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Use of gas liquid chromatography in combination with pancreatic lipolysis and multivariate data analysis techniques for identification of lard contamination in some vegetable oils

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Abstract

A study was conducted to investigate the use of gas liquid chromatography (GLC) to identify lard (LD) contamination in palm oil (PO), palm kernel oil (PKO), and canola oil (CLO). Vegetable oils were deliberately adulterated with animal fats such as LD, beef tallow (BT), and chicken fat (CF) in varying proportions. In order to monitor the fatty acid (FA) compositional changes due to adulteration, GLC analyses of fatty acid methyl esters (FAME) were performed on 2-monoacylglycerol (2-MG) and neutral triacylglycerol (TAG) isolated from each sample. For the evaluation of FA data, multivariate statistical techniques were employed. The results showed that canonical discriminant (CANDISC) analysis was the most effective technique for discriminating LD-adulterated samples from those adulterated with other animal fats. Additionally, mathematical equations obtained by simple regression analysis could be used for quantification of LD contents in admixtures.

Keywords: Adulteration; Animal fat; Gas liquid chromatography; Lard; Vegetable oils; Multivariate data analysis

1. Introduction

Authentication of food materials and detection of adulteration are important issues from a nutritional point of view. A sample is considered as adulterated when the determined value for a certain parameter deviates significantly from the range reported for the genuine product. Ways of authentication of food materials may vary widely, depending on the nature of the substance. They can be physical and chemical or various biochemical methods. In oils and fats, the procedures that are available usually depend on the identification and determination of certain characteristic constituents. Of these, FAME analysis by gas liquid chromatography (GLC) is an important method for authentication purposes. To date, it has provided satisfactory means of detection for a number of adulteration cases. However, this practice yielded positive results, mostly when the two oils differed widely in their fatty acid (FA) composition, whereas the detection became more difficult when the contaminant had a composition approaching that of the original oil (Rossell, King, & Downes, 1983).

Because of the usefulness of the GLC technique for determining adulteration, the Codex Committee on Fats and Oils compiled FA distributional ranges for various edible oils and fats. Subsequently, this became the international basis for establishing authenticity of oils and fats (Rossell, 1998). Even though the Codex Specification could be helpful in checking adulterations of suspected samples of oils/fats, it may not be able to trace the source of the adulterant. For the purpose of certain food regulations, determining the nature of adulterant is highly important. For instance, detection of lard (LD) as an adulterant in food systems is a major concern for

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many countries due to religious prohibitions and health reasons. But LD detection may become more difficult when it is present as a minor component in other oils and fats. Therefore, methods dealing with the overall FA composition may sometimes not be useful for detecting LD. This necessitated the need to look into the FA distribution pattern within the triacylglycerol (TAG) molecules. Hence, determination of positional distribution of fatty acids was considered as an alternative option for this purpose.

In previous reports, application of lipases in structural studies of natural TAG molecules has been discussed (Dourtoglou, Stefanou, Lalas, Dourtoglou, & Poulos, 2001). Pancreatic lipase hydrolysis is a useful technique in positional analysis of FA distribution, particularly at the *sn*-2 position of TAG molecules. Application of this technique has shown that in most fats and oils, the middle (*sn*-2) position is preferentially occupied by unsaturated fatty acids and the only exception is LD, in which the *sn*-2 position is predominantly occupied by saturated fatty acids, particularly palmitic acid (Christie, 1986). Therefore, this unique feature of LD was used to determine LD adulteration in food systems (Norris, 1982). Saeed, Ali, Rahman, and Sawaya (1989) used this technique to detect adulteration of beef, mutton and chicken products with pork. Similarly, Soliman and Younes (1986) have demonstrated the usefulness of the technique for determining adulteration of butterfat with either beef tallow (BT) or cottonseed oil.

In this study, the pancreatic lipolysis technique was adopted to monitor the compositional variations in *sn*-2 positional fatty acids in some vegetable oils after adulteration with animal depot fats such as GLD, BT, and CF. Unlike previous reports (Saeed et al., 1989; Soliman & Younes, 1986), this study attempted to use a multivariate data analysis approach to evaluate results and find a way to discriminate LD adulterations from other animal fat (AF) adulterations.

