Compositional and Thermal Characterization of Genuine and Randomized Lard: A Comparative Study

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ABSTRACT: Composition and thermal characterization of genuine and randomized lard were investigated comparatively in an attempt to find common merits that assess lard detection. The investigation included compositional and positional distribution of fatty acids, triacylglycerol profiling by gas chromatography (GC) and reversed-phase high-performance liquid chromatography (RP-HPLC), as well as thermal behavior by differential scanning calorimetry (DSC) of both samples. Individual and total saturated and unsaturated fatty acid composition in total fats of both genuine and randomized lard were identical. On the other hand, the results of pancreatic lipolysis/GC analysis showed that the average percent palmitic acid [PAEF(%)] and myristic acid [MAEF(%)] enrichment factors of genuine (280 and 270) and randomized lard (110 and 98) were quite different. Thus, application of PAEF to detect randomized lard is of no value. However, normalization of fatty acid distribution by randomization in 2-monoacylglycerols made the individual and total saturated and unsaturated fatty acids almost identical to that of total fat and neutral triacylglycerols (TG) of lard. TG compositional analysis by GC revealed that both genuine and randomized lard had six dominant TG (C_{46}, C_{48}, C_{50}, C_{52}, C_{54}, and C_{56}) with quite different concentrations. TG with C_{52} represent the major constituent of genuine and randomized lard. TG profiling of samples was also carried out by RP-HPLC with a refractive index detector. The same peaks were eluted in both samples, but the area % of major peaks changed due to randomization. 2-Palmitooleostearin (SPO) was found in high proportion in lard. However, the ratios of SPO to 2-palmitooleolinolein of both genuine and randomized lard are close (0.6 ± 0.05) and significantly distinguishable from that of beef (4.24), mutton (6.17), chicken (0.21), and turkey (0.14) fats. The DSC thermogram and thermodynamics of phase transitions of both samples were quite different and do not reveal common characteristic(s) that could be used for immediate detection of lard substances in fat admixtures.

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Dietary lipids have been shown to alter the lipid composition of adipocytes in muscle and in other tissues (1). Laboratory animal model studies have demonstrated that the fatty acid composition in lipids is the determining factor in nutrient transport, risk for intestinal diseases, and colon tumors (1,2). Diets rich in lard are known to cause hypercholesterolemia, aortic lesions, aortoiliac atherosclerosis, coronary heart disease, and multiple sclerosis (3–7). Lard is also associated with the risk of breast, pancreas, and colon cancer (2,8–11), and appears to act both at initiation and during promotion of carcinogenesis (12). A Food and Agricultural Organization (FAO) survey of 36 countries showed significant correlations between dairy and lard intake and the incidents of cancer in different organs, such as breast, prostate, rectum, colon, and lungs (13). It also showed a significant relationship between lard-caused site-specific cancer mortality and ischemic heart disease mortality (6,13). Therefore, several scientists have recommended that the general U.S. population reduce the consumption of dietary lipids to diminish the risk of chronic diseases (1,6,13). The Islamic and Orthodox Jewish religions prohibit the consumption of both pork and lard derived from pigs in any products. In view of the biological complications and risk of diseases associated with pork and lard and the restrictions on their consumption by some religions, a reliable method is required for the detection of lard in its various forms to enforce restriction of such products.

Pig fats, both genuine and industrially-modified randomized lard (shortening), are the cheapest fats. Commercial shortenings are usually prepared by interesterification of the fat samples. Randomization of lard improves physical properties because it changes the arrangement of the esterified acyl groups within the components of the mixed triacylglycerols (TG) of naturally-derived fats (14–16). Randomization is effectively done by metal alkoxide, such as sodium methoxide (14). The currently utilized methods of lard detection have been designed and carried out only on genuine fat without considering interesterification of lard (17–25). These methods include crystallographic microscopy (17), gas chromatography (GC) (18–23), high-performance liquid chromatography (HPLC) (16,24), and differential scanning calorimetry (DSC) (25).

The analysis of randomized lard by DSC, HPLC, and GC (for TG analysis) has not yet been reported. This paper describes thorough comparative investigations of compositional and positional distribution of fatty acids, TG profiling by GC and reversed-phase (RP)-HPLC/refractive index (RI) detector, as well as thermal behavior by DSC of both genuine and randomized lard.

