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Compositional and thermal analysis of RBD palm oil adulterated with lipase-catalyzed interesterified lard

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Abstract

A study was conducted to determine the presence of enzymatically-randomized lard (ERLD) as an adulterant in RBD palm oil using three different analytical techniques, namely gas liquid chromatography (GLC), reversed phase high performance liquid chromatography (RP–HPLC) and differential scanning calorimetry (DSC). Lard extracted from the adipose tissues of pig was enzymatically interesterified using non-specific lipase from *Pseudomonas* sp. Compositional and thermal characteristics of ERLD were compared with those of genuine lard (GLD). RBD palm oil samples, adulterated separately with various levels of GLD and ERLD, were analyzed using GLC, HPLC and DSC. Neither GLC nor HPLC showed any characteristic adulteration peaks to enable RBD palm oil, adulterated with either GLD or ERLD, to be distinguished from those adulterated with chicken fat (CF). However, DSC provided a better means for identification of lard, with a detection limit of 1%.

Keywords: Adulteration; Animal fat; Lard; Enzymatically-randomized lard; RBD palm oil

1. Introduction

Quality control has increasingly become an essential part of the fats and oils industry. Recently, considerable efforts have been directed towards the development of analytical methodology, resulting in a variety of methods for the analysis and characterization of fats and oils. Quality control methods are of two kinds: some are traditional or classical while others are based on advanced instrumentation. These quality control methods are widely used in international trade, process control and research and development activities. Application and development of new methods, to check adulterations in fats, oils and fat-based products, is of paramount importance in order to protect consumers and food industries. There has been a great deal of scientific investigation of adulteration in fats and oils. The GLC analysis of fatty acid methyl esters has been used for the compilation of fatty acid composition of authentic samples of fats and oils. As a result, the Codex Alimentarius Specification for Fats and Oils, which list fatty acid ranges for various fats and oils, has become the international basis for checking adulteration and purity of fats and oils. Similarly, GLC has also been shown to be useful for detecting adulteration, based on the analysis of triacylglycerols (TAGs) according to their carbon number (Padley & Timms, 1980; Timms, 1980). In addition, HPLC has also been used to check adulteration in fats and oils on the basis of TAG composition or plant sterol composition (Rashood, Abdel-Moety, Rauf, Abou-Shaaban, & Al-Khamis, 1995; Rossell, 1998; Rossell, King, & Downes, 1983; Saeed, Ali, Rahman, & Sawaya, 1989).

Many workers have used differential scanning calorimetry (DSC) to deal with adulteration problems associated with edible fats and oils, and fat-based products. Lambelet, Singhal, and Ganguli (1980) have reported that goat body-fat adulteration of ghee could be detected by DSC, while Lambelet and Ganguli (1983) have used DSC to detect ghee adulterated with animal body

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fats such as pig fat and buffalo body fat. Kowalski (1989) has demonstrated a method based on DSC, to differentiate lard and lard contaminated by cow tallow. DSC has also been used to detect the presence of added animal fats, such as chicken fat in butter (Coni, Coppolelli, & Bocca, 1994). In their work on cheeses, Tunic and Malin (1997) used DSC profiling as a way of distinguishing between two mozzarella cheese types made out of cow's milk and water buffalo milk, and found it possible to detect the presence of mixtures of milk fats in these products.

Palm oil may also pose similar adulteration problems to animal fats such as lard. In fact, lard or industrially-modified lard could be effectively blended with other vegetable oils to produce shortenings, margarines and other speciality food oils. According to Gillies (1974), a plastic shortening in the beta phase may be prepared from a partially-hydrogenated soybean oil, blended with hydrogenated lard, while a margarine can be made with a fat phase consisting of 60–70% of topped palm oil, 15–25% lard and the remainder ground nut oil. The blending may also be done using randomized lard in place of genuine lard, as randomization has been shown to improve the physical properties of lard (Sreenivasan, 1978). However, mixing of lard, in either genuine form or modified form, with other vegetable oils, such as palm oil may not be desirable for certain health reasons discussed elsewhere (Rashood, Abou-Shaaban, Abdel-Moety, & Rauf, 1996). Therefore, in this study we focus attention on the compositional and thermal analysis of RBD palm oil adulterated with lipase (nonspecific *Pseudomonas* sp. lipase)-catalyzed interesterified lard using GLC, HPLC and DSC.

2. Materials and methods

2.1. Materials

RBD palm oil (Slip Melting point: 30.5 °C; Iodine Value: 54.0) was purchased from a local refinery. The fat was stored at 4 °C. Prior to use, it was melted at 60 °C in the oven. All chemicals used in this experiment were of analytical or HPLC grade. Fatty acid methyl esters (FAME), triacylglycerol (TAG) and 2-monoacylglycerol (2-MAG), used as authentic standards, were purchased from Sigma Chemical Co. (St. Louis, MO). Amano Pharmaceutical Co. (Nagoya, Japan) donated the *Pseudomonas* sp. lipase in the powder form. Celite, used as a carrier for the immobilization of the *Pseudomonas* sp. lipase, was purchased from BDH Ltd, UK. Lipase from hog pancrease was obtained from Fluka Chemie (Buchs, Switzerland). Lard samples were extracted by rendering (90–100 °C for 2 h) adipose tissues of pig collected from a local slaughter house. The extracted lard was filtered through double-folded muslin cloth and anhydrous sodium sulphate was added to the extract to remove residual moisture, and then filtered through Whatman No. 2 filter paper and stored at 4 °C (Lambelet et al., 1980). Similarly, chicken fat was also extracted from the adipose tissues of chicken using the above method.