2. Materials and methods

2.1. Materials

Three different edible oils of plant origin were used in this study. Palm oil (PO) (slip melting point: 30.5 °C; iodine value: 54.0) and palm kernel oil (PKO) (slip melting point: 28.0 °C; iodine value: 19.8) were purchased from a local refinery. Canola oil (iodine value: 113) was separately purchased from a local supermarket. The oils were stored at 4 °C. Prior to use, the oils were melted at 60 °C in an oven. Lipase from hog pancreas was obtained from Fluka Chemie (Buchs, Switzerland). Animal body fats were obtained using the adipose tissues of animals collected from local slaughterhouses. All chemicals used in this experiment were of analytical or HPLC grade.

2.2. Standards

FAME used for peak identification were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). Standards include the following FAME: caprylic (8:0), capric (10:0), lauric (12:0), myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0), and gadoleic (20:1).

2.3. Blend preparations

PO, PKO and canola oil (CLO) were spiked with lard (GLD), BT and chicken fat (CF) in varying proportions, ranging from 2 to 20%. Altogether, fifteen blends were prepared for each oil: 98:2, 95:5, 90:10, 85:15, 80:20, (w/w), identified by the mass ratio of vegetable oil (VO) to AF (VO:AF). Only in the case of PO, was one additional series of blends prepared by spiking MT with the proportions shown above.

2.4. Isolation of neutral TAG and preparation of 2-MAG

Isolation of neutral TAG and preparation of 2-MAG of the oil samples were carried out according to procedures described in our previous report (Marikkar, Lai, Ghazali, & Che Man, 2002).

2.5. Fatty acid compositional analysis of neutral TAG and 2-MAG by GLC

This was performed according to the method described in the previous report (Marikkar et al., 2002).

2.6. Statistical analysis

Data were statistically analyzed by analysis of variance (ANOVA) with the SAS (Version 6.0) software package (SAS, 1989). Pearson correlation was applied to evaluate the relationships among variables. Canonical discriminant (CANDISC) analysis was used for distinguishing LD-adulterated samples from those adulterated with other animal fats. Variable selection for CANDISC analysis was based on the multiple comparison test via least significant difference (LSD) on treatments, and by the use of order of means of variables and step-wise procedure (Hair, Anderson, Tatham, & Black, 1998).

3. Results and discussion

3.1. Fat composition and statistical evalution

In this study, application of pancreatic lipolysis on animal fats and vegetable oils allowed the determination of FA composition at the *sn*-2 position [Tables 1A and 1B]. By making use of the FA data from 2-monoacyl-

Table 1A
Fatty acid composition (%) of lard and other animal fat samples in neutral triacylglycerol (TAG) and 2-monoacylglycerol (2-MG)[a]

Fat sample		Fatty acid (methyl esters) (%)						
		14:0	16:0	18:0	18:1	18:2	Others	PAEF
LD	TAG	1.3	24.0	9.2	42.5	18.2	4.8	284
	2-MG	3.6	68.2	3.3	16.1	4.4	4.4	
CF	TAG	1.3	24.0	4.1	41.3	17.9	11.4	50.0
	2-MG	1.9	12.0	7.2	51.5	17.9	9.5	
BT	TAG	3.8	27.9	28.3	28.2	2.1	9.7	57.4
	2-MG	8.4	16.0	14.9	40.3	2.8	17.5	
MT	TAG	3.3	25.3	22.8	34.9	2.5	11.2	56.9
	2-MG	7.2	14.4	12.8	48.2	3.0	14.5	

[a] Each value in the Table represents the mean of triplicate analysis. Abbreviations: PAEF, palmitic acid enrichment factor; LD, lard; CF, chicken fat; BT, beef tallow; MT, mutton tallow.

