

## Principal Component Analysis of Fatty Acid Data to Detect Virgin Coconut Oil Adulteration by Palm Olein

J.M.N. Marikkar<sup>1\*</sup> and N.A.M. Yanty<sup>2</sup>

### Abstract

Authentication of virgin coconut oil (VCO) is important to safeguard customers from adulteration practices. A study was carried out to distinguish VCO from VCO adulterated with palm olein (PO) using principal component analysis (PCA) of fatty acid (FA) compositional data. Six samples of VCO, and six samples of palm olein were obtained from oil producers' companies in Malaysia. Six samples of adulterated VCO were prepared by mixing with palm olein in 5% increment of adulteration. Fatty acid compositions of all oil samples were determined individually and the data were analyzed statistically. PCA analysis showed that lauric, palmitic and oleic acids were the most influencing parameters to discriminate VCO from adulterated VCO. Out of the thirteen FA variables investigated, ten were found to display high correlation with increasing adulteration. Predictive models showing higher coefficient of determination ( $R^2$ ) and good confidence limits were useful for quantification purposes.

**Key words:** Adulteration, Fatty acid data, Chemometrics, PCA, Virgin coconut oil

### Introduction

Virgin coconut oil (VCO) is a premium product that commands higher prices in the edible oil market. In coconut growing countries, VCO is produced hygienically using either a wet process or cold press extraction method to preserve its natural quality from high-heat treatment (Bawalan and Chapman, 2006). Being an edible oil, VCO finds several uses in food, pharmaceutical and cosmeceutical industries (Marina *et al.*, 2009a). VCO is also known to be a potential base oil for aroma therapies in alternative medicine. Owing to its high market value and short supply that exceeds demand, VCO has been vulnerable to adulteration with less expensive oils such palm olein. Palm olein, which is the liquid fraction of palm oil is relatively cheap to purchase due to low cost of production. This type of economic fraud will definitely alter the chemical composition of VCO affecting its nutritional and therapeutic values. According to several previous reports, the natural goodness and healing power of VCO are mainly due to its unique chemical composition dominated by medium chain triacylglycerols (Marina *et al.*, 2009b; Marikkar and Madurapperuma, 2012).

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Detection of adulteration in VCO has been the interest of several researchers in the past. They have employed instrumental techniques such as gas chromatography (GC) (Xu *et al.*, 2011), high performance liquid chromatography (HPLC), differential scanning calorimetry (DSC) (Marina *et al.*, 2009c), and Fourier transform infrared spectroscopy (FTIR) (Manaf *et al.*, 2007) for this purpose. VCO authentication by GC analysis would require a comparison of the fatty acid profile of an authentic VCO sample with that of the test sample. Deviations occurring in fatty acid profiles of fraudulent samples would become obvious at higher levels of adulterations. However, it is crucial to determine fatty acids showing high-sensitivity to adulteration at lower levels. The same goes true when considering triacylglycerols profile as a tool for detection of adulterations in VCO. However, there is a paucity of research studies that has been done to determine fatty acid parameters that reveal the presence of palm olein (PO) as adulterant in VCO. Hence, the objective of this study is to investigate the PCA application into fatty acid data to determine VCO adulterated with PO. In addition, effort was also undertaken to determine differences among component fatty acids of VCO that shows correlation with different levels of adulteration.

### Materials and Methods

**Materials:** Six samples of VCO used in this study were obtained from reliable suppliers located in Selangor, Malaysia. Six samples of PO were purchased from Lam Soon Edible Oils Sdn. Bhd, Selangor, Malaysia. All chemicals used in this study were of analytical grade unless otherwise specified (Sigma-Aldrich). For quantitative analysis, different mixture samples (w/w %) of virgin coconut oils with adulterant oil were prepared.

**Blend preparation:** VCO and adulterant (PO) were mixed together in differing ratios to form a set of samples. A total of six samples were prepared: (A<sub>1</sub>) 95:5, (A<sub>2</sub>) 90:10, (A<sub>3</sub>) 85:15, (A<sub>4</sub>) 80:20, (A<sub>5</sub>) 75:25, (A<sub>6</sub>) 70:30 (w/w), and identified by the mass ratio of VCO to PO.

