Detection of Lard and Randomized Lard as Adulterants in Refined-Bleached-Deodorized Palm Oil by Differential Scanning Calorimetry

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ABSTRACT: A study was conducted to assess the use of differential scanning calorimetry (DSC) for detecting the presence of lard/randomized lard as adulterants in refined-bleached-deodorized (RBD) palm oil. Lard extracted from the adipose tissues of pig was chemically interesterified using sodium methoxide as catalyst. DSC thermal profiles of both genuine lard and randomized lard were compared with those of other common animal fats such as beef tallow, mutton tallow, and chicken fat. Lard and randomized lard were then blended with RBD palm oil in two series, in proportions ranging from 0.2 to 20%, and DSC analyses were obtained. The DSC cooling profiles of adulterated RBD palm oil samples showed an adulteration peak corresponding to lard/randomized lard in the low-temperature region. This peak was confirmed as an indicator of the presence of lard in RBD palm oil since similar experiments carried out using other common animal fats such as mutton tallow, beef tallow, and chicken fat showed that the lard adulteration peak could be distinctly identified. Using this method, a detection limit of 1% lard/randomized lard was reached ($P < 0.0001$).

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KEY WORDS: Adulteration, animal fat, differential scanning calorimetry, lard, randomized lard, RBD palm oil, thermal behavior.

Food adulteration is a commonly encountered problem in food trade and industry. Almost all foodstuffs present a quality problem but even more so when they have a high intrinsic commercial value. Therefore, monitoring of adulteration practices has become essential in order to protect consumers and food industries. The fats and oils industry is no exception to this. Adulteration has been a problem in the oils and fats trade for a long time (1). It is sometimes deliberate and sometimes accidental (2). In the past, there were reports concerning cottonseed oil adulteration with palm oil (3), contamination of groundnut oil with sunflower oil, and adulteration of both groundnut and sunflower oils with cheaper oils such as soy or rape (2). Similarly, palm oil was reported to have been adulterated with relatively cheaper palm stearin (2,3). At times, traders had imported palm oil fractions, namely, palm olein and palm stearin, from the producing country and recombined them for export to Europe and North America. Unfortunately, the blending was seldom in the same proportions as when the fractions had first been generated and the quality of the "palm oil" therefore varied considerably. This led to gross manufacturing difficulties in food-processing industries (3). Palm oil may also pose similar adulteration problems with commonly found animal fats such as lard. According to previous studies, both genuine and randomized lard are found to possess high amounts of palmitic and oleic acids that are also the predominant fatty acids in refined-bleached-deodorized (RBD) palm oil (3,4). After adulteration, RBD palm oil may become inferior in quality due to the difference in the triacylglycerol (TAG) composition of lard. Apart from this, the Islamic and the Orthodox Jewish religions prohibit the consumption of products containing pork and lard (4).

The detection of pork and lard as adulterants has gained considerable importance and interest in many parts of the world. As a result, a number of detection methods have been reported by several workers. Lambelet and Ganguli (5) have studied the application of differential scanning calorimetry (DSC) to detect adulteration of ghee (a popular dairy product in India) with lard. A method based on fractional crystallization followed by gas chromatographic (GC) analysis of fatty acid methyl esters has been demonstrated by Farag *et al.* (6) to detect butterfat adulteration with lard. Saeed *et al.* (7) have used high-performance liquid chromatography (HPLC) analysis of derivatized TAG to detect pork in adulterated samples of mutton and beef. Rashood *et al.* (4) have shown that the HPLC analysis of TAG could be used as a method to distinctly identify genuine/randomized lard from other animal body fats. Recently, Che Man and Mirghani (8) developed a method for detecting lard in mixtures of body fats such as chicken, lamb, and cow based on Fourier transform infrared (FTIR) spectroscopy. The objective of this study is to investigate the use of DSC for monitoring the presence of genuine and randomized lard as adulterants in RBD palm oil.

