International Food Research Journal 19(4): 1497-1501 (2012)
Journal homepage: *http://www.ifrj.upm.edu.my*

Discrimination of plant and animal derived MAG and DAG by principal component analysis of fatty acid composition and thermal profile data

[1]Nasyrah A.R., [1,2*]Marikkar J.M.N. and [1]Dzulkifly M.H.

[1]Halal Products Research Institute and [2]Department of Biochemistry
Universiti Putra Malaysia,
43400 UPM Serdang, Selangor D.E.,
Malaysia

Article history

Received: 8 December 2011
Received in revised form:
14 February 2012
Accepted:14 February 2012

Keywords

Cooling curve
diacylglycerol
fatty acids
monoacylglycerol
PCA
thermal analysis

Abstract

A study was carried out to distinguish mono- (MAG) and di-acylglycerol (DAG) from plant lipids such as sunflower, rapeseed and soybean oil, from those derived from animal fats such as lard, goat fat and beef fat using fatty acid and thermal profile data. MAG and DAG of both plant and animal lipids were synthesized according to a chemical glycerolysis method catalyzed by sodium hydroxide. MAG and DAG of individual lipid were isolated and purified using the standard column chromatography method and subjected to fatty acid analysis by gas chromatography (GC) and thermal analysis by differential scanning calorimetry (DSC). The application of principal component analysis (PCA) to the data collected from the individual instrumental technique showed that it was possible to distinctly classify MAG and DAG of plant lipids from those derived from animal fats.

Introduction

Mono-(MAG) and di-acylglcerol (DAG) have long been recognized as ingredients in food formulation. They are produced through transesterification of lipids with glycerol in a catalytic environment (Hasenhuettl, 2008). The lipid source for the production of MAG and DAG can be either plant or animal material. Although MAG and DAG derived from plant-based raw materials are preferred, there has always been possibility for fraudulent practices to include those derived from animal sources (Mcneill *et al*., 1992; Stevenson *et al*., 1993; Soe, 2008). For instance, some previous studies suggested that some of the commercially available MAG and DAG could have been derived from hydrogenated lard (Sudraud *et al*., 1981). As the use of animal lipids may not be desirable as raw material for production of MAG and DAG due to certain religion restrictions (Riaz and Chaudhary, 2004), it has become necessary to avoid MAG and DAG originating from animal lipid sources. Hence, analytical approaches to distinguish plant and animal derived MAG and DAG are very much demanded for halal quality assurance purposes. According to the current literature, only a few studies have been carried out to distinguish MAG and DAG of plant lipids from those of animal fats. In a previous study, Indrasti *et al*. (2010) used gas chromatograph-time of flight-mass spectrometry (GC-TOF-MS) to discriminate partial acyl glycerols of lard from those of sunflower oil, corn oil, butter and palm oil. However, use of thermal profiles of MAG and DAG to determine their source of origin has been scantily investigated. According to the past literature, thermal profile obtained using DSC has been successfully employed to distinguish lard from other animal fats (Marikkar *et al*., 2001) and classify lipids originated from different plant sources (Tan and Che Man, 2000). Hence, the objective of this study is to investigate the application of PCA to fatty acid and thermal analysis data to discriminate plant and animal derived MAG and DAG for halal authentication purposes.

Materials and Methods

Materials

Lard (LD), beef (BF) and goat fat (GF) were extracted from the adipose tissue of animals collected from three different locations in West Malaysia using a microwave extraction method as reported by De Pedro *et al*. (1997). Oils of rapeseed (RS), soybean (SB) and sunflower (SF) seed were purchased from three different local supermarkets in Malaysia. Analytical grade chemicals of glycerol, hexane, diethyl ether, chloroform and sodium hydroxide pellets were obtained from Merck Chemicals, Germany. A set of

*Corresponding author.
Email: *nazrim_marikkar@putra.upm.edu.my*

FAME standard comprising 37 fatty acids (C_4 to C_{24}) and a monoglyceride stock solution were purchased from Sigma–Aldrich Chemicals (Deisenhofen, Germany).