**Fatty acid methyl esters (FAME) analysis:** Samples for the detection of fatty acid methyl

esters (FAME) were prepared by dissolving aliquots of oil (50 mg) with petroleum ether (0.8 ml) and sodium methoxide (1M, 0.2 ml) (PORIM Test Method, 1995) and analyzed (in triplicate) on an Agilent 4890D gas chromatograph (Agilent Technologies, Singapore) equipped with a Flame Ionization Detector (GC-FID). A non-polar capillary column HP-5 MS (0.25mm internal diameter, 30m length and 0.25µm film thickness, Hewlett Packard Company, Singapore) was used at a column pressure of 10 bars. The initial temperature of the column was at 100°C and was programmed to increase to 220°C at 4 °C/ min and then remain at 220°C for 15min. The temperatures of the injector and detector were maintained at 250°C and 275 °C, respectively (Marikkar *et al.*, 2013). The identification of the FA of the samples was done with reference to a chromatographic profile containing FAME standards. The percentage of individual fatty acid was calculated using heptadecanoic acid as the internal standard.

**Statistical analysis:** Data were statistically analyzed by one way analysis of variance (ANOVA) using the MINITAB (version 14; Minitab Inc., PA, USA) statistical package. Statistical significance was declared at 0.05 probability level. Principal Component Analysis (PCA) was carried out using Unscrambler 9.7 (Camo, USA) software for grouping and classification models.

### Results and Discussion

Edible oils and fats comprises FA which are esterified into glycerol. In the food industry, FA compositions of oils and fats are used as an indicator of nutritional status as well as purity. Since VCO has a unique FA composition, the analysis of FAME might help provide information regarding deviations resulting from adulteration practices. Data presented in Table 1 compares the fatty acid composition of authentic VCO with those of samples adulterated with different levels of PO. VCO samples consisted of 88.2 – 91.6 % saturated fatty acids (SFA) and 11.8 – 8.4 % unsaturated fatty acid (USFA). The major fatty acids of the pure samples were lauric (46.9 – 49.3 %) and myristic (19.1 – 20.5 %)



acids and their relative proportions were often found to exceed those of the same found in PO. According to Table 1, the total of these two FA found in PO was 1.0 – 2.5 %, which was extremely low when compared to those of VCO. VCO having greater content of shorter-chain fatty acids such as caprylic (C8:0) and capric (C10:0) is another interesting property. The proportions of lauric acid of VCO samples used in this study were comparably similar to findings reported previously by Marina *et al.* (2009b) who stated that lauric acid is the most dominant fatty acid found in VCO and ranged from 46 – 48%. In the adulterated samples, the total content of MCFA decreased in response to the increasing proportion of PO in the blends. According to data presented in Table 1, lauric acid (C12:0) is the most affected by adulteration as its percentage fell considerably. Since PO contained higher percentages of monounsaturated fatty acid (MUFA) mainly oleic acid (C18:1), an increasing pattern of MUFA was observed as the percentage of adulteration increased. In addition, the introduction of PO into VCO caused an increase in the amount of polyunsaturated fatty acids (PUFA) consisting of linoleic (C18:2) and linolenic (C18:3) acids and long-chain fatty acids consisting of arachidic (C20:0), behenic (C22:0) and lignoceric (C24:0) acids. These observable changes in fatty acid data are preliminary indicators to suspect possible adulteration in VCO. However, more concrete evidence of suspected adulteration in VCO may be obtained through application of chemometrics classification techniques such as principal component analysis.

### Principal component analysis

As shown in Table 1, GC analysis of FAME showed that samples were found to possess caprylic, capric, lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic, behenic and lignoceric as the constituent fatty acids. This study assumes that these thirteen fatty acids could be used as independent variables in PCA to distinguish VCO from samples with adulteration. Previously, fatty acid data has been used as variables while applying PCA to authentication

of commercial edible oils (Brodnjak-Voncina *et al.*, 2005) and oils extracted from different peanut cultivars (Shin *et al.*, 2011). PCA has the capability to identify patterns in data, and express the data in such a way as to emphasize their similarities and differences in the form of score plot. The score plot shown in Figure 1 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 Figure 1, a clear separation between VCO and PO samples were seen along the PC1 component. While samples of PO are located in the negative side of PC 1, samples of VCO and those adulterated with PO spotted in the positive side of PC 1. When coming to VCO samples and adulterated VCO samples, all adulterated VCO samples (A<sub>1</sub> to A<sub>6</sub>) were grouped together in upper-right quadrant while all VCO samples except C1 were grouped together in lower-right quadrant. Hence, PCA of the fatty acid compositional data in this case helped discriminate adulterated VCO samples from authentic VCO. Interestingly, all VCO adulterated with PO in the range of 5 to 30% were clustered into one block.

