Fredrick et al., (2008) the melting profiles give an indication of the amount of crystallised fat and the occurrence of polymorphic transitions. Thus, it has been considered as a very useful tool in determining melting points and various polymorphic forms associated with fat crystals (Naryati, 1999; Lovegaren et al., 1976).

The DSC melting profile of BT and CF mixed with LD are shown in Figure 10 and Figure 11. From Figure 10 in BT mixed with LD, it is very easy to characterize two major endotherms peaks. The first event was detected at $\sim (-44^{\circ}\text{C})$. This peak was due to the low melting TAGs such as POO, PLO and OOO contents; while the second one appeared at $\sim (-6^{\circ}\text{C})$ and it was because of the high melting TAGs including PPO, PPP and PPS.

The addition of LD into BT as shown in Figure 10 had numerous effects on both endothermic peaks. The first result was the changing in the peaks area where they came smaller while the concentration of LD increased. The second effect was on the position

of those peaks, where they have shifted slightly to the high melting side once the concentration of lard was increased. For instance, the onset temperature moved from $\sim (-42^\circ\text{C})$ to $\sim (-38^\circ\text{C})$ when LD was 0.5 % and 5 % respectively; meanwhile the end set temperature also moved from $\sim 26^\circ\text{C}$ to $\sim 30^\circ\text{C}$ when LD concentration was increased from 0.5 % and 5 % respectively. Moreover, the third consequence of LD addition into BT was observed at the enthalpy level. The interaction energy during melting phenomena changed as well and it augmented with the increasing of LD concentration. For example, it (enthalpy) increased from -69.10 (J/g) to -19.86 (J/g) when lard concentration increased from 0 % to 5 % respectively.

However, contrary changes were observed in the melting curve of CF and LD mixtures in Figure11 including the size of both endothermic peaks, the position of those peaks, and the physical parameters. For instance, the onset temperature decreased from $\sim (-42^\circ\text{C})$ to $\sim (-50^\circ\text{C})$ when LD was increasing from 0.5 % to 5 % respectively. Meanwhile the end set temperature also reduced from $\sim 31^\circ\text{C}$ to $\sim 27^\circ\text{C}$ when lard was 0.5 % and 5 % respectively. In addition, the level of energy decreasing changed as well; where it moved from (-16.38 J/g) to (-19.82 J/g) when lard went up from 0 % to 5 % respectively.

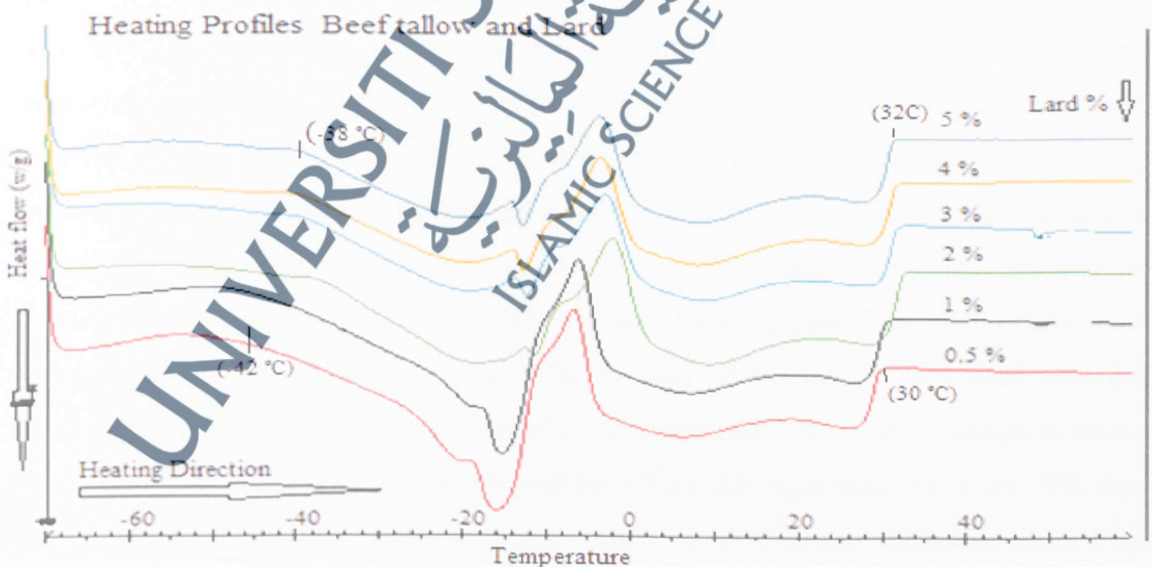


Fig. 10 DSC melting thermograms of beef tallow adulterated with different concentrations of lard.