MAG and DAG preparation

Chemical glycerolysis of each lipid were performed in triplicate according to the previous study as reported by Indrasti *et al*. (2010). For glycerolysis reaction, 35-g oil sample was mixed with 15 g glycerine solution and 0.2 g sodium hydroxide catalyst. The mixture was then heated at 250 °C with vigorous mixing for 60 min. The separation of glycerolysis reaction products to MAG and DAG was carried out according to AOCS method Cd 11c-93 (AOCS, 2007) using a glass column filled with Davison 923 type silica gel (Sigma Aldrich). TLC was run subsequently to verify the purity of the fractions collected through column chromatography.

GLC analysis of fatty acid methyl esters (FAME)

FAME were prepared by dissolving 50 mg portion of oil in 0.8 ml of hexane and adding 0.2 ml portion of 1M solution of sodium methoxide (PORIM, 1995) and analyzed on a gas chromatograph (Agillent Technologies, Singapore) fitted with a FID detector. The polar capillary column RTX-5 (0.32 mm internal diameter, 30 m length and 0.25 µm film thickness (Restex Corp., Bellefonte, PA) was used. The oven temperature was programmed as follows: initial temperature of 50°C (for 1 min), and programmed to increase to 200 °C at 8 °C/min. Both injector and detector temperatures were maintained at 200 °C throughout the analysis. The carrier gas (helium) flow rate was 1.0 mL/min and the split ratio was 58:1. The identification of the peaks of the samples was done with reference to a chromatographic profile containing FAME standards. The percentage of fatty acid was calculated as the ratio of the partial area to the total area (Yanty *et al*., 2011).

Thermal analysis

Thermal analysis was carried out on a Mettler-Tolledo differential scanning calorimeter. Nitrogen (99.999% purity) was used as the purge gas at a rate of 20 mL/min. Approximately 6–8 mg of melted sample was placed in a standard DSC aluminum pan and then hermetically sealed. An empty, hermetically-sealed DSC aluminum pan was used as the reference. The MAG and DAG samples were subjected to the following temperature program: 70 °C isotherm for 1 min, cooled at 5 °C/min to -70 °C (Yanty *et al*., 2011).

Statistical analysis

Data were statistically analyzed by one-way analysis of variance (ANOVA) using MINITAB (version 14) statistical package at 0.05 probability level. For the grouping and classification models, Principal Component Analysis (PCA) was carried out using Unscrambler 9.7 (Camo, USA) software.

Results and Discussion

Fatty acid profiles of MAG

The overall fatty acid distributional patterns of MAG derived from plant (RS, SF and SB) and animal (LD, BF and GF) lipids are compared in Table 1. The data shows that MAG of SF and SB are very much distinguishable from those derived from all three animal fats as they are found to possess linoleic (48-59%) as the most dominant fatty acid, followed by oleic acid (22-38%). Meanwhile, MAG derived from all three animal fats are found to possess either palmitic (BF) or oleic (LD and GF) as the major fatty acid. According to data in Table 1, MAG derived from GF, LD and RS share a common characteristic, with oleic being the major fatty acid. Hence, a cursory examination of fatty acid data does not always reveal a common characteristic that would help to discriminate MAG of animal fats from those derived from plant lipids. Therefore, PCA is suggested as an alternative approach to establish classification among MAG originating from plant and animal lipids. The score plot of MAG derived from BF, GF, LD, SB, RS and SF as shown in Figure 1(A) represent the projection of samples defined by principal component 1 (PC 1) and principal component 2 (PC 2). PC 1 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. According to the Figure 1(A), MAG derived from LD, BF and GF could be distinguished clearly from those derived of plant based lipids along PC1 axis which explained 76% of the variances in the data set. While LD, BF and GF were located in the negative scores of PC 1, those of RS, SF and SB exhibited positive scores along PC 1 axis. Analysis of loading plot (Figure 1B) indicates the variables, which give high influence on the separation of the samples in the score plot. Generally, variables that are located further away from the origin contribute the most variation to the principal component model. According to the loading plot in Figure 1(B), palmitic, stearic and linoleic acids are the most discriminating variables that separate MAG of plant oils with those derived from animal lipid in the PC 1 components.