2.2. Lipase immobilization

The immobilization of lipase on Celite was done by first dissolving 100 mg of lipase powder in 100 μl of cold deionized water, followed by thorough mixing with 250 mg of Celite. The preparation was lyophilized for 6 h prior to the transesterification of lard (Ghazali, Hamidah, & Che Man, 1995).

2.3. Transesterification reaction

Ten grams of genuine lard were added and thoroughly mixed with Celite-bound *Pseudomonas* sp. lipase (0.1 g) in the immobilized form in a 50 ml conical flask. The reaction mixture was then agitated for 8 h in an orbital shaker at 200 rpm and 40 °C. Transesterification was done in triplicate. At the end of the reaction, the immobilized enzyme was removed from the reaction mixture via hot filtration using Whatman No. 1 filter paper. Transesterification was carried out according to the method reported by Lai, Ghazali, France Cho and, Chong (2000).

2.4. Blend preparation

Liquefied RBD palm oil and enzymatically-randomized lard (ERLD)/genuine lard (GLD) were mixed in proportions ranging from 0.2 to 1% lard in 0.2% increments, 1 to 5% lard in 1% increments and from 5 to 20% lard in 5% increments (w/w). Altogether, 12 blends were prepared: 99.8:0.2, 99.6:0.4, 99.2:0.8, 99:1, 98:2, 97:3, 96:4, 95:5, 90:10, 85:15, 80:20, (w/w) identified by the mass ratio of RBD palm oil to lard (RBD palm oil:ERLD). Another series of five blends was prepared by mixing palm oil with chicken fat (CF): 98:2, 95:5, 90:10, 85:15, 80:20, (w/w) and identified by the mass ratio of RBD palm oil to chicken fat (RBD palm oil:CF).

2.5. HPLC analysis of TAG composition

TAG composition was determined according to the method described by Haryati, Che Man, Ghazali, Asbi, and Buana (1998). The system used was a Shimadzu LC-10 AD liquid chromatograph, equipped with a Shimadzu SIL-10 AD auto injector, Shimadzu system controller SCL-10A, and RID-6A Shimadzu refractive index detector (Shimadzu Corporation, Kyoto, Japan). The analysis of TAG was performed on a LiChroCART 100-RP-18 (5 μm) column (12.5 cm × 4 mm i.d.; Merck,

Darmstadt, Germany). The mobile phase was a mixture of acetone–acetonitrile (63.5:36.5) and the flow rate was 1 ml/min at 30 °C. The injector volume was 10 μl of 5% (w/w) oil in chloroform. Sensitivity of the detector was adjusted to 16×10^4 RI units.

2.6. Isolation of neutral TAG

Isolation of neutral TAG was based on the AOCS method Cd 20-91 (AOCS, 1987). A 2.5 g portion of melted fat was dissolved in 50 ml of elution solvent [petroleum ether and diethyl ether 87/13: (v/v)]. A glass column (45 cm×2.1 cm, i.d.) was properly packed with a slurry of 25-g silica gel 60 with particle size 0.063–0.200 mm (70–230 mesh) in 80 ml of elution solvent. Once the silica had settled, about 4 g of sea sand was slowly added on to the top of the column. After that, 20 ml of the fat solution was carefully introduced into the top of the column. The solution was allowed to drain off to the level of the sand layer (flow rate 2.1–2.5 ml/min). The column was then eluted with 150 ml portions of eluting solvent over a period of 60 min. Eluted portions were collected and the solvent was removed under vacuum. The purity of the isolate was confirmed by running a co-TLC with a standard TAG. A portion of the isolated neutral TAG was used to prepare 2-monoacylglycerol and the remainder was used to obtain fatty acid profiles of lard samples by GLC.

2.7. Preparation of 2-MAG

Preparation of 2-MAG was carried out according to the International Union of Pure and Applied Chemistry (IUPAC) standard method No. 2.210 (Paquoec, 1979). Melted samples of neutral TAG (100 mg) were taken in a centrifuged tube and mixed with hexane (200 μl). This solution was treated with 20 mg of pancreatic lipase, 2 ml of 1 M Tris-HCl buffer (pH 8), 0.5 ml of 0.05% bile salt and 0.2 ml $CaCl_2$ solution. After shaking, this mixture was incubated in a water bath kept at 40 °C for 1 min, followed by vigorous vortexing. After cooling under running water, 1 ml of 6 N HCl and 1 ml diethyl ether were added into the mixture. The tube was stoppered and vortexed vigorously. The diethyl ether extract was applied on a TLC plate and the plate was set in a well-saturated developing tank containing a developing solvent, hexane/diethyl ether/acetic acid (50:50:1). The plate was then dried and placed in a I_2 chamber. The band corresponding to 2-MG was scraped off and extracted with $CHCl_3/CH_3OH$ (95:5). The purity of the isolate was confirmed by running a co-TLC with a standard 2-MAG.

