

DIFFERENTIATION OF FATTY ACID COMPOSITION OF BUTTER ADULTERATED WITH LARD USING GAS CHROMATOGRAPHY MASS SPECTROMETRY COMBINED WITH PRINCIPAL COMPONENT ANALYSIS

Article history

Received
28 December 2014
Received in revised form
4 Mac 2016
Accepted
15 January 2016

Nurrulhidayah Ahmad Fadzillah^{a,d*}, Abdul Rohman^c, Arieff Salleh Rosman^d, Farahwahida Mohd Yusof^d, Amin Ismail^e, Shuhaimi Mustaffa^b, Ade Erawan Minhat^f, Alfi Khatib^g

*Corresponding author
nurrillahi@gmail.com

^aInternational Institute for Halal Research and Training, International Islamic University Malaysia, 50728 Kuala Lumpur, Malaysia

^bLaboratory of Halal Science Research, Halal Products Research Institute, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

^cResearch Center of Halal Products and Faculty of Pharmacy, Gadjah Mada University, 55281 Yogyakarta, Indonesia

^dCenter of Research for Fiqh Science and Technology, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia

^eDepartment of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia 43400, Selangor, Malaysia

^fDepartment of Microelectronic Engineering, Faculty of Electrical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia

^gDepartment of Pharmaceutical Chemistry, Kulliyah of Pharmacy, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia

Abstract

Butter is high priced product; as a consequence, butter can be subjected for adulteration with low price components such as lard. The presence of lard in any products is not allowed for Muslim and Jewish, therefore, its presence must be identified. Gas chromatography-mass spectrometry was successfully used to detect and discriminate butter from adulterated with lard. Results were presented in the form of chromatogram. Principal component analysis (PCA) was used to interpret the data and provided a good grouping of samples with 55.8% of the variation accounted for by PC 1 and 21.5% were accounted for by PC 2. All the lard containing samples formed a separate group from the samples that were free of lard. This method can be developed into a rapid method for detecting the presence of lard in food samples for Halal authentication.

Keywords: Lard, Gas Chromatography- mass spectrometry, adulteration, halal, principal component analysis (PCA)

Abstrak

Mentega merupakan bahan mentah yang mahal, akibatnya mentega boleh tercemar dengan bahan yang lebih murah seperti lemak babi. Kehadiran lemak babi di dalam mana-mana produk tidak dibenarkan terutamanya bagi penganut muslim dan yahudi. Oleh sebab itu, penting untuk identifikasi mentega yang telah dicemari oleh lemak babi. Kromatografi Gas Spektrometri Jisim telah berjaya digunakan untuk mengesan dan membezakan mentega dari dicemari dengan lemak babi. Keputusan digambarkan dalam bentuk kromatogram. Analisis komponen utama (PCA) digunakan untuk menganalisis data dan mengklasifikasi kumpulan mentega dan bahan pencemarnya. PC 1 menyumbang kepada komposisi asid lemak yang paling bervariasi (55.8%), PC 2 menyumbang kepada variasi besar berikutnya (21.5%). Semua lemak yang mengandungi lemak babi membentuk satu kumpulan yang berasingan daripada sampel yang bebas lemak. Kaedah ini boleh berkembang menjadi satu kaedah yang cepat untuk mengesan kehadiran lemak babi di dalam sampel makanan untuk pengesahan Halal.

Kata kunci: Lemak babi, Kromatografi Gas Spektrometri Jisim, Pencemaran, halal, Analisis komponen utama (PCA)

© 2016 Penerbit UTM Press. All rights reserved

1.0 INTRODUCTION

Fatty acid analysis in terms of its single composition and functionality has continually gain recognition mainly due to its multiple roles in humans and other organism. Fatty acids are defined as compounds that contain an aliphatic chain with a carboxylic acid group [1]. They greatly influence both the physical and chemical properties of the glycerides because of their preponderant weight in the glyceride molecule which contribute to 94-96% of the total weight of the triacylglycerol molecule [2]. It is essential as energy substrates comprising around 30% of total energy intake for humans. FA can be stored in excess amounts in adipose tissue, especially when increased dietary intake of fat and energy occurs resulting in obesity. Most importantly, FA is substantial part of lipids, one of the three major components of biological matter (along with proteins and carbohydrates) [3, 4].