Table 1. Fatty acid composition of MAG and DAG from some plant and animal lipids

Fat types		Fatty acid (%)										
		C14:0	C15:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C18:3	∑SFA	∑USFA
BF	MAG	4.5± 0.18 ^{A}	1.19 ± 0.05 ^{A}	31.99 ± 0.84 ^{A}	1.87 ± 0.04 ^{A}	1.75 ± 0.13 ^{A}	29.66 ± 0.28 ^{A}	27.34 ± 0.67 ^{C}	1.70 ± 0.01 ^{E}	-	69.09	30.91
	DAG	3.34 ± 0.2 ^{B}	1.54 ± 0.02 ^{B}	30.12 ± 0.94 ^{B}	1.7 ± 0.11 ^{B}	1.92 ± 0.05 ^{B}	35.6 ± 1.12 ^{B}	24.01 ± 0.20 ^{F}	1.77 ± 0.01 ^{E}	-	72.52	27.48
GF	MAG	4.12 ± 0.41 ^{A}	1.13 ± 0.09 ^{A}	27.79 ± 1.49 ^{B}	1.55 ± 0.58 ^{A}	1.77 ±0.02 ^{A}	23.30 ± 1.65 ^{B}	36.80 ± 0.97 ^{B}	1.59 ± 0.11 ^{E}	1.95 ± 0.04 ^{B}	58.11	41.89
	DAG	2.53 ± 0.18 ^{D}	-	30.79 ± 1.31 ^{B}	1.6 ± 0.11 ^{B}	1.48 ± 0.02 ^{B}	28.01 ± 1.15 ^{D}	32.56 ± 0.42 ^{E}	1.73 ± 0.04 ^{E}	1.3 ± 0.54 ^{D}	62.81	37.19
LD	MAG	1.82 ± 0.00 ^{E}	-	24.77 ± 0.09 ^{C}	1.68 ± 0.04 ^{A}	-	11.24 ± 0.3 ^{C}	36.81 ± 0.12 ^{B}	23.68 ± 0.31 ^{D}	-	37.83	62.17
	DAG	1.23 ± 0.05 ^{E}	-	20.84 ± 0.31 ^{D}	1.45 ± 0.03 ^{B}	-	15.51 ± 0.49 ^{E}	39.95 ± 0.61 ^{D}	21.02 ± 0.23 ^{E}	-	37.58	62.42
SF	MAG	-	-	7.98 ± 0.05 ^{E}	-	-	5.21 ±0.27 ^{D}	37.92 ± 0.28 ^{B}	48.89 ± 0.3 ^{B}	-	13.19	86.81
	DAG	-	-	8.63 ± 0.28 ^{E}	-	-	5.54 ± 0.28 ^{E}	37.11 ± 0.29 ^{E}	48.72 ± 0.09 ^{B}	-	14.17	85.83
SB	MAG	-	-	12.86 ± 0.11 ^{D}	-	-	5.4 ± 0.11 ^{D}	22.97 ± 0.16 ^{D}	58.77 ± 0.05 ^{A}	-	18.26	81.74
	DAG	-	-	12.31 ± 0.06 ^{E}	-	-	5.5 ± 0.07 ^{E}	26.85 ± 0.21 ^{E}	55.34 ± 0.2 ^{B}	-	17.81	82.19
RS	MAG	-	-	4.97 ±0.05 ^{F}	-	-	2.13 ± 0.03 ^{E}	56.63 ± 0.14 ^{A}	26.49 ± 0.30 ^{C}	9.78 ± 0.25 ^{A}	7.1	92.9
	DAG	-	-	5.77 ± 0.08 ^{E}	-	-	2.64 ± 0.26 ^{E}	65.07 ± 0.03 ^{A}	19.87 ± 0.03 ^{E}	6.65 ± 0.09 ^{B}	8.41	91.59

[1]Each value represents the means and standard deviation of three analyses. Means within column with different superscripts are significantly different (p<0.05%)
[2]Abbreviations: MAG, monoacylglycerol: DAG, diacylglycerol; BF, beef fat; GF, goat fat; LD, lard; SF, sunflower seed oil; SB, soybean oil; RS, rapeseed oil, SFA, saturated fatty acid; USFA, unsaturated fatty acid.

