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Detection of butter adulteration with lard using differential scanning calorimetry

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Abstract

Differential scanning calorimetry (DSC) is developed and used for detection of butter adulteration with lard. Butter has the similar characteristics to lard makes lard a desirable adulterant in butter. DSC provides unique thermal profiling for lard and butter. In the heating thermogram of the mixture, there was one major endothermic peak (peak A) with a smaller shoulder peak embedded in the major peak that gradually smoothed out to the major peak as the lard percent increased. In the cooling thermogram, there were one minor peak (peak B) and two major exothermic peaks, peak C which increased as lard percent increased and peak D which decreased in size as the lard percent increased. From Stepwise Multiple Linear Regression (SMLR) analysis, two independent variables were found to be able to predict lard percent adulteration in butter with R^2 (adjusted) of 95.82. The SMLR equation of lard percent adulteration in butter is 293.1 - 11.36 (Te A) - 2.17 (Tr D); where Te A is the endset of peak A and Tr D is the range of thermal transition for peak D. These parameters can serve as a good measurement parameter in detecting lard adulteration in butter. DSC is a very useful means for halal screening technique to enhance the authenticity of Halal process.

Introduction

Butter is one of the most valuable edible fats having more than 500 different fatty acids comprising of saturated fatty acids, monounsaturated fatty acids, and small amounts of polyunsaturated fatty acids (PUFA). Besides, butter has more than 1300 individual triacylglycerols (TAG) (Barron *et al.*, 1990). In the sector of dairy product, butter is a good sources of fat soluble vitamins, namely vitamin A, D, E, K; consequently, butter is taken into account as high valuable price from other dairy products. This phenomenon has attracted unscrupulous fats and oils industry players to adulterate butter with other animal fats like chicken fat, mutton fat and lard to gain economic profit (Shukla *et al.*, 1983). The food adulteration practice is common issue in food industry, which has caused uneasiness amongst consumer belonging to certain religion groups.

Due to the Muslim, Christian, and Jewish scientist awareness, the major concern lies in detecting non-halal components especially pig derivatives like pork and lard in the food. This has gained recognized attention globally as the Muslim and Jewish markets have grown rapidly due to the increasing awareness of their needs (Mansor *et al.*, 2012). Muslim scholars are prohibited to eat pork and its derivatives. This is based on the verses of the Quran: "He hath only forbidden you dead meat, and blood, and the flesh of swine, and that on which any other name hath been invoked besides that of Allah." (Quran, al-Baqarah, 2:173). Any substance which is prohibited by Islam to be eaten, then any changes made to the form that originates from it also prohibited. As Prophet Muhammad said, "God cursed the Jews because they melted the banned animal fat and sell it." Narrated by Bukhari (2002: 2/45) and Muslim (1998: 3/48). Khattabi said, "God cursed the Jews because they thaw the banned fat and make it into liquid until it changed from the banned fat. This hadith stated that any fraudulent purposes to change the substance that being prohibited thing into other name are forbidden.

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This forbidden does not changed even the prohibited animal have changed into different name." (Ibn Majah, 1998: 4/72).

The Christian is likely to be convinced by his religious scriptures. The Bible prohibits the consumption of pork, in the book of Leviticus "And the swine, though he divide the hoof, and be cloven footed, yet he cheweth not the cud; he is unclean to you". "Of their flesh shall ye not eat, and their carcass shall ye not touch, they are unclean to you." (Leviticus 11:7-8). Pork is also prohibited in the Bible in the book of Deuteronomy "And the swine, because it divideth the hoof, yet cheweth not the cud, it is unclean unto you. Ye shall not eat of their flesh, nor touch their dead carcass." (Deuteronomy 14:8). A similar prohibition is repeated in the Bible in the book of Isaiah chapter 65 verse 2-5.

The kosher (kashrus) dietary laws determine which foods are "fit or proper" for consumption by Jewish consumers who observe these laws. The laws are Biblical in origin, coming mainly from the original five books of the Holy Scriptures, the Torah, which has remained unchanged. The kosher dietary laws are based on commandments found in the Torah that has been interpreted and refined by the Jewish religious leaders known as rabbis; this system of Jewish law is referred to as "halacha." In these laws food are categorised into four: meat (fleishig); dairy (michig); neutral (pareve); and unacceptable (traif) (Regenstein & Regenstein, 1991).The reviews of halal and kosher dietary laws can be found in Al-Qaradawi (1960), Regenstein, Chaudry, and Regenstein (2003), and Kamali (2008a, 2008b, 2008c).

Therefore, food industries should be sensitive towards the current issue of the presence of pork or lard in food product. Animal fats including lard, have been used as adulterants in fats components including butter in order to gain economical profits, since lard is one of the cheapest fats available in the market. Indeed, for assuring the halal authenticity of food products, it is necessary for developing analytical techniques capable of determining of lard (Mursyidi, 2013). Rohman and Che Man (2012) have reviewed some physicochemical techniques for detecting non-halal components (pig derivatives) in some food products for halal authentication, including DSC technique.

For the analysis of non-halal components such as lard, some instrumental techniques have been developed and continuously used. Such methods are FTIR spectroscopy for analysis of lard in chocolate and chocolate products (Che Man *et al*., 2005), in cake formulation (Syahariza *et al.*, 2005), and in meatball broth (Kurniati *et al*., 2014), electronic nose for analysis of lard profile in some food products (Nurjuliana *et al*., 2011; Mansor *et al*., 2011), two dimensional gas chromatography copuled with TOF-mass spectrometry (Indrasti *et al*., 2010), differention of lard using Gas Chromatography Mass Spectrometry (GC-MS) and Elemental Analyzer–Isotope Ratio Mass Spectrometry (Nizar *et al.*, 2013), high performance liquid chromatography coupled with multivariate data analysis for differention of lard from some vegetable oils (Marikkar *et al*., 2005), polymerase chain reaction (Aida *et al.*, 2005), proton nuclear magnetic resonance for classification of lard and other fats and oils (Fang *et al*., 2013), as welll as differential scanning calorimetry.