2.8. GLC analysis of fatty acid methyl esters

FAME were prepared according to the PORIM test method (PORIM Test Methods, 1995, p3.4) by dissolving oil sample (50 mg) with petroleum ether (0.8 ml) and sodium methoxide (1M, 0.2 ml) and analyzing on a gas chromatograph (Shimadzu GC-14 A) fitted with a FID detector. A polar capillary column BPX70 (0.32 mm i.d., 30 m length and 0.25 μm film thickness; SGE International Pty, Ltd., Victoria, Australia) was used at a column pressure of 10 psi. The temperature of the column was 90 °C, programmed to increase to 220 °C at 15 °C/min (for 5 min), 2 °C/min and 15 °C/min (for 1 min). The temperature of the injector and detector was maintained at 240 °C. Standard methyl esters of fatty acids were used as authentic samples and peak identification was done by comparing relative retention times. The peak areas were obtained from the computer and the percentage of the fatty acid was calculated as the ratio of the partial area to the total area.

2.9. DSC thermal analysis

A Perkin–Elmer Model DSC-7 DSC (Norwalk, CT) was used for analyzing the thermal characteristics of the oil samples. The instrument was calibrated with indium and dodecane. Samples of ca. 8–10 mg were weighed into aluminium pans and covers were crimped into

Table 1
Fatty acid compositions (%) of GLD and ERLD in total fats, neutral triacylglycerols (NTG), 2-monoacylglycerols (2-MAG), and calculated 1,3-diacylglycerols (1,3-DAG)[a]

Fat sample		Fatty acid (methyl esters) composition (%)											
		C12:0	C14:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2
GLD	Total fat	0.24	1.46	25.20	1.69	0.35	8.72	42.40	17.70	0.69	0.20	0.73	0.52
ERLD	Total fat	0.22	1.48	25.90	1.98	0.38	8.70	43.50	15.80	0.56	0.19	0.73	0.50
GLD	NTG	0.21	1.29	24.00	1.60	0.38	9.24	42.50	18.20	0.77	0.26	0.86	0.60
	2-MAG	0.50	3.57	68.20	2.77	0.55	3.26	16.10	4.35	0.15	0.10	0.24	0.13
	1,3-DAG[b]	0.07	0.15	1.96	1.02	0.30	12.20	55.80	25.10	1.08	0.34	1.17	0.84
ERLD	NTG	0.21	1.41	26.20	1.76	0.33	8.80	43.30	16.00	0.57	0.18	0.72	0.50
	2-MAG	0.46	3.14	58.70	2.77	0.57	5.49	23.60	5.02	–	–	0.32	–
	1,3-DAG[b]	0.09	0.55	9.96	1.26	0.21	10.50	53.20	21.50	0.86	0.27	0.92	0.75

[a] Each value in the table represents the means of triplicate analyses.
[b] 2×1,3-DAG = 3×TAG−2−MAG. Abbreviations: GLD, genuine lard; ERLD, enzymatically-interesterified lard.

place. An empty covered pan was used as a reference. Both were placed in the instrument sample chamber. The following temperature program was used to obtain the cooling measurements on each sample: 80 °C isotherm for 5 min, cooled from 80 to −80 °C at a rate of 5 °C/min. The manufacturer's software (7 Series/UNIX DSC software library) program was used to analyze and plot the thermal data. The crystallization characteristics of each sample in a DSC scan were obtained using the normalized thermogram. Start (°C) and End (°C) are the starting and ending temperatures of each crystallization transition. The temperature maximum of a crystallization transition is denoted by Max (°C). Onset (°C) is the temperature where the extrapolated leading edge of the endotherm intersects with the baseline (Tan & Che Man, 2000; 7 Series/UNIX DSC7 Users Manual, 1995).

2.10. Statistical analysis

Three replicates of each sample were analyzed. The SAS/STAT (SAS,Cary, NC) release 6.08 program was used for stepwise multiple linear regression (SMLR) analysis (SAS, 1989). The significance level of the stepwise variable for entry in the calibration model was set to 0.15 during execution of the stepwise variable selection in SAS procedure "REG".

3. Results and discussion

3.1. General

Interesterification and hydrogenation are the two common methods employed for modification of lard in order to increase its range of application in the food industry. However, interesterification of fats, either by chemical or enzymatic means, is an attractive alternative to hydrogenation since it can provide low *trans* fat products (de Man, 1999). Even though, at present, chemical interesterification is the cheaper process for the purpose of randomization, the enzymatic interesterification may represent the way of the future (Rousseau, Forestiere, Hill, & Marangoni, 1996). Much work has been published on chemical interesterification of lard but there is a paucity of information concerning characteristics of ERLD. Therefore, a comparison of GLD and ERLD, with regard to chemical composition and thermal characteristics would be useful for the purpose of this study.

