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Differentiation of lard and other animal fats based on triacylglycerols composition and principal component analysis

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Abstract: The composition of triacylglycerol (TAG) for the differentiation of lard and other animal fats (beef, mutton, and chicken fats) as well as cod liver oil (CLO) has been investigated using high performance liquid chromatography with refractive index detector. The authenticity of the used samples was determined based on their fatty (FA) acid profiles. FA compositions of the studied fats and oils were determined with gas liquid chromatography. The main TAGs composing lard are palmitooleoolein (POO), palmitooleostearin (POS), and palmitooleopalmitin (POP) accounting of 21.55 ± 0.08, 14.08 ± 0.04, and 5.10 ± 0.04%, respectively. The TAG composition was further subjected to principal component analysis in order to classify lard and others. Lard was separated along negative side, either in the first principle components (PC1) and second principle components (PC2). Using score plot of PCA, lard has the similarity with chicken fat in term of TAG composition.

Keywords: : Lard, triacylglycerol, HPLC, principal component analysis

Introduction

Lard, one of the pig derivatives, is obtained from the rendering of adipose tissue of pig. In some countries, lard is one of the cheapest edible fats and oils; consequently, lard is deliberately added into the food products to reduce the production cost (Che Man and Sadzili, 2010). From the religious point of view, the presence of lard in any food products is not allowed. For this reason, several analytical methods either physical or chemical based-methods have been developed to identify lard (Rohman *et al*., 2010).

The animal fats, including lard, and vegetable oils were mainly composed from triacylglycerols (TAG), diacylglycerols (DAGs), free fatty acids ant other minor components like phospholipids, sterols, tocopherols, carotenoids, and fat soluble vitamins (Gunston, 2004), however, the main classes found in fats and oils are TAGs (Andrikopolous *et al*., 2002). Therefore, this study was directed to differentiate lard and other animal fats based on the levels of TAGs in fats and oils. Che Man *et al*. (2011) have differentiated lard from other animal fats using Fourier transform infrared spectra in combination with chemometrics of principal component analysis and cluster analysis. However, using literature searching, there is no available report related to the application of principal component analysis using TAG data as matrix variables of lard and other animal fats. For this reason, in this study, we exploited the TAG composition for such differentiation.

Several analytical methods have been developed to analyze TAGs quantitatively. One of the most used techniques is high performance liquid chromatography (HPLC) (Rashood *et al*., 1996; Yoshida *et al*., 2009). Using HPLC technique, the parameter used for characterization and separation of TAGs was "equivalent carbon number" or ECN, which is defined by the following equation:

ECN = the number of carbon – 2n

where n is the number of double bonds per TAG present in three fatty acids composed TAG (Buchgraber *et al*., 2004; de la Mata-Espinosa *et al*., 2011). The objective of the present study is to differentiate lard from other animal fats (chicken, beef, and mutton fats) as well as cod liver oil) using TAG composition in combination with the chemometrics of principal component analysis.

Materials and Methods

Sample preparation

All reagents and solvents were bought from E. Merck (Darmstat, Germany). Lard and other animal fats were prepared by rendering adipose tissues of the corresponding animals, obtained from some

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slaughtered houses in Yogyakarta. The rendering process was carried out according to previously reported publication by Rohman and Che Man (2009a). Adipose tissues of pig (lard), chicken, mutton, and beef were cut into small pieces, mixed, and melted at 90–100°C for 2 h in the conventional oven. The melted fat was strained through triple-folded muslin cloth, dried by the addition of anhydrous Na_2SO_4 and then centrifuged at 3000 rpm for 20 min. The fat layer was decanted, shaken well and centrifuged again before being filtered through Whatman paper containing Na_2SO_4 anhydrous to remove the trace of water residue. The filtered samples were directly subjected to chemical analysis or kept in tightly closed container under a nitrogen blanket in -20°C. Before being used for analysis, all animal fats were thawed at water bath at 60°C until they melt.