MATERIALS AND METHODS

Materials. RBD palm oil (slip melting point: 30.5ºC; iodine value: 54.0) was purchased from a local refinery. All chemicals used in this experiment were of analytical or HPLC grade. Lard samples were extracted by rendering adipose tis-

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sues of pig collected from a local slaughterhouse at 90–100°C for 2 h. The extracted lard was filtered through double-folded muslin cloth, and anhydrous sodium sulfate was added to the extract to remove residual moisture. The extract was filtered through Whatman No. 2 filter paper and stored at 4°C (9). The same procedure was followed in the extraction of other animal fats such as mutton tallow (MT), beef tallow (BT), and chicken fat (CF).

Blend preparations. Liquified RBD palm oil and genuine lard (GLD)/chemically randomized lard (CRLD) were mixed in proportions ranging from 0.2 to 1% lard, in 0.2% increments, 1 to 5% lard, in 1% increments (w/w), and from 5 to 20% lard, in 5% increments (w/w). A total of 12 blends were prepared—99.8:0.2, 99.6:0.4, 99.4:0.6, 99.2:0.8, 99:1, 98:2, 97:3, 96:4, 95:5, 90:10, 85:15, 80:20 (w/w)—and these were identified by the mass ratio of RBD palm oil to lard (RBD palm oil/GLD and RBD palm oil/CRLD). For other animal fat studies, three series of blends were prepared by mixing palm oil separately with BT, MT, and CF. In each case, five blends were prepared: 98:2, 95:5, 90:10, 85:15, 80:20 (w/w), identified by the mass ratio of RBD palm oil to animal fat (RBD palm oil/BT, RBD palm oil/MT, and RBD palm oil/CF).

Chemical transesterification. Lard (80 g) was dried in an oven (Memmert 854, Schwahbach, Germany) at 100°C for 30 min, and 16 g of anhydrous sodium sulfate was added to remove any residual moisture. The sample was filtered through Whatman No. 2 filter paper and flushed with nitrogen gas before being covered with a glass stopper. Transesterification was carried out using 0.5% sodium methoxide as the catalyst under a constant agitation (for 340 min) in a water bath kept at 80 ± 2°C. To terminate the reaction, the flask was placed in a lukewarm water bath, followed by the addition of 2 g of citric acid to neutralize the catalyst. The citric acid and sodium methoxide were removed with warm water washes (2 × 100 mL). Residual moisture was removed with excess anhydrous sodium sulfate, followed by filtration through a Whatman No. 2 filter paper (10).

GC analysis of fatty acid methyl esters (FAME). FAME were prepared by dissolving the oil (50 mg) with petroleum ether (0.8 mL) and sodium methoxide (1 M, 0.2 mL) (11), and were analyzed on a gas chromatograph (Shimadzu GC-14 A, Kyoto, Japan) fitted with a flame-ionization 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 (for 20 min), and 15°C/min (for 1 min). The temperatures of the injector and detector were maintained at 240°C.

DSC thermal analysis. A PerkinElmer Model DSC-7 (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 aluminum pans, and covers were crimped into 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 (12). The crystallization characteristics of each sample in a DSC scan were obtained using the normalized thermogram. Start (°C) and End (°C) were the starting and ending temperatures of each crystallization transitions. The temperature maximum of a crystallization transition was denoted by Max (°C). Onset (°C) was the temperature where the extrapolated leading edge of the endotherm intersected with the baseline. The value of each DSC parameter used in this work was obtained as illustrated in Figure 1 of Tan and Che Man (15).

Statistical analysis. Three replicates of each sample were analyzed. The SAS/STAT release 6.08 program (13) was used for the stepwise multiple linear regression (SMLR) analysis. The significance level of an independent variable for entry and stay in the calibration model was set to 0.15 during execution of the stepwise variable selection in the SAS procedure "REG." A least significant differences (LSD) test was applied to determine the significant differences between the means of lard adulteration peak temperature and chicken fat adulteration peak temperature at a level of $P < 0.05$.

