

# Principal Component Analysis (PCA) on Multivariate Data of Lard Analysis in Cooking Oil

Nor Aishah Mohd Salleh<sup>1</sup> and Mohd Sukri Hassan<sup>1,2</sup>

1. Faculty of Science and Technology, Universiti Sains Islam Malaysia (USIM), Bandar Baru Nilai, 71800 Nilai, Negeri Sembilan, Malaysia.

2. Institute of Halal Research & Management (IHRAM), Universiti Sains Islam Malaysia (USIM), Bandar Baru Nilai, 71800 Nilai, Negeri Sembilan, Malaysia.

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**Abstract:** Discrimination of fatty acids (FAs) of lard in used cooking oil is important in *halal* determination. The aim of this study was to find the information related to the changes FAs of lard when frying in cooking oil. Quantitative analysis of FAs composition extracted from a series of experiments which involving frying cooking oil spiked with lard at three different parameters; concentration of spiked lard, heating temperatures and period of frying. The samples were analyzed using Gas Chromatography (GC) and Principal Components Analysis (PCA) technique. Multivariate data from chromatograms of FAs were standardized and computed using Unscrambler X10 into covariance matrix and eigenvectors correspond to Principal Components (PCs). Results have shown that the first and second PCs contribute to the FAs mapping which can be visualized by scores and loading plots to discriminate FAs of lard in used cooking oil

**Key words:** Fatty acids, lard, gas chromatography, Principal Components Analysis

## 1. Introduction

The development of method for the detection of adulteration in used cooking oil by non- *halal* substances such as lard is very challenging because of the similarity of Fatty Acids (FAs) composition for lard, animals and plants. In addition the degrading of the chemical structures during frying at high temperatures. The FAs composition are identified in the form of Fatty Acid Methyl Esters (FAMES) using Gas Chromatography Flame-Ionization (GC-FID) [1].

Chemometrics has been often used for the classification and comparison of different edible fats and oils. Some examples of recent studies are the combination of Fourier Transform Infrared spectroscopy (FTIR) with chemometrics for analysis of lard in the mixtures with body fats of lamb, cow,

and chicken [2], profiling FAs of lard using comprehensive Gas Chromatography hyphenated with Time of Flight mass spectrometry (GC-ToF) [3], the differentiation of lard from other animal fats in admixtures of some vegetable oils using Liquid Chromatographic (LC) data coupled with multivariate data analysis [4].

Principal Component Analysis (PCA) is an application of chemometrics used as a tool of exploratory analysis using algorithms and designed to reduce large complex data sets or rearranges the data to exploit linear structure. PCA is a technique using mathematically procedures as on orthogonal linear transformation from original data. The transformation of new data must have correlation between the new variable that called as Principal Components (PCs). The PCs is known as latent variable or factor and eigenvector. PC is a linear transformation (axis) of original data set and calculated based on coefficient

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**Corresponding author:** Mohd Sukri Hassan, Ph.D., research field: Analytical Chemistry and Chemometrics: E-mail: mohdsukri@usim.edu.my.

among variables. PC is defined as per equations below. Given example of  $n$  observation on a vector of  $p$  variables;

$$X = (x_1, x_2, \dots, x_p) \quad (1)$$

Define first principal component of the sample by linear transformation;

$$Z_i = a_1^T x = a_{1i} x_i \quad (2)$$

Where the vector  $a_1 = a_{11}, a_{21}, \dots, a_{p1}$  is chosen then variance  $[z_i]$  is maximum. The simplified equation (1), (2) of first principal component;

$$PC1 = a_1 x_1 + a_2 x_2 + a_3 x_3 \quad (3)$$

$$X = \text{mean} + b_1 PC1 + b_2 PC2 + \text{Error} \dots \dots \quad (4)$$

The model equation of PCA (3), (4) can be described as;

$$X = TP^T + E \quad (5)$$

From equation (5)  $X$  is the data matrix.  $TP^T$  is the structure and  $E$  is residual or noise which not "explained" by PC model [5, 6, 7].

Results of PCA are usually discussed by viewing graphical of component Scores called Scores plot; the transformed variable values corresponding to a particular data point and Loadings plot; the weight by which each standardized original variable should be multiplied to get the component score [8].

In this research, we studied the changes of FAs composition of lard during frying at different conditions of three parameters; percentages of lard in cooking oil, temperatures and time of cooking. Palm oil was selected due to the most abundant cooking oil in Malaysian market. Multivariate data of FAME obtained from GC-FID were analyzed using PCA and the changes were observed in Scores and Loadings plot.