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.* (2003), a variable which exited farther from the origin of axis contributed to the most variation in the statistical model generated by the PCA. This is in agreement with reports published by other researchers (Shin *et al.*, 2011; Brodnjak-Voncina *et al.*, 2005). According to the loading plot in Figure 2, out of the thirteen fatty acid variables lauric (C12:0), palmitic (C16:0) and oleic acids (C18:1) were most discriminating variables that influence group separation into three different clusters.

### Correlation analysis and regression models

The data presented Table 2 compared the linearity between individual component fatty acid and the different percentage level of the



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Correlation analysis and regression models

The data presented Table 2 compared the linearity between individual component fatty acid and the different percentage level of the

Table 1: Fatty acid composition of virgin coconut oil, palm olein and adulterated samples

Samples	Variable												
	C8:0	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C22:0	C24:0
C1	6.1±0.2	5.6±0.0	46.9±0.2	19.1±0.3	10.0±0.1	0	3.9±0.2	7.0±0.1	1.2±0.0	0	0	0	0
C2	5.7±0.1	5.5±0.0	48.0±0.1	20.2±0.4	9.0±0.3	0	0.1±0.0	8.5±0.2	3.0±0.0	0	0	0	0
C3	5.1±0.0	5.3±0.0	48.0±0.4	20.5±0.6	9.2±0.2	0	0.1±0.0	8.8±0.6	2.9±0.0	0	0	0	0
C4	5.0±0.0	4.5±0.0	48.5±0.1	21.1±0.5	9.1±0.4	0	2.8±0.0	6.6±0.2	2.6±0.0	0	0	0	0
C5	5.1±0.1	5.5±0.0	49.3±0.3	20.5±0.4	9.1±0.2	0	1.0±0.0	6.8±0.1	2.7±0.0	0	0	0	0
C6	4.8±0.0	5.4±0.0	48.6±0.5	20.3±0.3	9.2±0.1	0	0.4±0.0	8.2±0.3	3.0±0.0	0	0	0	0
P1	0	0	0.1±0.0	0.9±0.0	37.9±0.7	0.1±0.0	4.0±0.0	40.7±0.7	10.4±0.2	0.1±0.0	0.2±0.0	0	0
P2	0	0	1.1±0.0	1.4±0.0	41.7±0.5	0.4±0.0	4.8±0.0	43.9±0.5	13.4±0.1	0.6±0.1	0.5±0.0	0	0
P3	0	0	0.2±0.0	0.8±0.0	36.3±0.6	0.2±0.0	3.7±0.0	47.7±0.6	10.4±0.1	0.3±0.0	0.1±0.0	0	0
P4	0	0	0.7±0.1	1.0±0.0	38.1±0.8	0.4±0.0	3.4±0.0	43.8±0.6	12.0±0.2	0.4±0.0	0.2±0.0	0	0
P5	0	0	0.3±0.0	1.1±0.0	37.0±0.2	0.2±0.0	4.0±0.0	44.6±0.4	11.8±0.4	0.6±0.0	0.5±0.0	0	0
P6	0	0	0.3±0.0	1.0±0.0	35.4±0.6	0.3±0.0	3.8±0.0	45.1±0.3	13.4±0.3	0.3±0.0	0.3±0.0	0	0
A1	5.6±0.0	5.2±0.0	43.6±0.2	18.0±0.2	11.8±0.2	0	3.9±0.0	9.5±0.0	1.9±0.1	0	0.1±0.0	0.2±0.0	0.2±0.0
A2	5.2±0.0	4.8±0.0	40.9±0.0	17.2±0.0	13.5±0.3	0	4.0±0.1	11.5±0.1	2.4±0.1	0	0.1±0.0	0.2±0.0	0.2±0.0
A3	5.5±0.0	4.8±0.4	39.0±0.2	16.1±0.0	14.5±0.1	0	3.9±0.0	13.0±0.2	2.8±0.2	0	0.2±0.0	0.1±0.0	0.2±0.0
A4	5.0±0.0	4.5±0.3	36.5±0.4	15.1±0.0	16.1±0.0	0.1±0.0	3.9±0.3	15.2±0.4	3.4±0.1	0.1±0.0	0.2±0.0	0.1±0.0	0.0
A5	4.5±0.0	4.1±0.2	33.6±0.2	14.2±0.0	17.6±0.1	0.1±0.0	4.0±0.2	17.3±0.3	4.0±0.0	0.1±0.0	0.2±0.0	0.2±0.0	0.3±0.0
A6	4.1±0.0	3.7±0.1	30.6±0.1	12.9±0.0	19.3±0.2	0.1±0.0	4.0±0.1	19.9±0.5	4.7±0.3	0.1±0.0	0.2±0.0	0.2±0.0	0.2±0.0