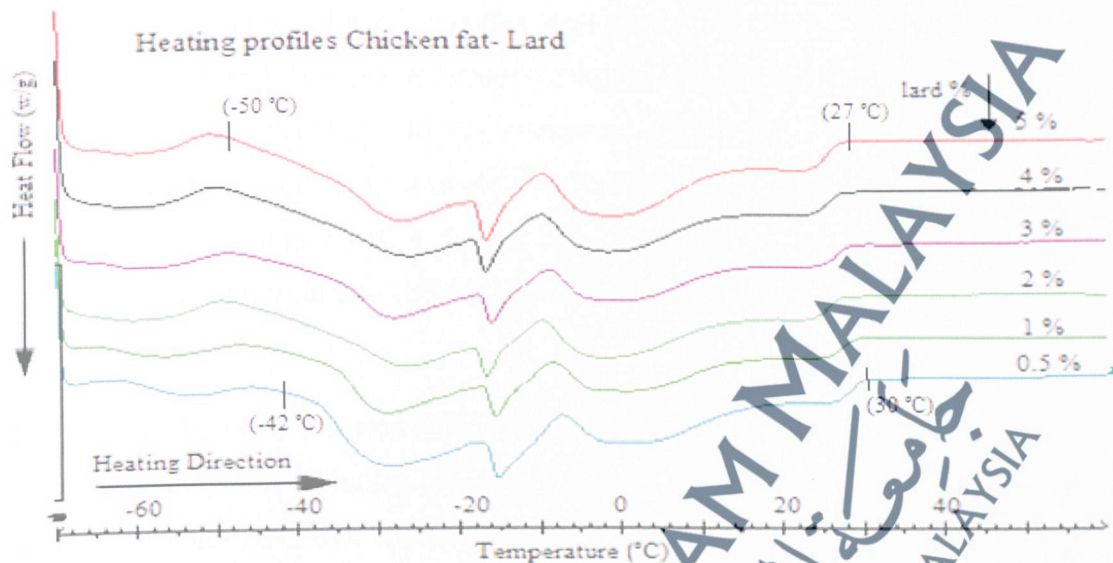


Fig.11 DSC melting thermograms of chicken fat adulterated with different concentrations of lard.

3.5 Chemometric Results: Principal Component Analysis

In order to differentiate and classify lard (LD) from beef tallow (BT), chicken fat (CF) and their different blending, the multivariate data matrix of 15 samples (i.e., the pure BF, LA, and CF as well as their mixtures) targeting DSC parameters including onset temperature, enthalpy, and end set temperature was subjected to principal component analysis (PCA). Shin et al. (2010) has reported that PCA is a method of identifying patterns in data and expressing the data in such a way as to emphasize their similarities and differences. The authors also reported that PCA could designate interactions between groups of variables in a data set and show relationships that might exist between objects. In addition, to reduce the number of descriptors associated with the data set without losing the information of the original data. Scores and loadings matrices were generated using 4 principal components (PCs). An eigenvalue of about 99% was achieved using four PCs where PC1 accounted for 95% of the variation, while PC2 described 4% of the variation; therefore, the remaining two ($\leq 1\%$ total) did not explain significant variability in the data.

On the other hand, PCA results of score plot of cooling thermograms in Figure12 do not give conclusive information about the separation and the distribution of the samples. For instance, in the scores plot the samples were displayed only in two groups. The first group (A) (1, 2, and 3) which referred to the pure samples including beef tallow, lard, and chicken fat respectively. It was located in the positive side of the X-axis. The distribution of this group helped to observe that LD sample located far from BT and CF samples. The second group B (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15) referred to all adulterated samples with lard (BT+LD, and CF+LD). It was located in the negative side of the X-axis.