## 2. Materials and Methods

### 2.1 Chemicals and Reagents

The analytical solvents used for fat extraction and GC analysis were methanol 99.9 %, acetone 99.9 %, *n*-hexane 99.9 %, chloroform 99.9 % and sodium methoxide 1 % solution. All mentioned solvents were purchased from Sigma Aldrich USA. Certified

Reference Standards (CRM) of FAME Mix were purchased from Supelco, Sigma–Aldrich, USA. A total of 17 FAs standards were involved for identifying the FAs in the fried oil samples containing spiked lard; The FAs were; Caproic (C6:0), Caprilic (C8:0), Capric (C10:0), Lauric (C12:0), Myristic (C14:0), Palmitic (C16:0), Palmitoleic (C16:1), Oleic (C18:0), *Trans*-Oleic (C18:1n9t), *Cis*-Oleic (C18:1n9c), *Trans*-Linoleic (C18:2n6t), *Cis*-Linolenic (C18:2n6c), Linolenic (C18:3n3), Docosadienoic (C22:2) Behenic, (C20:0), Eicosapentaenoic (C20:5n3) and Nervoic (C24:1).

### 2.2 Samples Preparation

Lard (rendered from the belly pork) and refined palm oils were purchased from a local supermarket. Lard was heated at 90 °C for 15 min to melt the fat and later it was blended with the palm oil. The samples of spiked lard (0 %, 1 %, 5 % and 10 %) in palm oil were prepared and heated on a digital hot plate at selected temperatures (120 °C, 180 °C, 240 °C) at interval times (15, 45, 75 min). The fat was extracted according to the Bligh-Dryer method (1959) [9] with slight modification. The summary of the prepared samples and its labeling are as in TABLE 1.

### 2.3 Analysis of Fatty Acid Methyl Esters (FAME) in Lard Using GC-FID

FAs composition were determined by conversion of the fatty acid methyl esters (FAME) following to the method of Cocks and Rede (1966) [10] with a slight modification. FAMES were prepared by adding 950  $\mu$ L of *n*-hexane into 50 mg of fat followed by 5  $\mu$ L sodium methoxide. The mixtures were vortexed for 5 s and allowed to settle for 40 min. A volume of 1  $\mu$ L was collected from the top layer of the settled mixture and injected into a gas chromatography. The gas chromatography-flame ionization detector (Agilent 7980) was fixed with a polar capillary column HP 88 with specifications of 100 m, 25 mm and 25  $\mu$ m. The temperature of the column was set up at 140 °C (for 4

**Table 1** Samples Treatments and its Labeling.

Time of frying (min)	Temperatures °C								
	120			180			240		
0% lard in palm oil (a)	a1	a2	a3	a4	a5	a6	a7	a8	a9
1% lard in palm oil (b)	b1	b2	b3	b4	b5	b6	b7	b8	b9
5% lard in palm oil (c)	c1	c2	c3	c4	c5	c6	c7	c8	c9
10% lard in palm oil (d)	d1	d2	d3	d4	d5	d6	d7	d8	d9

min), increased to 240 °C at a rate of 5 °C/min (for 15 min). The temperature of the injector was set up at 230 °C and the detector at 280 °C. The run time was set up for 44 min for a sample. The FAME peaks were identified by comparing their retention times with certified reference standards of FAME. Percentage of FAs were calculated based on the peak area of a fatty acid to the total peaks of all the fatty acids in the oil samples.

#### 2.4 Data Analysis

GC-FID chromatograms of fried samples containing spiked lard at different concentrations and frying condition produced a matrix (36 x 15) which each element consist of the area of FAs. Each peak area of FAs were normalized by the maximum area of FA in each chromatogram before subjected to PCA. PCA was performed by using Unscrambler software (X10). The multivariate data set of FAMES were subjected to PCA in order to reduce the data set to Scores and Loadings matrices.

### 3. Results and Discussion

#### 3.1 Principal Components Analysis (PCA)

PCA was performed to find correlation among the 36 samples. Samples were divided into 3 groups represent the frying times; 15 min labeled as (1, 4, 7), 45 min labeled as (2, 5, 8) and 75 min labeled as (3, 6, 9).