Fatty acid composition

The composition of fatty acids in lard and other studied edible fats and oils was determined using gas chromatography using flame ionization detector. The sample preparation and the condition of GC used can be seen in Rohman and Che Man 2009[b].The column and carrier gas was DB-5 from Restex and helium with the purity of 99.99%, respectively. Derivatization of fatty acids was carried out by methylation technique using sodium methoxyde as methylation agents.

Quantification of Triacylglycerol

Quantitative analysis of triacylglycerols (TAGs) was performed according to Marikkar *et al*. (2011). Liquid chromatograph used was Knauer (Belin Germany) equipped with refractive index detector (Knauer, Germany). The quantitative analysis of TAGs was accomplished on an Eurospher100-5 C-18 Column (Knauer, Germany) packed with a particle size of 5 µm (25 cm x 4.6 mm i.d., Merck, Darmstadt, Germany). The mobile phase was a mixture of acetone–acetonitrile (63.5: 36.5 v/v) and the flow rate was 1 ml/min at 30°C. The injector volume was 10 µL of 5% (w/w) the studied fats and oil in chloroform. The peak was treated with the software of Chromgate version 3.1.6. Each sample was chromatographed three times, and the data were quantified using internal normalization technique, as described in Rohman and Che Man (2011).

Statistical analysis

In order to differentiate and to classify lard, animal fats and cod liver oil, the chemometrics of principal component analysis using triacylglycerols composition as matrix variable was performed with the aid of The Unscrambler software (version 9.7) from Camo, USA.

Results and Discussion

Fatty acid composition

Fatty acids (FAs), one of the lipid classes, are the most vital components of edible fats and oils in which they are typically found in the ester form with glycerol backbone (triglycerides). Due to the nutritional value, quantitative analysis of FAs composition is a pivotal work in food research area, especially in lipid studies (Carrosco-Pancorbo *et al*., 2009). In addition, FAs composition can vary from one source to another or even from one organ to others; Therefore, FAs profiles can be used for determining the purity or authenticity of animal fats (Hauff and Vetter, 2010). For this reason, we initially determine the level of FAs in the studied samples (lard and others).

The fatty acid composition of lard and other animal fats (beef, chicken, and mutton) as well as cod liver oil as determined using gas chromatography with flame ionization detector is shown in Table 1. These results were in agreement with those obtained by several authors (Chin *et al.*, 2009; Indrasti *et al*., 2011). Compared with other evaluated samples, lard was differentiated by higher level of unsaturated fatty acids. The main fatty acids composed of lard are palmitic, stearic, oleic and linoleic acids. In addition, the level of lard is also in accordance with that listed in Codex Allimentarius (2003). Based on this result, it is also can be stated that the used animal fats as well as CLO meet the fats specification.

Triacylglycerol (TAG) composition

TAG composition was determined with HPLC using refractive index detector. Figure 1 showed the chromatogram of lard describing the TAG composition along with its retention time. Table 2 compiled the TAG composition of lard and others (beef fat, mutton fat, chicken fat, and cod liver oil).

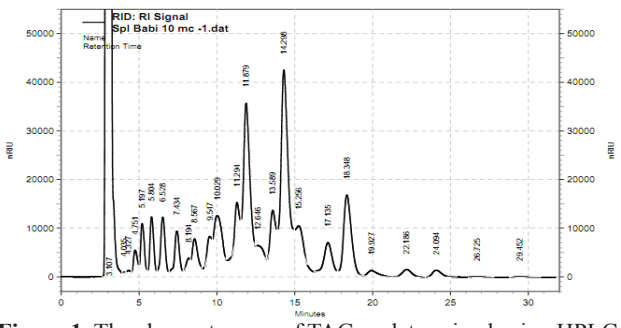

Figure 1. The chromatogram of TAG as determined using HPLC with refractive detector. The retention time corresponding to TAG composition is described in Table 2

