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Differentiation of Lard, Chicken Fat, Beef Fat and Mutton Fat by GCMS and EA-IRMS Techniques

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Abstract: A study was conducted to differentiate lard, chicken fat, beef fat and mutton fat using Gas Chromatography Mass Spectrometry (GC-MS) and Elemental Analyzer–Isotope Ratio Mass Spectrometry (EA-IRMS). The comparison of overall fatty acid data showed that lard and chicken fat share common characteristics by having palmitic, oleic and linoleic acid as major fatty acids while beef and mutton fats shared common characteristics by possessing palmitic, stearic and oleic acid as major fatty acids. The direct comparisons among the fatty acid data, therefore, may not be suitable for discrimination of different animal fats. When the fatty acid distributional data was subjected to Principle Component Analysis (PCA), it was demonstrated that stearic, oleic and linoleic acids as the most discriminating parameters in the clustering of animal fats into four subclasses. The bulk carbon analysis of animal fats using EA-IRMS showed that determination of the carbon isotope ratios ($\delta^{13}C$) would be a good indicator for discriminating lard, chicken fat, beef fat and mutton fat. This would lead to a faster and more efficient method to ascertain the source of origin of fats used in food products.

Key words: animal fats, food authentication, lard, GC-MS, EA-IRMS, PCA

1 INTRODUCTION

Authenticity is an important issue for the food industry due to legal compliance, economic reasons, guarantee of a constant quality, use of safe ingredients, and religious regulations[1]. In this connection, adulteration and misbranding of meat species from cow, lamb, chicken, and swine is a particular concern for followers of certain religious practices. Because of this, there has been a great deal of interest among researchers to develop analytical methodologies to authenticate different meat species. For instance, Marikkar *et al.*[2] investigated the differentiation of lard, chicken fat, beef fat, and mutton fat using differential scanning calorimetric analysis. Che Man *et al.*[3] used fourier transform infrared spectroscopy to study the possibility of discriminating these four animal fats. Chin *et al.*[4] followed by Dias *et al.*[5] employed GCxGC-Time of Flight (ToF) MS to differentiate lard from other animal fats using branched fatty acids containing carbon atoms more than 18. Although GCxGC-Time of Flight (ToF) MS is based on more advanced technology, the longer analysis time taken makes it less attractive technique, as the industry and analytical laboratories demand for high throughput and fast-turnaround analytical approaches. The most important shortcoming of GCxGC technique is that it demands higher sorting time for the identification of individual components as there would be too many peaks to be detected by the MS. Alternatively, GC-MS could offer a faster method of analysis without jeopardizing the credibility of the data. If problems are encountered in the classification, the fatty acid data could be processed using multivariate data analysis techniques such as Principal Component Analysis (PCA)[6]. PCA has been well-known for its capability to identify patterns in data, and express the data in such a way as to emphasize their similarities and differences[7]. Eventually, it helps to establish the authenticity of various kinds of foodstuffs and other agricultural produces. For instance, application of PCA to fatty acid data has been studied for authentication of commercial edible oils[8] and oils extracted from different peanut cultivars[7]. In a recent study, the fatty acid profiles of 119 oil samples were determined by GC and the correlation among peanut, soybean, rapeseed and palm oils were elucidated using PCA whereby computations proved that the samples form clusters according to the type of oil[9].

Stable isotope mass spectrometry (SIRMS) is another potential analytical tool to cross-check food authenticity[10, 11].

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Over the years, SIRMS has been used successfully to obtain specific information about the authenticity and source of origins of different biological substances such as beef[12], poultry[13], and lamb meat[14]. The ability to determine the source of an organic substance stems from the relative isotopic abundances of the elements which comprise the material. As elements such as carbon, hydrogen, oxygen, sulfur, and nitrogen can become locally enriched or depleted through a variety of kinetic and thermodynamic factors, measurement of the isotope ratios can be used to differentiate between samples which otherwise share identical chemical compositions. Many past reports highlighted the use of stable isotope ratio in the detection of adulteration in edible oils. For instance, the potential use of SIRMS has been investigated for detection of sesame oil adulteration with corn oil[15], adulteration of ganoderma spore lipid with cheaper vegetable oils[16] as well as adulteration of maize oil with other vegetable oils[17]. According to our literature search, the carbon isotope ratios determination has been scantily used for differentiation of animal fats such as lard, chicken fat, mutton fat and beef fat. Hence, the objective of this study is to compare the GC-MS and SIRMS approaches to differentiate lard, chicken fat, mutton fat, and beef fat for halal authentication purposes.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

Samples of animal fats namely, lard, beef fat, mutton fat, and chicken fat were extracted by rendering of adipose tissues of animals collected from local slaughter houses located in three different places of Sri Serdang, Malaysia. Melted animal fats were filtered through double-folded muslin cloth to remove impurities. After adding the samples with small proportions of anhydrous sodium sulphate to remove residual moisture, they were filtered through Whatman No. 2 filter paper and stored at 4℃ for 24 h[2]. All chemicals used in this experiment were of either analytical or HPLC grade.