First observation at frying time for 15 min with 0 % (a), 1 % lard (b), 5 % lard (c) and 10 % lard (d) at temperatures of 120 °C (1), 180 °C (4), 240 °C (7). Scores plot in Fig. 1 showed that samples c7, b7, d1,

d4 and d7 located at negative side on PC1 but oppositely to the other samples (a1, a4, a7, b1, b4, b7, c1, c4). Loadings plot in Fig. 2 showed that FAs C18:2n6t and C18:1n9c are anti-correlated with positive values for PC2. FA C18:2n6t (trans linoleic acid) is significantly different from others FA which mostly contributed by samples spiked with 10 % lard. It also contributed by spiked of 1 % and 5 % at higher temperatures 240 °C. Other FAs such as C16:0, C18:1n9, C18:2n6c, C24:1, C14:0 and C18:0 are located near 0 on PC1.

Second observation at time of frying for 45 min with 0 % lard (a), 1 % lard (b), 5 % (c) lard and 10 % lard (d) at temperatures of 120 °C (2), 180 °C (5), 240 °C (8). Scores plot in Fig. 3 showed that samples d2, d5 and d8 located at negative side on PC1 and oppositely to the other samples (a2, a5, a8, b2, b5, b8, c2, c5 and c8). Loadings plot in Fig. 4 showed that FAs C18:2n6t and C18:1n9c are anti-correlated. FA C16:0 located between the two FAs. FA C18:2n6t (trans linoleic acid) is significantly different from others FA contributed by samples spiked with 10 %. Samples spiked with 1 % and 5 % of lard also contributed significantly different to the trans linoleic acid. The others FAs (C18:0, C18:2n6c, C24:1, C18:0 C18:3n3) are slightly separated in the middle of PC2. Eder (1982) [11] evaluated the formation of geometric isomers in various oils on the laboratory, scale, with unbleached soy bean oil at 240 °C, the formation of C18:3 isomers (determined by GLC) is insignificant especially linoleic acid. This FA is used as parameter to detect chemical changes resulting from severe processing conditions in cis-trans isomerization.

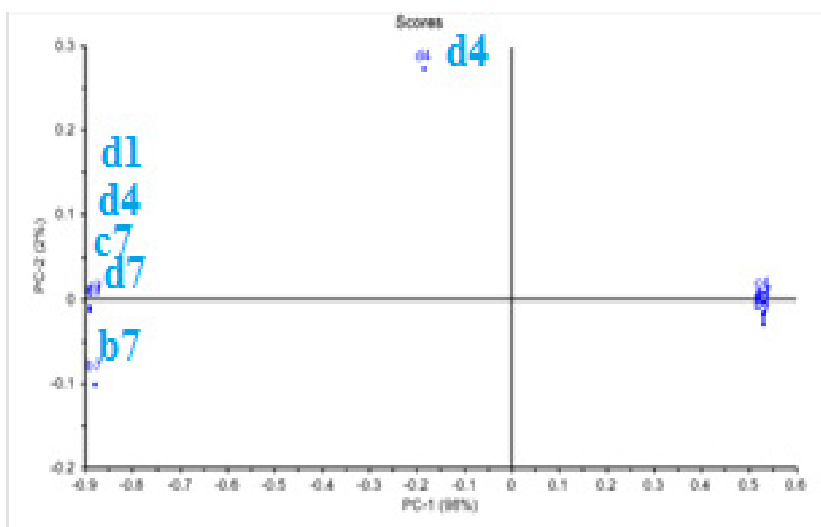


Fig. 1 Scores plot of PC1 versus PC2 at time of heating for 15 min.

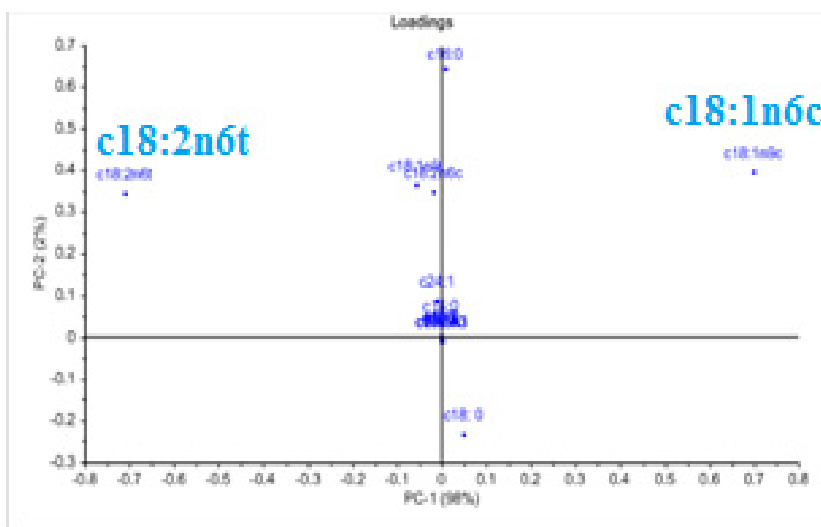


Fig. 2 Loadings plot of PC1 versus PC2 at time of frying for 15 min.