A comparative fatty acid composition (%) of GLD and ERLD in total fat, neutral TAG, 2-monoacylglycerol (2-MAG) and the calculated 1,3-diacylglycerol (1,3-DAG), is presented in Table 1. Although GLD and ERLD are found to be rather similar with regard to the fatty acid composition of the total fat and the neutral TAG, the pancreatic lipolysis of the neutral TAG showed that there are considerable differences between the positional distributions of fatty acids. It is very clear that the palmitic acid occupation in the C-2 position is partly replaced by oleic acid in the ERLD sample. As a result, the ratio of saturated to unsaturated acids in the C-2 position was lower for ERLD (S/U: 2.15) than for GLD (S/U: 3.21).

Table 2
TAG compositions (peak area %) of GLD and ERLD[a]

Peak No.	Retention time (min)	GLD	ERLD
1	10.7	1.23	1.22
2	11.5	0.89	0.74
3	13.0	4.09	3.35
4	13.8	6.53	4.53
5	15.1	0.34	0.35
6	16.2	6.84	7.25
7	17.2	22.40	17.70
8	18.5	2.42	4.00
9	20.4	5.47	7.01
10	21.7	26.20	24.50
11	23.1	8.81	11.40
12	25.2	0.16	0.47
13	27.2	2.66	5.28
14	29.2	10.60	10.60
15	31.6	0.52	0.80
16	36.8	0.41	0.89
17	39.8	0.54	–

[a] Each TAG % in the table represents the means of triplicate analyses. Abbreviations see Table 1.

With regard to HPLC analysis, comparative TAG profiles of GLD and ERLD are shown in Fig. 1A, B. Based on this, the TAG compositions (peak area %) of both GLD and ERLD are tabulated in Table 2. It is apparent that the interesterification reaction was accompanied by the formation of diacylglycerols (peaks before 10 min on the chromatogram; Fig. 1B) because they are unavoidable intermediates in the reaction (Ghazali et al., 1995). According to Table 2, Peaks 7 and 10 are found to be the predominating TAGs in both GLD and ERLD. Peaks 3, 4, 7 and 10 were undergoing decrease in peak area while area increases were observed for peaks 6, 8, 9, 11 and 13.

A comparison of thermal profiles of GLD and ERLD is presented in Fig. 2A, B. It is very clear that the thermal profile of ERLD is significantly different from that of GLD. While ERLD exhibited four major transition steps at 15.91, 6.81, −28.09 and −40.55 °C, GLD has only two major exothermic peaks of transition at 4.93 and −16.85 °C. Based on this, the basic differences in thermodynamic parameters of GLD and ERLD could be summarized as shown in Table 3. The observed differences in thermodynamic parameters of the two types of lard could be attributed to the changes in TAG profiles of GLD and ERLD, as shown in Table 2.