Each value in the table represents the mean ± standard deviation of triplicate analyses.

Abbreviations: VCO (C1 to C6), virgin coconut oil; PO (P1 to P6), palm olein; C<sub>8:0</sub>, caprylic; C<sub>10:0</sub>, caproic; C<sub>12:0</sub>, lauric; C<sub>14:0</sub>, myristic; C<sub>16:0</sub>, palmitic; C<sub>16:1</sub>, palmitoleic; C<sub>18:0</sub>, stearic; C<sub>18:1</sub>, oleic; C<sub>18:2</sub>, linoleic; C<sub>18:3</sub>, linolenic; C<sub>20:0</sub>, arachidic; C<sub>22:0</sub>, behenic; C<sub>24:0</sub>, lignoceric.



**Table 2: Pearson correlation coefficient between individual fatty acid and % level of adulterant**

Fatty Acid	Correlation Coefficient
Caprylic (C <sub>8:0</sub> )	-0.960 (p<0.001)
Caproic (C <sub>10:0</sub> )	-0.989 (p<0.0001)
Lauric (C <sub>12:0</sub> )	-0.998 (p<0.0001)
Myristic (C <sub>14:0</sub> )	-0.999 (p<0.0001)
Palmitic (C <sub>16:0</sub> )	+0.998 (p<0.0001)
Palmitoleic (C <sub>16:1</sub> )	+0.900 (p<0.006)
Stearic (C <sub>18:0</sub> )	+0.673 (p<0.098)
Oleic (C <sub>18:1</sub> )	+0.998 (p<0.0001)
Linoleic (C <sub>18:2</sub> )	+0.998 (p<0.0001)
Linolenic (C <sub>18:3</sub> )	+0.903 (p<0.005)
Arachidic (C <sub>20:0</sub> )	+0.996 (p<0.0001)
Behenic (C <sub>22:0</sub> )	-0.710 (p<0.074)
Lignoceric (C <sub>24:0</sub> )	-0.612 (p<0.144)

**Table 3: Stepwise regression analysis of individual fatty acid parameter versus % level of adulterant**

Model	Regression equation	R <sup>2</sup>	SE
1	Y = -6.06E-04C <sub>8:0</sub> + 6.05E-02	0.923 (p < 0.001)	0.00207753
2	Y = -6.037E-04C <sub>10:0</sub> + 5.57E-02	0.978 (p < 0.001)	0.00106962
3	Y = -5.23E-03C <sub>12:0</sub> + 0.47	0.996 (p < 0.001)	0.00382392
4	Y = -2.02E-03C <sub>14:0</sub> + 0.19	0.998 (p < 0.001)	0.00104585
5	Y = 3.02E-03C <sub>16:0</sub> + 0.10	0.996 (p < 0.001)	0.00224223
6	Y = 2.80E-05C <sub>16:1</sub> - 1.54E-04	0.809 (p < 0.006)	0.000160618
7	Y = 3.90E-05C <sub>18:0</sub> + 3.91E-02	0.453 (p < 0.098)	0.000414773
8	Y = 4.13E-03C <sub>18:1</sub> + 7.15E-02	0.996 (p < 0.001)	0.00309457
9	Y = 1.13E-03C <sub>18:2</sub> + 1.20E-02	0.996 (p < 0.001)	0.000840665
10	Y = 3.24E-05C <sub>18:3</sub> - 1.82E-04	0.815 (p < 0.005)	0.000182755
11	Y = 3.01E-05C <sub>20:0</sub> + 1.14E-03	0.991 (p < 0.001)	0.0000332835
12	Y = -5.40E-05C <sub>22:0</sub> - 1.60E-03	0.503 (p < 0.074)	0.000634074
13	Y = -3.70E-06C <sub>24:0</sub> + 8.00E-05	0.375 (p < 0.144)	0.0000564679