Table 1B
Fatty acid composition (%) of palm kernel oil, palm oil, canola oil samples in neutral triacylglycerol (TAG) and 2-monoacylglycerol (2-MG)[a]

Fat sample		Fatty acid (methyl esters) (%)							
		12:0	14:0	16:0	18:0	18:1	18:2	Others	PAEF
PKO	TAG	54.9	14.4	6.00	1.23	11.4	2.10	9.91	63.3
	2-MG	55.5	15.7	3.80	0.47	19.5	2.50	2.50	
PO	TAG	0.21	1.02	46.8	3.88	37.9	9.42	0.77	36.3
	2-MG	0.31	0.63	17.0	1.00	62.1	18.5	0.55	
CLO	TAG	–	–	5.30	1.92	55.3	26.5	11.0	24.5
	2-MG	–	–	1.30	0.30	52.8	34.6	11.0	

[a] Each value in the Table represents the mean of triplicate analysis. Abbreviations: PKO, palm kernel oil; PO, palm oil; CLO, canola oil. For other abbreviations see Table 1A.

glycerol (2-MG) and neutral TAG, one can calculate palmitic acid enrichment factor (PAEF) [PAEF (%): the percent ratio of palmitic acid in 2-MAG to its overall percent in TAG] for different animal fats and vegetable oils [Tables 1A and 1B]. Due to the high concentration of palmitic acid at the *sn*-2 position, LD was found to have a very high PAEF value (284%) as compared to other animal fats [BT (57.4%); MT (56.9%); CF (50%)] and vegetable oils [PO (36.3%); PKO (63.3%); CLO (24.5%)]. As mentioned previously, in earlier studies (Saeed et al., 1989), PAEF was used as a parameter to deal with pork and LD adulteration in food systems. Therefore, a higher value recorded for PAEF was taken as an indication of LD contamination in food systems. However, according to the data presented in Tables 1A and 1B, it is obvious that adulteration of other animal fats, such as CF, BT, and MT could also cause an increase in PAEF values of PKO, CLO and other highly unsaturated oils. As such, a broader approach was necessary, where the whole FA profile could be taken into consideration, rather than relying on a single FA as the criterion. Therefore, multivariate data analysis techniques were adopted to evaluate the major and minor changes in FA profile of PO, PKO, and CLO due to different AF adulterations.

Multivariate data analysis generally refers to those statistical methods, which analyze multiple measurements on each sample under investigation. Therefore, it helps to extract more subtle information that may not be available from a cursory examination of data. Out of the different methods examined in the multivariate context, CANDISC analysis was found to show promising results.

CANDISC is a powerful technique, which allows multiple variables to be evaluated by creating mathematical models utilizing all variables for each observation. Unique linear combinations could be created which can be used to define model characteristics for each type of adulteration. Further manipulation of these values creates canonical variables which are ranked so that the first canonical variable represents the greatest variance of the sample from the assigned model, the second canonical variable the next greatest variance, and so on. Consequently, samples that are very similar in their characteristics will appear to be tightly grouped in the canonical plots while those having dissimilar characteristics will appear far apart (Dyszel & Baish, 1992).

3.2. Compositional changes of sn-2 position of oils after adulteration with AF

PO is distinguished from other plant oils by having a high level of palmitic acid (Rossell, King, & Downes, 1985). However, oleic acid is the predominant FA at the

Table 2
Changes in fatty acid composition of *sn*-2 position of palm oil after adulteration with different concentrations (%) of animal fats[a]

Sample	Treatment	Adulteration level (%)	Fatty acid (%)								
			12:0	14:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3
1	BT	2	0.31	0.80	17.0	0.55	–	1.03	61.3	18.1	1.03
2	BT	5	0.30	0.90	16.9	0.90	0.11	1.30	60.6	17.6	1.40
3	BT	10	0.40	1.50	16.9	1.01	0.20	2.03	59.2	16.0	2.70
4	BT	15	0.51	2.10	16.9	1.45	0.15	2.45	58.5	15.4	2.61
5	BT	20	0.60	2.80	16.8	2.03	0.20	2.60	57.7	14.6	2.51
6	CF	2	0.30	0.55	17.0	0.36	–	1.11	61.9	18.3	0.45
7	CF	5	0.30	0.60	16.8	0.40	–	1.30	61.6	18.1	0.61
8	CF	10	0.31	0.60	16.6	0.70	–	1.51	61.3	18.2	0.81
9	CF	15	0.35	0.65	16.5	0.90	–	1.50	61.2	18.1	0.85
10	CF	20	0.40	0.70	16.3	1.10	–	1.60	61.0	18.0	0.90
11	LD	2	0.40	0.71	17.0	0.31	–	1.05	62.0	22.9	0.71
12	LD	5	0.30	0.70	19.6	0.51	–	1.00	60.9	15.8	1.20
13	LD	10	0.51	1.03	23.4	0.80	–	1.10	57.3	14.5	1.40
14	LD	15	0.51	1.50	27.5	0.91	0.11	1.10	54.8	12.4	1.21
15	LD	20	0.61	1.70	31.9	1.03	0.31	1.20	52.8	10.1	0.41