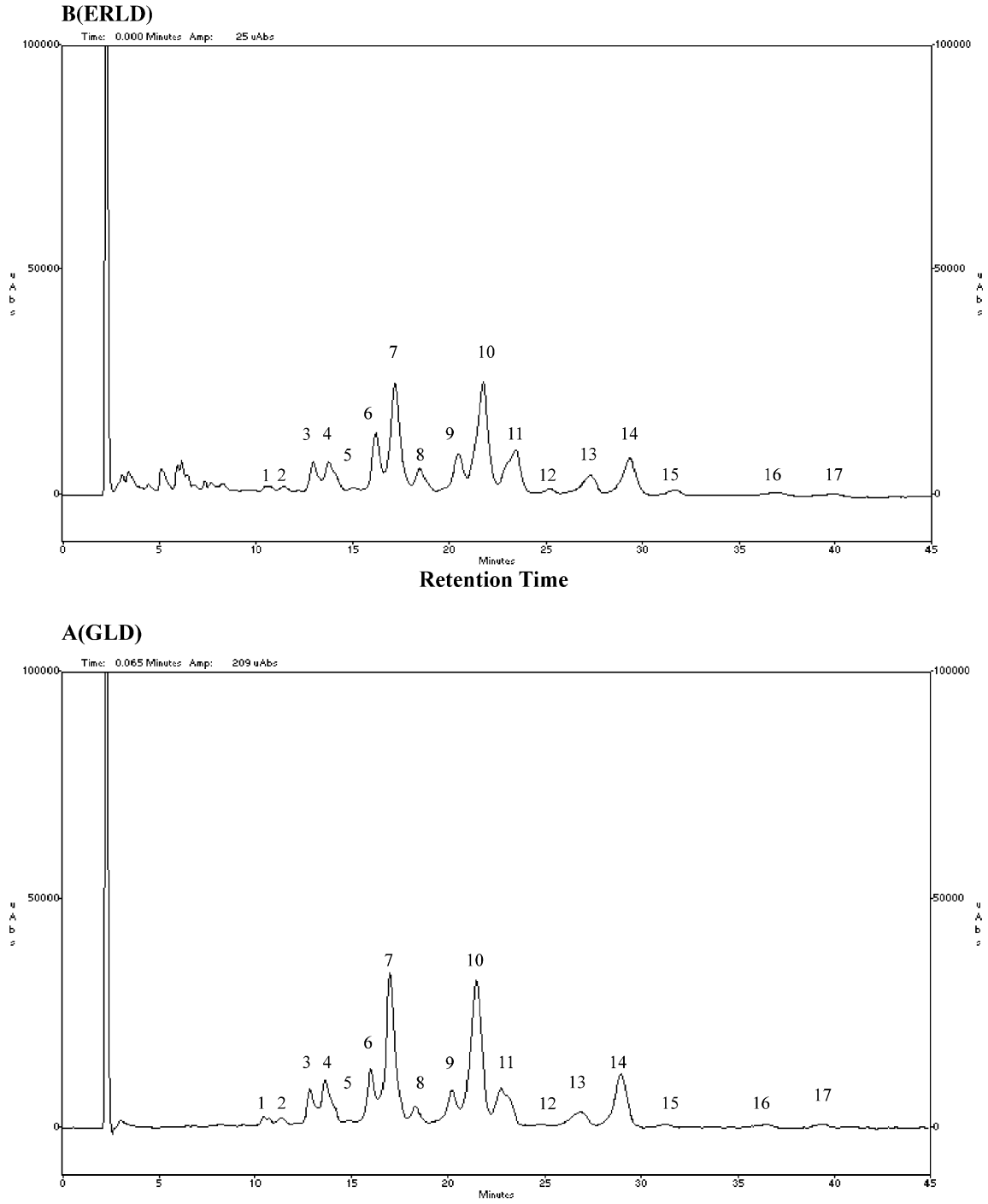

Fig. 1. HPLC chromatograms of (A) genuine lard (GLD), and (B) enzymatically-randomized lard.

3.2. Compositional changes in RBD palm oil due to adulteration with ERLD

3.2.1. GLC analyses of FAME

Relative percentage distributions of various fatty acid components of RBD palm oil and a series of RBD palm oil samples adulterated with ERLD are summarized in Table 4. RBD palm oil is found to have C16:0, C18:0, C18:1 and C18:2 in higher amounts, with C16:0 being the predominant one. On the other hand, as shown in Table 1, the prevalent fatty acids of ERLD are C16:0, C18:0, C18:1 and C18:2, with C18:1 being the predominant fatty acid. As a result, a comparison of fatty acid distribution between RBD palm oil and adulterated RBD palm oil revealed that there is a gradual decrease and increase in the amounts of C16:0 and, C18:1 and C18:2, respectively as the adulterant is increased in concentration. According to Table 5, a similar trend

Table 3
Comparison of thermodynamic parameters of phase transitions of GLD and ERLD[a]

Sample	Peak No.	Temperature of peak(s) transition(s) (°C)				Peak height (W/g)	Peak are ΔH(J/g)
		Start	Onset	Max.	End		
GLD	1	7.85	6.62	4.93	−4.62	−0.51	−15.25
	2	−9.02	−14.20	−16.85	−35.79	−0.56	−34.30
ERLD	1	21.05	18.19	15.91	12.98	−0.11	−5.38
	2	12.98	10.19	6.81	−21.12	−0.20	−40.70
	3	−21.12	−22.73	−28.09	−32.49	−0.06	−7.30
	4	−32.49	−34.86	−40.55	−50.82	−0.11	−16.62

[a] Each value in the table represents the means of triplicate analyses. Abbreviations see Table 1.

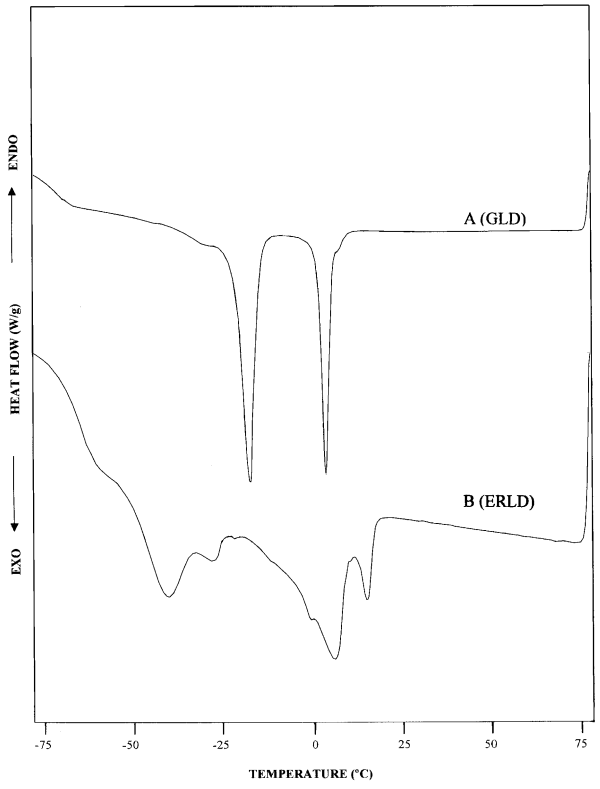

Fig. 2. DSC cooling thermograms of (A) genuine lard (GLD), and (B) enzymatically randomized lard.