In addition, Figure13 showed the loading plot for the different DSC parameters of cooling thermograms including onset temperature, enthalpy, and end set temperature of the pure samples (BT, LD, and CF) as well as mixtures by the first (PC1) and second (PC2) components. The PCA results of loadings plot described the different distribution of variables. It was well shown the non-homogenized location of the three parameters where the onset (number 1) and the end set temperatures (number 2) were located in the negative side of the figure. At the same time, the enthalpy was located at the positive side. This distribution due to physical characteristics of these measured parameters.

However, PCA results for the melting thermograms in Figure14 showed different results compared with the cooling thermograms. Figure14 demonstrated the scores plot of the different concentrations of samples (BT+LD, and CF+LD) as well as the pure samples BT, CF and LD. The PCA results of the scores plot described the different distribution of variables (concentrations) in three main groups. The first group A (1, 2, and 3) which referred to pure beef tallow (BT) and pure lard (LD), and pure chicken fat (CF) respectively are very isolated from the other two groups and their distance is convergent; and it was located under of the X-axis. Meanwhile, the second group B (4, 5, 6, 7, and 8) referred to BT+LD and the third group C (9, 10, 11, 12, 13, 14, and 15) which referred to CF+LD was located up of X-axis.

This grouping of samples was because of the distinction in their physico-chemical characteristics; and it is known that TAGs play extremely important role in this differentiation (Wood et al., 2008). Therefore, Figure14 helped to differentiate BT and

CF from LA, and it aided to distinguish very low adulterated concentration (0.5%) of lard in BT and CF.

In addition, Figure15 showed the loadings plot for the different DSC parameters of melting thermograms including onset temperature, enthalpy, and end set temperature of the pure samples (BT, LD, and CF) as well as mixtures. The PCA result of the loadings plot of heating thermograms appeared similar to that one from cooling results, where it described the different distribution of variables. The allocation of the three parameters was very separately. For instance, the onset (number 1) and the end set temperatures (number 2) were located in the negative side of the figure. At the same time, the enthalpy was located at the positive side. This distribution due to physical characteristics of these measured parameters.