EXPERIMENTAL PROCEDURES

Materials. Genuine pork was obtained from the Amon Factories (Shoubra, Cairo, Egypt) for pork and processed foods. Fats were extracted from the minced samples by a modified Folch *et al.* (16) procedure with $CHCl_3/CH_3OH$ (2:1, vol/vol) as solvents. Fatty acid methyl esters (FAME) were prepared by alkali-catalyzed (1% CH_3ONa in anhydrous CH_3OH) esterification. Fatty acids, FAME, and TG, used as authentic standards, were purchased from Nu-Chek-Prep (Elysian, MN) and Sigma Chemical Co. (St. Louis, MO). Lipase (E.C. 3.1.1.3) from hog pancreas was obtained from Fluka Chemie (Buchs, Switzerland). HPLC-grade solvents were used. All reagents were of reagent grade.

Randomization of lard. Dehydration of the lard sample (~12.5 g) was completed by heating with vigorous stirring at about 105°C for 30 min. The melted fat, in a suction flask, was allowed to cool to ~80°C before adding 6.2 mL of 1% CH_3ONa in freshly prepared absolute CH_3OH. Thermally controlled heating, at 80°C for 30 min, was allowed under vacuum before adding an equal volume of H_2O and extracting with $(C_2H_5)_2O$ (4 × 15 mL). The combined ether extract was washed with warm H_2O (5 × 25 mL), then dried over anhydrous Na_2SO_4. Evaporation of the organic solvent was undertaken under vacuum at room temperature.

Isolation of neutral TG. A small chromatographic column (14 cm × 2 cm, i.d.) was properly packed with a slurry of 6 g silica gel 100 (70–230 mesh) in n-C_6H_{14}. Once the silica had settled, 0.5 g of the dried fat in 0.5 mL n-C_6H_{14} was carefully introduced into the top of the column, and the sample was allowed to adsorb on the surface of the silica. The column was eluted with 20 mL n-C_6H_{14} followed by 5% $(C_2H_5)_2O$ in n-C_6H_{14} until the TG were completely eluted. Fractions (20-mL each) were collected, and the solvent was removed under vacuum. The purity of the TG fractions was assessed on thin-layer chromatography (TLC) plates.

Pancreatic lipolysis of neutral TG. Pancreatic lipolysis of a part of TG was carried out according to the International Union of Pure and Applied Chemistry (IUPAC) standard method No. 2.210 (18). The 2-monoacylglycerols (2-MG) were separated on H_3BO_3–TLC plates with $CHCl_3/(CH_3)_2CO$ (96:4, vol/vol). The developed plates were air-dried and visualized under ultraviolet light spraying with 0.2 g % solution of 2′,7′-dichlorofluorescin in 95% C_2H_5OH. TLC reference standard (Code No. 18-1; Nu-Chek-Prep) was used to identify the 2-MG band. The silica layer carrying 2-MG was scraped and eluted with $CHCl_3/CH_3OH$ (95:5, vol/vol). The solvent was removed under a stream of N_2. The FAME of 2-MG were prepared by alkali-catalyzed transesterification.

Analytical methods. The purity of FAME and TG was assessed on TLC plates (silica gel 60, 5 cm × 10 cm, 0.25 mm) with n-$C_6H_{14}/(C_2H_5)_2O/CH_3COOH$ (90:10:1, vol/vol/vol). Visualization of the spots was accomplished by charring the plates after spraying with 20% $HClO_4$ (aqueous). Preparative boric acid-impregnated TLC plates (20 cm × 20 cm) of silica gel G (Fluka AG) were made in 0.4 N solution of boric acid. Preactivation of the plates at 110°C for 30 min was done on the day of fractionation.