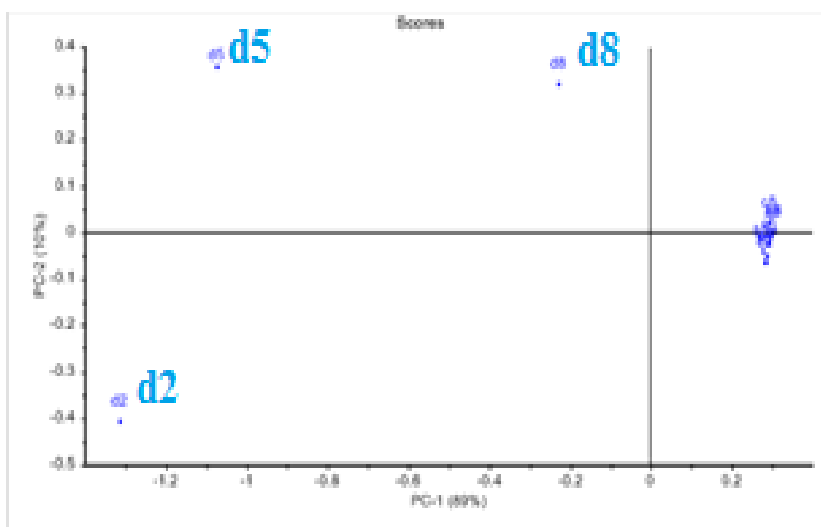


Fig. 3 Scores plot of PC1 versus PC2 at time of frying for 45 min.

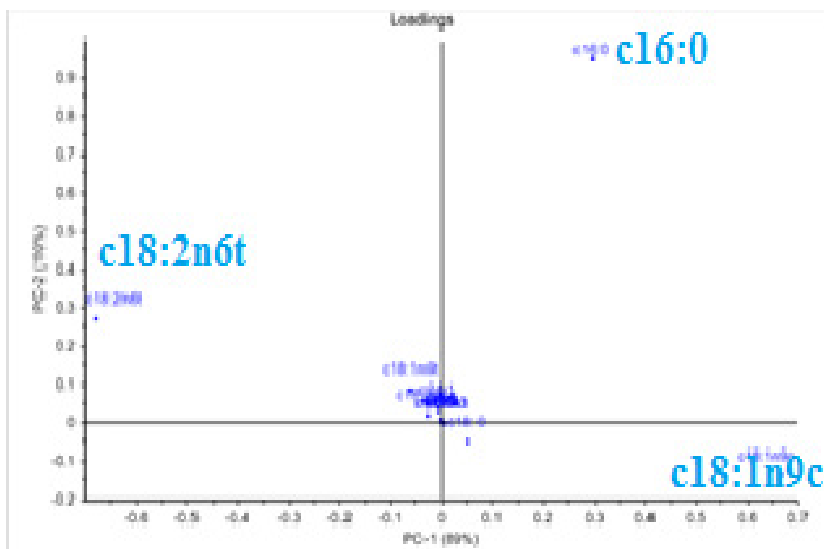


Fig. 4 Loadings plot of PC1 versus PC2 at time of frying for 45 min.

Third observation at time of frying for 75 min with 0 % (*a*), 1 % lard (*b*), 5 % lard (*c*) and 10 % lard (*d*) at temperatures of 120 °C (3), 180 °C (6), 240 °C (9). Scores plot in Fig. 5 showed that samples *c*3, *d*6 and *d*9 located at negative side on PC1 and oppositely to the other samples (*a*3, *a*6, *a*9, *b*3, *b*6, *b*9, *c*6, *c*9 and *d*3). Loadings plot in Fig. 6 showed that FAs C18:2n6t and C18:1n9c are anti-correlated and the first FA has positive value of PC2 but the second FA has a negative value. The position of FA C16:0 moves to the right side from its initial point. The position of FA C18:2n6t (trans linoleic acid) is significantly different from the others FAs which contributed by samples

spiked with 10 % of lard. Samples spiked with 5 % of lard also contributed significantly different to the trans linoleic acid at frying time of 75 min at 120 °C. The other FAs located very close to each other in the middle of the plots which are near 0 values of PC1 and PC2. It is significantly different from the first observation. It can be explained that at longer heating times the percentage of others FAs is decreasing while the percentage of FA C18:2n6t is not. This result suggests that the increase of generated trans isomers of C18:2, since the *cis* isomer C18:1 is little or non-reactive as it has been reported by Medina-Juárez et al., (2000) [12]. During the heating process, other

