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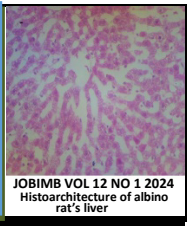

Physicochemical Differentiation of Lard and Fats of Beef and Chicken

Sahar Abbasiliasi[1,2], Marliana Azir[1], Tan Joo Shun[2,3], Tengku Azmi Ibrahim[4], Awis qurni Sazili[1] and Shuhaimi Mustafa[1,5,*]

[1]Halal Products Research Institute, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[2]Bioprocessing and Biomanufacturing Research Center, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[3]Bioprocess Technology, School of Industrial Technology, Universiti Sains Malaysia, 11800 Gelugor, Malaysia.
[4]Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[5]Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

*Corresponding author:
Prof Dr. Shuhaimi Mustafa
Halal Products Research Institute,
Universiti Putra Malaysia,
43400 UPM Serdang,
Selangor,
Malaysia.
Email: shuhaimi@upm.edu.my

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ABSTRACT

This study differentiates, based on their physicochemical properties, the fats of pork, beef and chicken. Fats from the three animal species were extracted and assessed for their iodine, peroxide and acid values and moisture/volatile matter contents. Triacylglycerol (TAG) and fatty acid compositions of the oils were determined, and their structural and thermal properties analysed. Results showed that chicken fat had higher iodine value compared to that of lard and beef fat indicating that avian fat has more double bonds and less oxidative stability. There was no significant difference (P>0.05) between the acid values of lard and beef fat. Similarly, there was no significant differences (P>0.05) either in the moisture/volatile matter content of lard, and fats of beef and chicken. Highest unsaturated fatty acids were present in chicken fat (68%) followed by lard (55.06%) and beef fat (46.86%). Chicken fat has the lowest cooling and melting temperatures (~ -38 ºC and ~ -27 ºC, respectively). Fourier transform infrared (FTIR) spectrum of beef fat could be differentiated from that of lard and chicken fat in existing peak at frequency ~1127 cm^{-1}. Highest total saturated TAG and fatty acids were observed in beef fat (~84% and ~ 53%) while chicken fat showed the highest total unsaturated TAGs and fatty acids (~81% and ~68%). Results from this study could serve as a basis for authenticity determination of food products and quantification of adulteration.

INTRODUCTION

Edible animal fats are lard, beef dripping and chicken fats. Consumed from time immemorial animal fats provided the essential nutrients required by the body. Animal fat is a natural and beneficial part of a balanced diet supplying energy, vitamins and fatty acids [1]. Edible animal fats are devoid of the unnatural trans-fatty acids and "fast" carbohydrates which are widely linked to coronary heart diseases and stroke. With significant levels of oleic acid animal fat is widely associated with health benefits and its acceptance is further enhanced by its delicious taste apart from its excellent baking and cooking properties [2]. To further augment the beneficial properties of animal fats is the commendable effort on the part of researchers to understand the functionality of different fats and oils in relation to food quality. Food quality is principally dependent on the functionality of fats and oils in food itself which ultimately is the combined result of its physical, chemical, and rheological properties [3]. Fats and oils have a major influenced on the finished products texture through the formation of structures of the crystalline networks and additionally through interruption of the structure on account of their interaction with non-fat components. Further, fats and oils can affect the structural integrity and the shelf life of the ultimate product [4]. It has also been reported that the selection of fats and oils should concur with their precise performance within the end product in addition to the nutritional profiles. The type of fatty acids at every position profoundly influences on the physical behaviour of fats and oil [5]. Moreover, the comparative proportions of every triacylglycerol in fats and oil are crucial to their overall performance and stability.

The importance of fats and oils is from the perspective of their functionality attributed to their chemical and structural composition [3]. In spite of the fact that the physicochemical

properties of fats and oils are rather complex and not fully understood both however have a remarkable effect on the texture of the final product. Each fat and oil have a range of physical, chemical and structural compositions. The latter have a major role in deciding the food quality especially cookies and bakery products. In view of the importance of the highly sought after animal fats in the food industry the general objective of this study is aimed at characterizing lard, beef and chicken fats based on their physiochemical properties focusing on their iodine, peroxide and acid values and determination of their moisture/volatile matter contents. In this study triacylglycerol (TAG) and fatty acid composition, determination of their functional group and structural organization and their thermal profiles are further elucidated.