Structure and composition of fatty acids in fats and oils could be an indicator for determination the source of lipid. Information on fatty acid composition is important for health awareness and religious commitment. However, the determination of fatty acid compositions, especially from animal fats, results show a great challenge due to their complexity in chain length, branching, degree of unsaturation, geometry and position of the double bonds [5].

Butter is a fairly expensive raw material, and from an economic point of view, it could be attractive to modify its composition by partly replacing the high priced milk fat with low priced of vegetable or animal fats like lard without labeling the product accordingly. Untill now the issues on the necessity of labeling this product accordingly is still debatable [6, 7].

Analytical methods for assessing the authenticity of milk fat based on fatty acid composition have a long tradition [8, 9]. A review of most commonly used method for detection of the triglyceride composition of butter has been compiled by Lipp [10]. At though certain techniques of physical analysis such as scanning calorimetry have been successfully applied [11-13] most current analytical approaches to detection adulterant issues in butter constituents are essentially based on the Gas Chromatography (GC) separation of its component. GC is the most commonly used method of analyzing milk fat FAs. It has gained wide spread favor due to its versatility, high sensitivity and relatively low cost.

An important factor that contributing to the success of chromatography is chemometrics [14]. Chemometrics is the discipline of extracting chemically relevant information from data produced in chemical experiments by means of statistical and mathematical tools [15]. There are numerous chemometric technique used in the study of fats and oils such as principal component analysis (PCA), principle component regression (PCR), partial least square (PLS) and cluster analysis (CA). Such methods are employed to solve several problems related to the analysis of fats and oils [6, 16]. However, there is no information available related to the use of GC combined with chemometrics for analysis butter adulterated with lard. Therefore, in this research, we established developed gas chromatography mass spectrometer with headspace analyzer (GCMS-HS) combined with PCA for quantitative analysis of butter adulterated with lard.