2.2 Methods

2.2.1 Preparation of fatty acid methyl esters（FAME）

Preparation of fatty acid methyl esters of animal fat was done according to AOAC method 969.33. A 50-mg portion of animal fat was weighed into a 20-ml test tube（with screw cap）. After adding 2ml portion of 2N sodium hydroxide in methanol, the sample tube was closed and heated at 80℃ for 1 hour. After allowing the tube to cool down for a few minutes, a 2 ml portion of 25% borontrifluoride solution in methanol was added. The tube was closed and heated again for 1 hour at 80℃. Subsequently, 5 ml portions of water and hexane were added into this. The contents of the tube were shaken well and allowed to undergo phase separation. The clear supernatant of the solution was transferred into a 2-ml auto-sampler vial[18].

2.2.2 Determination of fatty acid composition

The top hexane layer of the FAME solution was injected on an Agilent 6890N gas chromatograph（Agilent Technologies, Singapore）equipped with a polar capillary column RTX-5（0.32 mm internal diameter, 30 m length and 0.25 μm film thickness; Restex Corp., Bellefonte, PA）and a Flame Ionization Detector（FID）. Split injection was conducted with a split ratio of 58:1 using nitrogen as a carrier gas at a flow-rate of 1.00 mL/min. The temperature of the column was 50℃（for 1 min）, and programmed to increase to 200℃ at 8℃/min. The temperatures of the injector and detector were maintained at 200℃[19].

2.2.3 Determination $\delta^{13}C$ of bulk animal fats

About 0.2 μg of each animal fat was weighed and loaded into a clean tin capsule to determine their $\delta^{13}C$ values. The capsules containing samples were placed in auto-sampler system of elemental analyzer（Europa Scientific, UK）for burning in an O_2 atmosphere of the combustion CuO tube with its temperature set at 960℃. Combustion gases were eluted through a reduction column by a stream of He gas and passed into the gas chromatograph where CO_2, still in the He stream, was separated from the other gases. The gas stream was then entered into the IRMS system（Sercon Ltd., Crewe, U.K.）where the CO_2 gas was analyzed by comparison with NBS-22 reference material（with a $\delta^{13}C$ value of －30.03‰）. During every batch of analyses, an empty tin capsule was analyzed as the blank to check the background[16]. Results are referenced to Vienna Pee Dee Belemnite（V-PDB）. The isotopic values were calculated against the international isotope reference standards: NBS-22（International Atomic Energy Agency, Vienna, Austria）for 13C/12C measurements[20].

2.2.4 Statistical analysis

Data were statistically analyzed by one-way analysis of variance（ANOVA）using MINITAB（version 14）statistical package at 0.05 probability level. The relationships between individual unsaturated fatty acid and $\delta^{13}C$ values of animal fats were determined by Pearson's correlation analysis. For grouping and classification models, PCA was carried out using Unscrambler 9.7（Camo, USA）software.

3 RESULTS AND DISCUSSION

Fatty acid composition

Fatty acid distributional patterns of lard, chicken fat, mutton fat and beef fat are presented as shown in Table 1. Lard and chicken fat were found to have more unsaturated fatty acids（60.98 to 67.52%）than saturated fatty acids（32.48 to 67.52%）. On the other hand, beef and mutton fats were found to possess more saturated fatty acids（55.15 to 68.05%）than unsaturated fatty acids（44.85 to

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Table 1 Fatty acid compositions (%, peak area) of lard, chicken fat, beef fat and mutton fat[1].