MATERIALS AND METHODS

Sample and supplies

Adipose tissues from chicken, beef and pig were collected from the local market at Sri Serdang, Selangor, Malaysia. Acetone (C_3H6O_2, ≥99.9%), acetonitrile (CH_3CN, ≥99.0%, chloroform ($CHCl_3$, 99.8%), anhydrous sodium sulphate (Na_2SO_4), cyclohexane (C_6H_{12}), Wij's reagent (iodine trichloride solution), potassium iodide (KI), sodium thiosulphate pentahydrate ($Na_2S_2O_3$,5H_2O) and acetic acid glacial (CH_3CO_2H, ≥99%) were obtained from Qrec, New Zealand. TAG standards and sodium methoxide solution (CH_3ONa, 25 wt. % in methanol) were sourced from Sigma- Aldrich, USA. All chemicals used in this study were of analytical grade.

Extraction of oils from lard, beef and chicken fats

Samples of animal fats from chicken, beef and pig were diced into small pieces (0.5 cm × 0.5 cm), heated at 90-100 ºC for 2 h, strained through a triple folded cloth and filtered using Whatman no. 2 filter paper. The filtered fats were flushed with nitrogen gas to extend its shelf life and kept in a tightly closed container at 4 ºC for subsequent use [6].

Chemical properties of lard, beef and chicken fats

Iodine value (IV) was evaluated as described by Lan et al. [7] with some modification. 20 mL cyclohexane was added to 0.5 g of fat and the mixture warmed until the fat was dissolved. 25 mL of the Wijs reagent was then added and the mixture agitated in a shaker incubator in the dark for 1 h at 25 ºC. Following incubation, 20 mL of the 10% (w/v) of KI solution and 100 mL of distilled water was added and solution titrated with 1 N $Na_2S_2O_3$5H_2O solution until the colour of the solution changed to yellow. A mixture of cyclohexane, Wij's, potassium iodine, sodium thiosulphate and starch were used as a blank sample under the same conditions. Iodine value was determined using equation (1):

$$\boldsymbol{IV} = \frac{12.69N \times V_2 - V_1}{W} \qquad \text{Eqn. 1}$$

Where;

N presents the normality of the $Na_2S_2O_3$ solution,V_2 presents the volume of the $Na_2S_2O_3$ solution used for the blank test (mL), V_1 presents the volume of the $Na_2S_2O_3$ solution used for the determination (mL) and W presents the weight of fat sample used for analysis (g). The method of Zhang et al. [8] was used to prepare the sample for of determination of Peroxide value (PV). 5 g of fat samples was mixed with 30 mL of the acetic-chloroform solution (3:2 v/v). The mixture was agitated in an incubator shaker at room temperature till the sample was dissolved. 0.5 mL of 5M potassium iodide was added to the solution and swirled for 1 min and topped up with 30 mL of distilled water. 1 to 2 mL of 1% (w/v) starch solution was added and then titrated with 0.01N $Na_2S_2O_3$ solution. Blank contain acetic-chloroform, potassium thiosulphate, potassium idodide and starch. The PV was calculated using equation (2):

$$\boldsymbol{PV} = \frac{V_s - V_b}{W} \times 1000 \qquad \text{Eqn. 2}$$

Where;

V_s is the volume of the $Na_2S_2O_3$ in the sample (mL), N is the normality of $Na_2S_2O_3$, V_b is the volume of $Na_2S_2O_3$ used in blank (mL) and W is the weight of sample (g)

Acid value of fats were determined as descibed by Horwitz and Latimer [9] and calculated using equation (3) as follow:

$$\boldsymbol{AV} = \frac{\text{Titre value (mL)}}{Weight\ of\ sample\ used\ (g)} \times 5.61 \qquad \text{Eqn. 3}$$

Volatile matter/moisture of fats were evaluated as explained by Latimer [10]. 2 g of oil samples was dried in an oven at 105 ºC and kept in desiccator until a constant weight was achieved. The weight of empty petri dish and the final constant weight were recorded, and the percentage of volatile matter was calculated using equation (4).

$$\%\ \textbf{Volatile matter/moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad \text{Eqn. 4}$$

Where;

W_1 presents the weight of empty dish (g), W_2 presents the initial weight of dish with fat sample (g) and W_3 presents the final weight of dish with fat sample (g)

Determination of TAG composition

The method of Haryati et al. [11] with some modifications was used to prepare the sample for determination of TAG composition of lard and fats of chicken and beef. Operational conditions of the HPLC were as described in a previous study by Azir et al. [12].