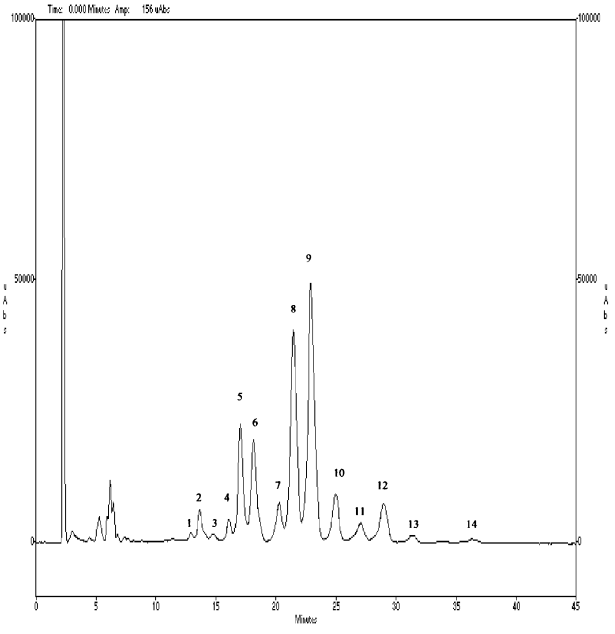

Fig. 3. HPLC chromatogram of RBD palm oil.

was also observed for RBD palm oil adulterated with genuine lard (GLD). However, these changes in fatty acid composition could not be used for the immediate detection of GLD or ERLD in RBD palm oil since similar changes were also observed for RBD palm oil adulterated with chicken fat (Table 6).

3.3. HPLC analyses of TAG

A sample chromatogram of RBD palm oil is presented in Fig. 3. The identification and assignment of TAG peaks is based on the previous study of Haryati et al. (1998). The peaks were identified as 1:MMM, 2:PLL, 3:MPL, 4:OOL, 5:PLO, 6:PPL, 7:OOO, 8:OOP, 9:PPO, 10:PPP, 11:OOS, 12:POS, 13:PPS and 14:SOS, where M stands for myristic, P for palmitic, O for oleic, L for linoleic, and S for stearic. A comparison of Fig. 1B and Fig. 3 shows the differences in TAG profiles of RBD palm oil and ERLD. The changes in TAG composition of RBD palm oil due to adulteration with ERLD and GLD are illustrated in Table 7 and Table 8, respectively. ERLD and GLD seemed to show similar trends with regard to the changes in TAG composition. They did not show any additional peak indicating the presence of the adulterant but, instead, peak increases were observed for peaks 2(PLL), 4(OOL), 5(PLO), 7(OOO) and 8(OOP) while peaks 6(PPL), 9(PPO) and 10(PPP) were found to decrease in size. Hence, in general, the lard adulteration in RBD palm oil, whether it is by GLD or ERLD, caused a slight increase in oleic acid-containing TAGs, while the palmitic acid-containing TAGs decreased slightly. However, these changes in RBD

Table 4
Fatty acid composition of RBD palm oil after adulteration with ERLD[a]

% ERLD	C12	C14	C16	C16:1	C18	C18:1	C18:2	C18:3	C20
0	0.22	1.09	45.74	0.21	3.75	38.63	9.43	0.21	0.35
2	0.23	1.14	45.31	0.21	3.89	38.75	9.62	0.20	0.26
5	0.21	1.05	44.82	0.29	4.09	39.00	9.92	0.23	0.27
10	0.23	1.10	44.13	0.36	4.32	39.22	10.11	0.23	0.27
15	0.22	1.08	42.80	0.40	4.58	39.53	10.53	0.25	0.27
20	0.22	1.09	41.83	0.45	4.80	39.83	10.83	0.26	0.27

[a] Each value in the table represents the means of triplicate analyses. Abbreviations see Table 1.

Table 5
Fatty acid composition of RBD palm oil after adulteration with GLD[a]

% GLD	C12	C14	C16	C16:1	C18	C18:1	C18:2	C18:3	C20
0	0.22	1.09	45.74	0.21	3.75	38.63	9.43	0.21	0.35
2	0.21	1.09	44.74	0.28	4.07	39.00	9.81	0.25	0.31
5	0.21	0.98	44.65	0.28	4.13	39.20	9.90	0.25	0.30
10	0.22	1.02	43.30	0.34	4.34	39.60	10.34	0.24	0.28
15	0.21	1.04	42.05	0.41	4.61	39.90	10.80	0.31	0.26
20	0.22	1.08	41.24	0.50	4.80	40.20	11.11	0.32	0.25

[a] Each value in the table represents the means of triplicate analyses. Abbreviations see Table 1.

Table 6
Fatty acid composition of RBD palm oil after adulteration with CF[a]

% CF	C12	C14	C16	C16:1	C18	C18:1	C18:2	C18:3	C20
0	0.22	1.09	45.74	0.21	3.75	38.63	9.43	0.21	0.35
2	0.22	1.10	45.44	0.41	4.17	38.76	9.52	0.29	0.29
5	0.23	1.09	44.90	0.53	4.11	38.94	9.68	0.29	0.28
10	0.21	1.06	44.00	0.82	4.18	39.30	9.89	0.32	0.27
15	0.21	1.05	43.50	1.08	4.40	39.40	9.78	0.39	0.22
20	0.20	1.08	42.45	1.35	4.39	39.83	10.20	0.35	0.24

[a] Each value in the table represents the means of triplicate analyses. Abbreviation: CF, chicken fat.