RESULTS AND DISCUSSION

The measuring principle of DSC is to compare the enthalpy of heat flow to the sample and to the reference materials that are heated or cooled at the same rate. This technique measures the net changes in enthalpy per weight unit and is particularly useful for indicating the temperature range and the rate of thermal processes as well as for giving considerable information on physical and chemical changes. Changes in the sample that are associated with the absorption or evolution of heat cause a change in the heat flow, which is then recorded as a peak. Naturally occurring fats and oils, such as an edible oils composed of a wide variety of TAG, melt and crystallize over a wide range of temperatures (14). Thermal properties of edible oils are closely related to those of TAG. In the heating thermogram of an edible oil, complex features are not easily interpretable. This is a consequence of a known phenomenon of polymorphism of fats and oils that is strongly dependent on the thermal history of the sample (15). Therefore, in this work we focused our attention on the cooling behavior of fats and oils that was found to be mostly influenced by the chemical composition of the sample.

In Figure 1, the DSC cooling profiles of both GLD and CRLD are compared with other common animal fats such as BT, MT, and CF. Based on the information obtained from the cooling profiles, the basic differences in thermodynamic parameters between lard and other animal fats could be summarized as shown in Table 1. Both GLD and CRLD exhibited two major exothermic peaks at 4.9 and –16.9°C, and 10.4 and –16.1°C, respectively. It is apparent from Figure 1, lines A and B that randomization caused a slight peak broadening as

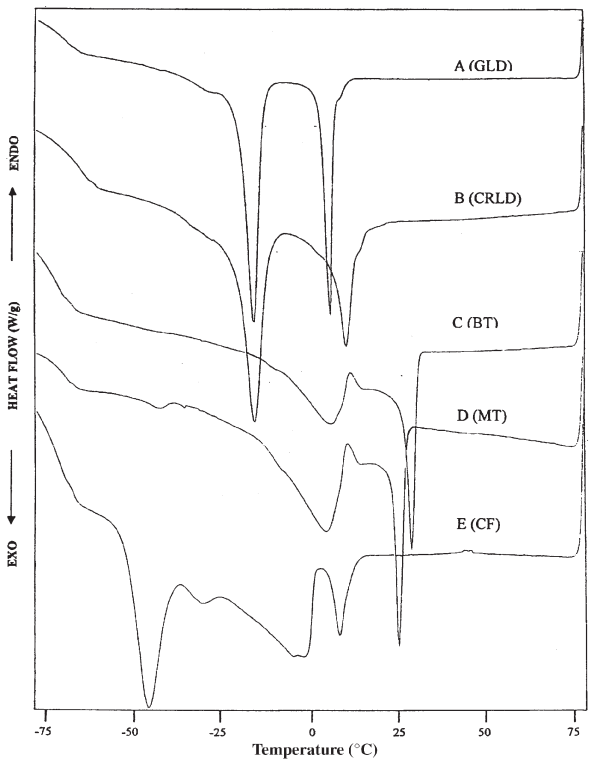

FIG. 1. Differential scanning calorimetry (DSC) cooling thermograms of (A) genuine lard (GLD), (B) chemically randomized lard (CRLD), (C) beef tallow (BT), (D) mutton tallow (MT), and (E) chicken fat (CF).