Evaluation of Fatty acid Composition

The method of Marina et al. [13] was used for evaluation of fatty acids methyl ester (FAME) composition. The detailed procedures for the preparation of samples and operational condition of the GC were performed according to a previous study by Azir et al. [12].

Determination of functional and structural properties of lard, beef and chicken fats using FTIR

The detailed procedure for the determination of functional and structural properties of lard, beef and chicken fats were based on a previous study by Che Man et al. [14] .

Determination of thermal properties of lard and fats of beef and chicken

Scanning calorimeter (DSC, Mettler Toledo Star System, Columbus, USA) was used to assess the thermal properties of the fat samples. The procedure and operational properties are as reported in a previous study by Tan and Che Man [15].

Statistical analysis

The means of all calculated parameters were compared by analysing the data using SAS (version 10). To identify the differences between data, a one-way analysis of variance (ANOVA) with a post-hoc Tukey's honestly significant difference (HSD) test was carried out.

RESULTS AND DISCUSSION

Chemical properties of lard, chicken and beef fats

The chemical properties of lard, chicken and beef fats are presented in **Table 1**. The highest iodine value was observed in chicken fat as reflected by the presence of high percentage of unsaturated fatty acids which concur with that of Farmani and Rostammiri [16]. It was reported that the oxidative and chemical properties of oil during storage could be influenced by an increase in free fatty acid content and/or a decrease in total unsaturated fatty acids [17]. The higher iodine values could be attributed the greater number of double bonds, higer content of unsaturated FAs and less oxidative stability [18].

Table 1. Chemical properties of lard, chicken and beef fats.

Sample	Iodine Value (I_2g/100g)	Peroxide Value (mEq/kg)	Moisture/Volatile Matter (%)	Acid value (mg KOH/g)
Chicken fat	74.45±0.39^a	2.04±0.07^a	0.45± 0.02^a	0.45± 0.02^a
Beef fat	39.41±0.40^c	2.07± 0.11^a	0.50±0.00^a	0.50±0.00^a
lard	63.95±0.91^b	1.70±0.10^b	0.48±0.06^a	0.52±0.06^a

Note: Mean (± S.E.) of results from three separate experiments.
a, b and c: Values with the different superscript letters in the same column are statistically significantly different (P<0.05

The peroxide value of fats is dependent on the degree of oxidation as indicated by the presence of the amount of hydroperoxides, the primary product of oxidation. It was reported that the quality and stability of fats and oils is associated with the peroxide value [8]**.** Peroxides value of lard was significantly different from that of chicken and beef fats. Peroxide value is dependent on storage time, temperature, air and light in contact with the sample. Peroxide value is an indication of the extent of oxidation of oil or rancidity [19].

Lard, beef and chicken fats contain a small amount of moisture (<1%) which is desirable in maintaining the quality and shelf life of oil [20]. Water is responsible for hydrolysis of oil which generates free fatty acids and glycerol products [21]. Acid value is a measurement of the amount of free fatty acids, a split from triglycerides which represent hydrolytic breakdown of triglyceride molecules. There were no significant differences (P<0.05) among the acid values of lard, beef and chicken fats and all were within the range of maximum limit based on Codex Alimentarius guidelines [22].

Triglycerides composition of lard, chicken and beef fats

Distinction of the nature of triglycerides (TAGs) is the principal factor which differentiates one animal fat from the other and this variation affect TAG separation [12]. TAGs profile of fats which is indicative of their fatty acid compositions and separation using a reverse-phase silica column has a major influence on the degree of unsaturation of TAGs present in oil or fat samples. The TAG profile of lard, beef and chicken fats are as shown in **Table 2**.

Chicken fat contain the highest total di- and tri-unsaturated TAGs while beef fat contains the highest di- and tri-saturated fats. TAGs profile of chicken fat reveals a number of features which are similar to that of lard and the latter concurred with that of Marikkar et al., [23]. O, P and S dominated the chromatograms of beef fat while L, Ln and O are present in lard and chicken fat as considered by the shorter retention times of LLLn, LLL, OLL, OOL, PLL, OLL, POL and POO (**Fig. 1**). It was reported that the retention time of di-saturated TAGs (SSU) species could be higher than that of a monosaturated TAG (SUU) species [24]. Three compounds which are dominant in each profile are POL, POS and POO in lard, POS, PPS and POP in beef fat and POL, POO and POP in chicken fat. PPP and PSS in the class of trisaturated TAGs of chicken fat and lard are absent while beef fat contain all the trisaturated TAGs. Furthermore, most of the trisaturated (LLLn, LLL, OLL, OOL and OOO), diunsaturated (PLL and POL) and disaturated (PPL) TAGs were not detected in beef fat.