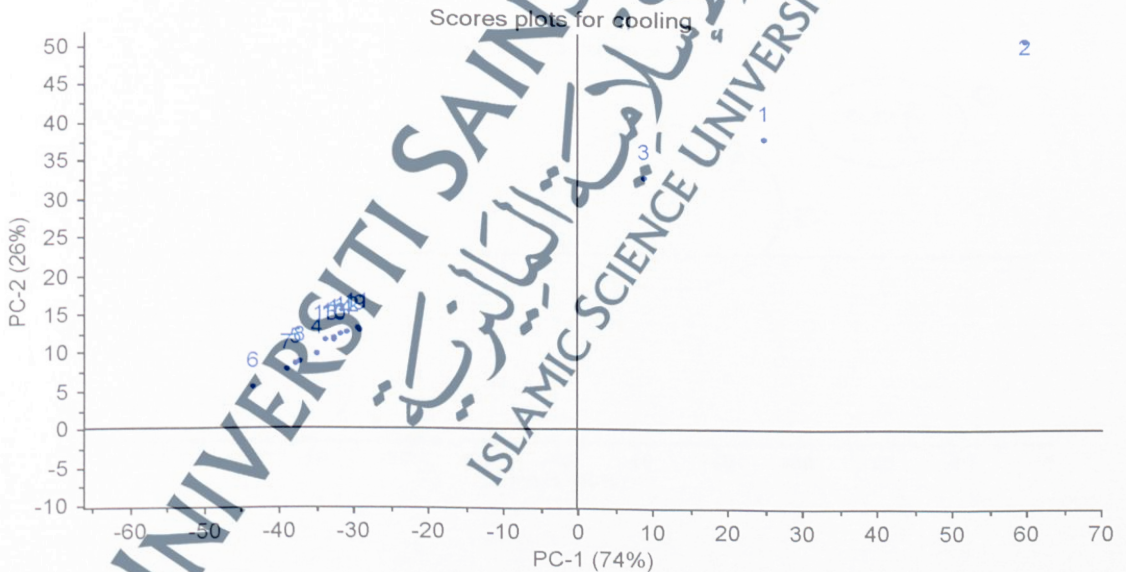


Fig. 12 Principal component analysis (PCA) scores plot of PC1 versus PC2 showing the cooling thermograms results of the pure and the adulterated samples, where: 1 refers to beef tallow (BT); 2 lard (LD); 3 chicken fat (CF); 4 BT+0.5 % LD; 5 BT+1 %LD; 6 BT+2 %LD; 7 BT+3 %LD; 8 BT+4 %LD; 9 BT+5 %LD; 10 CF+0.5 %LD; 11 CF+1 %LD; 12 CF+2 %LD; 13 CF+3%LD; 14 CF+4%LD; and 15 CF+ 5% LD.

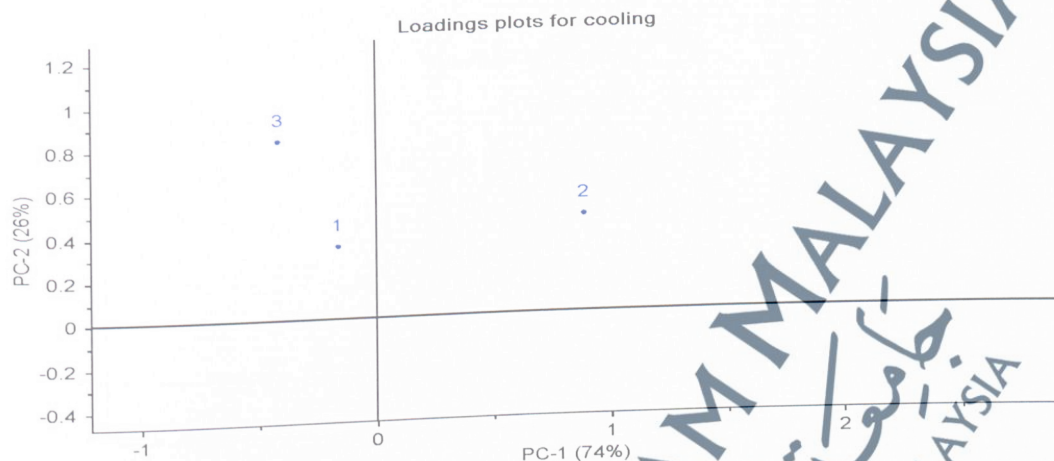


Fig.13 The loadings plot (cooling thermograms) for the first two principal components (PCs) for DSC parameters of the studied samples where 1 refers to onset temperature, 2 for enthalpy, and 3 for end set temperature.

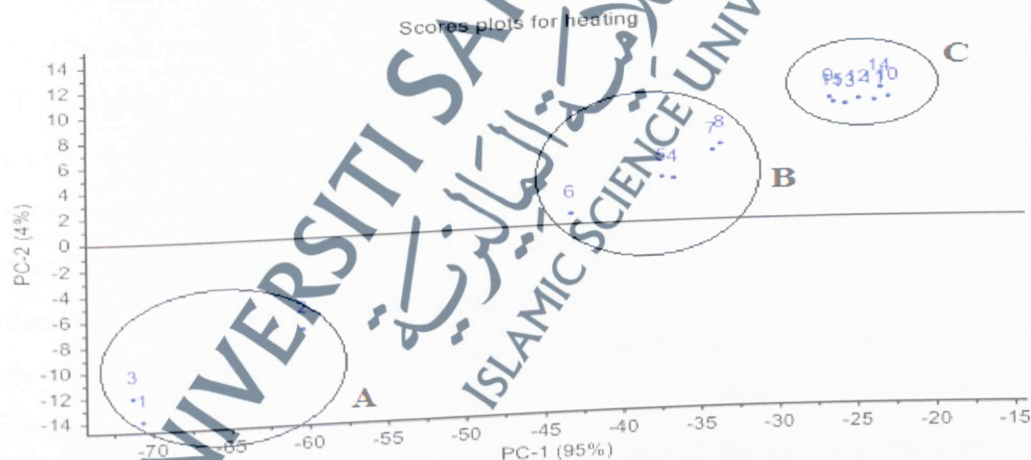


Fig. 14 Principal component analysis (PCA) scores plot of PC1 versus PC2 showing the melting thermograms results of the pure and the adulterated samples, where: 1 refers to beef tallow (BT); 2 lard (LD); 3 chicken fat (CF); 4 BT+0.5 %LD; 5 BT+1 %LD; 6 BT+2 %LD; 7 BT+3 %LD; 8 BT+4 %LD; 9 BT+5 %LD; 10 CF+0.5 %LD; 11 CF+1 %LD; 12 CF+2 %LD; 13 CF+3%LD; 14 CF+4%LD; and 15 CF+ 5% LD.