In fats and oils analysis, thermal analysis (TA) is one of the analytical techniques used by researchers in food science (Cebula and Smith, 1992; Biliaderis, 1983). Among TA methods, differential scanning calorimetry (DSC) is the most versatile ones for a range of applications (Griffin and Laye, 1992). The principle of DSC is to keep the sample and the reference at the same temperature in separate micro-ovens. The electrical power require for the compensation is equivalent to the calorimetric effect (Coni *et al*., 1994). DSC offers a direct method for studying the thermal properties of various materials and has a possibility to be developed as a quality control procedure for food adulteration (Tan and Che Man, 2002). Therefore, some scientist has developed some analytical methods for authentication of butter. Our group have used FTIR spectroscopy in combination with chemometrics for authentication of butter from animal fats (Nurrulhidayah *et al*., 2012; Nurrulhidayah *et al.*, 2013).

In fats and oils authentication, DSC is used for authentication of virgin coconut oil from lard (Mansor *et al.*, 2011), for analysis of lard in RBD palm oil (Marikkar *et a*l., 2001), for determination of lard in some food products deep fried with lard (Marikkar *et al*., 2003), lard and other animal fats in sunflower oil (Marikkar *et al*., 2012). In this study, we used DSC for analysis of lard as adulterant in butter.

Materials and Methods

Sample preparation

Lard was obtained by rendering the adippose tissue of pig. The preparation and rendering process is similar with that previously reported by Rohman and Che Man (2009). The lard obtained was placed into a container, flushed with nitrogen and stored until further use. The chemicals and solvents used were of analytical grade, unless otherwise specified. TAG standards were purchased from Sigma Aldrich

(St. Louis, MO, USA). Butter sample was extracted according to AOAC official method 920.118 (2000). The extracted samples were kept in glass vials under refrigerated conditions (-20°C) until used for analysis.

Preparation of blends

The blends of lard and butter were prepared according to percentage of lard in buffer (v/v), namely 1.0, 3.0, 5.0, 10.0, 20.0, 30.0, 40.0, and 50.0 % of lard in butter. All blend were prepared in triplicate and analyzed for TAG composition and thermal profile using HPLC and DSC, respectively.

Analysis of triacylglycerol (TAG) cmpositional by high performance liquid chromatography (HPLC)

The composition of TAG was analyzed using reverse-phase HPLC (Waters, Milford, MA) coupled with refractive index detector (Waters, Milford, MA). The oil samples were diluted in acetone (1: 9 v/v) and directly injected into HPLC instrument. The column used in this study was a LiChroCART 100-RP-18 (12.5 cm x 4 mm i.d.; thickness 5 µm, Merck, Darmstadt). The mobile phase consisting of acetone: acetonitrile (63.5: 36.5 v/v) was delivered isocratically. For each analysis, 10-µL of sample solutions were injected into HPLC system. TAG peaks were analyzed by the Empower software (Milford, MA) and identified based on the retention time of TAG standards, which were then presented as percentage areas. Quantification of TAG composition was done using internal normalization technique.

Thermal analysis by differential scanning calorimetry (DSC)

Into an aluminum pan, an approximately of 9 mg of each oil sample was accurately weighed using an analytical balance with a sensitivity of 0.01 mg, and sealed into place. Thermal analyses were carried out using a DSC 823e Mettler Toledo instrument equipped with a sample robot (Julabo FT400 intracooler). The instrument was equipped with STARe excellence software for data interpretation. For the instrument calibration, indium and n-dodecane were used. The reference used was an empty covered aluminum pan of the same size as used in the samples. The samples were subjected to the following programmed temperature ramp, namely: 60°C isotherm for 5 min, cooled at 5°C/min to -60°C and helard for 5 min. It was subsequently heated from -60 to 60°C at 5°C/ min. The scanning rate was further programmed at 5°C/min to reduce the lag in output response from the DSC instrument as well as to preserve the minor peaks and to reduce the peak smoothing tendencies, which can occur at a high scanning rate. The thermal characteristics determined in our study are the temperatures of melting and cooling transition (measured from the DSC curve as the maximum peak temperature), as well as the temperatures of onset (T_o) and endset (Te) (measured as the point where extrapolation of the leading and ending curve edge intersects with the baseline) and the temperature range for cooling and melting phase (Tr) (determined from the differences between the onset and endset temperatures).

Statistical analysis

All experiments were carried out in triplicate and analyzed using one-way analysis of variance (ANOVA) using SPSS sofware Version 17.0 (Armonk, New York,

USA). Tukey's test was utilized to ascertain the significant differences among means at the level of $p < 0.05$. DSC data were further evaluated by the stepwise multiple linear regression model using Minitab version 16 (Minitab Inc., State College, PA, USA). The significant difference of the independent variables was set at 0.05 for the entry and stay of the calibration model. The coefficient of determination (R^2) (adjusted) were chosen for this study to reduce the chance variation of predictors given by the R^2.