Fatty acids	Lard	Chicken fat	Beef fat	Mutton fat
C10:0	0.08 ± 0.01[a]	–	–	0.29 ± 0.06[b]
C12:0	0.19 ± 0.11[a]	0.64 ± 0.93[a]	0.11 ± 0.05[a]	0.46 ± 0.06[a]
C14:0	2.28 ± 1.10[a]	1.62 ± 0.65[a]	6.15 ± 0.31[b]	6.40 ± 0.49[b]
C15:0	0.05 ± 0.04[a]	–	0.46 ± 0.24[b]	0.76 ± 0.02[c]
C16:0	24.64 ± 1.90[a]	25.39 ± 1.01[a]	31.07 ± 0.78[b]	27.38 ± 1.22[ab]
C16:1	1.07 ± 0.46[a]	5.32 ± 0.48c	2.56 ± 0.07[b]	0.52 ± 0.18[a]
C17:0	0.25 ± 0.23[a]	–	0.82 ± 0.62[a]	1.85 ± 0.13[b]
C18:0	11.53 ± 1.67[b]	4.84 ± 0.18[a]	16.53 ± 1.25[c]	30.90 ± 0.50[d]
C18:1	42.62 ± 0.74[c]	43.94 ± 1.77[c]	35.70 ± 1.71[b]	29.82 ± 1.04[a]
C18:2	17.29 ± 3.11[b]	18.26 ± 1.64[b]	6.59 ± 0.61[a]	1.61 ± 0.06[a]
ΣSFA	39.02	32.48	55.15	68.05
ΣUSFA	60.98	67.52	44.85	31.95

[1] Each fatty acid value in the table represents the mean ± standard deviation of three replicates.
Means within each row with different superscripts (a-d) are significantly different ($p < 0.05$).

31.95%). The difference in the degree of unsaturation of these animal fats could be due to the diverse pattern of distribution of individual fatty acids. The most predominant fatty acid of lard was oleic acid (42.62%), followed by palmitic (24.66%) and linolenic (17.29%), which was in accordance with the findings reported by previous workers[2, 21, 22]. Chicken fat was also found to have oleic acid (43.94%) as the most dominant fatty acid, followed by palmitic (25.39%) and linolenic (18.26%) acids. In addition, lard and chicken fat having lower levels of myristic acid was a common characteristic feature, when compared to the proportion of myristic in mutton and beef fats. These similarities in fatty acid distribution would cause a difficulty in making a clear distinction between lard and chicken fat.

The proportional distributions of individual fatty acids of mutton and beef fats in this study were somewhat comparable to those reported previously by other research workers[23, 24]. Although these two animal fats were also found to have oleic and palmitic as their most prominent fatty acids, they might tend to differ from lard and chicken fat with regard to the third and fourth most abundant fatty acids. While linoleic was the third most fatty acid in lard and chicken fat, stearic was the third most fatty acid in beef and mutton fats. This difference could be probably due to the differences in the metabolic process taking place in ruminant and non-ruminant animals. For instance, Wood *et al.*[25] emphasized that linolenic acid in lard may have been derived entirely from the diet, which could be passed through the pig's (non-ruminant) stomach unchanged, and absorbed into the blood stream through the small intestine. From there, it could be incorporated into

tissue lipids, resulting in higher levels of linolenic acid in lard. In contrast to this, linolenic acid in ruminants may be degraded into saturated and monounsaturated fatty acids through a process known as microbial bio-hydrogenation. As a result, only a small proportion of dietary linolenic acid (around 10%) would be incorporated into their tissue lipids.

As beef and mutton fat share common characteristics in the way lard and chicken fat share common characteristic with regard to major fatty acids, it might be difficult to find differentiation between them using mere comparison of the overall fatty acid data. In such situations, it would be more appropriate to use multivariate data analysis techniques such as PCA. As shown in Table 2, fatty acids namely lauric (C12:0), myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids were found to occur in variable amounts in all animal

Table 2 δ^{13}C values of different animal fats[1].

Animal Fat	n	δ^{13}C (‰)	
		Mean	Sd
Chicken fat	3	− 20.3[d]	0.93
Lard	3	− 23.2[c]	0.97
Beef fat	3	− 29.5[b]	0.87
Mutton fat	3	− 33.2[a]	0.32