Table 1 Fatty acid compositions of butter added with different levels of lard

FA [†]	Ratio (Lard: Butter)											
	(0%: 100%)	(1%: 99%)	(3%: 97%)	(5%: 95%)	(10%: 90%)	(20%: 80%)	(30%: 70%)	(40%: 60%)	(50%: 50%)	(60%: 40%)	(80%: 20%) ^a	(100%: 0%)
C4:0	2.54± 0.03 ^{a,b}	2.45± 0.05 ^a	2.34± 0.07 ^{a,b}	2.25± 0.05 ^{a,b}	2.05± 0.05 ^{a,b}	1.88± 0.39 ^{b,c}	1.73± 0.03 ^c	1.67± 0.04 ^d	1.55± 0.04 ^d	1.38± 0.09 ^d	1.01± 0.18 ^e	0.00± 0.00 ^f
C6:0	1.67± 0.02 ^{a,b}	1.55± 0.02 ^a	1.47± 0.04 ^a	1.32± 0.01 ^a	1.12± 0.02 ^{a,b}	1.08± 0.07 ^b	0.97± 0.01 ^e	0.89± 0.02 ^c	0.75± 0.03 ^{c,d}	0.63± 0.07 ^d	0.40± 0.12 ^f	0.00± 0.00 ^g
C8:0	1.04± 0.01 ^a	1.03± 0.01 ^a	1.03± 0.02 ^a	1.03± 0.01 ^a	0.94± 0.011 ^a	0.94± 0.08 ^a	0.74± 0.00 ^d	0.59± 0.01 ^b	0.54± 0.02 ^{b,c}	0.46± 0.05 ^c	0.25± 0.07 ^e	0.00± 0.00 ^f
C10:0	2.34± 0.01 ^a	2.26± 0.02 ^a	2.23± 0.04 ^a	2.24± 0.02 ^a	2.06± 0.02 ^a	1.97± 0.20 ^a	1.62± 0.01 ^d	1.34± 0.03 ^b	1.19± 0.05 ^{b,c}	1.03± 0.10 ^e	0.58± 0.16 ^f	0.00± 0.00 ^g
C12:0	9.79± 0.03 ^a	9.27± 0.09 ^a	9.02± 0.17 ^a	8.43± 0.07 ^a	8.34± 0.10 ^a	8.12± 0.73 ^a	6.57± 0.01 ^d	5.49± 0.11 ^b	4.74± 0.19 ^{b,c}	4.10± 0.40 ^c	2.18± 0.67 ^e	0.05± 0.04 ^f
C14:0	13.63± 0.02 ^a	13.4± 0.12 ^a	13.29± 0.23 ^{a,b}	13.0± 0.08 ^{a,b}	12.5± 0.16 ^b	11.30± 0.63 ^b	10.15± 0.02 ^d	9.70± 0.16 ^e	7.55± 0.25 ^c	6.72± 0.53 ^c	4.01± 0.91 ^f	1.12± 0.03 ^g
C15:0	0.00± 0.00 ^d	0.99± 0.01 ^a	0.96± 0.01 ^{a,b}	0.97± 0.01 ^{a,b}	0.90± 0.01 ^b	0.87± 0.02 ^b	0.73± 0.08 ^e	0.63± 0.01 ^f	0.54± 0.02 ^c	0.48± 0.04 ^c	0.29± 0.07 ^g	0.06± 0.05 ^d
C16:0	31.29± 0.05 ^c	30.6± 0.09 ^a	29.50± 0.09 ^{a,c}	29.1± 0.04 ^a	28.7± 0.06 ^b	28.18± 0.07 ^{a,b}	27.75± 0.07 ^d	27.09± 0.11 ^d	26.22± 0.56 ^e	25.11± 0.19 ^e	24.37± 0.43 ^f	23.37± 0.22 ^g
C16:1	0.00± 0.00 ^h	1.88± 0.00 ^a	1.88± 0.01 ^a	1.89± 0.00 ^c	1.82± 0.00 ^b	1.78± 0.03 ^{a,c}	1.71± 0.01 ^{b,c,d}	1.69± 0.00 ^{d,e}	1.62± 0.04 ^{e,f}	1.59± 0.02 ^{e,f}	1.50± 0.02 ^g	1.47± 0.02 ⁱ
C17:0	0.65± 0.00 ^c	0.63± 0.02 ^a	0.62± 0.00 ^{a,b}	0.61± 0.01 ^{a,b}	0.59± 0.01 ^a	0.58± 0.01 ^{a,b}	0.57± 0.01 ^b	0.56± 0.01 ^b	0.55± 0.01 ^{a,b}	0.54± 0.00 ^{a,b}	0.53± 0.00 ^{a,b}	0.50± 0.00 ^{b,c}
C17:1	0.50± 0.00 ^f	0.48± 0.00 ^a	0.42± 0.00 ^a	0.41± 0.00 ^a	0.39± 0.00 ^a	0.37± 0.00 ^b	0.35± 0.00 ^b	0.33± 0.00 ^c	0.31± 0.01 ^{c,d}	0.29± 0.00 ^d	0.28± 0.00 ^e	0.26± 0.01 ^g
C18:0	11.62± 0.04 ^{a,b,c}	11.6± 0.04 ^a	11.6± 0.07 ^{a,b}	11.7± 0.01 ^a	11.75± 0.02 ^{a,b}	11.87± 0.64 ^{a,b,c}	11.97± 0.20 ^{b,c,d}	12.01± 0.10 ^{d,e}	12.23± 0.25 ^{a,d,e}	12.35± 0.15 ^{d,e}	12.85± 0.23 ^{e,f}	13.26± 0.18 ^f
C18:1	22.77± 0.13 ^a	22.8± 0.18 ^a	23.4± 0.30 ^a	23.2± 0.09 ^a	24.90± 0.17 ^a	25.07± 0.79 ^c	27.19± 0.08 ^d	29.48± 0.29 ^b	30.70± 0.55 ^b	32.68± 0.80 ^e	36.70± 1.42 ^f	40.15± 0.31 ^g
C18:2	1.99± 0.00 ^a	2.02± 0.02 ^a	2.10± 0.28 ^a	2.23± 0.07 ^a	2.34± 0.06 ^a	2.50± 0.07 ^a	6.69± 0.11 ^c	8.38± 0.20 ^b	10.32± 0.10 ^b	12.65± 0.61 ^d	14.22± 0.96 ^e	17.29± 0.09 ^f
C18:3	0.00± 0.00 ^{b,c}	0.00± 0.00 ^a	0.00± 0.00 ^b	0.00± 0.00 ^a	0.06± 0.00 ^d	0.17± 0.01 ^a	0.31± 0.01 ^{a,c}	0.45± 0.01 ^{a,b,c}	0.59± 0.02 ^{a,b,c}	0.65± 0.02 ^{a,c}	0.72± 0.04 ^a	0.90± 0.02 ^{a,b}