Results and Discussion

Triacylglycerol composition

All edible fats and oils including lard and butter are mostly composed of triacylglycerol (TAG) and fatty acid (FA), with small amounts of minor components such as tocopherols, carotene, sterols and free fatty acids. TAG is the main components of edible fats and oils, which correlates to the cooling and melting behavior as seen in thermal analysis. Table 1 shows the profiles of TAG composition of pure butter, lard and admixtures between them. The dominant TAGs present in butter fat is LaLaLa and CCLa (La = lauric; C = capric) accounting of 15.8±0.18 and 12.99±0.02%, respectively. It is also observed, that the level of LaLaLa and CCLa was decreased significantly as the adulteration level of lard was increased. While, the main TAGs observed in lard are palmitooleoolein (POO) 23.37%, palmitooleolinolenin (POL) 21.6%, and palmitooleostearin (POS) 18.14%. This results were in agreement with those reported by Rashood *et al.* (1996) which were 19.3% of POO and 13.2% of POS and Yanty *et al*., (2011) reported the major TAG molecular species of lard are POL, POO and POS comprising 61.5%. As expected, the increase level percentage of lard adulteration will result in the

Table 1. Triacylglycerol (TAG) composition of butter adulterated with different concentration of lard (v/v)

TAG†	Adulteration level							
	(0 %)	(5%)	(10%)	(20%)	(30%)	(40%)	(50%)	(100%)
CCpCp	4.57±0.52^{a,d}	4.27±0.87^{d}	3.57±0.25^{a}	2.46±0.03^{b}	2.19±0.03^{b,c}	1.36±0.03^{b,d}	0.18±0.02^{b,c,d}	0.00±0.00^{a}
CpCpLa	6.06±0.42^{a,c}	5.80±1.16^{b,c}	4.39±0.05^{a}	3.93±0.41^{b}	3.74±0.59^{b}	2.59±0.03^{c}	0.7±0.21^{b,c}	0.00±0.00^{a}
CpCLa	5.02±0.64^{a,b,c}	4.87±0.62^{a,b,c}	4.54±0.05^{d}	4.16±0.08^{a}	3.18±0.43^{a,b}	3.17±0.19^{c}	1.59±0.12^{c}	0.00±0.00^{e}
CCLa	12.99±0.02^{b}	12.43±0.01^{a}	11.79±0.84^{a}	11.31±0.05^{a}	10.09±0.01^{a}	9.31±0.03^{a}	8.81±0.01^{a}	0.00±0.00^{a}
CLaLa	12.34±0.14^{b}	6.41±0.82^{a,b,c}	6.16±0.52^{a}	5.2±0.02^{b}	4.53±0.24^{b}	4.5±0.36^{a,c}	4.17±0.43^{a}	0.00±0.00^{d}
LaLaLa	15.8±0.18^{e}	8.05±0.99^{b,c,d}	7.43±0.41^{a}	7.33±0.16^{a,b}	7.09±0.29^{a,c}	6.84±0.37^{a,d}	5.13±0.96^{a,d}	0.00±0.00^{f}
LaLaM	10.46±0.11^{c}	6.15±0.76^{a,b}	5.55±0.36^{a}	4.99±0.04^{b}	4.72±0.28^{a,b}	4.5±0.19^{a}	4.13±0.18^{a}	0.00±0.00^{d}
LaLaO	5.02±0.6^{a,d,f}	4.57±0.32^{a,b,c,d,e}	4.4±0.08^{a}	3.65±0.28^{b}	3.55±0.21^{b,c}	3.38±0.13^{c,d}	2.97±0.04^{a,c,d,e,f}	0.00±0.00^{g}
LaMM	7.37±0.08^{d}	5.91±0.73^{a,b,c}	5.71±0.11^{a}	5.36±0.2^{b}	4.77±0.26^{a,b,c}	4.72±0.12^{a,b,c}	4.63±0.07^{a,c}	0.00±0.00^{e}
LaLaP	3.65±0.06^{a}	3.17±0.27^{b}	2.91±0.21^{a}	2.85±0.15^{b}	2.15±0.15^{b}	1.68±0.23^{b}	1.51±0.01^{a}	0.00±0.00^{c}
LaMO	5.38±0.07^{e}	5.03±0.47^{a,b}	4.68±0.21^{a}	4.38±0.08^{a,b}	4.32±0.02^{a,b,c}	3.81±0.13^{a,b,c,d}	3.24±0.04^{c,d,e}	0.00±0.00^{f}
LaMP	5.07±0.06^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
LaOO	4.4±0.00^{b}	3.66±0.02^{c}	2.69±0.00^{a}	2.64±0.04^{a}	2.43±0.05^{b}	2.3±0.02^{d}	2.33±0.04^{b}	0.00±0.00^{e}
LaPO	6.2±0.02^{c}	5.97±0.02^{a,b}	4.76±0.02^{a}	4.02±0.09^{b}	3.47±0.01^{c}	3.34±0.04^{b}	3.2±0.08^{c}	0.00±0.00^{d}