Abbreviation: Y, Percentage of adulterant; E, x10; C<sub>8:0</sub>, caprylic proportion; C<sub>10:0</sub>, caproic proportion; C<sub>12:0</sub>, lauric proportion; C<sub>14:0</sub>, myristic proportion; C<sub>16:0</sub>, palmitic proportion; C<sub>16:1</sub>, palmitoleic proportion; C<sub>18:0</sub>, stearic proportion; C<sub>18:1</sub>, oleic proportion; C<sub>18:2</sub>, linoleic proportion; C<sub>18:3</sub>, linolenic proportion; C<sub>20:0</sub>, arachidic proportion; C<sub>22:0</sub>, behenic proportion; C<sub>24:0</sub>, lignoceric proportion; R<sup>2</sup>, coefficient of determination; SE, standard error

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5	Y = 3.02E-03C <sub>16:0</sub> + 0.10	0.996 (p<0.001)	0.00224223
6	Y = 2.80E-05C <sub>16:1</sub> - 1.54E-04	0.809 (p<0.006)	0.000160618
7	Y = 3.90E-05C <sub>18:0</sub> + 3.91E-02	0.453 (p<0.098)	0.000414773
8	Y = 4.13E-03C <sub>18:1</sub> + 7.15E-02	0.996 (p<0.001)	0.00309457
9	Y = 1.13E-03C <sub>18:2</sub> + 1.20E-02	0.996 (p<0.001)	0.000840665
10	Y = 3.24E-05C <sub>18:3</sub> - 1.82E-04	0.815 (p<0.005)	0.000182755
11	Y = 3.01E-05C <sub>20:0</sub> + 1.14E-03	0.991 (p<0.001)	0.0000332835
12	Y = -5.40E-05C <sub>22:0</sub> - 1.60E-03	0.503 (p<0.074)	0.000634074
13	Y = -3.70E-06C <sub>24:0</sub> + 8.00E-05	0.375 (p<0.144)	0.0000564679

Abbreviation: Y, Percentage of adulterant; E, x10; C<sub>8:0</sub>, caprylic proportion; C<sub>10:0</sub>, caproic proportion; C<sub>12:0</sub>, lauric proportion; C<sub>14:0</sub>, myristic proportion; C<sub>16:0</sub>, palmitic proportion; C<sub>16:1</sub>, palmitoleic proportion; C<sub>18:0</sub>, stearic proportion; C<sub>18:1</sub>, oleic proportion; C<sub>18:2</sub>, linoleic proportion; C<sub>18:3</sub>, linolenic proportion; C<sub>20:0</sub>, arachidic proportion; C<sub>22:0</sub>, behenic proportion; C<sub>24:0</sub>, lignoceric proportion; R<sup>2</sup>, coefficient of determination; SE, standard error