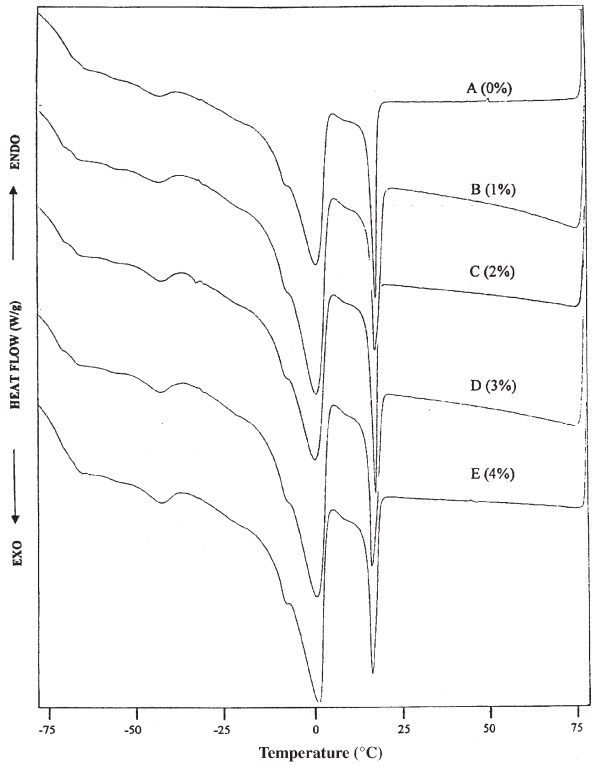

FIG. 2. DSC cooling thermograms of (A) refined-bleached-deodorized (RBD) palm oil, and RBD palm oil adulterated with (B) 1% GLD, (C) 2% GLD, (D) 3% GLD, and (E) 4% GLD. See Figure 1 for other abbreviations.

well as changes in the peak height and position of both peaks. The DSC cooling profiles of other animal fats were totally different from those of lard/randomized lard. BT showed two major peaks at 29.8 and 5.9°C (Fig. 1, line C), while MT was found to have two major peaks at 26.5 and 4.9°C and a minor peak at –43.9°C (Fig. 1, line D). In the case of CF (Fig. 1, line E), there were two major transitions at –1.9 and –47.1°C, while two minor transitions were observed at 8.4 and –27.2°C.

Meanwhile, the DSC cooling thermogram of RBD palm oil, as illustrated in Figure 2, line A, exhibited two major exothermic peaks at 17.8 and 1.3°C. In addition, two small shoulder peaks appeared at –6.8 and –43.9°C. The shoulder peak at –43.9°C is of particular interest since it was found to be sensitive to lard/randomized lard adulteration in RBD palm oil (Figs. 2–5). As lard/randomized lard adulteration level went up from 1 to 20%, this peak was found to gradually increase in

TABLE 1
Comparison of Thermodynamic Parameters of Phase Transition of Genuine and Randomized Lard with Other Animal Fats[a]

		Temperatures of peak(s) transition(s)					
Sample	Peak no.	Start (°C)	Onset (°C)	Max (°C)	End (°C)	Peak height (W/g)	Peak area ΔH (J/g)
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
CRLD	1	24.35	13.50	10.37	–6.46	–0.24	–19.61
	2	–6.82	12.23	–16.11	–32.49	–0.38	–29.78
BT	1	33.51	31.66	29.84	16.65	–0.55	–24.85
	2	11.88	11.21	5.90	–14.16	–0.16	–23.93
MT	1	30.21	28.84	26.49	17.01	–0.36	–12.65
	2	11.15	10.42	4.86	–17.09	–0.15	–25.47
	3	–39.09	–40.30	–43.91	–53.02	–0.01	–0.93
CF	1	16.65	11.60	8.38	3.08	–0.08	–3.75
	2	2.71	1.13	–1.85	–25.89	–0.09	–15.14
	3	–25.89	–27.04	–31.48	–37.99	–0.01	–0.82
	4	–36.89	–41.24	–47.12	–59.62	–0.16	–15.20

[a]Abbreviations: GLD, genuine lard; CRLD, chemically randomized lard; BT, beef tallow; MT, mutton tallow; CF, chicken fat.

size and shift in peak position (peak temperature maximum) toward a higher temperature region. The changes in DSC cooling profile of RBD palm oil due to adulteration with GLD and CRLD are illustrated in Figure 2, lines A–E and Figure 3, lines A–D, and Figure 4, lines A–E and Figure 5, lines A–D, respectively. It is believed that a particular group of lower-melting TAG common to RBD palm oil and lard/randomized lard was the cause for the enlargement of the shoulder peak appearing at –43.9°C, while the shift in peak temperature of the adulteration peak was due to the fact that the oil samples behaved as a binary mixture after having been adulterated with lard/randomized lard. A similar observation was previously reported in the study of lard adulteration in ghee (5).