LaMP+OLL	6.02±0.04^{c,d}	5.35±0.11^{a}	5.3±0.11^{a}	5.15±0.22^{a,b}	4.81±0.11^{b}	0.00±0.00^{a,b}	0.00±0.00^{c}	0.00±0.00^{c,d}
LaPP+MMO	1.67±0.02^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
MMP	2.32±0.03^{a,b}	0.00±0.00^{b}	0.00±0.00^{a}	0.06±0.08^{b}	0.1±0.01^{b}	3.72±0.05^{b}	0.00±0.00^{b}	0.00±0.00^{b}
MOO	2.88±0.03^{c}	1.99±0.13^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{d}	0.00±0.00^{a,b}
MPO+POL	1.94±0.02^{b}	3.8±0.01^{c}	4.82±0.11^{d}	2.53±0.18^{a}	2.01±0.05^{b}	2.54±0.23^{a}	2.29±0.03^{a,b}	0.00±0.00^{e}
OOO	0.93±0.01^{d}	1.63±0.33^{a,b,c}	1.97±0.06^{a}	2.29±0.12^{b}	2.42±0.16^{a,b,c}	2.44±0.01^{a}	2.96±0.29^{a,c}	5.24±0.01^{a}
POO	1.64±0.02^{e}	3.82±0.18^{b,e}	6.35±1.82^{a}	8.1±0.41^{a,b}	8.36±0.57^{a,b,c}	9.26±0.71^{a,b,c,d}	10.65±0.75^{a,c,d}	23.37±0.07^{f}
PPO	1.8±0.02^{a}	1.74±0.25^{a}	2.78±0.01^{b}	3.13±0.66^{a}	3.48±1.77^{a}	4.12±0.00^{a}	4.23±0.37^{a}	8.05±0.12^{a}
PPP	0.78±0.01^{b}	0.00±0.00^{a}	0.00±0.00^{c}	0.00±0.00^{a}	1.67±0.01^{a}	3.15±0.18^{a}	0.00±0.00^{d}	0.00±0.00^{a}
SOO	0.14±0.2^{a}	0.00±0.00^{a}	1.65±0.12^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
SPO	0.4±0.01^{d}	1.62±0.01^{d}	4.11±0.58^{a}	4.34±0.2^{a}	4.84±0.48^{a,b}	4.7±0.29^{a,b,c}	6.07±0.61^{b,c}	18.14±0.37^{e}
PPS	0.19±0.00^{d}	1.15±0.04^{b,c,d}	0.78±0.28^{a}	0.78±0.11^{a,b}	0.8±0.07^{b,c}	1.64±0.26^{c,d}	0.56±0.03^{b,c,d}	0.91±0.03^{b,c,d}
OLL	0.00±0.00^{a,c,d,e}	0.00±0.00^{a,b,c,d}	0.00±0.00^{a}	0.00±0.00^{a,b}	0.00±0.00^{a,b}	0.00±0.00^{a,b,c,d}	0.00±0.00^{a,b,c,d,e,f}	4±0.23^{f}
PLL	0.00±0.00^{d}	2.62±0.04^{c}	0.85±0.08^{e}	1.84±0.04^{a}	2.12±0.04^{a,b}	3.34±0.22^{f}	2.25±0.09^{b,c}	5.75±0.13^{g}
OOL	0.00±0.00^{a}	5.56±0.04^{b}	4.78±0.21^{c}	3.33±0.15^{d}	2.32±0.13^{e}	1.49±0.01^{f}	0.00±0.00^{a}	7.32±0.3^{g}
POL	0.00±0.00^{a,b}	0.91±0.8^{a,b}	1.14±0.35^{a}	1.16±0.03^{a,b}	4.21±0.78^{c}	6.07±0.4^{d}	8.89±0.49^{c}	21.6±0.58^{e}
PPL	0.00±0.00^{b}	6.87±0.18^{c}	5.79±0.03^{d}	3.49±0.04^{a}	4.52±0.28^{a}	3.19±0.32^{e}	0.00±0.00^{b}	2.69±0.07^{a}
SSO	0.00±0.00^{a,d}	0.00±0.00^{a,d}	0.59±0.04^{a}	1.35±0.08^{b}	1.24±0.24^{b}	1.29±0.23^{b,c}	1.71±0.29^{b,c}	4.84±0.05^{e}
SOS	0.00±0.00^{e}	0.53±0.08^{e}	0.14±0.01^{a}	0.5±0.04^{a,b}	0.42±0.02^{a,b,c}	0.37±0.06^{a,b,c,d}	0.55±0.1^{a,b,c,d}	1.76±0.02f
UUU	**0.93±0.54**	**2.42±1.40**	**3.46±1.03**	**3.95±1.19**	**6.29±1.83**	**7.22±2.39**	**7.85±2.79**	**16.56±1.68**
SUU	**6.96±1.28**	**13.74±1.68**	**12.68±2.26**	**13.74±3.03**	**17.12±3.14**	**22.33±3.58**	**27.8±4.19**	**50.72±11.11**
SSU	**11.75±1.54**	**20.9±1.99**	**25.93±1.61**	**26.88±1.68**	**27.23±1.66**	**28.24±2.41**	**30.21±6.05**	**33.59±2.86**
SSS	**74.24±4.89**	**44.33±2.79**	**43.07±2.88**	**42.76±2.80**	**39.32±1.86**	**37.51±2.51**	**35.22±2.33**	**0.91±0.25**
others	6.12	1.75	14.35	9.74	8.95	12.07	9.56	1.6

†Each value in the table represents the mean of three replicates ± standard deviation. Means within each row bearing different superscripts are significantly ($p<0.05$) different.

Abbreviations: Cp caproic, C capric, La lauric M myristic, O oleic, L linoleic, P palmitic, S stearic, SSS trisaturated triacylglycerol, SSU monounsaturated triacylglycerol, SUU diunsaturated triglycerol.

concentration increase significantly of POO, POL and POS.