The TAG identification was carried out based on the previous reports (Rashood *et al.*, 1996; Marikkar *et*

Table 1. Fatty acid compositions of lard and other animal fats of beef fat (BF), chicken fat (CF), mutton fat (MF), and cod liver oil (CLO)

Animal Fats‡	Fatty acids (% w/w)†													
	C14:0		C16:0		C16:1		C17:0		C18:0		C18:1		C18:2	
	Exp	Ref	Exp	Ref	Exp	Ref	Exp	Ref	Exp	Ref	Exp	Ref	Exp	Ref
LD	1.30± 0.03^{b}	1.0 – 2.5	20.66 ± 0.24^{b}	20 - 30	1.98 ± 0.01^{b}	2.0 – 4.0	0.48 ± 0.02^{c}	< 1.0	10.91± 0.12^{c}	8 -22	39.13± 0.09^{d}	35-55	19.56± 0.04^{e}	4-12
BF	2.93± 0.03^{d}	-	24.52 ± 0.09^{c}	-	2.81± 0.01^{c}	-	1.33 ± 0.01^{d}	-	17.85± 0.07^{d}	-	38.63± 0.15^{c}	-	1.84± 0.01^{c}	-
CF	0.89± 0.01^{a}	-	28.36 ± 0.13^{d}	-	5.75± 0.01^{d}	-	0.20 ± 0.00^{a}	-	6.70± 0.09^{b}	-	41.12± 0.29^{e}	-	14.32± 0.05^{d}	-
MF	2.65± 0.01^{c}	-	20.73 ± 0.08^{b}	-	0.96 ± 0.01^{a}	-	2.02 ± 0.02^{e}	-	28.06± 1.38^{e}	-	33.94± 0.19^{b}	-	1.36± 0.06^{b}	-
CLO	4.16± 0.02^{e}	-	11.89 ± 0.05^{a}	-	6.85 ± 0.28^{e}	-	0.22 ± 0.00^{b}	-	2.30± 0.01^{a}	-	21.16± 0.04^{a}	-	0.42± 0.01^{a}	-

Animal Fats‡	Fatty acids (% w/w)†											
	C18:3		C20:0		C20:1		C20:5n3		C20:6n3		C22:6n3	
	Exp	Ref	Exp	Ref	Exp	Ref	Exp	Ref	Exp	Ref	Exp	Ref
LD	1.21± 0.06^{d}	< 1.5	0.91± 0.01^{d}	< 1.0	0.96± 0.04^{d}	< 1.5	0.12± 0.00^{bc}	-	0.14± 0.01^{c}	-	0.20± 0.00^{b}	-
BF	0.83± 0.01^{b}	-	0.12± 0.00^{a}	-	0.21± 0.01^{c}	-	0.06± 0.00^{a}	-	0.12± 0.00^{b}	-	0.16± 0.04^{b}	-
CF	0.63± 0.03^{a}	-	0.15± 0.02^{b}	-	0.01± 0.00^{a}	-	0.09± 0.01^{ab}	-	0.31± 0.02^{d}	-	0.07± 0.00^{a}	-
MF	0.83± 0.01^{d}	-	0.55± 0.01^{c}	-	0.11± 0.01^{b}	-	0.15± 0.00^{c}	-	0.07± 0.01^{a}	-	0.19± 0.02^{b}	-
CLO	1.98± 0.07^{e}	-	0.12± 0.01^{a}	-	11.44± 0.08^{e}	-	16.74± 0.05^{e}	-	1.22± 0.01^{e}	-	8.82± 0.08^{c}	-

‡nd= not detected; LD = lard; BF = beef fat; CF = chicken fat; MF = mutton fat; and CLO = cod liver oil. † Each value in the table represents the means of triplicate analysis; SD is given after ±. Each column with different letter is significantly different ($P < 0.05$). Ref = this value was taken from reference as stated in Codex Allimentarius (2003).