FA = fatty acid; [†]Each value in the table represents the means of triplicate analysis; SD is given after ±. Means within each row with different letters are significantly different at P < 0.05

2.0 EXPERIMENTAL

2.1 Sample Preparation

Lard was extracted by rendering process of adipose tissues of pig. Lard was prepared by rendering the adipose tissues of corresponding animals according to Che Man [17] as follows: the tissues were cut into small pieces, mixed, and melted at 90 - 100 °C for 2 h in the oven (Memmert, Germany). The melted fat was strained through triple-folded muslin cloth, dried by addition of anhydrous sodium sulfate (Na_2SO_4) and subsequently centrifuged at 3000 rpm (1500 xg) for 20 min. The fat layer was decanted, shaken well, and centrifuged again before being filtered through Whatman filter paper Number 2 containing anhydrous Na_2SO_4 to remove traces of water. The filtered samples were further used for analysis. Sampling was done triplicates and consists of three pig samples. The rendering was done at 90–100 °C for 2 h in the oven. The melted fat was collected and filtered with glass wool, dried by addition of anhydrous Na_2SO_4 , and then centrifuged at 3,000 rpm for 20 min. Fat was dried over anhydrous sodium sulphate and kept in a freezer at (-20°C) before further analysis.

Fresh milk from Universiti Putra Malaysia (UPM), Serdang dairy farm field was used as a raw material. The heavy cream was taken out from the milk by using cream separator. The heavy cream then was shaken in the mixer with a medium-high speed till the colour of cream change to pale yellow. The pale yellow buttermilk was collected and kept at -20°C before further usage.

2.2 Calibration and Validation

The calibration samples composed a number of standard or training sets consisting of lard in butter at concentration ranges of 1% – 80% v/v were prepared. For validation, a series of independent sample was built to evaluate the predictive ability of the developed calibration model. The chromatogram of pure butter and lard as well as their mixtures were analyzed using gas chromatography-mass spectrometry.

2.3 Fatty Acid Analysis

Fatty acid (FA) compositions of butter, lard and the mixture of butter-lard were determined using a headspace auto sampler (Model G1888, Agilent Technologies, Palo Alto, CA, USA). The transfer line from the headspace sampler was directly connected to the injector of the gas chromatograph (GC). The oven was set at 110 °C. The extraction conditions in the headspace autosampler were programmed as follows: 20.0 min for vial equilibration, 0.20 min for vial pressurization, 0.20 min for filling the injection loop, 0.05 min for loop equilibration and 1.0 min for sample injection. Helium with a purity of 99.999% was used for vial pressurization and as carrier gas. The volatile

compounds were analyzed using a GC MS (Model 7890, Agilent Technologies, Palo Alto, CA, USA) equipped with a non polar column (J&W Scientific DB-5; 30 m, ID 0.25 mm, film thickness 0.25 μm). The column temperature was kept at 40 °C for 10 min, increased at 6°C/min to 240°C and isothermally maintained for 20 min. The mass selective detector (Model MSD59556, Agilent Technologies, Palo Alto, CA, USA) was used in electron ionization mode. A mass range between 30 and 550 m/z was scanned. The mass spectra obtained were compared to the National Institute of Standards and Technology (NIST) Mass Spectral Search Program for compound identification. Thirty seven standard FAME (Sigma St. Louis, MO) were used as authentic samples to calculate the percentage of fatty acids based on peak area. Quantification of FAME was performed using a normalization internal technique.

2.4 Statistical and Chemometrics Analysis

For fatty acid analysis, the statistical treatment using one-way analysis of variance (ANOVA), followed with Duncan multiple comparison using SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA) was used for differentiation fatty acid composition levels in butter adulterated with lard. The significance value (P) of less than 0.05 was considered statistically different. All sample analyses (fatty acid composition and chromatogram) were performed as three replicates and averaged using Microsoft Excel software 2007. The chemometrics analysis of PCA was generated using Minitab software (Release 15; Minitab, State College, PA).