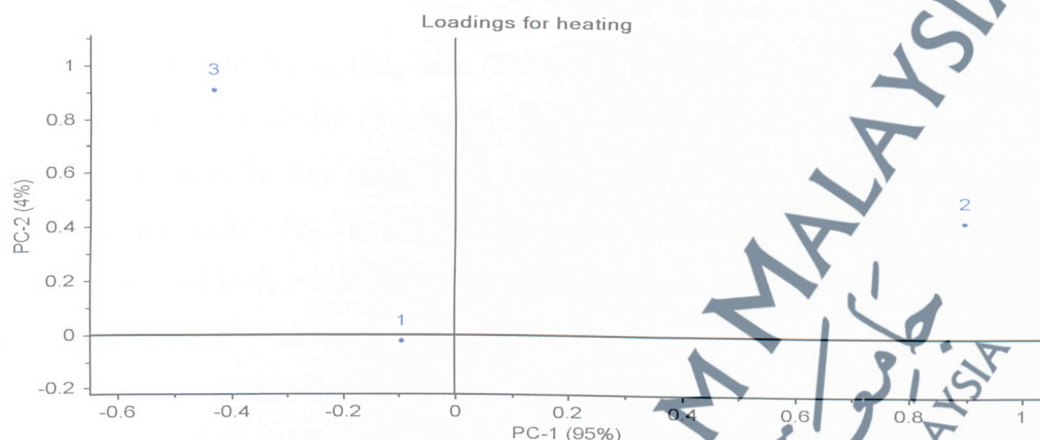


Fig. 15 The loadings plot (melting thermograms) for the first two principal components (PCs) for DSC parameters of the studied samples where 1 refers to onset temperature, 2 for enthalpy, and 3 for end set temperature.

3.6 Cluster Analysis

The original data set (Table 12), $X (15, 3)$ consisted of 3 variables including onset temperature, enthalpy, and end set temperature as measured in 15 samples (three samples are pure animal fats: BT, LD and CF and other twelve are BT+LD, CF+LD respectively), where each entry matrix was an average value of three replications. The data was standardized, normalized, and mean-centered prior to the analysis, and then they were subjected to cluster analysis (CA). The K-mean cluster analysis results for cooling and melting data are given in (Table 12 and Table 13) respectively. For the cooling results K mean cluster (Table 12) has classified the samples into three classes. The first class (class 1) referred to the pure lard, meanwhile the pure beef tallow and pure chicken fat was grouped in the second class (class 2). The last group (class 3) represented all other adulterated samples including beef tallow with lard, and chicken fat with lard.

Based on K mean cluster results from Table 12, cooling thermograms from DSC cannot differentiate slight incremental adulteration from 0.5, 1, 2, 3, 4, and 5 % of lard in both

beef tallow and chicken fat. The grouping class 3 indicates this for all adulterated BT and CF with LD at respective percentages (Table 12) using onset, enthalpy, and endset data points.

K mean cluster results for melting data (Table 13) have shown different results as the cooling ones. K mean cluster (Table 13) has categorised the samples into three main classes. For instance, the first class (class 1) referred to the pure samples including beef tallow, lard, and chicken fat. The second class (class 2) indicated the adulterated samples of beef tallow and lard; while, the third group (class 3) was specified for the mixed samples of chicken fat with lard.

For melting thermograms, K mean cluster cannot differentiate pure lard (LD), pure beef tallow (BT), and pure chicken fat (CF). Adulterated BT with 0.5 to 4 % of LD can be differentiated against CF adulterated with 0.5 to 5 % of LD.