Table 2. TAG composition of lard and other animal fats

Time retention	TAG	Animal fats				
		Lard	Chicken	Beef	Lamb	Cod liver oil
4.33	LLLn	0.19 ± 0.08	0	0	0.28 ± 0.10	0
4.75	LLL	1.80 ± 0.42	3.48 ± 0.23	4.00 ± 0.50	4.10 ± 0.89	18.32 ± 1.26
5.19	MOL	3.45 ± 0.03	0.08 ± 0.03	0	0	0
5.8	OOL	4.56 ± 0.01	0.11 ± 0.01	0.07 ± 0.00	0.09 ± 0.04	2.95 ± 0.17
6.52	POO	5.39 ± 0.01	0.08 ± 0.01	0.02 ± 0.00	0.09 ± 0.02	7.04 ± 0.49
7.43	POL	4.26 ± 0.01	0.64 ± 0.00	0.01 ± 0.00	0.07 ± 0.00	1.55 ± 0.08
8.19	PPO	1.49 ± 1.55	5.74 ± 0.07	0.03 ± 0.00	0.08 ± 0.04	3.24 ± 0.18
9.54	MOP	1.12 ± 0.01	3.38 ± 0.05	0.13 ± 0.01	0.12 ± 0.02	3.42 ± 0.05
10.02	PLP	5.13 ± 0.01	8.43 ± 0.09	0.88 ± 0.04	0.34 ± 0.01	3.43 ± 0.14
13.58	OOO	3.29 ± 0.13	8.37 ± 0.10	1.00 ± 0.11	1.03 ± 0.04	5.78 ± 0.45
14.29	POO	21.55 ± 0.08	23.52 ± 0.06	10.76 ±0.18	9.66 ± 0.25	11.71 ± 0.80
15.25	PLS	2.35 ± 0.04	14.15 ± 0.04	13.51 ± 0.32	7.52 ± 0.08	3.83 ± 0.31
17.13	POP	5.10 ± 0.04	3.09 ± 0.13	0	6.48 ± 0.25	7.34 ± 0.45
18.34	POS	14.08 ± 0.04	5.77 ± 0.01	21.01 ± 0.44	19.44 ± 0.26	1.89 ± 0.06
19.92	PPS	1.27 ± 0.01	1.68 ± 0.01	11.83 ± 0.31	10.56 ± 0.18	4.94 ± 0.21
22.18	SOS	1.71 ± 0.01	0.66 ± 0.01	10.22 ± 0.23	16.12 ± 0.02	2.65 ± 0.30
24.09	PSS	1.49 ± 0.01	0.30 ± 0.00	8.17 ± 0.21	11.42 ± 0.40	2.48 ± 0.10
29.45	SSS	0.17 ±0.00	0	0	5.19 ± 0.16	0.46 ± 0.25

Each value in the table represents the means of triplicate analysis; SD is given after ±.

al., 2011) and based on TAG standard coming from Sigma, Aldrich, USA. Lard can be differentiated based on its TAG composition in which the level of palmitooleoolein (POO), palmitooleostearin (POS), and palmitoolepalmitin (POP) were predominantly present compared with other TAGs. In lard, these three TAGs composed for 21.55 ± 0.08, 14.08 ± 0.04, and 5.10 ± 0.04%, respectively.

In order to make the differentiation and classification, the TAGs profiles of lard and others were subjected to the chemometrics technique of principal component analysis (PCA). PCA is an unsupervised pattern recognition technique widely used in chemometrics study. PCA projects the original data in reduced dimensions defined by the principal components (PCs). This technique is useful when there are correlations present among studied data (Cserhati *et al*., 2009).