3.0 RESULTS AND DISCUSSION

3.1 Fatty Acid Composition

The chromatogram of the FAs derived from pure butter and lard are shown in Figure 1. Fatty acids composition results are useful for fingerprinting fats and oils purposes and adulteration process detection by comparing area and heights of the chromatogram peak. The fatty acid composition was determined based on the normalized peak area are listed in Table 1. The experimental values of lard were in good agreement reported by Marikkrar [18]. Butter is known as medium chain fats because of high contain of medium chain FAs. Lauric acid (C12:0) was the predominant FA in butter following other medium chain FAs myristic acid (C14:0) and palmitic acids (C16:0) as presented in Figure 1(A).

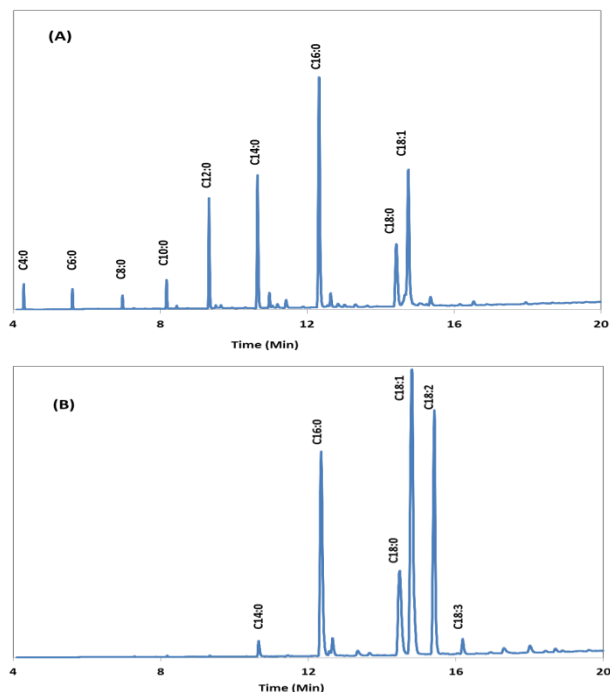


Figure 1 Fatty acids composition of pure butter (A) and pure lard (B)

The bulk of FAs are mostly from medium chain FAs (containing 12-16 carbons), which is 50.06% of the total FAs. In contrast to butter, lard contains more long chain FAs which are stearic acid and other unsaturated FAs such as oleic, linoleic and linolenic acids as shown in Figure 1(B). As lard concentration rises in the sample admixtures, the proportion of the saturated to unsaturated FA had decreased. This is in line with the inherent FA structure of lard, which had high unsaturated FAs and lower lauric acid thus increase the oleic acid percentage in mixtures [19].

Table 1 shows the changes of FA composition of butter subjected to adulteration with lard. As shown in Table 1, the major fatty acid of lard was oleic acid 40.15%, followed by palmitic 23.37% and linoleic 17.29% acids. This is in accordance with the findings reported by several researchers [18, 20-22]. Lard is generally found to have more unsaturated fatty acids (USFA) than saturated fatty acids (SFA) [23, 24]. As indicated in Table 1, there was no significant difference among the concentration of these FAs ($P > 0.05$)

3.2 Principal Component Analysis of Butter and Butter Adulterated with Lard

Fats and oils are mainly consisted from fatty acids esterified with glycerol. Previous study [10] stated that FA composition of butter was more similar to animal fats than vegetable oils. This may be due to butter mainly contain of milk fat from animal not from vegetables. For this reason, in this study we determined animal fats (lard) as adulterants in butter.

In order to classify butter and its adulterants, FA profile of butter and lard were subjected to principal component analysis (PCA).

Figure 2(a) shows the PCA score plot of butter adulterated with lard representing the projection of samples defined by the first principal component (PC 1) and the second principal component (PC 2). PC 1 accounts for the most variation in FA composition (55.8%), PC 2 accounts for the next large variation (21.5%), and PC3 contributes to 15.1 % variation. Therefore, more than 90 % variables (fatty acid composition) can be extracted using 3 PCs. Based on PCA score plot, it is evident that lard makes one cluster, while butter also makes one cluster. Due to its capability to describe the similarity among analytes based on score plot, PCA score plot is considered as latent variables.