Table 7
TAG composition of RBD palm oil after adulteration with ERLD[a]

% ERLD	MMM	PLL	MPL	OOL	PLO	PPL	OOO	OOP	PPO	PPP	OOS	POS	PPS	SOS
0	0.44	2.54	0.61	1.61	10.40	10.31	4.19	23.00	31.20	5.28	2.30	5.19	0.97	0.34
2	0.47	2.57	0.49	1.78	10.74	10.26	4.38	23.91	31.30	5.41	2.50	5.22	0.84	0.17
5	0.51	2.65	0.48	1.92	11.10	10.21	4.47	24.16	31.10	5.18	2.23	5.43	0.46	0.13
10	0.69	2.82	0.47	2.25	11.60	9.96	4.56	24.42	30.34	4.90	2.08	5.24	0.52	0.15
15	0.77	2.70	0.43	2.49	11.80	9.68	4.71	24.22	29.30	4.78	2.52	5.70	0.70	0.25
20	0.85	2.73	0.26	2.82	12.36	9.54	4.95	24.45	28.54	4.59	2.50	5.85	0.55	0.12

[a] Each value in the table represents the means of five replicate analyses. Abbreviation see Table 1.

Table 8
TAG composition of RBD palm oil after adulteration with GLD[a]

% GLD	MMM	PLL	MPL	OOL	PLO	PPL	OOO	OOP	PPO	PPP	OOS	POS	PPS	SOS
0	0.44	2.54	0.61	1.61	10.40	10.31	4.19	23.00	31.21	5.28	2.30	5.19	0.97	0.34
2	0.59	2.91	0.76	1.89	10.75	10.17	4.23	23.30	31.11	5.25	2.38	5.41	1.08	0.18
5	0.63	2.94	0.69	2.00	11.17	10.11	4.32	23.28	30.56	5.31	2.56	5.50	0.84	0.11
10	0.77	3.07	0.55	2.24	11.84	9.73	4.32	23.64	29.61	4.77	2.47	5.84	0.92	0.22
15	0.86	3.25	0.59	2.52	12.25	9.41	4.56	23.60	28.41	4.94	2.60	5.99	0.88	0.14
20	1.17	3.73	0.73	2.71	12.83	9.05	4.33	23.50	27.22	4.66	2.69	6.40	1.02	–

[a] Each value in the table represents the means of five replicate analyses. Abbreviation see Table 1.

Table 9
TAG composition of RBD palm oil after adulteration with CF[a]

% CF	MMM	PLL	MPL	OOL	PLO	PPL	OOO	OOP	PPO	PPP	OOS	POS	PPS	SOS
0	0.44	2.54	0.61	1.61	10.40	10.31	4.19	23.00	31.21	5.28	2.30	5.19	0.97	0.34
2	0.56	2.76	0.69	1.85	10.62	10.34	4.36	23.17	31.10	5.47	2.59	5.29	0.91	0.35
5	0.66	2.94	0.63	2.09	11.10	10.26	4.40	23.27	30.64	5.07	2.34	5.24	0.96	0.39
10	0.80	3.00	0.58	2.50	11.37	10.02	4.76	22.74	29.37	5.28	2.79	5.38	1.28	0.17
15	0.97	3.37	0.67	2.89	12.02	9.81	4.92	23.00	28.82	4.87	2.44	4.94	0.94	0.39
20	1.15	3.43	0.42	3.34	12.47	9.54	5.15	22.85	27.84	4.73	2.60	5.05	0.96	0.32

[a] Each value in the table represents the means of five replicate analyses. Abbreviation see Table 6.

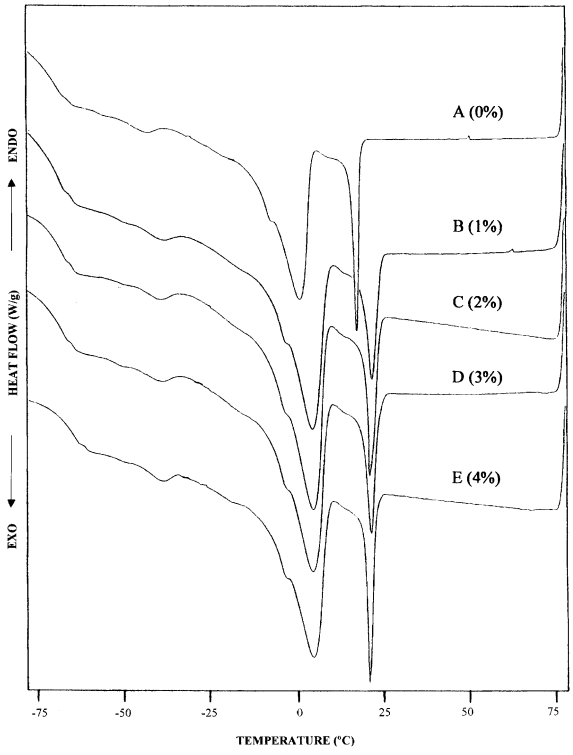

Fig. 4. DSC cooling thermograms of (A) RBD palm oil, and RBD palm oil adulterated with (B) 1% ERLD, (C) 2% ERLD, (D) 3% ERLD, and (E) 4% ERLD.