Further calorimetric analyses were also carried out on mixtures of palm oil adulterated with other common animal fats such as BT (Fig. 6), MT (Fig. 7), and CF (Fig. 8). According to Figure 6, lines A–F and Figure 7, lines A–F, both BT and MT were not found to give adulteration peaks in the temperature region of the lard adulteration peak when blended in the range of 2 to 20% with RBD palm oil. But in the case of CF adulteration in RBD palm oil, the adulteration peak was found to be at a position closer to the lard adulteration peak (Fig. 8, lines A–F). Accordingly, a statistical evaluation of the range of adulteration peak temperatures was done by LSD test and is presented in Table 2. The lard adulteration peak was found to be sufficiently wide apart and could still be distinguishable from the CF adulteration peak. In addition, lard and CF fat

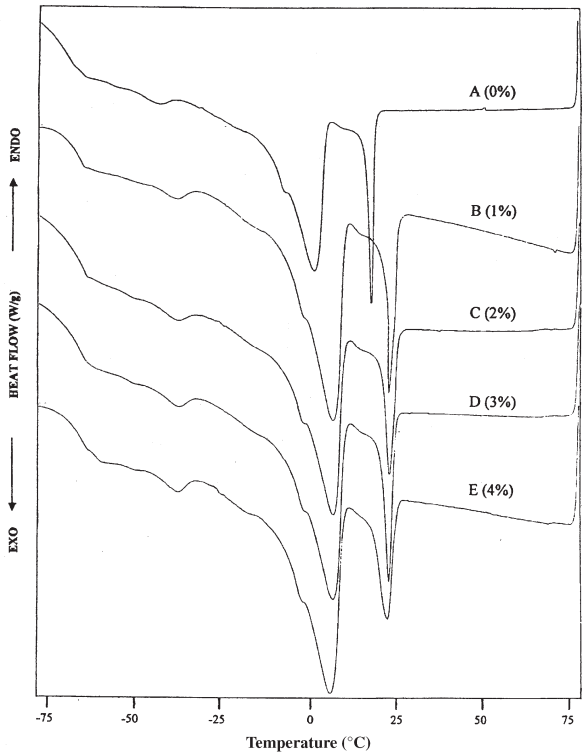

FIG. 4. DSC cooling thermograms of (A) RBD palm oil, (B) 1% CRLD, (C) 2% CRLD, (D) 3% CRLD, and (E) 4% CRLD. See Figures 1 and 2 for abbreviations.

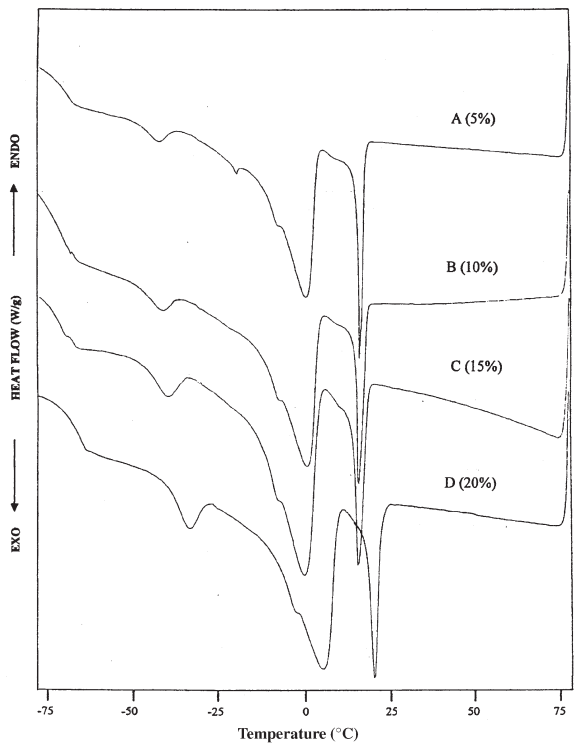

FIG. 3. DSC cooling thermograms of (A) RBD palm oil adulterated with 5% GLD and (B) 10% GLD, (C) 15% GLD, and (D) 20% GLD. See Figures 1 and 2 for abbreviations.