In this study, PCA was accomplished using TAG composition as variable, as shown in Table 2. Figure 2 demonstrates the score plot of PCA of lard and others describing the projection of samples defined

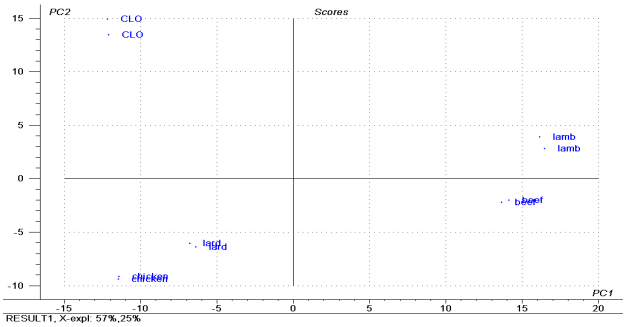

Figure 2. The score plot of PCA model using TAG composition of lard and others as matrix variables

by the first (PC1) and second (PC2) components. PC1 accounts for the most variation in FTIR spectra absorbances, while PC2 accounts for the next largest variation. PC1 accounted for 57% of the variation, while PC2 explained 25% of variation, and PC3 contributed to 13% of variation; therefore, it can be stated that using PCA, the 18 variables of TAG data can be described by three PCs because these three first PCs can describe more than 95% of variation. Based on the score plots, it is known that lard can be separated from others in which lard has negative side either in PC1 and PC2. In addition, chicken fat has similar TAG composition with lard, among others, as shown by the close distance of chicken fat to lard.

In order to know the number of PCs suggested by software to be used in PCA model, the residual analysis was constructed. Based on the predicted residual error sum of square values (Figure 3), it can be stated that 4 PCs is necessary for PCA model, because at this PC number, PRESS value reach minimal (Sedman *et al.*, 1997).

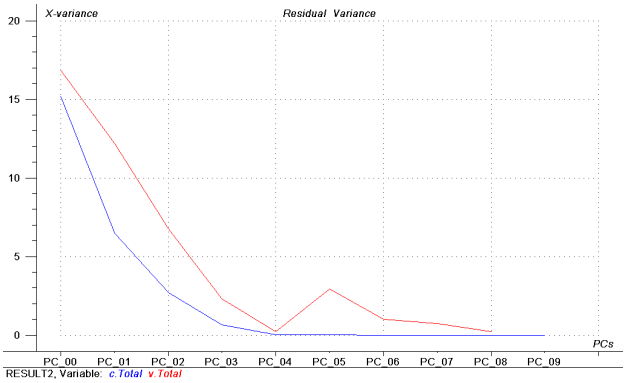

Figure 3. The residual variance of PCA model for determination of optimum principle components used in PCA model

Figure 4 shows the loading plot for the determination of variables (TAG composition) contributing to the differentiation and separation of the samples (lard and others). The PCA loading plot describes the projection of variables in the same plane as the score plot. The absolute value of loading plot in the TAG composition explains the importance of the contribution of each TAG. Therefore, the further away a TAG composition from the origin of variable point, the larger the contribution of that variable to the PCA model (Marina *et al.*, 2010). From Figure 4, it is known that POO and POS make a larger contribution to PCA model.

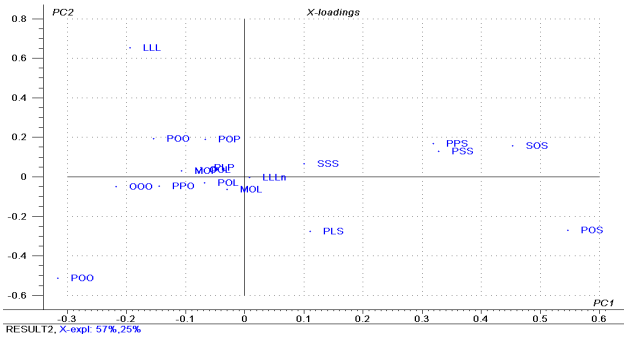

Figure 4. The loading plot of PCA model describing the distribution of TAG

Conclusion

It can be concluded that TAG composition in combination with chemometrics of PCA can be exploited for differentiation and classification of lard from others. The developed method is fast and reliable due to the capacity of HPLC to separate the sudied samples into its TAG components.

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