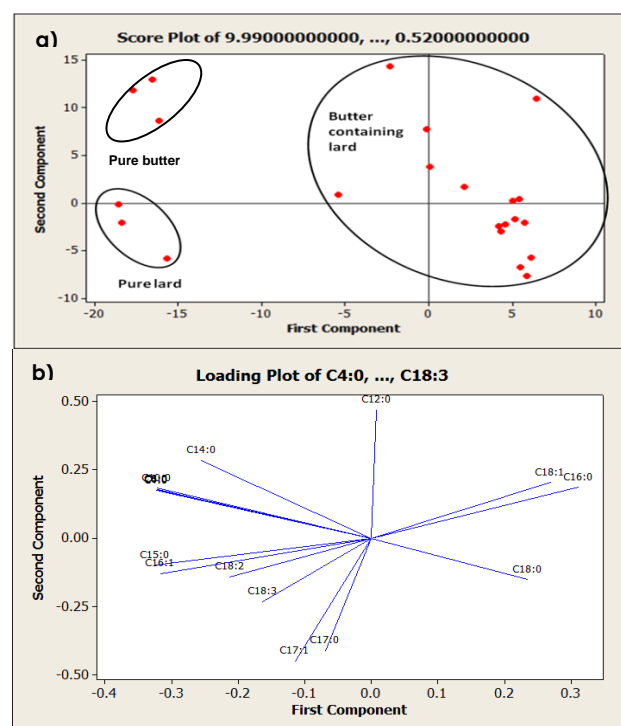


Figure 2 PCA classification for butter and lard, projected by PC1 and PC2: a) score plot b) loading plot

3.3 Analysis of Butter Adulteration Based on Fatty Acid Composition

Determination of FA profiles using GC-MS seems to be a very useful technique for controlling the authenticity of butter from animal fats such as analysis of fatty acids [25-28]. Figure 3 shows the FA composition changes of butter mixed with lard up to final concentrations of 1, 3.5, 10, 20, 30, 40, 50, 60, 80% (v/v) of lard.

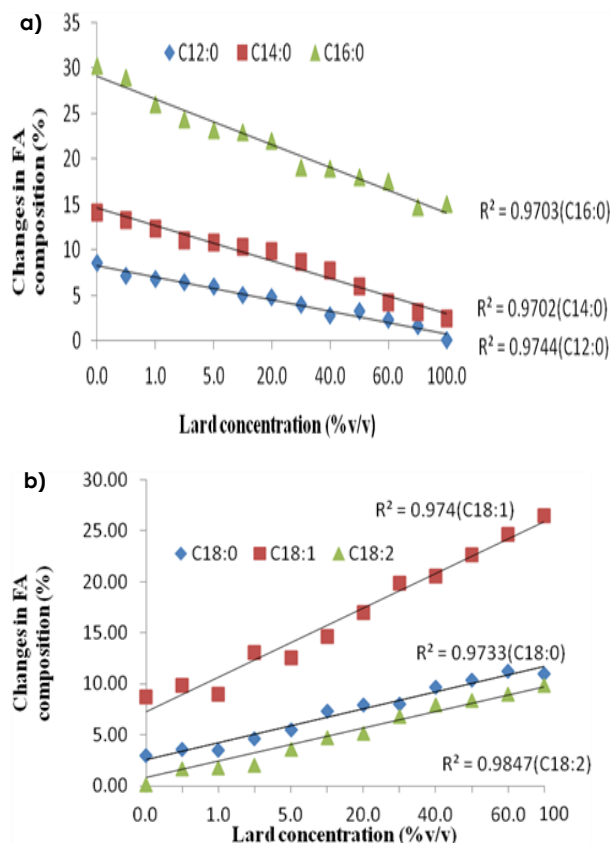


Figure 3 Composition changes of fatty acids (FAs) in butter adulterated with different levels of lard: a) FA with decreased level; and b) FA with increased level, with increasing concentration of lard