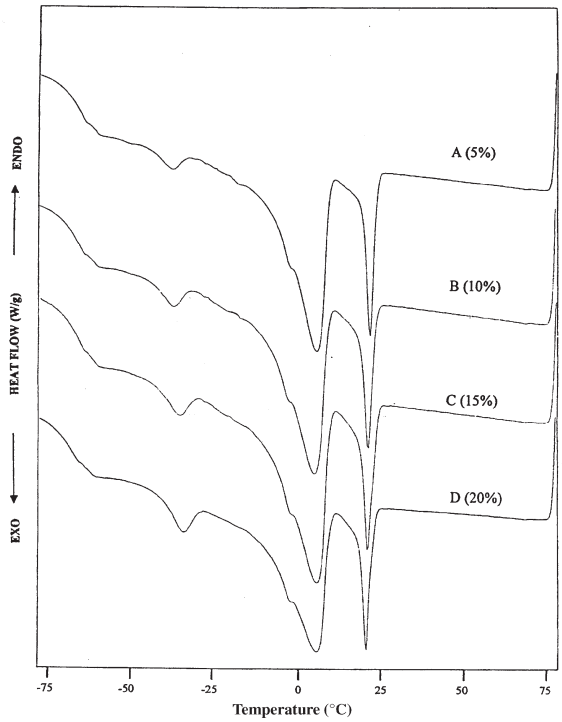

FIG. 5. DSC cooling thermograms of (A) RBD palm oil adulterated with 5% CRLD and (B) 10% CRLD, (C) 15% CRLD, and (D) 20% CRLD. See Figures 1 and 2 for abbreviations.

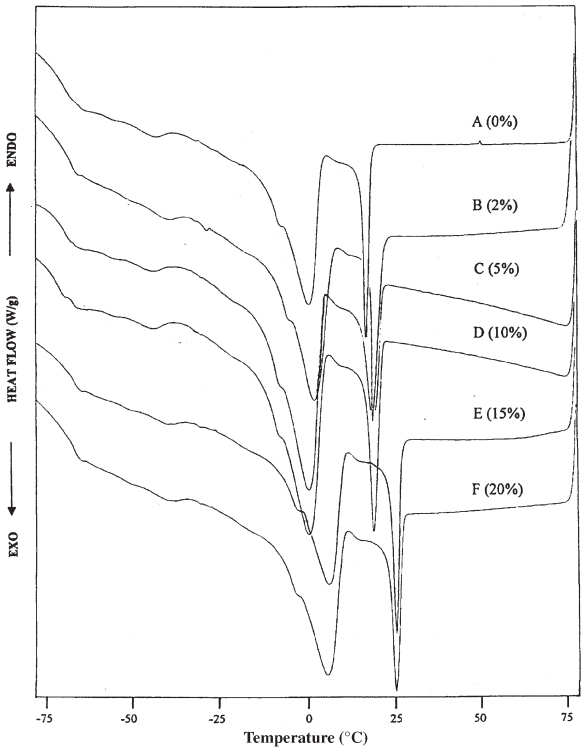

FIG. 6. DSC cooling thermograms of (A) RBD palm oil and (B) RBD palm oil adulterated with 2% BT, (C) 5% BT, (D) 10% BT, (E) 15% BT, and (F) 20% BT. See Figures 1 and 2 for abbreviations.