[a] Each value in the Table represents the mean of triplicate analysis. Abbreviations: see Table 1A.

sn-2 position of PO [Table 1B]. Linoleic and palmitic acids are other FA occurring in higher amounts at the *sn*-2 position. Table 2 shows the FA compositional changes taking place at the *sn*-2 position after adulteration with different concentration of AF. Since changes occurred in a series of FA, each component FA of the *sn*-2 position could be considered as a variable. Hence, a variable assignment, as follows, was needed: C12:0 (P_1), C14:0 (P_2), C16:0 (P_3), C16:1 (P_4), C17:0 (P_5), C18:0 (P_6), C18:1 (P_7), C18:2 (P_8), C18:3 (P_9).

PKO is classified as a lauric oil. It is an oil characterized by high contents of lauric, myristic and oleic acids in its *sn*-2 position [Table 1B]. Fatty acid compositional changes caused by different AF adulterants at the *sn*-2 position are shown in Table 3. According to Table 3, changes are taking place in ten different fatty acids and therefore, for the purpose of multivariate data analysis, each of the FA could be taken as a variable and denoted as: $C_{8:0}$ (Q_1), $C_{10:0}$ (Q_2), $C_{12:0}$ (Q_3), $C_{14:0}$ (Q_4), $C_{16:0}$ (Q_5), $C_{16:1}$ (Q_6), $C_{18:0}$ (Q_7), $C_{18:1}$ (Q_8), $C_{18:2}$ (Q_9), $C_{18:3}$ (Q_{10}).

CLO is classified as an oleic oil and it is characterized by its high content of oleic and linoleic acids in its *sn*-2 position [Table 1B]. The compositional variations of FA at the *sn*-2 position due to AF adulterations are presented in Table 4 and the data presented show that adulteration has brought about changes in nine FA. For the purpose of multivariate data analysis, these FA were considered as variables and denoted as: $C_{12:0}$(R_1), $C_{14:0}$ (R_2), $C_{16:0}$ (R_3), $C_{16:1}$ (R_4), $C_{17:0}$ (R_5), $C_{18:0}$ (R_6), $C_{18:1}$ (R_7), $C_{18:2}$ (R_8), $C_{18:3}$ (R_9).

3.3. CANDISC analysis to distinguish lard contamination in oils

3.3.1. Palm oil

ANOVA was performed by considering GLD, BT, and CF as the three treatments involved in this study. According to ANOVA, all the variables in PO except P_5 and P_8 showed significant differences with regard to treatments. Multiple comparison showed that the LD-adulterated series was significantly ($p < 0.05$) different from other AF adulterated series with respect to the P_3 variable only and the rest of the variables did not show any positive discriminating power to identify the LD adulterated series. However, use of the stepwise procedure and order of means of variables suggested that P_1, P_6, and P_7 could also be included, along with P_3, for the purpose of discrimination of LD. Therefore, P_1, P_3, P_6 and P_7 were the four variables finally selected to perform the CANDISC analysis. The outcome of the CANDISC, when plotted for the first two canonical variates, showed adequate discrimination for identification of the LD-adulterated series (Fig. 1).

3.3.2. Palm kernel oil

According to the results of ANOVA, Q_5, Q_8, Q_9, and Q_{10} were the variables that showed significant differences with regard to treatments. The multiple comparison test via LSD showed that treatment-LD was significantly ($p < 0.05$) different from treatment-BT and treatment-CF with respect to variables Q_5, Q_8, and Q_9. However, based on the order of means of variables and by the use of stepwise procedure, variables Q_1, Q_2, and Q_3 were also found to be useful for the purpose of discriminating admixtures of LD from those of other animal fats. Consequently, Q_1, Q_2, Q_3, Q_5, Q_8, and Q_9 were the final set of variables considered for performance of CANDISC analysis. By plotting the first and second canonical values associated with each sample, a two-dimensional representation of the grouping by characteristic types was obtained (Fig. 2). This clearly showed that adulterated samples belonging to each different AF type lie in a particular spatial region and hence, there is adequate discrimination of the LD-adulterated series from other AF adulterations.