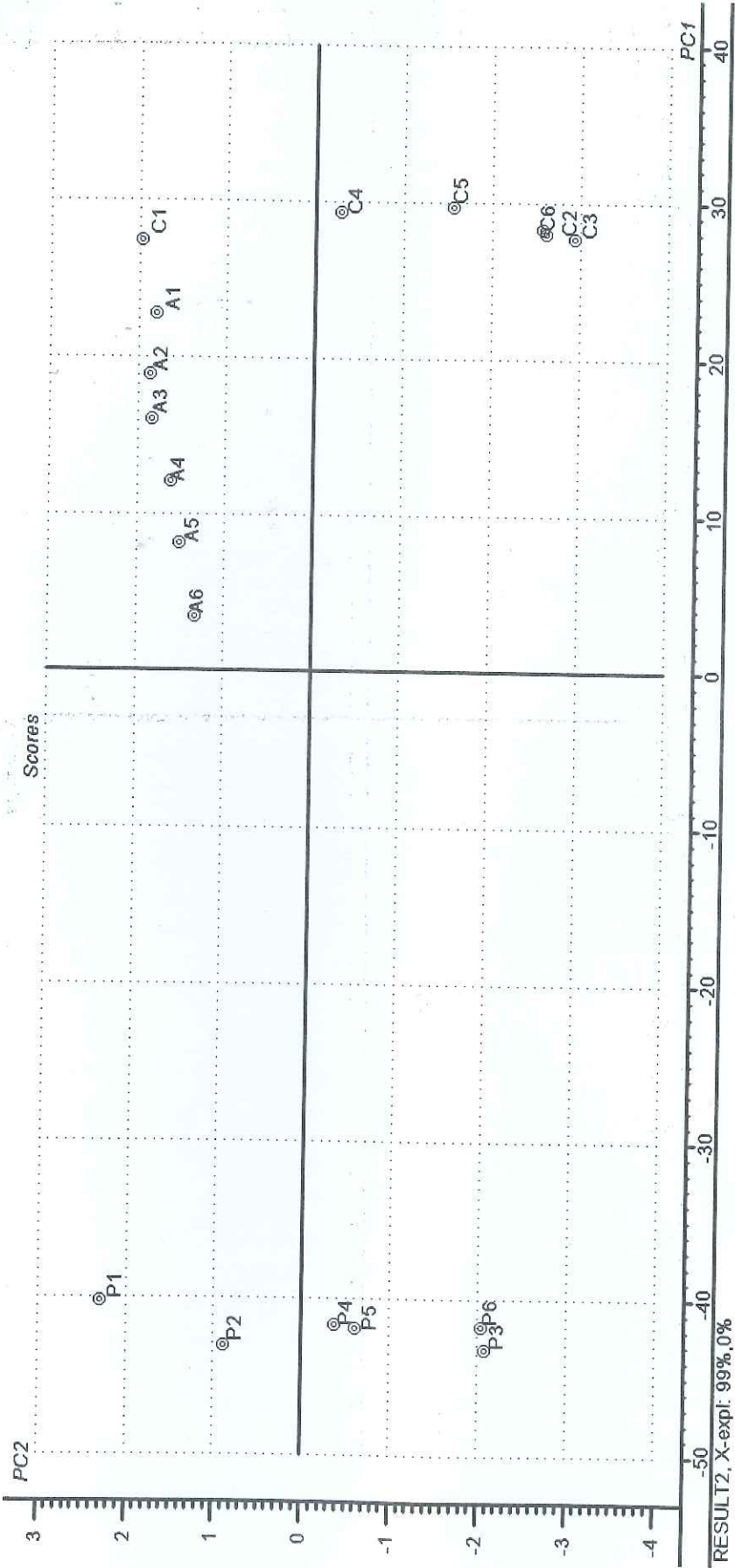


Figure 1. Score plot of principal component analysis applied to fatty acid composition data Abbreviations: A1 – A6, adulterated VCO samples; C1 – C6, pure VCO samples; P1 – P6, palm olein samples.