GC analysis was carried out on a Varian (Sunnyvale, CA) model 3700 gas chromatograph with a Hewlett-Packard (Palo Alto, CA) HP 3396 Series II curve integrator. GC conditions: System 1 (for FAME), 15% OV-275 on Chromosorb-W (80/100 mesh) stainless-steel packed column (2 m × 2 mm i.d.; Varian); programmed oven temperature, 140–220°C (10°C/min); carrier gas N_2 (30 mL/min); flame-ionization detector (FID), 290°C. System 2 (for TG), glass column (50 cm × 2 mm i.d.) packed with 3% OV-1 on 100/120 mesh Gas Chrom Q (Custom Fabricated; Altech Associates, Inc., Deerfield, IL); glass column conditioning, first, column was maintained at 50°C for 15 min with 40 mL/min N_2 flow, then heated up to 355°C for 1°C/min at 10 mL/min N_2 and held at 355°C for 15 h; operating conditions, oven temperature 210°C for 1 min, then programmed at 6°C/min to 340°C, final hold, 7 min; FID, 370°C. Resolved peaks were identified by matching with standard mixtures.

HPLC analysis of TG was performed on a LiChrospher 100-RP-18 (5 μm) column (12.5 cm × 4 mm i.d.; E. Merck, Darmstadt, Germany), an RI detector (differential refractometer R401; Waters, Milford, MA) and CH_3CN/CH_2Cl_2 (58:42, vol/vol) as the mobile phase (flow rate 1 mL/min at ambient temperature). Shimadzu's (Kyoto, Japan) HPLC system was used, consisting of a solvent delivery module (LC-10AD) connected to a solvent mixer (FCV-10AL), a degasser (DGU-3A), a column embedded in a column oven (CTO-10A), and a C-R4A chromatopac multifunctional data processor.

DSC measurements. DSC measurements were done on a DuPont (Boston, MA) TA 9900 computer/thermal analyzer. The analysis was done after calibration of the instrument with 3–5 mg of the sample under constant flow of N_2 (150 mL/min) and heating rate (10°C/min). The semi-solid fat samples were heated to about 70°C in a closed glass container and placed by a disposable 10-μL pipette into the weighed pan. The sample was placed in the middle of the pan. The pan was then covered, cramped, and weighed again to ensure that there was no weight loss during pan cramping. The data were analyzed with the General Program (V 2.2; DuPont) to give directly the DSC scan and thermodynamic parameters. The perpendicular drop technique was used to determine the partial enthalpy (ΔH_i) of each step, as shown in Figure 1. The percentage of peak area was calculated from Equation 1 as the ratio of the partial enthalpy (ΔH_i) to the total enthalpy (ΔH_t).

$$\text{percentage of peak area} = \frac{\Delta H_i}{\Delta H_t} \times 100 \quad [1]$$

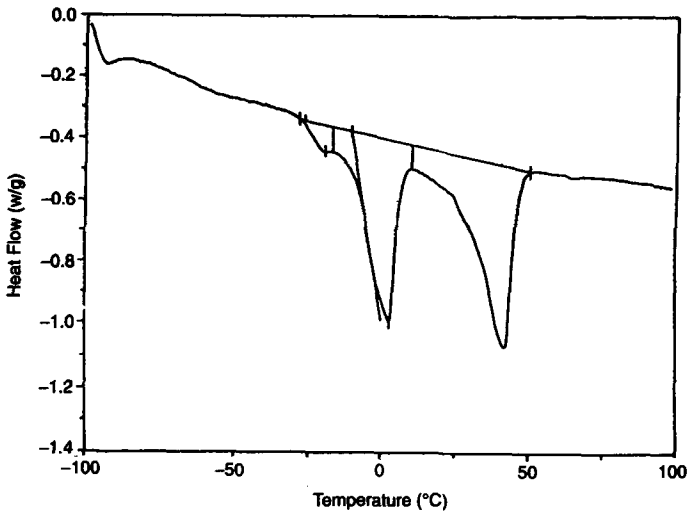

FIG. 1. Differential scanning calorimetry thermogram of free fatty acid content of lard illustrates the perpendicular drop technique used for calculation of percentage peak area.

RESULTS AND DISCUSSION

To identify the presence of lard in its genuine or randomized forms, investigators must find common compositional or thermal characteristics that can be used for its detection. Current methods of detection (17–25) do not consider interesterification of lard. Therefore, comparative investigation of compositional and thermal parameters of both genuine and randomized lard is undertaken in this article to find common characteristic merits (or ratios) in fatty acids, their distribution in the glycerol molecule, and TG profiling by both GC and RP-HPLC/RI methods.