Table 3
Changes in fatty acid composition of *sn*-2 position of palm kernal oil after adulteration with different concentrations (%) of animal fats[a]

Sample	Treatment	Adulteration level (%)	Fatty acid (%)									
			8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
1	BT	2	0.81	1.60	55.2	15.0	3.80	–	0.70	19.9	3.03	0.31
2	BT	5	0.60	1.40	52.5	14.1	4.30	0.31	1.20	21.3	3.90	0.40
3	BT	10	0.70	1.41	50.6	13.4	4.60	0.41	1.40	22.7	4.21	0.61
4	BT	15	0.61	1.31	49.9	13.0	4.80	0.40	1.50	23.4	4.30	0.70
5	BT	20	0.60	1.30	48.2	12.7	5.20	0.40	1.70	24.5	4.61	0.80
6	CF	2	0.83	1.60	55.1	15.1	3.90	–	0.51	20.0	3.02	0.15
7	CF	5	0.81	1.51	53.5	14.5	4.30	0.11	0.60	20.7	3.70	0.21
8	CF	10	0.61	1.41	50.3	14.0	5.00	0.31	0.71	23.1	4.41	0.20
9	CF	15	0.51	1.30	48.9	13.5	5.50	0.40	0.71	23.6	5.11	0.40
10	CF	20	0.51	1.22	47.4	13.0	6.00	0.71	0.72	24.0	5.90	0.50
11	LD	2	0.70	1.50	54.1	15.3	5.60	0.21	0.51	19.0	2.70	0.40
12	LD	5	0.70	1.60	51.8	14.0	7.50	0.10	0.60	19.0	3.80	0.61
13	LD	10	0.90	2.03	50.8	13.3	9.00	0.31	0.60	18.9	3.40	0.51
14	LD	15	0.60	1.51	49.8	13.7	11.20	0.31	0.70	18.7	3.21	0.30
15	LD	20	0.50	1.30	46.6	13.5	14.00	0.40	0.80	18.5	4.00	0.30

[a] Each value in the Table represents the mean of triplicate analysis. Abbreviations: see Table 1A.

Table 4
Changes in fatty acid composition of *sn*-2 position of canola oil after adulteration with different concentration (%) of animal fats[a]

Sample	Treatment	Adulteration level (%)	Fatty acid (%)								
			12:0	14:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3
1	BT	2	–	–	1.40	0.40	–	0.50	52.7	34.4	10.5
2	BT	5	–	0.50	1.70	0.40	0.11	0.80	52.5	33.1	10.9
3	BT	10	–	0.90	2.90	0.51	0.10	1.70	51.5	32.0	10.3
4	BT	15	–	1.30	3.70	0.60	0.20	2.10	51.0	31.0	10.0
5	BT	20	–	1.60	4.50	0.70	0.21	2.90	50.5	29.9	9.70
6	CF	2	–	–	1.35	0.65	–	0.60	52.7	34.1	10.5
7	CF	5	–	0.20	1.80	1.03	0.11	0.90	52.6	33.2	10.1
8	CF	10	–	0.21	3.10	1.03	0.10	0.80	52.3	32.8	9.70
9	CF	15	–	0.30	3.60	1.05	0.10	0.90	52.2	32.3	9.50
10	CF	20	–	0.31	4.90	1.03	0.10	1.02	52.1	31.6	8.90
11	LD	2	–	0.21	3.60	0.20	–	0.20	52.3	33.7	9.70
12	LD	5	–	0.30	6.60	0.50	–	0.80	51.0	32.0	8.70
13	LD	10	–	0.50	9.80	0.70	0.11	0.60	48.6	31.0	8.50
14	LD	15	0.31	0.91	14.4	0.90	0.20	0.80	44.4	29.3	9.20
15	LD	20	0.30	1.31	18.1	1.03	0.21	0.80	41.3	28.6	8.30

[a] Each value in the Table represents the mean of triplicate analysis. Abbreviations: see Table 1A.