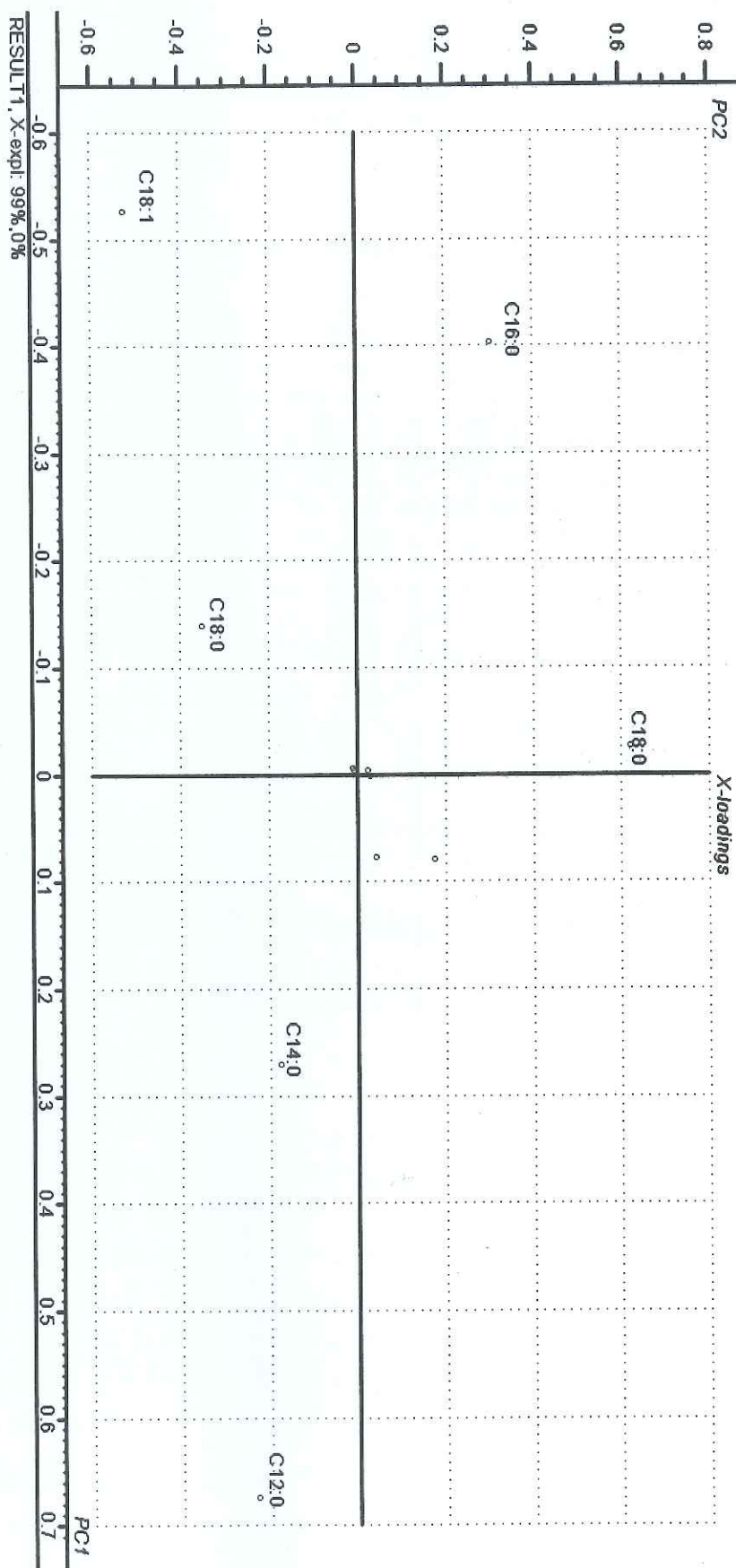


Figure 2. Loading plot applied to fatty acid composition data adulterant. Pearson's correlation coefficient (PCC) indicated that only ten out of thirteen FA.



parameters displayed strong correlations with the increasing level of adulteration (Table 2). FA namely stearic, behenic, and lignoceric showed weaker correlation ( $<0.71$ ) while the rest of the FA parameters showed strong correlations ( $>0.90$ ). The highest positive correlation was displayed by myristic acid ( $-0.999$ ;  $p < 0.0001$ ), followed by lauric ( $-0.998$ ;  $p < 0.0001$ ), palmitic ( $+0.998$ ;  $p < 0.0001$ ), oleic ( $+0.998$ ;  $p < 0.0001$ ) and linoleic acid ( $+0.998$ ;  $p < 0.0001$ ). Hence, these FA are useful as parameters in stepwise procedures to develop predictive models for quantification of adulterations. Data presented in Table 3 shows the outcome of the stepwise regression analysis. Altogether there were thirteen regression models based on individual fatty acid to predict the levels of adulteration. Predictive models showing higher coefficient of determination ( $R^2$ ) and good confidence limits were useful for quantification purposes. As such model number 7, 11, and 13 were not considered as suitable for quantification.