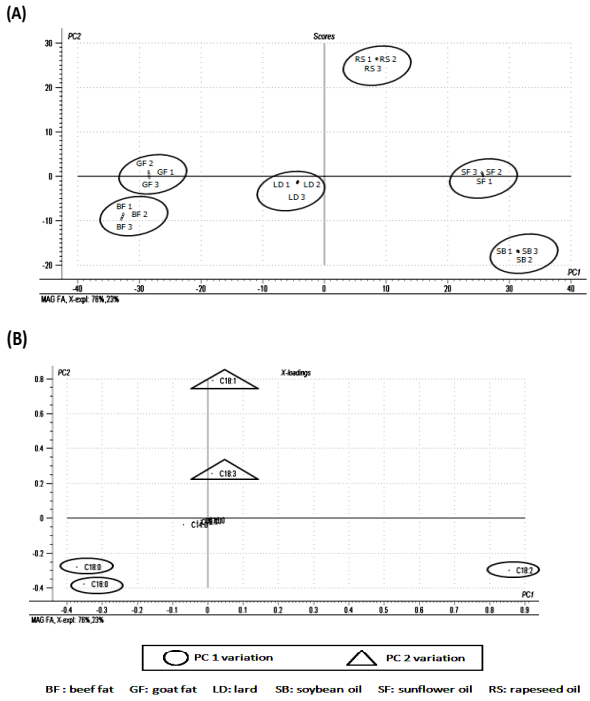

Figure 1. Principal component analysis of MAG based on FA compositions: (A) score plot and (B) loading plot

Fatty acid profiles of DAG

The overall fatty acid distributional patterns of DAG derived from plant (RS, SF and SB) and animal (LD, BF and GF) lipids are compared as shown in Table 1. Finding differentiation between DAG of SF and SB is much easier as they are found to possess completely different fatty acid distributional pattern from DAG of animal fats. While DAG of SF and SB are found to possess linoleic (48-55%) as the most dominant fatty acid, DAG derived from all three animal fats are found to possess either stearic (BF) or oleic (LD and GF) as the major fatty acid. However, DAG derived from RS, GF and LD are found to possess similar major fatty acid by having oleic (32-65%) as the most dominant fatty acid. Hence, a cursory examination of fatty acid data does not always reveal a common characteristic that would help to discriminate DAG of animal fats from that derived of RS. These fatty acid data were further analyzed using PCA to get a better discrimination of DAG derived from different lipid classes. The PCA score plot as shown in Figure 2(A) displays six clusters representing DAG derived from BF, GF, LD, SB, RS and SF. The two first principal components showed all together 89% variance of which PC1 accounted for 72% of the variation while PC2 described 27% variation. PC1 shows clear separation of DAG derived from of animal fats from those derived from plant lipids. While DAG of LD, BF and GF clusters were laid on the negative side of PC1, the DAG of SF, RS and SB were on the positive side of PC 1. According to the loading plot in Figure 2(B), palmitic, stearic and linoleic acids were the most discriminating variables that separate DAG of plant oils with those derived from animal lipid in the PC 1 components.

Cooling profiles of MAG

An overlay of cooling curves in Figure 3(A) shows the nature of thermal profiles displayed by MAG derived from BF (curve-BF), GF (curve GF), LD (curve LD), SB (curve-SB), SF (curve-SF) and RS (curve-RS). By overall, the thermal profiles displayed by the MAG of RS and SF are distinctly different from those derived from animal fats. While the cooling curve of MAG derived from BF, GF and LD are found to display thermal transitions in both the high (> 0 °C) and low temperature regions (< 0 °C), the cooling curve of SF and RS are not found to display any thermal peak in a high temperature region (>5°C). These two types of MAG possessing extremely high amount of unsaturated fatty acid (86-93%) could be considered as a probable reason for them to be different from those of the three animal fats (Table 1). The cooling profile displayed by SB (Curve -SB, Figure 3A) is found to possess thermal transitions in both low (< 0 °C) and high (> 0 °C)