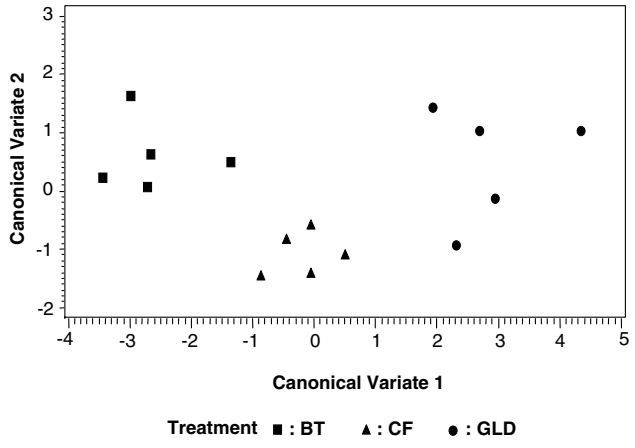

Fig. 1. Canonical discriminate (CANDISC) analysis plot of Canonical variate2 vs. Canonical variate1 values for PO samples adulterated with LD, BT, and CF. Abbreviations: PO, palm oil, LD, lard; BT, beef tallow; and CF, chicken fat.

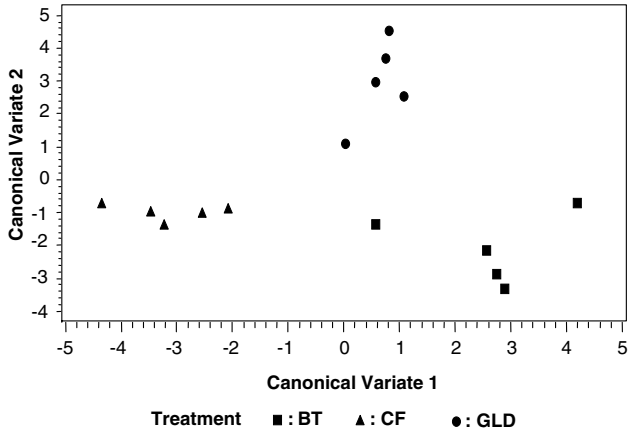

Fig. 2. CANDISC plot of Canonical variate2 vs. Canonical variate1 values for PKO samples adulterated with LD, BT, and CF. Abbreviations: PKO, palm kernel oil. For other abbreviations see Fig. 1.

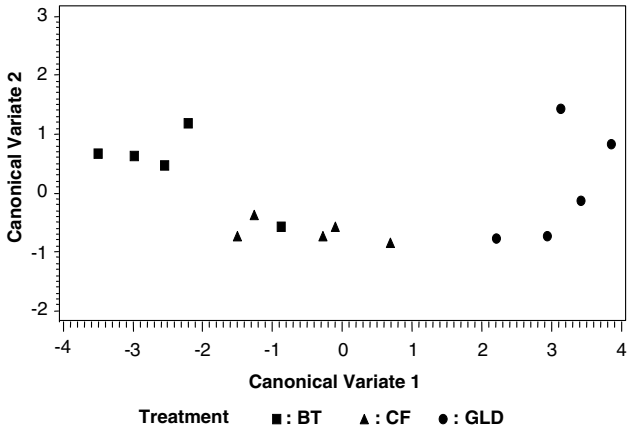

Fig. 3. CANDISC plot of Canonical variate2 vs. Canonical variate1 values for CLO samples adulterated with LD, BT, and CF. Abbreviations: CLO, canola oil. For other abbreviations see Fig. 1.

3.3.3. Canola oil

The ANOVA procedure showed that R_2, R_3, R_4, R_6, R_7, R_8, and R_9 were the only variables which showed significant differences with regard to treatments. Multiple comparison test via LSD showed that the LD-treated sample series was significantly ($p < 0.05$) different from the other AF treated series with respect to variables R_3, R_7, R_8, and R_9. Subsequent treatment of data by stepwise procedure and order of means of variables also confirmed that R_3, R_7, R_8, and R_9 were the most suitable variables to perform CANDISC analysis. The outcome of CANDISC analysis showed an adequate discrimination of the LD-adulterated series from those adulterated with other animal fats (Fig. 3).