#### Conclusion

This study demonstrated the effectiveness of chemometrics approach to differently classify VCO adulterated by PO from VCO. Among the fatty acids, lauric, palmitic and oleic were most influential discriminating parameters in clustering VCO and adulterated VCO separately. This approach can classify even samples with adulteration level as low as 5%. Predictive models showing higher coefficient of determination ( $R^2$ ) and good confidence limits were useful for quantification purposes. As such model number 7, 11, and 13 were not considered as suitable for quantification.

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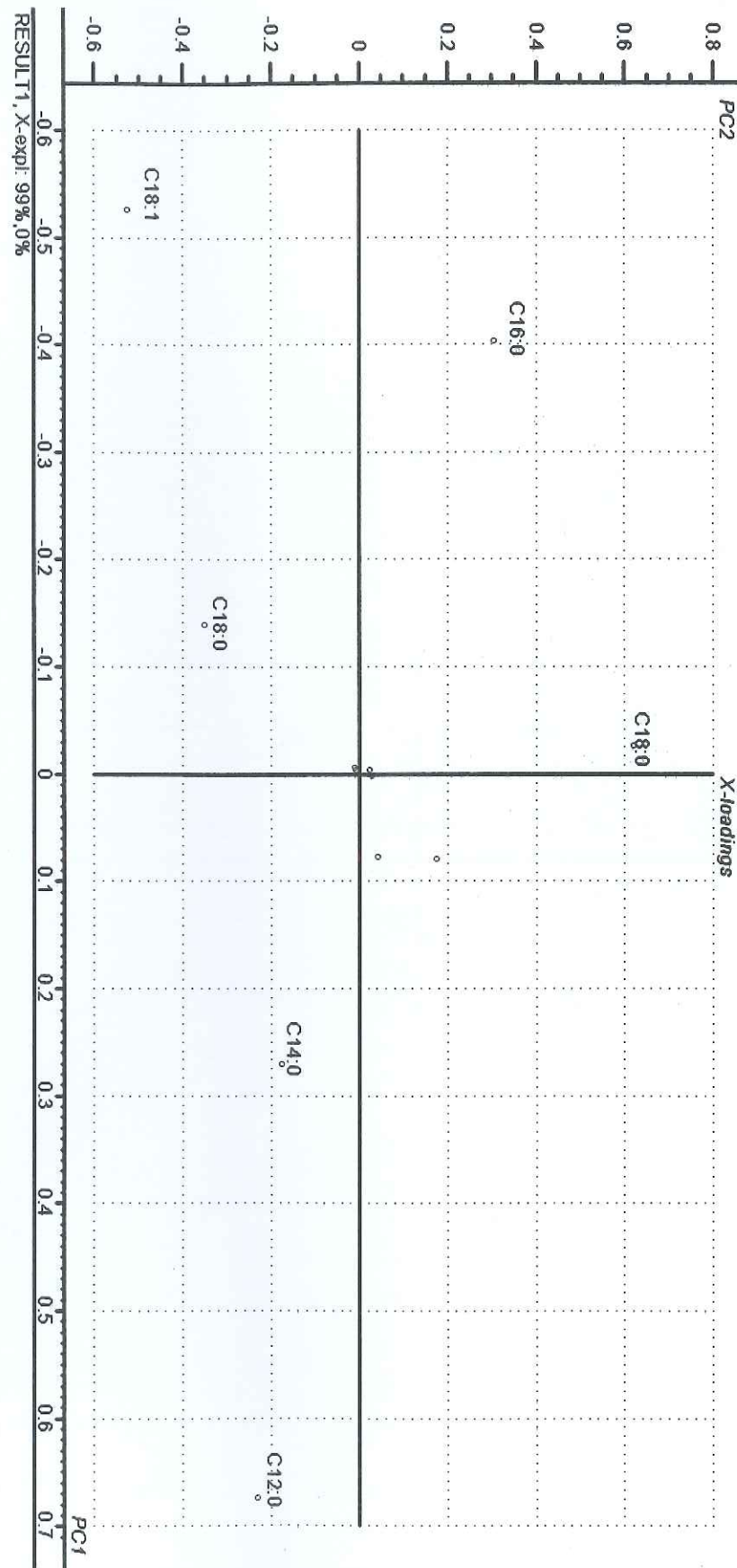


Figure 2. Loading plot applied to fatty acid composition data adulterant. Pearson's correlation coefficient (PCC) indicated that only ten out of thirteen FA.



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