Due to the addition of lard into butter, some fatty acids namely lauric (12:0), myristic (14:0) and palmitic (C16:0) were decreased linearly. The increased concentration of lard in adulterated butter sample caused the FA levels of C18:0, C18:1 and C18:2 to increase linearly with coefficient of determination of 0.973 – 0.984. At a concentration of 5% lard in butter, these FAs were significantly different ($p < 0.05$), as analyzed using one way analysis of variance. Figure 4 also shows the R^2 value for the relationship between FA composition changes and the level of lard (%v/v). The higher R^2 indicates the closer the relationship. Based on this result, it can be stated that lard with a level down to 5% (v/v) in butter can be detected using FA composition.

4.0 CONCLUSION

The presence of lard as an adulterant in butter can be detected using the changes of fatty acid profiles as determined using GC-MS. Principal component analysis can successfully classified lard and butter. Based on fatty acid composition, the addition of lard into butter has decreased the levels of lauric (12:0),

myristic (14:0) and palmitic (C16:0) acids. The increased concentration of lard in adulterated butter sample caused the FA levels of C18:0, C18:1 and C18:2 to increase linearly with coefficient of determination of 0.973 – 0.984.

Acknowledgement

The first author acknowledges to MyPhD for its scholarship to pursue PhD programme in Halal Products Research Institute, Universiti Putra Malaysia (UPM). This research was supported by Universiti Putra Malaysia (UPM) for providing the funding support awarded to Prof. Dr. Russly Ab Rahman through RUGS 9316900 grant. The author also acknowledges the Research Management Centre, Universiti Teknologi Malaysia for providing her with a postdoctoral research fellowship (Project No. QJ130000.21A2.02E45).

References

- [1] D. J. McClements, T. Waraho, V. Cardenia, M. T. Rodriguez-Estrada, and E. A. Decker. 2009. Prooxidant Mechanisms Of Free Fatty Acids In Stripped Soybean Oil-In-Water Emulsions. *Journal of Agricultural and Food Chemistry*. 57: 7112-7117.
- [2] N. Sonntag. 1979. Fat Splitting. *Journal of the American Oil Chemists' Society*. 56: 729A-732A.
- [3] E. Tvřicka, L.-S. Kremmyda, B. Stankova, and A. Zak. 2011. Fatty Acids As Biocompounds: Their Role In Human Metabolism, Health And Disease—A Review. Part 1: Classification, Dietary Sources And Biological Functions. *Biomedical Papers*. 155: 117-130.
- [4] D. Nelson and M. Cox. 2005. *I. Ehninger Principles Of Biochemistry*. New York: WH Freeman.
- [5] D. Indrasti, Y. B. Che Man, S. Mustafa, and D. M. Hashim. 2010. Lard Detection Based On Fatty Acids Profile Using Comprehensive Gas Chromatography Hyphenated With Time-Of-Flight Mass Spectrometry. *Food Chemistry*. 122: 1273-1277.
- [6] M. A. De La Fuente and M. Juárez. 2005. Authenticity Assessment Of Dairy Products, Critical Reviews In Food Science And Nutrition. 45: 563-585.
- [7] E. Schlimme, D. Martin, and H. Meisel. 2000. Nucleosides And Nucleotides: Natural Bioactive Substances In Milk And Colostrum. *British Journal of Nutrition*. 84: 59-68.
- [8] M. Juárez. 1990. Test For Fraudulent Identification. In *Proceedings of the XXIII International Dairy Congress*. 1285-1300.
- [9] M. Collomb and M. Spahni. 1991. Adulteration Of Dairy Products. Review Of Analytical Methods For The Determination Of Vegetable And Animal Fats In Butterfat. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene*. 82: 615-662.
- [10] M. Lipp. 1995. Review Of Methods For The Analysis Of Triglycerides In Milk Fat: Application For Studies Of Milk Quality And Adulteration. *Food Chemistry*. 54: 213-221.
- [11] E. Coni, M. Di Pasquale, P. Coppolelli, and A. Bocca. 1994. Detection Of Animal Fats In Butter By Differential Scanning Calorimetry: A Pilot Study. *Journal of the American Oil Chemists' Society*. 71: 807-810.
- [12] V. Yakovlev. 2003. Long Flying Component Produced By Protons At Energies Above 12 Tev In The Ionization Calorimeter. *Nuclear Physics B-Proceedings Supplements*. 122: 417-421.