Thus, K-mean cluster results helped to distinguish the pure lard from other fats. As there are variable results of K mean cluster in melting and cooling thermograms, it is not recommended for detection of lard adulteration in beef tallow as well in chicken fat unless if lard was mixed in high amounts for more than 5 %. Another consideration is, in future studies instead of three data sets (onset, enthalpy, and endset), five or seven data sets may yield better results.

Table 12: K-mean cluster of cooling thermograms results for all samples

Samples	Onset	Enthalpy	End set	Class
BT	-40.140	39.123	8.613	2
LD	-45.410	76.065	15.895	1
CF	-41.033	22.625	16.315	2
BT1	-40.580	36.870	8.895	3
BT2	-40.350	31.820	10.070	3
BT3	-40.180	30.170	12.147	3
BT4	-38.020	29.340	12.180	3
BT5	-37.850	26.220	12.310	3
BT6	-37.160	20.450	12.460	3
CF1	-41.420	20.900	3.395	3
CF2	-41.530	21.440	3.270	3
CF3	-41.680	21.820	3.147	3
CF4	-41.850	22.740	3.080	3
CF5	-41.980	23.770	2.460	3
CF6	-42.160	25.060	1.930	3

BT is pure beef tallow; CF is pure chicken fat; LD is pure lard;

BT 1: 99.5 % BT + 0.5 % LD

BT 2: 99 % BT + 1 % LD

BT 3: 98 % BT + 2 % LD

BT 4: 97 % BT + 3 % LD

BT 5: 96 % BT + 4 % LD

BT 6: 95 % BT + 5 % LD

CF 1: 99.5 % BT + 0.5 % LD

CF 2: 99 % BT + 1 % LD

CF 3: 98 % BT + 2 % LD

CF 4: 97 % BT + 3 % LD

CF 5: 96 % BT + 4 % LD

CF 6: 95 % BT + 5 % LD

Table 13:K-mean cluster of melting thermograms results for all samples

Samples	Onset	Enthalpy	End set	Class
BT	-44.505	-69.100	36.500	1
LD	-50.390	-56.940	30.390	1
CF	-46.140	-69.753	32.650	1
BT1	-52.200	-31.571	28.720	2
BT2	-48.260	-32.160	29.080	2
BT3	-45.370	-38.716	30.325	2
BT4	-42.810	-28.457	32.585	2
BT5	-40.050	-27.808	32.760	2
BT6	-38.210	-19.850	32.920	3
CF1	-42.260	-16.390	31.240	3
CF2	46.240	-17.310	29.420	3
CF3	-47.180	-18.075	28.980	3
CF4	-48.760	-19.093	28.170	3
CF5	-50.420	-16.620	28.050	3
CF6	-50.600	-19.820	27.435	3

BT is pure beef tallow; CF is pure chicken fat; LD is pure lard;

BT 1: 99.5 % BT + 0.5 % LD CF 1: 99.5 % BT + 0.5 % LD
 BT 2: 99 % BT + 1 % LD CF 2: 99 % BT + 1 % LD
 BT 3: 98 % BT + 2 % LD CF 3: 98 % BT + 2 % LD
 BT 4: 97 % BT + 3 % LD CF 4: 97 % BT + 3 % LD
 BT 5: 96 % BT + 4 % LD CF 5: 96 % BT + 4 % LD
 BT 6: 95 % BT + 5 % LD CF 6: 95 % BT + 5 % LD

4. Conclusion

From the results have shown above, DSC combined with the chemometric technique such as PCA and K-mean cluster can be employed to differentiate lard from beef tallow and chicken fat based on their onset temperature, enthalpy, and end set temperature in other meaning cooling and melting thermograms. In addition, it was found that by using melting thermograms, it was possible to detect lard adulteration in beef tallow and chicken fat even less than 1 %. However, it was difficult by using cooling thermograms to do so, especially in chicken fat with lard. PCA has showed the ability of detection of lard within the mixtures of beef tallow- lard and chicken fat-lard as low as 0.5 % of lard especially with melting data. K-mean cluster was only able to differentiate between pure beef tallow, pure chicken fat and pure lard. In future continuation from this study will be look into more replications; more data set points as well as the percentages of lard will be higher in the mixtures with other animal fats to elaborate more information in intention of detection of lard in food matrix in very small possible amounts.