Table 2. TAG Composition of lard, beef and chicken fats.

	Animals fats		
TAG	LD	BF	CF
Tri-unsaturated			
LLLn	0.88± 0.06^b	nd	2.19±0.20^a
LLL	1.24±0.09^a	nd	0.89±0.01^b
OLL	2.96±0.06^a	nd	7.40±0.03^b
OOL	4.38±0.05^b	nd	10.07±0.06^a
OOO	2.28±0.06^c	5.10±0.04^a	8.57±0.07^b
Sub total	11.72±1.40	5.10±0.01	29.12±4.05
Di-unsaturated			
PLL	7.36±0.03^b	nd	8.87 ±0.09^a
POL	20.21±0.09^b	nd	19.96± 0.11^a
POO	17.25±0.06^b	3.86±0.18^c	19.93±0.05^a
SOO	3.21±0.06^b	6.60±0.09^a	3.06±0.10^b
Sub Total	48.03±2.03	10.46±1.94	51.82±8.41
Di-saturated			
POP	18.56±0.06^c	12.77±0.05^a	11.83±1.42^b
PPL	3.46±0.06^b	nd	2.02±0.06^b
POS	51.68	26.37±0.02^a	3.87± 0.01^c
SOS	1.32±0.08^b	8.36±0.04^a	0.42±0.09^c
Sub Total	135.39±7.68	47.50±11.02	18.14 ±5.02
Tri-saturated			
PPP	nd	9.08±0.07^a	nd
PPS	1.99±0.18^b	14.71±0.07^a	0.78±0.04^c
SSS	2.89±0.01^a	2.26±0.03^b	0.13±0.07^c
PSS	nd	10.90±0.02^a	nd
Sub Total	4.88±1.46	36.95±5.21	0.91±0.37
Total Saturated	40.27±2.57	84.45±7.46	87.50
Total Unsaturated	59.75±5.68	15.56±3.79	12.50

Note:
Mean (± S.E.) of results from three separate experiments.
a, b and c: Values with the different superscript letters in the same row are statistically significantly different (P<0.05)
TAG, triacylglycerol; BF, beef fat; LD, lard; CF, chicken fat; P, palmitic; O, oleic; L, linoleic; S, stearic; Ln, linolenic; nd: not detected.

Fatty acid methyl ester composition of lard, chicken and beef fats

Fatty acid methyl ester (FAME) compositions of lard, chicken and beef fats are shown in **Table 3**. The most abundant FAMEs in lard, beef and chicken fats are oleic acid (C18:1) followed by palmitic acid (C16:0) as reported by Hasan [25] is dependent on the type of animal. Linoleic acid (C18:2) is high in lard and chicken fat while C18:0 is high in lard and beef fat. C18:2 is the only fatty acid that cannot be synthesized by the pig and must be supplied in the diet for the animal to grow normally. Ruminants have low level of C18:2 and C18:3 due to the activities of microorganism in the rumen which effectively saturate the polyunsaturated plant oils present in the animal's natural diets. In lard only Gadoleic acid (C20:1) and eicosadienoic acid (C20:2) are present while pentadecylic acid (C14:0) is absent.

Chicken fat is different from beef fat and lard by virtue of the absence of pentadecanoic acid (C15:0), margaric acid (C17:0), margaroleic acid (C17:1), C20:1 and C20:2. Lard and chicken fat were found to have higher total unsaturated fatty acids as compared to that of beef fat. Results from this study is in agreement with that of Lee and Foglia [26] who reported that chicken fat contains about 60% unsaturated fatty acids and has higher degree of unsaturated fatty acids compared to beef [27]. The major fatty acids of lard is oleic acid, followed by palmitic and linoleic acids which concur with the report by Nizar et al. [28]. The amount of fatty acids increased with increased fat content of the animal from young to the time of slaughter [29]. It was also showed that species, sex, age, health status, genetic, type, freshness and diet have effects largely on fatty acid composition [30].