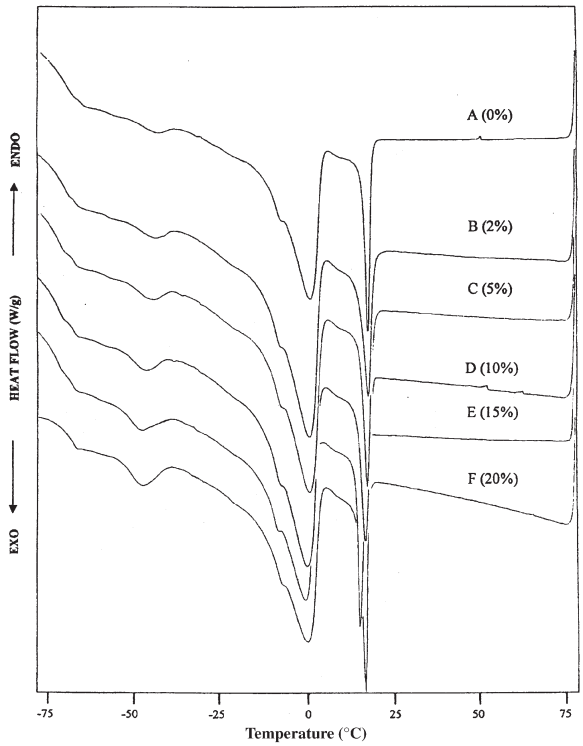

FIG. 8. DSC cooling thermograms of (A) RBD palm oil and (B) RBD palm oil adulterated with 2% CF, (C) 5% CF, (D) 10% CF, (E) 15% CF, and (F) 20% CF. See Figures 1 and 2 for abbreviations.

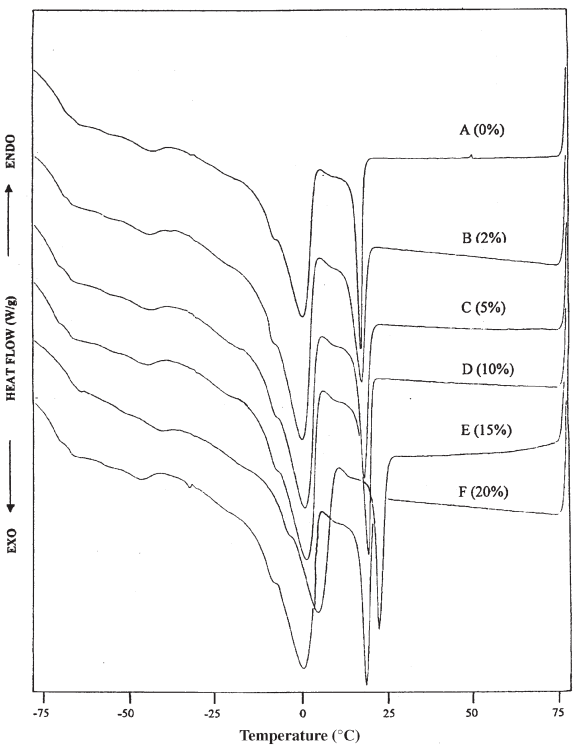

FIG. 7. DSC cooling thermograms of (A) RBD palm oil and (B) RBD palm oil adulterated with 2% MT, (C) 5% MT, (D) 10% MT, (E) 15% MT, and (F) 20% MT. See Figures 1 and 2 for abbreviations.

were found to be different in their fatty acid compositions while there were not many differences in fatty acid composition of GLD and CRLD samples (Table 3). As reported by de Man (16), lard and CF were also quite different in the composition of diunsaturated and triunsaturated TAG. According to Chacko and Perkins (17), the pancreatic lipase hydrolysis technique was commonly used to show the unique composition of pork TAG. In contrast to other common animal fats, lard TAG are mostly esterified by saturated fatty acid (especially palmitic acid) at the *sn*-2 position, making it possible for the adulteration of lard to be detected in RBD palm oil using the DSC cooling thermogram.

In addition to qualitative identification of GLD/CRLD lard in an adulterated oil sample, the particular crystallization peak mentioned earlier may be used for the quantitative determination of GLD/CRLD adulteration in RBD palm oil, as good correlations were observed for different peak parameters against the GLD ($r = 0.9967$, $P < 0.0001$) and CRLD ($r = 0.9892$, $P < 0.0001$) adulteration levels ranging from 1 to 20%. However, the oil samples containing less than 1% GLD/CRLD did not show good correlation with any of the peak parameters. For each sample, three DSC parameters (peak area, A; peak height, HT; and peak onset, ON) were derived from the adulteration peak. These three parameters served as independent variables in the SMLR analysis, with percentage lard (added into palm oil) as the dependent variable. The summary of the SMLR analyses for RBD palm oil blended with GLD and the same blended with CRLD are pre-

TABLE 2
Comparison of Adulteration Peak Temperatures of DSC Cooling Thermograms of RBD Palm Oil Adulterated with GLD, CRLD and CF[a]