3.4. Quantitative estimation of lard content in admixtures of vegetable oils

In addition to the qualitative detection, it is also possible to make use of the data in Tables 2–4 to estimate the LD content in the admixtures of vegetable oils by applying simple regression analysis to palmitic acid content. Palmitic acid was considered for the regression analysis because it tended to show a significant variation, even at low levels of adulteration. Thus, the correlation plots obtained for admixtures of PO, PKO, and CLO are shown in Figs. 4(a), 5(a) and 6(a), respectively. Although, the results show high coefficients of determination (R^2) for all three cases, it is pertinent to check

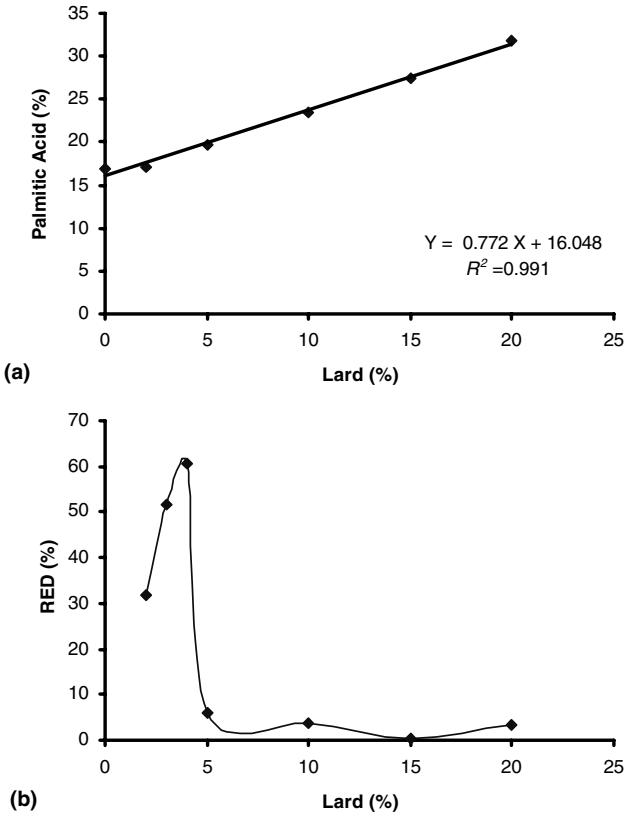

Fig. 4. (a) Correlation between palmitic acid content at *sn*-2 position and LD content in admixtures of PO, and (b) Relationship between relative error of GLC determination (RED) and LD content in admixtures of PO. Abbreviations: GLC, gas liquid chromatography, RED, relative error of determination. For other abbreviations see Fig. 1.

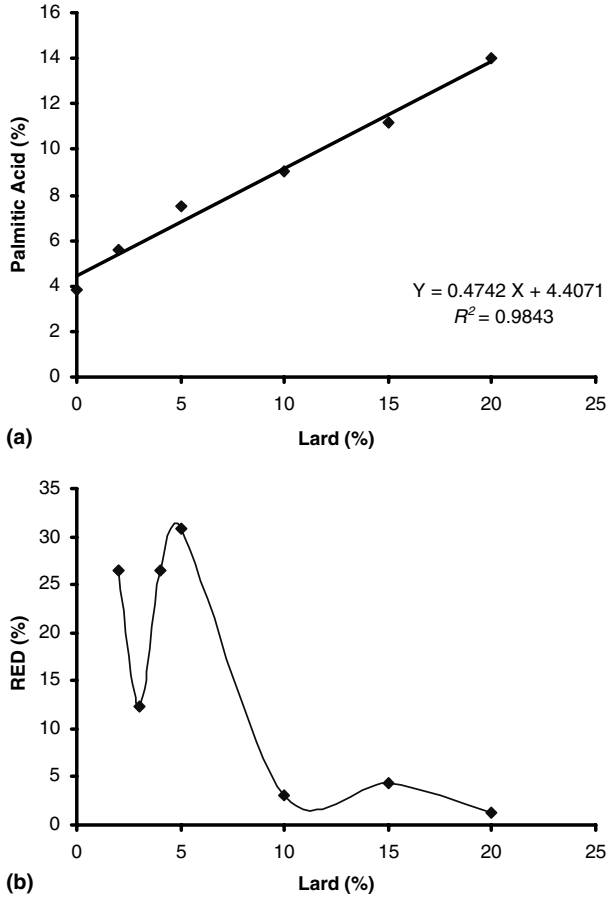

Fig. 5. (a) Correlation between palmitic acid content at *sn*-2 position and LD content in admixtures of PKO, and (b) Relation between RED and LD content in admixtures of PKO. Abbreviations: see Figs. 1 and 4.