- [13] M. H. Tunick, J. J. Basch, B. E. Maleeff, J. F. Flanagan, and V. Holsinger. 1989. Characterization Of Natural And Imitation Mozzarella Cheeses By Differential Scanning Calorimetry. *Journal of Dairy Science*. 72: 1976-1980.
- [14] B. K. Lavine, A. Moores, and L. K. Helfend. 1999. A Genetic Algorithm For Pattern Recognition Analysis Of Pyrolysis Gas Chromatographic Data. *Journal of Analytical and Applied Pyrolysis*. 50: 47-62.
- [15] S. C. Rutan. 1989. Fast On-Line Digital Filtering, Chemometrics And Intelligent Laboratory Systems. 6: 191-201.
- [16] A. A. Christy and P. K. Egeberg. 2006. Quantitative Determination Of Saturated And Unsaturated Fatty Acids In Edible Oils By Infrared Spectroscopy And Chemometrics. *Chemometrics And Intelligent Laboratory Systems*. 82: 130-136.
- [17] Y. Che Man and M. Mirghani. 2001. Detection Of Lard Mixed With Body Fats Of Chicken, Lamb, And Cow By Fourier Transform Infrared Spectroscopy. *Journal of the American Oil Chemists' Society*. 78: 753-761.
- [18] J. Marikkar, H. Ghazali, K. Long, and O. Lai. 2003. Lard Uptake And Its Detection In Selected Food Products Deep-Fried In Lard. *Food Research International*. 36: 1047-1060.
- [19] T. S. T. Mansor, Y. B. Che Man, and M. Shuhaimi. 2012. Employment of Differential Scanning Calorimetry in Detecting Lard Adulteration in Virgin Coconut Oil. *Journal of the American Oil Chemists' Society*. 89: 485-496.
- [20] Y. B. Che Man, S. Z. Abidin, and A. Rohman. 2011. Discriminant Analysis Of Selected Edible Fats And Oils And Those In Biscuit Formulation Using FTIR Spectroscopy. *Food Analytical Methods*. 4: 404-409.
- [21] M. Nurjuliana, Y.C. Man, and D.M. Hashim. 2011. Analysis Of Lard's Aroma By An Electronic Nose For Rapid Halal Authentication. *Journal of the American Oil Chemists' Society*. 88: 75-82.
- [22] L.-Z. Cheong, H. Zhang, Y. Xu, and X. Xu. 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.
- [23] K. A. Al-Rashood, R. R. Abou-Shaaban, E. M. Abdel-Moety, and A. Rauf. 1996. Compositional And Thermal Characterization Of Genuine And Randomized Lard: A Comparative Study. *Journal of the American Oil Chemists' Society*. 73: 303-309.
- [24] N. Yanty, J. Marikkar, Y.C. Man, and K. Long. 2011. Composition And Thermal Analysis Of Lard Stearin And Lard Olein. *Journal Of Oleo Science*. 60: 333-338.
- [25] P. Toppino, G. Contarini, G. Amelotti, A. Traversi, and A. Gargano. 1982. Parametri gascromatografici di valutazione della genuinita del burro, *Rivista Italiana delle Sostanze Grasse*.
- [26] M. Iwadia. 1979. Y. Ito, I. Tongai, M. Toyoda, T. Watanabe, Detection Of Adulterated Milkfats By Gas-Liquid Chromatographic Determination Of Butyrate And Caproate. *J. Food Hyg. Soc. Jpn*. 20: 328.
- [27] A. Echizen and M. Diki. 1975. Determination Of Butterfat In Mixed Fats. Relation Between Butyric Acid And Butterfat Content In Mixed Fats. *Rep. Cent. Customs Lab*. 15: 111.
- [28] R. Farag, S. Abo-Raya, F. Ahmed, F. Hewedi, and H. Khalifa. 1983. Fractional crystallization And Gas Chromatographie Analysis Of Fatty Acids As A Means Of Detecting Butterfat Adulteration. *Journal of the American Oil Chemists Society*. 60: 1665-1669.