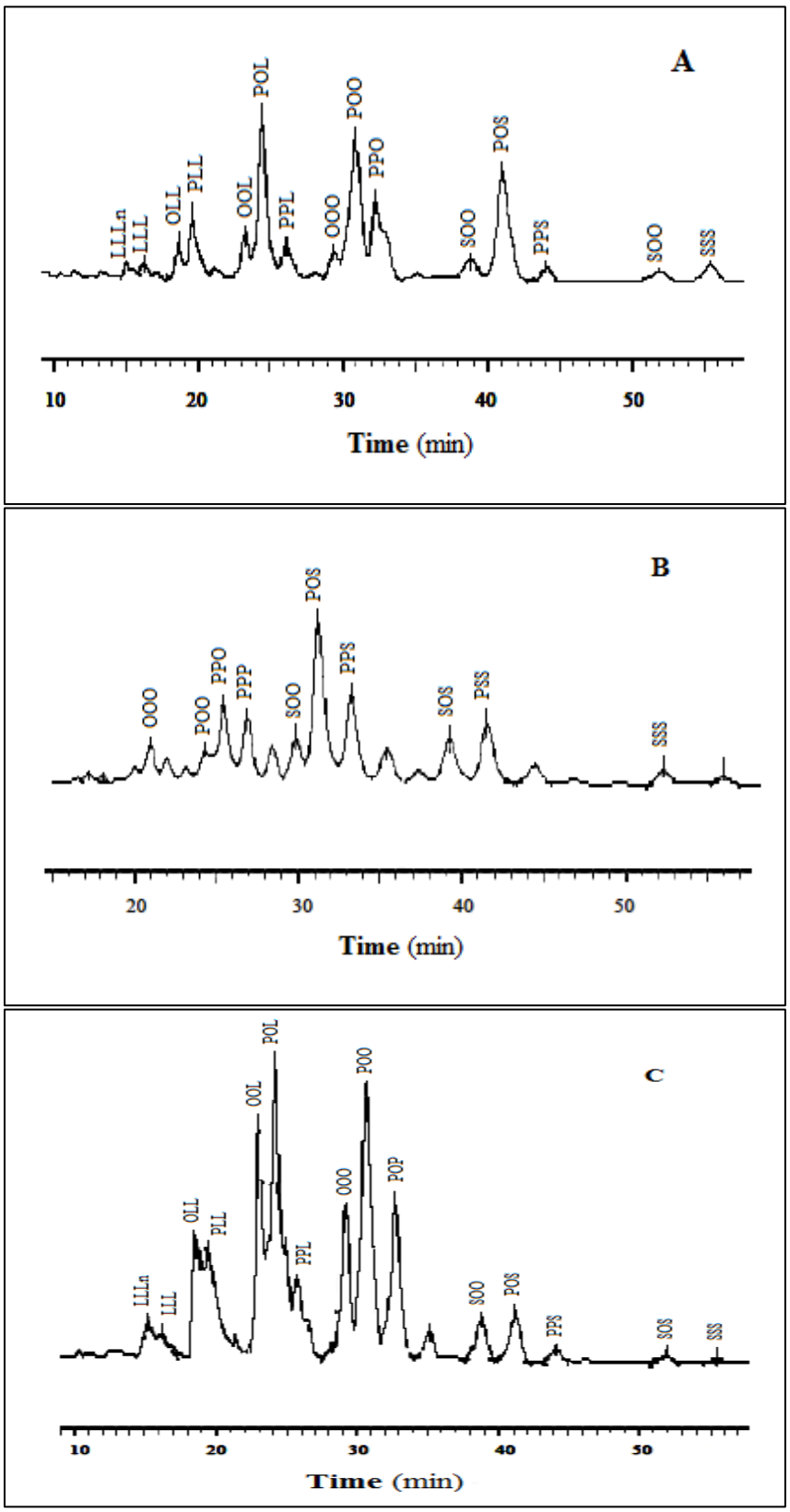

Fig. 1. TAGs profile of (A) lard, (B) beef and (C) chicken fats.