As shown in Table 1, the level of POO, POL and POS were the three TAGs having a significant increase by the increasing level of lard addition. Unlike the most edible fats and oils, lard has a unique saturated fatty acid, especially palmitic acid in sn-2 position (Kallio *et al.*, 2001). The presence of saturated fatty acid in edible fats and oils at sn-2 position has negative effect in risk increase of atheroscheloris because it can contribute to fat absorption increase and delays chylomicron clearance from the blood vessels (Kritchevsky *et al.*, 1998). However, the TAG analysis using conventional HPLC alone would not be able to differentiate regioisomerism in edible fats and oils, because there are positional isomers of fatty acids present in the glycerol backbone of TAG such as that observed in POO, POL and POS. Therefore, in order to identify the specific regioisomers in TAG would require prior analysis such as enzymatic hydrolysis or more advanced HPLC analysis such as HPLC in combination with mass spectrometry (Mansor *et al.*, 2012). This is particularly seen for POO TAG in our analysis, as butter also contain POO

at 1.64±0.02%. Even TAG of POO increases with increments of lard in butter, further analysis should be performed to ensure this TAG is POO of lard.

It is well recognized that the thermal behavior of edible fats and oils are correlated with TAG composition composed of fats and oils. Indeed, the variety of TAG present in fats and oils contributes to the melting and cooling phase to occur over a temperature span. As a consequence, analysis of TAG composition would compliment with the thermal behaviour of butter adulterated with lard.

Thermal Analysis by Differential Scanning Calorimetry: Heating thermogram

From the previous publication, it is reported that butter and lard have different levels of saturated and unsaturated fatty acids, although the physical properties of both are similar (Nurrulhidayah *et al*., 2013). The large range level of the arrangements and saturation levels of TAG present in butter and lard (Table 1), results to the formation of different multiple endothermic and exothermic peaks, as seen in DSC curves. The more saturated the TAG, the higher the melting temperature, and inversely, the less saturated TAG, the lower the melting temperature. Lard has more unsaturated fatty acids than saturated fatty acids (Yanty *et al*., 2011). Edible fats and oils with higher saturated FA and in turn saturated TAG would have higher melting points. Butter melted in three fractions resulting in three melting peaks. The peaks of butter were most distinct and the first pronounced fraction melted around 0°C. At around 23 to 25°C, in butter, there are two peaks indicating that major fraction melted, and butter appeared as a liquid though with visible crystals. Finally, butter was completely melted (offset) at approximately 32°C (Figure 1).

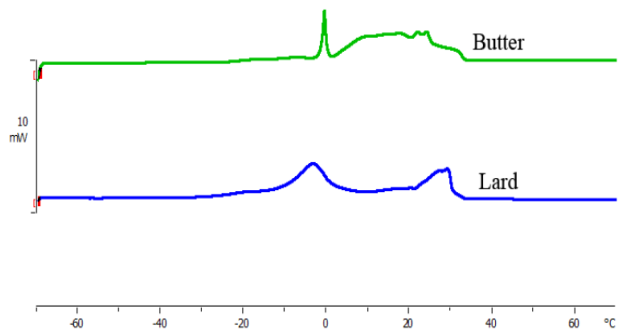

Figure 1. The heating thermogram of Differential scanning calorimetry (DSC) of butter and lard

The melting point peaks of pure lard were wide, either at around 0°C or around 23 to 25°C. This melting profile of lard agrees with that reported previously (Cheong *et al*., 2009; Svenstrup *et al*., 2005).

In lard, the first major peak at around 0°C is caused by mostly to the unsaturated TAG and FA and the second peak around 23 to 25°C is caused mostly by the saturated FA and TAG. Due to the higher ratio of unsaturated FA and TAG in lard, the first major peak is seen to be bigger than the second endothermic peak (Salwani *et al*., 2012). Figure 2A exhibit the melting curves of lard, butter and lard-butter blends containing 1 to 80% of butter. It is obvious that there is peak reduction at around 0°C with the increasing level of lard until concentration level of 60 %. While, the enlarged melting peak of heating can be seen in Figure 2B. This major peak was also called as peak A (Figure 2B).

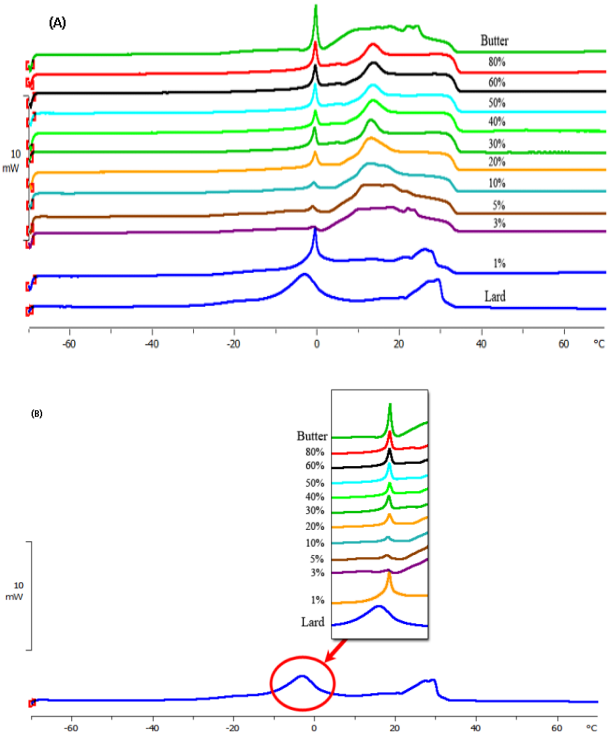

Figure 2. The melting curves of lard, butter and lard-butter blends containing 1–80 % of butter (A), and the enlarged melting profile at around 0°C (B).