Sample	Adulteration peak temperature (°C) (at different adulteration levels)					Mean adulteration peak temperature (°C)
	2%	5%	10%	`5%	20%	
RBDPO	—	—	—	—	—	–43.9
RBDPO + GLD	–39.0	–38.8	–37.7	–35.7	–33.8	–37.0[a]
RBDPO + CRLD	–39.1	–38.6	–37.9	–35.9	–34.0	–37.1[a]
RBDPO + CF	–45.3	–46.1	–47.8	–48.4	–49.2	–47.4[b]

[a]Mean adulteration peak temperatures with different superscripts are significantly ($P < 0.05$) different. Abbreviations: RBDPO, refined-bleached-deodorized palm oil; RBDPO + GLD, RBDPO adulterated with GLD; RBDPO + CRLD, RBDPO adulterated with CRLD; RBDPO + CF, RBDPO adulterated with chicken fat; DSC, differential scanning calorimetry; for other abbreviations see Table 1.

sented in Table 4. The SMLR analysis also showed that only the peak area and peak height were necessary to predict the lard adulteration level in RBD palm oil based on the cooling thermogram.

The regression models used to predict percent lard (GLD)/percent lard (CRLD), as based on the highest R^2 value, are shown in the following equations:

$$\% \text{ lard (GLD)} = 14.2675\ A - 479.9473\ \text{HT} - 10.159 \quad [1]$$

$R^2 = 0.9967$ and $P < 0.0001$. Also,

$$\% \text{ lard (CRLD)} = 14.4511\ A - 570.227\ \text{HT} - 8.6122 \quad [2]$$

$R^2 = 0.9892$ and $P < 0.0001$; A = peak area and HT = peak height of the peak at –37.0°C.

Even though DSC cannot reveal the fine details of the fatty acid composition or TG profiles of edible oils, it provides useful information regarding the nature of the thermodynamic changes that are associated with edible oils transforming from one physical state to another. These thermodynamic characteristics are sensitive to the general chemical composition of edible oils and fats, and thus can be used in qualitative and quantitative ways for identification of edible oils (18). It may be the main reason that DSC is sensitive enough to detect lard adulteration even at 1% level in RBD palm oil. In addition, DSC was found to be able to distinctly identify RBD palm oil samples adulterated with different animal body fats. It is also advantageous that both genuine lard and randomized lard samples were found to show their adulteration peaks in the same temperature region. Therefore, the use of DSC, as presented here, may offer an attractive alternative method to determine lard adulteration in RBD palm oil since it is rapid and requires no sample preparation or use of chemicals to carry out the analysis.

TABLE 3
Fatty Acid Composition of Genuine Lard, Randomized Lard, and Chicken Fat[a]

Fatty acid	GLD (%)	CRLD (%)	CF (%)
12:0	0.24	0.21	0.07
14:0	1.46	1.37	0.79
14:1	—	—	0.21
15:0	—	—	0.34
16:0	25.18	24.58	27.79
16:1	1.69	1.66	6.54
17:0	0.35	0.36	0.23
17:1	—	—	0.41
18:0	8.72	8.82	4.77
18:1	42.44	43.58	44.11
18:2	17.70	17.20	13.71
18:3	0.69	0.65	0.75
20:0	0.20	0.22	—
20:1	0.73	0.79	0.25
20:2	0.52	0.56	0.05

[a]Each value in the table represents the mean of triplicate analyses. For abbreviations, see Tables 1 and 2.

TABLE 4
Summary of Stepwise Regression Analysis with Differential Scanning Calorimetric Crystallization Peak Parameters as Independent Variables[a]

Sample	Step	Regression equation	R^2
RBDPO + GLD	1	% Lard = 8.3436 *A* – 9.7937	0.9864
	2	% Lard = 14.2675 *A* – 479.9473 *HT* – 10.159	0.9967
RBDPO + CRLD	1	% Lard = 8.1777 *A* – 9.1011	0.9837
	2	% Lard = 14.4511 *A* – 570.227 *HT* – 8.6122	0.9892

[a]Abbreviations: *A*, peak area at –37.0°C; *HT*, peak height at –37°C; R^2, coefficient of determination; for other abbreviations, see Table 2.

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