Table 3. Fatty acid compositions in lard, chicken fat and beef fat

FAMEs	LD	Reported range[1]	CF	Reported range[2]	BF	Reported range[3]
Capric acid (C10:0)	0.17±.05^{c}	-	0.27±0.03^{b}		0.37±0.07^{a}	<0.5
Lauric acid (C12:0)	1.44±0.03^{b}	-	0.77±0.02^{c}	-	6.21±0.01^{a}	<0.5
Myristoleic acid (C14:1)	nd	<0.2	0.19±0.05^{b}	0.3	1.72±0.04^{a}	-
Pentadecanoic acid (C15:0)	0.09±0.06^{b}	<0.1	nd		0.60±0.05^{a}	-
Palmitic acid (C16:0)	22.55±0.09^{c}	20-32	25.10±0.09^{b}	25.2	27.66±0.09^{a}	20-30
Palmitoleic acid (C16:1)	1.22± 0.12^{c}	1.7-5	6.08±0.09^{a}	7.8	4.02±0.08^{b}	1-5
Margaric acid (C17:0)	0.58±0.04^{b}	<0.5	nd		1.18±0.06^{a}	-
Margaroleic acid (C17:1)	0.36±0.07^{b}	<0.5	nd		0.89±0.10^{a}	-
Stearic acid (C18:0)	19.29±0.18^{a}	5-24	5.21±0.04^{c}	5.9	15.24±0.01^{b}	15-30
Oleic acid (C18:1)	32.41±0.31^{c}	36-62	44.56±0.06^{a}	40.5	39.92±0.03^{c}	30-45
Linoleic acid (C18:2)	18.78±0.32^{a}	3-16	16.60±0.03^{b}	18.4	1.26±0.01^{c}	1-6
Linolenic acid (C18:3)	1.09±0.32^{a}	<1.5	0.81±0.05^{b}	0.7	0.77±0.03^{c}	<1.5
Arachidic acid (C20:0)	0.82±0.09^{a}	<0.5	0.41±0.04^{b}		0.15±0.02^{c}	-
Gadoleic acid (C20:1)	0.85±0.10^{a}	<1	nd	0.5	nd	-
Eicosadienoic acid (C20:2)	0.35±0.0^{a}	-	nd	-	nd	-
∑SFA	44.94±2.03	-	31.95±1.53	-	53.13±3.83	-
∑USFA	55.06±1.03	-	68.05±2.03	-	46.86±2.18	-

Note:
[1], [2] and [3] sourced from Love, [31]; Selva et al.,[32] ; and Limmatvapirat et al. [33], respectively.
Mean (± S.E.) of results from three separate experiments.
a, b and c: Values with the different superscript letters in the same row are statistically significantly different (P<0.05)
BF, beef fat; LD, lard; CF, chicken fat; nd, not detected

Functional and structural properties of lard, beef and chicken fat using FTIR

FTIR spectra were used for detection of the chemical and functional groups of lard, beef and chicken fats within the range of 4000-600 cm^{-1} and the results are shown in **Fig. 2**. Similar absorption pattern and structural characteristics were observed in Lard, chicken and beef fats. Although the pattern of infrared spectral peaks in all fats are similar, but these peaks have different intensity of absorption peaks [34]. Absorption observed at ~3475 cm^{-1} (a) corresponded to the overtone of the glyceride ester carbonyl. The band in the region of ~2920 cm^{-1} (b) was assigned to asymmetric or symmetric stretching vibration of methylene (-CH2) band [35].

The peaks in the range of ~2850 cm^{-1} (c) could be attributed to the C-H stretching vibration which in turn resulted in a high proportion of polyunsaturated groups in the sample [36]. Peak intensity at ~1740 cm^{-1} (d) is attributed to carbonyl (C=O) functional group from the ester linkage of triacylglycerol [37]. Absorption at ~1450 cm^{-1} (e) are associated with the bending vibration of the CH_2 and CH_3 aliphatic group. Peaks in the range of ~1367 cm^{-1} (f) is attributed to symmetrical bending vibration of methyl group. Wavelength in the range of ~1167 cm^{-1} (g) corresponded with the stretching vibration of the C-O group in esters could be due to the proportion of monounsaturated and polysaturated acyl groups in the sample [35]. The FTIR spectrum of beef fat can be differentiated from that of lard and chicken fats in existing peak at frequency ~1127 cm^{-1} which is in agreement with the finding of Witjaksono et al. [38] who claimed that lard and chicken fat have unique peaks at wave numbers 1159.6 cm^{-1}, 1743 cm^{-1}, 2853 cm^{-1} and 2922 cm^{-1}.