The melting profile of the blend containing 1% has increased significantly the melting profile at around 23 to 25°C. This intensity was attributed to the addition in beta crystals in the blend (Miklos *et al*., 2013).

Thermal analysis by differential scanning calorimetry: cooling thermogram

The cooling thermograms of DSC curve for pure butter and pure lard are depicted in Figure 3. There are two minor exothermic peak at around 7 to 15°C. The existence of the two major exothermic peaks in butter can be correlated with crystal formation of

Table 2. Cooling and Melting partial enthalpy of butter adulterated with lard (v/v)

% Lard concentration	Cooling enthalpy (J/g)	Melting enthalpy (J/g)
100	-10.72±0.89	13.95±0.07
80	-11.18±2.33	14.96±0.05
60	-12.79±0.89	15.75±0.89
50	-13.11±0.68	16.69±1.48
40	-12.77±2.45	17.06±2.09
30	-13.37±1.23	16.33±1.77
20	-23.80±1.61	17.91±1.27
10	-21.10±0.99	19.41±0.90
5	-35.63±2.13	21.14±4.69
3	-46.37±2.29	20.52±0.98
1	-46.28±1.02	24.71±0.89
0	-54.06±8.87	35.97±4.18

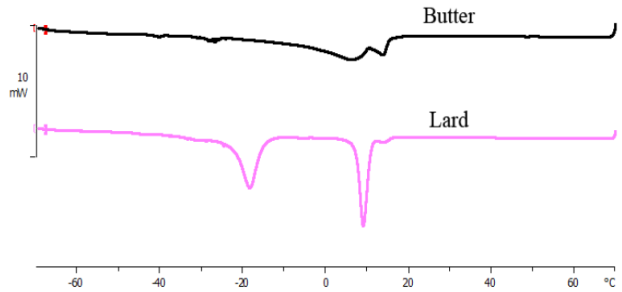

Figure 3. The cooling thermograms of Differential scanning calorimetry (DSC) of pure butter and pure lard

TAG (Marina *et al.*, 2009). The small exothermic peaks could be related to the unsaturated TAG. In addition, lard has two major exothermic peaks observed at around -18.5 and 9.0°C, slightly higher than -16.13 and 8.84°C those reported by Salwani *et al*. (2012). This can be attributed to the different nature of lard, the feeding type and the variety of fatty acid composition. These two peaks are well separated as compared to the major exothermic peaks in butter and that the phase transition has a wider temperature range than butter. This can be explaiined by the different crystal formation of each specific group of FA and TAG. The saturated FA and TAG will crystallize at high temperature, while the unsaturated FA and TAG will crystallize at low temperature. Fasina *et al*., (2008) have reported that the melting behavior of edible fats and oils varies due to the different characteristics of FA composition . This can be explored as a basis of using FA composition and heating and cooling profiles or food quality control besides correlating the TAG composition alone with DSC thermal parameters.

Figure 4 shows the cooling curves of lard, butter and lard-butter blends containing 1 to 80 % of butter (A), and the enlarged cooling profile at around 0°C (B), known as peak B. When butter was added with

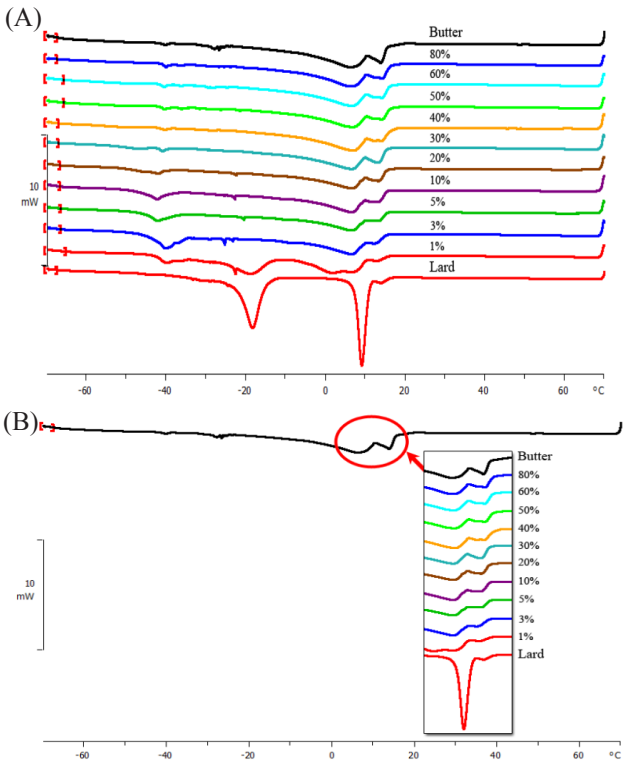

Figure 4. The cooling curves of lard, butter and lard-butter blends containing 1–80 % of butter (A), and the enlarged cooling profile at around 0°C (B), known as peak B

lard, the two cooling peaks in butter can still be observed with subtle morphological changes in the thermal curve as the lard concentration increases from 1 to 80%. Figure 4 shows the adulterant exothermic peak B, which was seen to decrease as the percentage of adulteration increases. Table 2 presents the cooling enthalpy and melting enthalphy (taken from the measurement of the area under the peaks in the cooling and melting curve) for butter is -54.06±8.87 J/g and 35.97±4.18 J/g respectively, as compared to the cooling enthalpy and melting enthalphy of lard of -10.72 ± 0.89 J/g and 13.95 ± 0.07 J/g respectively. The cooling enthalpy supports their comparative FA and TAG saturation levels, i.e. butter has a higher saturation FA and TAG, leading to a larger cooling enthalpy.