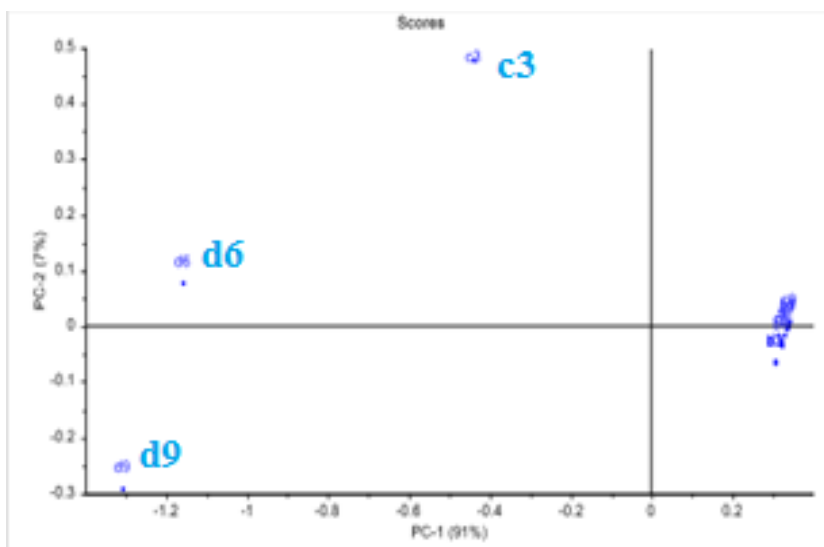


Fig. 5 Scores plot of PC1 versus PC2 at time of frying for 75 min.

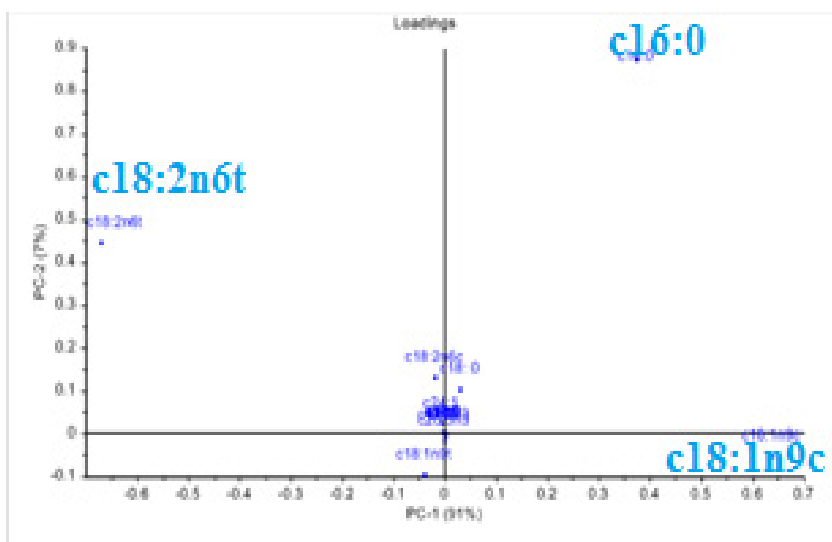


Fig. 6 Loadings plot of PC1 versus PC2 at time of frying for 75 min.

important components of the oils, such as tocopherols, tocotrienols, sterols and fatty esters, are also partially eliminated. In addition, the double bonds of FAs may isomerize from *cis* to *trans* [13].

#### 4. Conclusions

The multivariate data of FAs from 36 samples of fried oil containing lard at different percentages, times of frying and temperatures were analysed using PCA. A total of 17 FAs were studied and PCA has shown that three significant FAs; *trans* linoleic acid (C18:2n6t), *cis* oleic acid (C18:1n9c) and palmitic acid (C16:0) can be observed in all loading plots. However the other FAs are centered in the middle and located very close to each other when heating time is increased. It is observed that samples spiked with 10 % of lard contribute significantly from other samples in Scores plot.

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