[1] Means within each column with different superscripts (a-d) are significantly different ($p < 0.05$).
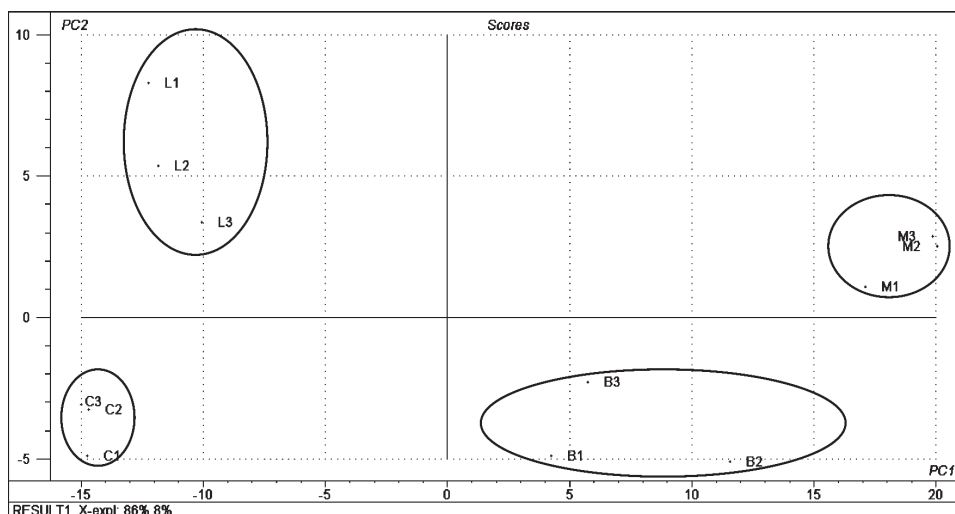Abbreviation: *Sd*, standard deviation.

Fig. 1 Score plot of PCA of animal fats based on fatty acid composition.
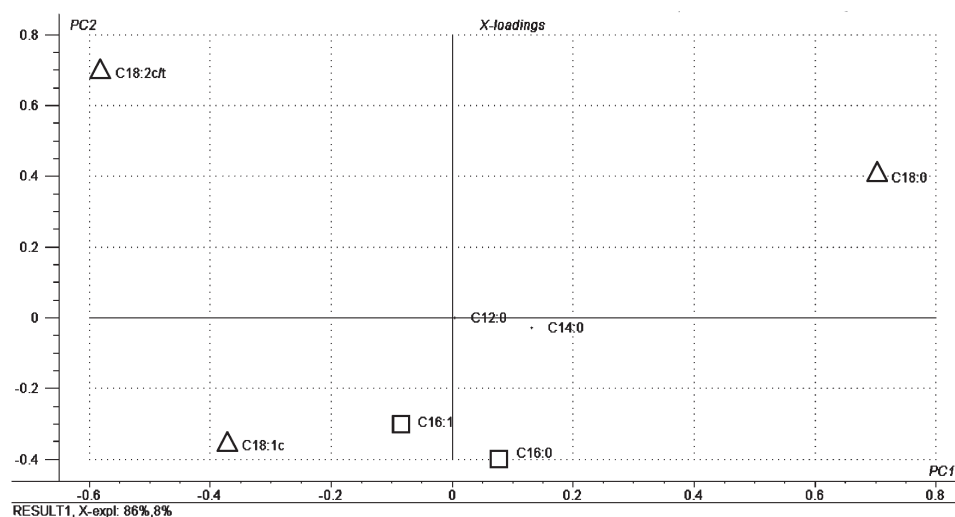Abbreviations: L, lard; C, chicken fat; B, beef fat; M, mutton fat.

Fig. 2 Loading plot of PCA of animal fats based on fatty acid composition (PC1: △, PC2: □).

fats and could be used as independent variables in PCA procedure. The score plot of fatty acids derived from the four animal fats as shown in **Fig. 1** represented the projection of samples defined by principle component 1 (PC1) and principle component 2 (PC2). PC1 is the linear combination of variables that explain the highest variation among the samples, while PC2 is orthogonal to PC1 and exhibited the second largest variation[26]. The score plot projected on PC1 described 86% of the variation while PC2 accounted for 8% of the variation, making up of 94% of variance for PC1 and PC2. According to the group separation illustrated in **Fig. 1**, lard was located in upper left quadrant, chicken fat in lower left quadrant, mutton fats in upper right quadrant, and beef fat in lower right quadrant. Fatty acid variables giving high influence on the group separation of the samples in the score plot could be traced from the analysis

of the loading plot. As explained by Cordella *et al.*[26], a variable which was farther from the origin of axis contributed to the most variation in the statistical model generated by the PCA. According to the loading plot in **Fig. 2**, out of the seven fatty acid variables, stearic, oleic, linoleic, palmitic and palmitoleic acids were the most discriminating variables that influence the group separation into four different clusters.