Conclusion

It was highlighted the use of differential scanning calorimetry (DSC) to detect changes in the cooling and heating curves of butter when butter is adulterated with lard. Qualitatively, the curves showed subtle differentiation between butter, lard and their blends. The main advantage of DSC for analysis of lard as adulterant in butter is that its provides a direct estimate of the overall enthalpy change of lard transitions and is relatively quick and simple to do with minimal

sample preparation and chemical free method.

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References

Aida, A.A., Che Man, Y.B., Wong, C.M.V.L., Raha A.R. and Son, R. 2005. Analysis of raw meats and fats of pigs using polymerase chain reaction for Halal authentication. Meat Science 69: 47–52.

Al-Qaradawi, Y. 1960. The lawful and the prohibited in Islam [Al-Haram Wal Haram Fil Islam]. Indianapolis, IN. USA: American Trust Publications.

AOAC. Association of Official Analytical Chemists.2000. Official methods of analysis of AOAC International. 17th ed. Gaithersbug, MD: AOAC International Official ed. Gaithersbug, MD: AOAC International.

Barron, L.J.R., Hierro, M.T.G. and Santa-María, G. 1990. HPLC and GLC analysis of the triglyceride composition of bovine, ovine and caprine milk fat. Journal of Dairy Research 57: 517-526.

Biliaderis, C.G. 1983. Differential Scanning Calorimetry in Food Research A Review. Food Chemistry 10: 239-265.

Bukhari, Abu 'Abd Allah Muhammad bin Ismail. 2002. *Sahih al-Bukhari*. 2nd Edition. Beirut: Dar al-Kutub al-'Ilmiyah.

Cebula, D.J. and Smith, K.W. 1992. Differential scanning calorimetry of confectinery fats: part II-effects of blends and minor components. Journal of the American Oil Chemists' Society 69: 992–998.

Che Man, Y.B, Syahariza, Z.A., Mirghani, M.E.S., Jinap, S. and Bakar, J. 2005. Analysis of potential lard adulteration in chocolate and chocolate products using fourier transform infrared spectroscopy. Food Chemistry 90: 815–819.

Cheong, L. Z., Zhang, H., Xu, Y. and Xu, X. B. 2009. Physical characterization of lard partial acylglycerols and their effects on melting and crystallization properties of blends with rapeseed oil. Journal of Agricultural and Food Chemistry 57: 5020–5027.

Coni, E., Pasquale, M. D., Cappolelli, P. and Bocca, A. 1994. Detection of animal fats in butter by SC: A pilot study. Journal of the American Oil Chemists' Society 71: 807–810.

Fang, G., Goh, J.Y., Tay, M., Lau, H.F. and Li, S.F.Y. 2013. Characterization of oils and fats by ^{1}H NMR and GC/MS fingerprinting: Classification, prediction and detection of adulteration. Food Chemistry 138: 1461–1469.

Farkas, J. and Mohacsi-Farkas, C. 1996. Application of differential scanning calorimetry in food research and food quality assurance. Journal of Thermal Analysis 47: 1787–1803.

Fasina, O.O., Craig-Schmidt, M., Colley, Z. and Hallman, H. 2008. Predicting melting characteristics of vegetable oils from fatty acid composition. LWT Food Science and Technology 41:1501–1505.

Griffin, V.J. and Laye, P.G. 1992. Differential thermal analysis and differential scanning calorimetry. In E. L. Charsley, and S. B. Warrington (Eds.), Thermal analysis (pp. 17–30). Cambridge: Royal Society of Chemistry.

Ibn Majah, Abu 'Abd Allah Muhammad bin Yazid al-Qazwini. 1998. *Sunan Ibn Majah.* Edt. Mahmud Muhammad Mahmud Hasan Nassar. Beirut: Dar al-Kutub al-'Ilmiyah.

Indrasti, D., Che Man, Y.B., Mustafa, S. and Hashim, D.M. 2010. Lard detection based on fatty acids profile using comprehensive gas chromatography hyphenated with time-of-flight mass spectrometry. Food Chemistry 122: 1273–1277.

Kallio, H., Yli-Jokipli, K., Kurvinen, J-H., Sjo¨vall, O. and Tahvonen, R. 2001. Regioisomerism of triacylglycerols in lard, tallow, yolk, chicken skin, palm oil, palm olein, palm stearin, and a transesterified blend of palm stearin and coconut oil analyzed by tandem mass spectrometry. Journal of Agriculture and Food Chemistry 49: 3363–3369.

Kamali, M. H. 2008a. Halal industry within Islamic principles. A shari'ah perspective on halal and haram. Part 1. Understanding the parameters of halal and haram. The Halal Journal, 40–43.

Kamali, M. H. 2008b. Halal industry within Islamic principles. A shari'ah perspective on halal and haram. Part 2. Revisiting the grey area. The Halal Journal (36,37–38, 40).

Kamali, M. H. 2008c. Halal industry within Islamic principles. A shari'ah perspective on halal and haram. Part 1. Of customs and science for determination of the lawful & the unlawful. The Halal Journal (36–38, 40).

Kritchevsky, D., Tepper, S.A., Kuksis, A., Eghtedary, K. and Klurfelard, D.M. 1998. Cholesterol vehicle in experimental atherosclerosis. Native and randomized lard and tallow. Journal of Nutrition and Biochemistry 9:582–585.

Kurniawati, E., Rohman, A. and Triyana, K. 2014. Analysis of lard in meatball broth using Fourier transform infrared spectroscopy and chemometrics. Meat Science 96: 94–98.