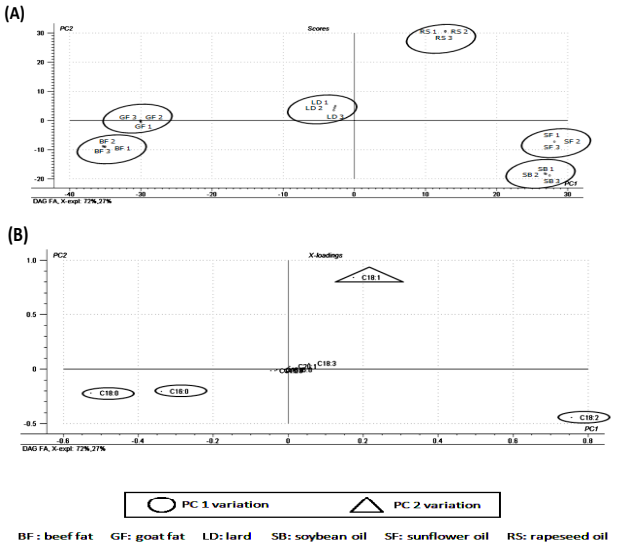

Figure 2. Principal component analysis of DAG based on FA compositions: (A) score plot and (B) loading plot

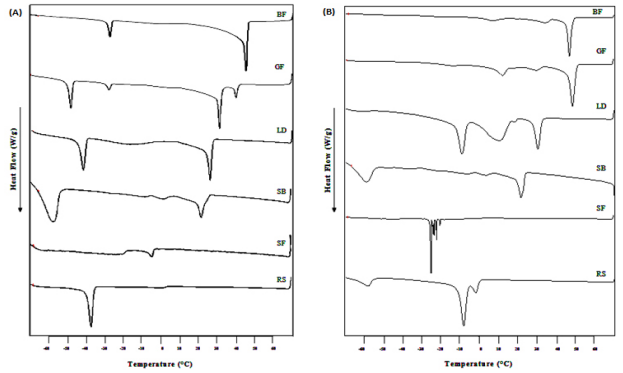

Figure 3. DSC cooling curve of (A) MAG and (B) DAG derived from beef fat (curve-BF), goat fat (curve GF), lard (curve-LD), soybean oil (curve-SB), sunflower oil (curve-SF) and rapeseed oil (curve-RS)

temperature regions as similar to the thermal profiles displayed by the three animal fats. As there is no clear differentiation between MAG derived from SB and those derived from animal based lipids, PCA was applied to DSC thermal data to find better discrimination between MAG derived from plant lipids and those derived from animal fats. In the application of PCA, DSC parameters namely, onset of crystallization temperature (T_{on}), peak temperature of largest peak (T p max), enthalphy (ΔH) of the largest peak, and total ΔH were used as a variables. The score plot of MAG based on the above mentioned DSC parameters has been given in Figure 4(A), which showed that classification between MAG of plant lipids and animal fats could be achieved by using the PC 1 components. The PC1 components accounted for 81% of the variation while PC2 described 14% variation. While MAG derived from animal fats is located in the upper negative side of PC1, RS, SF and SB are scattered in the negative side of PC1. According to the loading plot shown in Figure 4(B),

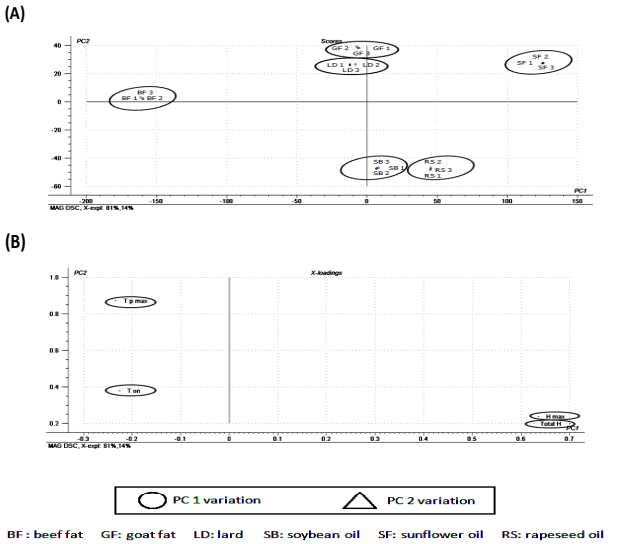

Figure 4. Principal component analysis of MAG based on cooling profiles: (A) score plot and (B) loading plot

ΔH of the largest peak and total ΔH were the most discriminating variables that separate MAG of plant oils with those derived from animal lipid in the PC 1 components.