$\delta^{13}C$ values of bulk animal fats

The data presented in **Table 2** was to compare the $\delta^{13}C$ values of lard, chicken fat, beef fat and mutton fat. According to **Table 2**, the highest $\delta^{13}C$ value was found with chicken fat (−20.3‰) while the lowest value of the same was found for mutton fat (−33.2‰.). The $\delta^{13}C$ values of lard (−23.2‰) and beef fat (−29.5‰) were within the range of these two extremes of the mean $\delta^{13}C$ values. Inter-

Table 3 Pearson's correlation coefficient between δ¹³C vales and unsaturated fatty acid contents of lard, chicken fat, beef fat and mutton fat.

Fatty acid	Correlation coefficient
Palmitoleic (C16:1)	r = + 0.683 ($p < 0.317$)
Oleic (C18:1)	r = + 0.987 ($p < 0.013$)
Linoleic (C18:2)	r = + 0.988 ($p < 0.012$)
∑USFA	r = + 0.997 ($p < 0.003$)

Abbreviation: USFA, unsaturated fatty acid

estingly, the δ¹³C values of these animal fats were found to show good correlation (+ 0.997; $p < 0.003$) with the increasing degree of unsaturation as shown in **Table 3**. When chicken fat with highest proportion of unsaturated fatty acids (67.52%) having the highest δ¹³C value, mutton fat with the lowest proportion of unsaturated fatty acids (31.92%) showed the lowest δ¹³C value. Among the three unsaturated fatty acids enlisted in **Table 1**, linoleic acid showed the best positive correlation (+ 0.988; $p < 0.012$) with the δ¹³C values of animal fats (**Table 3**). The δ¹³C values of beef fat, chicken fat and mutton fat obtained in this study were comparably similar to those reported in some previous investigations. For instance, Rhodes *et al.*[13] found that δ¹³C values of chicken fat obtained from Hubbard and Ross breeds were around −21‰, which was closely comparable to the δ¹³C value of chicken fat obtained in the present study. Likewise, Bojlul *et al.*[12] found that the δ¹³C value of beef fat was −29.2‰, which was closely similar to the δ¹³C value of beef fat obtained in the present study. Bojlul *et al.*[12] further pointed out that the δ¹³C value obtained for beef fat was in accordance with the previous findings reported by De Smet *et al.*[27]. In an attempt to authenticate lamb meat, Piasentier *et al.*[14] observed that δ¹³C values of mutton fat extracted from the lamb meat originated from Great Britain, France and Iceland were within a narrow range from −31.3 to −32.5‰. Interestingly, the δ¹³C value of mutton fat obtained in the present study was found to be roughly similar to the range of values reported by them. In the case of lard, there was hardly any report to compare the δ¹³C value, except the report of Gonzalez *et al.*[28], which indicated that the δ¹³C values of pigs' adipose tissues were within a narrow range from −22.14 and −23.87‰. The δ¹³C values of adipose tissue of pig may still be considered for comparison purpose as adipose tissue is the part of the animal where roughly about 80% of animal fats are deposited[29]. The statistical analysis of the data from the present study suggested that the determination of δ¹³C value for bulk animal fats can be a useful tool since the δ¹³C value of lard (−23.2‰) was significantly ($p < 0.05$) different from those of beef fat (−29.5‰), chicken fat (−

20.3‰), and mutton fat (−33.2‰) (**Table 2**). The observed variation in the δ¹³C values of animal fats could be attributed to their species difference[20], genetic factors[25] as well as the diet fed to the animals[12]. According to previous investigators, the variation in the δ¹³C values of oils and fats originated from different plant sources were due to isotopic fractionation during physical, chemical and biological processes in plants[30].

4 CONCLUSIONS

This study investigated the application of GC-MS and EA-IRMS techniques for discrimination of lard, chicken fat, beef fat, and mutton fat. Comparison of the overall fatty acid data showed that use of single fatty acid as parameter may not be suitable to classify animal fats into distinct subclasses. Hence, the application of multivariate statistical techniques such as PCA would be required to classify them to determine the source of origin. According to the outcome of PCA, stearic, oleic and linoleic acids were found to be the most discriminating parameters in the clustering of animal fats. Hence, this study showed that PCA of fatty acid data allowed separation of lard from other animal fats and could be a potential tool for differentiation of meat species. The significant differences in the values of carbon isotope ratios (δ¹³C) of all animal fats have been proven to be good indicators for discriminating lard, chicken fat, beef fat and mutton fat. This showed that determination of bulk carbon isotope ratio have considerable potential in classification of animal fats for halal authentication purposes.

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