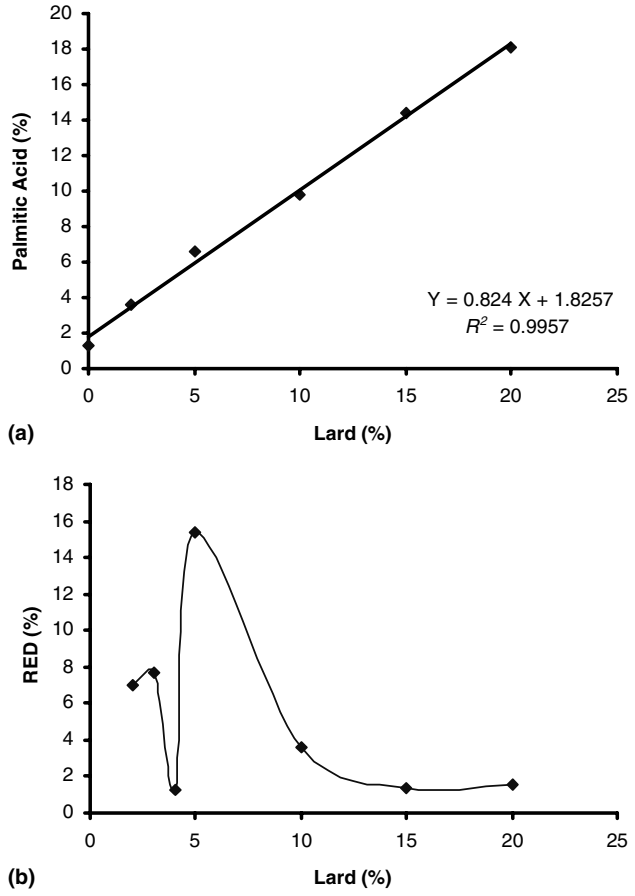

Fig. 6. (a) Correlation between palmitic acid content at *sn*-2 position and LD content in admixtures of CLO, and (b) Relation between RED and LD content in admixtures of CLO. Abbreviations: see Figs. 1 and 4.

the linearity of the calibration curve at lower levels of adulteration, particularly below 5%. Hence, admixture of oil samples containing 3% and 4% LD were taken as independent samples in respect of each oil in order to determine the accuracy of prediction of the calibration plots. The results show that, for PO and PKO, the relative errors of determination (RED) of these two independent samples were high [Figs. 4(b) and 5(b)]. However, in the case of CLO, these two samples showed lower RED values while the admixture containing 5% LD was found to have a higher RED value [Fig. 6(b)].

Table 5
Mathematical models for estimation of lard content in admixtures of palm oil, palm kernal oil, and canola oil[a]

Oil	Equation	R^2
PO	$LD_{Act} = 0.892LD_{Pre} + 1.496$	0.992
PKO	$LD_{Act} = 1.056LD_{Pre} - 0.889$	0.991
CLO	$LD_{Act} = 1.025LD_{Pre} - 0.298$	0.996

[a] Abbreviations: LD_{Act}, actual lard content; LD_{Pre}, predicted lard content; R^2, coefficient of determination. See Table 1B for other abbreviations.

Therefore, in order to predict closer values to the actual LD content (LD_{Act}) in fat admixtures, the predicted LD contents (LD_{Pre}) from calibration plots had to be adjusted by the use of simple regression. Hence, the adjusted models corresponding to PO, PKO, and CLO are presented in Table 5, along with their respective correlation coefficients.

4. Summary

This study has demonstrated that multivariate evaluation of FA profiles changes at the *sn*-2 position of PO, PKO, and CLO is a well-suited technique for distinguishing LD contamination. It is worth noting that, with the application of the CANDISC technique, oil samples that are contaminated with as little as 2% LD could be easily distinguished and no misclassification of other animal fats occurred within the spatial region of the LD-adulterated series. Additionally, simple regression analysis with appropriate adjustments would help to develop linear models for quantification of LD content in admixtures.

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