Cooling profiles of DAG

An overlay of cooling curves displayed by DAG derived from LD (curve-LD), BF (curve-BF), GF (curve-GF), SB (curve SB), RS (curve RS) and SF (curve SF) is presented in Figure 3(B). Similar to DSC profiles of MAG, the pattern of profiles displayed by DAG derived from RS and SF are distinctly different from those of the three animal fats (GF, LD and BF). Particularly, they do not display any thermal peak in the temperature region above 0°C as the onset of crystallization temperature for DAG of SF and RS are -0.8° C and -8.55°C, respectively. As mentioned previously, the observed difference between the thermal profiles of DAG derived from SF and RS and those of the three animal fats could be probably due to the differences in the distribution of fatty acids. In fact, the DAG derived SF and RS are found to possess extremely higher proportions of unsaturated fatty acid (85-91%) when compared to those derived from the three animal fats (Table 1). However, the cooling profile displayed by SB (curve-SB, Figure 3B) is found to possess thermal transitions in both low (< 0 °C) and high (> 0 °C) temperature regions as similar to the thermal profiles displayed by DAG of three animal fats. As there is no clear discrimination on the DAG of SB from those of the three animal fats, PCA was applied for grouping DAG according to the four thermal properties mentioned previously in the MAG. A score plot of the first two principal components (Figure 5A) showed that PCA of DAG was able to discriminate DAG of BF, GF and LD from the DAG

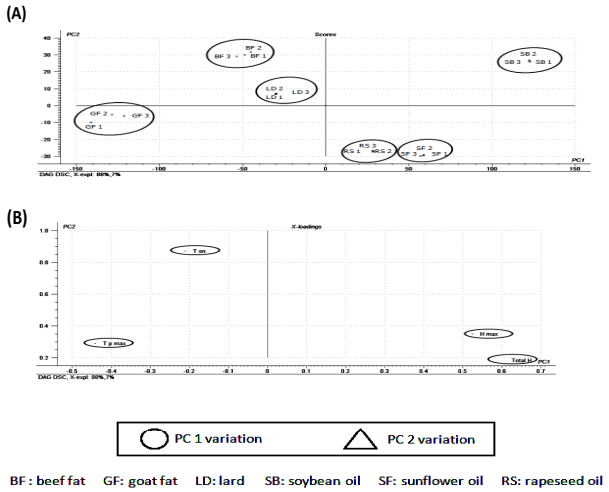

Figure 5. Principal component analysis of DAG based on cooling profiles: (A) score plot and (B) loading plot

of RS, SB and SF. The PC1 components accounted for 88% of the variation while PC2 described 7% variation. While clusters of DAG derived from SB, RS and SF are located in the positive side of PC1, the DAG derived from three animal fats are cluster together in the negative side of PC1. The loading plot in Figure 5(B) indicates that enthalpy (ΔH) of the largest peak, and total ΔH contribute the most variation in the sample classification in the PC 1 components.

Conclusions

The loading plots based on fatty acid data showed that palmitic, stearic and linoleic acids were the most discriminating parameters in the classification of both MAG and DAG derived from the two lipid classes. Likewise, there is adequate discrimination between the MAG and DAG derived from plant and animal lipids based on DSC thermal data. The loading plots of MAG and DAG based on DSC data indicate that the enthalphy (ΔH) of the largest peak, and total ΔH contribute the most variation in the sample classification. Hence, the present study demonstrated that the application of PCA to fatty acid and DSC thermal data have considerable potential for classification of MAG and DAG derived from plant and animal lipids.

Acknowledgments

Authors gratefully acknowledge a research grant (02-12-1010 FR) obtained under the Fundamental Research Grants Scheme of the Ministry of Higher Education, Malaysia.

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