The weak band at ~983 cm^{-1} indicated the presence of CH functional groups of isolated trans-olefin**.** The bands at ~720 cm^{-1} (h) is characteristics of overlapping of methylene rocking vibration and the out-of-plane bending vibration of cis-disubstituted olefins [39].

Thermal properties of lard, chicken and beef fats

Thermal properties of lard, chicken and beef fats are shown in **Fig. 3** (**a** & **b**). Different oils extracted revealed different onset of temperature of the initial endothermic phase. Likewise, endothermic peaks with differences in the enthalpy changes were detected for these three fat extracts. It has been reported that the melting behaviour of fats and oils and the phase transition depended on their compositional changes such as fatty acid chain length, degree of unsaturation and nature of distribution of fatty acids in TAG species [40].

Beef fat exhibited the higher T_{off} which may be associated with the higher saturated lipid fraction which melt at higher temperature. It was reported that the higher melting temperature occurred in more saturated TAG and tri-saturated (SSS) [41]. TAG melt at higher temperature than tri-unsaturated (UUU) TAG, mono-unsaturated (SSU) and di-unsaturated (SUU) triglycerides**.** Chicken fat has lowest cooling enthalpy due to free fatty acids and lipid oxidation products. These molecules were adsorbed into the crystal lattices of TAG forming mixed crystals which require lower enthalpy to undergo phase transition. The unsaturated fatty acids and TAGs crystallize at low temperature while the saturated fatty acids and TAGs crystallize at high temperature as reported by De Graef et al. [42].

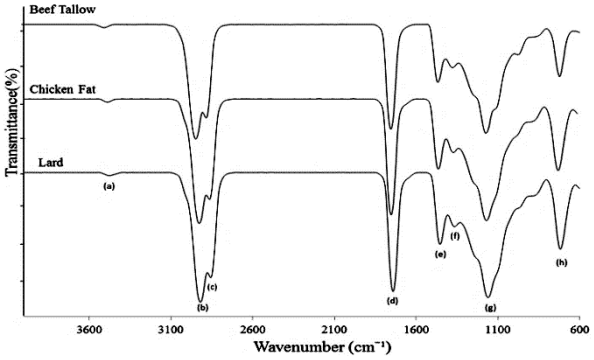

Fig. 2. FTIR spectra of lard, beef tallow and chicken fat.

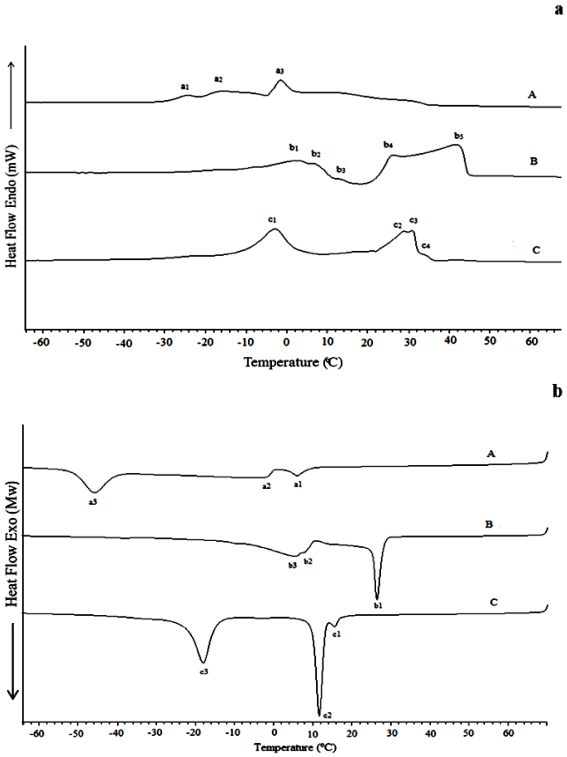

Fig. 3 DSC (a) heating and (b) cooling thermogram obtained from of (A) chicken fat, (B) beef fat and (C) lard.

CONCLUSION

Results from this study demonstrated that different animal fats have different physicochemical properties which could serve as a basis for the determination of authenticity in food products and detection and quantification of adulteration. However, their characteristics following mixing with other fats in the food system should be taken into account when evaluating their differentiation with other animal fats. However other advanced methods in view of sensitivity and time consumed to detect and quantifying adulteration in foods need to be considered.

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CONFLICT OF INTEREST

All authors declare no competing financial professional or personal interests that might have influenced the performance or presentation of the work described in this manuscript.

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