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.

Keywords

Adulteration Lard Butter Halal Differential Scanning calorimetry
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), differenten 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 differentiation 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 well 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 al., 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 adipose 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) compositional 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 (T_e) (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 (T_r) (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 software 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 palmitoleoolceolein (POO) 23.37%, palmitoleoolcinolein (POL) 21.6%, and palmitoleolestearin (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
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 atherosclerosis 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).

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).

The melting profile of the blend containing 1% has increased significantly the melting profile at around 0°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
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 explained 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.

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

<table>
<thead>
<tr>
<th>% Lard concentration</th>
<th>Cooling enthalpy (J/g)</th>
<th>Melting enthalphy (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>10.7±0.89</td>
<td>13.9±0.7</td>
</tr>
<tr>
<td>80</td>
<td>11.1±2.3</td>
<td>14.9±0.05</td>
</tr>
<tr>
<td>60</td>
<td>12.7±1.6</td>
<td>15.7±0.89</td>
</tr>
<tr>
<td>50</td>
<td>13.1±0.8</td>
<td>16.0±1.18</td>
</tr>
<tr>
<td>40</td>
<td>12.7±2.4</td>
<td>17.0±2.10</td>
</tr>
<tr>
<td>30</td>
<td>13.3±1.2</td>
<td>16.3±1.77</td>
</tr>
<tr>
<td>20</td>
<td>23.8±0.1</td>
<td>17.9±1.27</td>
</tr>
<tr>
<td>10</td>
<td>21.1±0.9</td>
<td>19.4±0.90</td>
</tr>
<tr>
<td>5</td>
<td>35.6±2.13</td>
<td>21.4±0.69</td>
</tr>
<tr>
<td>3</td>
<td>46.7±2.29</td>
<td>20.5±0.98</td>
</tr>
<tr>
<td>1</td>
<td>46.2±1.02</td>
<td>24.7±0.89</td>
</tr>
<tr>
<td>0</td>
<td>54.0±4.87</td>
<td>35.9±4.18</td>
</tr>
</tbody>
</table>

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

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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