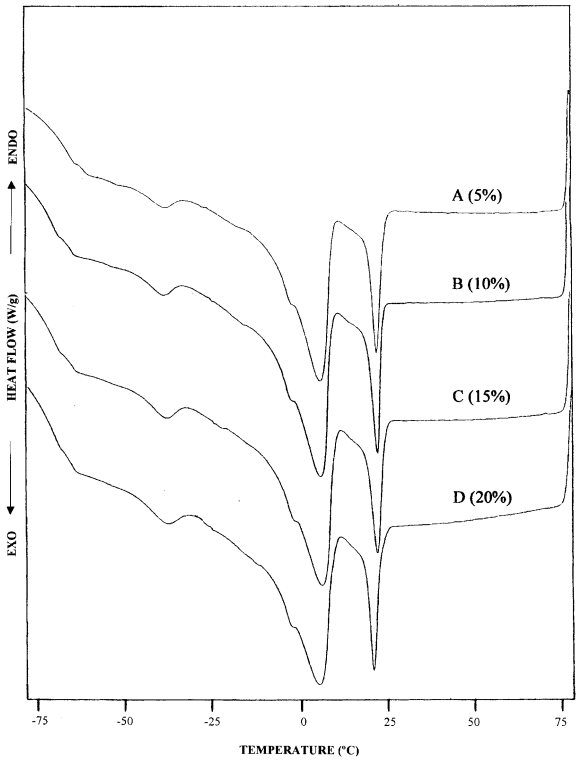

Fig. 5. DSC cooling thermograms of (A) RBD palm oil adulterated with 5% ERLD and (B) 10% ERLD, (C) 15% ERLD, and (D) 20% ERLD.

palm oil could not be used to detect the presence of lard in the form of GLD or ERLD since similar changes were also observed for RBD palm oil adulterated with chicken fat (Table 9).

3.4. Thermal analysis of RBD palm oil adulterated with ERLD

A typical cooling thermogram of unadulterated RBD palm oil is presented in Fig. 4A. It shows two major exothermic transition peaks at 17.75 and 1.25 °C, and two minor shoulder peaks at −6.82 and −43.86 °C. Our previous studies, using DSC (data not shown), showed that the shoulder peak appearing at −43.86 °C is sensitive to adulteration of the oil with either genuine or chemically-randomized lard. As the adulteration level went up from 1 to 20%, this particular peak was found gradually to increase in size and, as a result, a good correlation for concentration of lard in adulterated palm oil was observed with peak parameters such as peak area and peak height. In addition, the DSC thermal analyses of RBD palm oil samples adulterated with other common animal fats, such as beef tallow, mutton tallow and chicken fat showed that the particular lard adulteration peak in RBD palm oil could be distinguished from adulteration peaks due to other animal fats (data not shown). This finding served as the basis for the investigation of RBD palm oil adulterated with lipase-catalyzed interesterified lard.

As shown in Figs. 4 A–E and 5 A–D, the small shoulder peak originally appearing at −43.86 °C for unadulterated sample of RBD palm oil has undergone

Table 10
Adulteration peak temperatures of DSC cooling thermograms of RBD palm oil adulterated with ERLD[a]

% ERLD	Peak temperature (°C)
0	−43.86
1	−38.55
2	−38.93
3	−38.97
4	−38.92
5	−38.90
10	−38.93
15	−38.87
20	−38.87

[a] Each value in the table represents the means of triplicate analyses. Abbreviations see Table 1.

an enlargement with increasing concentration of ERLD, ranging from 1 to 20%. The peak position (peak temperature maximum) was also found to have shifted to a higher temperature region (Table 10). Therefore, a qualitative identification of RBD palm oil samples adulterated with ERLD, in the range 1–20%, could be achieved. In addition, this particular adulteration peak could be used for quantitative estimation of ERLD and also GLD present in RBD palm oil. For this purpose, three parameters (peak area, A; peak height, HT; and peak onset, ON) were derived from the adulteration peak of each sample and they served as independent variables in the SMLR analysis, with percent ERLD (added into palm oil) as the dependent variable. The SMLR analysis showed that percent of ERLD in oil samples could be predicted using the regression model given below

$$\%\ \text{ERLD} = 11.9058\ A - 12.1737$$

$$R^2 = 0.9583,\quad P < 0.0001$$

where A = peak area.

The peak area changes were hardly seen for samples containing less than 1% ERLD. Therefore, this method may not be applicable for adulteration levels below 1%.

In conclusion, this study shows that, even though GLC and HPLC are capable of providing fine details of fatty acid composition and TAG profile of adulterated RBD palm oil, they were found to be of very little use for qualitative identification and quantitative determination of adulterants such as ERLD. On the other hand, DSC is found to be a more sensitive technique for qualitative and quantitative determination of ERLD in palm oil. It has been shown that detection of samples of RBD palm oil adulterated with ERLD up to a 1% detection limit is still possible. Another useful advantage of DSC is that it does not require any sample pretreatment or chemicals for this analysis. In addition, the accuracy and speed of the DSC method for the detection and determination of ERLD in RBD palm oil makes it ideal for quality control purposes.

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