Mansor, T.S.T., Che Man, Y.B. and Rohman, A. 2011. Application of fast gas chromatography and Fourier transform infrared spectroscopy for analysis of lard adulteration in virgin coconut oil. Food Analytical Methods 4:365–372.

Mansor, T.S.T. Che Man, Y.B. and Shuhaimi M. 2012. Employment of Differential Scanning Calorimetry in Detecting Lard Adulteration in Virgin Coconut

Oil. Journal of the American Oil Chemists' Society 89:485–496.

Marikkar, J.M.N., Lai, O.M., Ghazali, H.M. and Che Man, Y.B. 2001. Detection of Lard and randomized lard as adulterants in refined-bleached-deodorized palm oil by differential scanning calorimetry. Journal of the American Oil Chemists' Society 78: 1113 – 1119.

Marikkar, J.M.N., Ghazali, H.M., Long, K. and Lai, O.M. 2003. Lard uptake and its detection in selected food products deep-fried in lard. Food Research International 36: 1047–1060.

Marikkar, J.M.N. Ghazali, H.M., Che Man, Y.B., Peiris, T.S.G. and Lai, O.M. 2005. Distinguishing lard from other animal fats in admixtures of some vegetable oils using liquid chromatographic data coupled with multivariate data analysis. Food Chemistry 91: 5–14.

Marikkar, J.M.N., Dzulkifly, M.H., Nadiha, M.Z.N. and Man, Y.B.C. 2012. Detection of animal fat contaminations in sunflower oil by differential scanning calorimetry. International Journal of Food Properties 15: 683-690.

Marina, A.M., Che Man, Y.B., Nazimah, S.A.H. and Amin, I. 2009. Monitoring adulteration of virgin coconut oil by selected vegetable oils using differential scanning calorimetry. Journal of Food Lipids 16:50–61.

Miklos, R., Zhang, H., Lametsch, R. and Xu, X. 2013. Physicochemical properties of lard-based diacylglycerols in blends with lard. Food Chemistry 138: 608–614.

Mursyidi, A. 2013. The role of analytical chemistry in Halal certification. Journal of Food and Pharmaceutical Sciences 1: 1–4.

Muslim, Ibn al-Hajjaj Abu al-Husayn al-Qusyairi al-Nisaburi. 1998. *Sahih Muslim*. Edt. Ahmad Syams al-Din. Beirut: Dar al-Kutub al-'Ilmiyah.

Nizar, N.N.A., Marikkar, J.M.N. and Hashim, D.M. 2013. Differentiation of Lard, Chicken Fat, Beef Fat and Mutton Fat by GCMS and EA-IRMS Techniques. Journal of Oleo Science 62: 459-464.

Nurjuliana, M., Che Man, Y. B. and Mat Hashim D. 2011b. Analysis of lard's aroma by an electronic nose for rapid halal authentication. Journal of the American Oil Chemists' Society 88: 75 – 82.

Nurrulhidayah, A. F., Rohman, A., Amin, I., Shuhaimi M. and Khatib, A. 2012. Authentication analysis of butter from beef fat using Fourier Transform Infrared (FTIR) spectroscopy couple with chemometrics. International Food Research Journal 20: 1383-1388.

Nurrulhidayah, A. F., Rohman, A., Amin, I., Shuhaimi M. and Khatib, A. 2013. Analysis of chicken fat as adulterant in butter using fourier transform infrared spectroscopy and chemometrics. Grasas y Aceites. 64: 349-355.

Rashood, K.A., Shaaban, R.R.A., Moety, E.M.A. and Rauf, A. 1995. Triacylglycerols-profiling by high performance liquid chromatography: A tool for detection of pork fat in processed foods. Journal of Liquid Chromatography 18: 2661.

Regenstein, J. M. and Regenstein, C. E. 1991. Current issues in kosher foods. Trends in Food Science and Technology March, 50–54.

Regenstein, J. M., Chaudry, M. M. and Regenstein, C. E. 2003. The kosher and halal food laws. Comprehensive Review in Food Science and Food Safety 2: 111–127.

Rohman, A. and Che Man, Y.B. 2009. Analysis of cod-liver oil adulteration using Fourier Transform Infrared (FTIR) spectroscopy. Journal of the American Oil Chemists' Society 86: 1149-1153.

Shukla, V.K.S., Schiotz Nielsen, W. and Batsberg, W. 1983. A Simple and Direct Procedure for the Evaluation of Triglyceride Composition of Cocoa Butters by High Performance Liquid Chromatography-A Comparison with the Existing TLC-GC Method. Fette Seifen Anstrichm. 85: 274-278.

Svenstrup, G., Bruggemann, D., Kristensen, L., Risbo, J. and Skibsted, L. H. 2005. The influence of pretreatment on pork fat crystallization. European Journal of Lipid Science and Technology 107: 607–615.

Syahariza, Z.A, Che Man, Y.B, Selamat, J. and Bakar, J. 2005. Detection of lard adulteration in cake formulation by Fourier transform infrared (FTIR) spectroscopy. Food Chemistry 92: 365–37.

Tan, C.P. and Che Man, Y.B. 2002. Recent development in differential scanning calorimetry for assessing oxidative deterioration of vegetable oil. Trends Food Science and Technology 13: 312–318.

Yanty, N. A. M., Marikkar, J. M. N., Che Man, Y. B. and Long, K. 2011. Composition and thermal analysis of lard stearin and lard olein. Journal of Oleo Science 60: 333-338.