Fatty acid compositional and distributional analyses. A comparative fatty acid composition (%) of genuine and randomized lard in total fat, neutral TG, 2-MG, and the calculated 1,3-diglyceride (1,3-DG) are shown in Table 1. The fatty acid composition in total fat of both genuine and randomized lard are almost identical. A low concentration (0.49%) of 11,14-eicosadienoic acid ($C_{20:2}$) was found in both genuine and randomized lard. The sum of saturated and unsaturated fatty acids are 39.27 and 60.73%, respectively. The percentage of myristic ($C_{14:0}$), palmitic ($C_{16:0}$), and stearic ($C_{18:0}$) acids of the total saturated fatty acids in total fats of both samples are 4.41, 59.61, and 35.8 respectively. On the other hand, the percentage of palmitoleic ($C_{16:1}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and linolenic ($C_{18:3}$) of both samples with respect to total unsaturated fatty acids are 6.77, 57.7, 29.7, and 3.38, respectively. Fatty acids in neutral TG of both genuine and randomized lard were also identical in total saturated (39.95), unsaturated (60.05), and the percentage of their components ($C_{14:0}$ = 4.27, $C_{16:0}$ = 59.56, $C_{18:0}$ = 35.96, $C_{16:1}$ = 6.81, $C_{18:1}$ = 58.9, $C_{18:2}$ = 28.43, and $C_{18:3}$ = 4.92).

Although the total fatty acid composition of both genuine and randomized lard is identical, results of pancreatic lipolysis of neutral TG showed that there is a significant difference in the positional distribution of fatty acids in the neutral TG of genuine and randomized lard. The TG in lard are esterified with 75.82% saturated and only 24.08% unsaturated fatty acids at the C-2 position of the glycerol molecule. In addition, the percentages of $C_{16:0}$ and $C_{18:1}$ in 2-MG of lard are 89.26 and 43.23 with respect to their corresponding total saturated and unsaturated fatty acid contents, respectively. However, randomization of lard changed the fatty acid distribution to 41.9% saturated and 58.1% unsaturated fatty acids at the C-2 position and made the fatty acid composition of 2-MG almost identical to that of total fat and neutral TG (Table 1).

The calculated 1,3-DG composition of lard also reveals that unsaturated fatty acids (76.48%) are concentrated at the 1,3-position of glycerol, of which 48.59% is oleic acid. In addition, the percentage of individual fatty acids of randomized lard in 2-MG and 1,3-DG are almost identical to that of the total fat and of the neutral TG of both genuine and randomized lard. Consequently, interesterification of lard normalizes

TABLE 1
Fatty Acid Compositions (%) of Genuine and Randomized Lard in Total Fats, Neutral Triacylglycerols (TG), 2-Monoacylglycerols (MG), and the Calculated 1,3-Diacylglycerols (1,3-DG)

		Fatty acids (methyl esters)										
Fat sample		$C_{12:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:2}$
Genuine	Total fat[a]	trace[b]	1.60	trace	23.67	4.00	trace	15.05	34.67	17.13	2.05	0.55
Randomized	Total fat[a]	trace	1.86	0.10	23.16	4.22	trace	13.10	35.36	18.89	2.06	0.43
Genuine	TG[c]	trace	1.62	trace	24.14	4.11	trace	15.15	35.86	16.50	1.55	0.37
	2-MG[c]	trace	4.37	0.12	67.68	7.27	trace	3.65	10.41	5.24	0.08	0.11
	1,3-DG[d]	—	0.25	—	2.37	2.53	—	20.9	48.59	22.13	2.29	0.50
Randomized	TG[c]	trace	1.79	0.16	23.45	4.07	0.50	13.58	34.88	17.65	2.81	0.54
	2-MG[c]	trace	1.77	0.73	25.86	3.55	1.74	13.54	33.08	15.86	2.73	0.50
	1,3-DG[d]	—	1.80	—	22.25	4.33	—	13.60	35.78	18.54	2.85	0.56

[a]Average of nine determinations, and some minor fatty acids are not included. [b]<0.1. [c]Average of three determinations, and some minor fatty acids are not included. [d]1,3-DG = {[(3 × TG) − (2-MG)]/2}.

the distribution of fatty acid in the three esterified sites of the glycerol molecule.

Application of pancreatic lipolysis/GC analysis showed that the percent palmitic acid enrichment factor [PAEF(%); percent ratio of palmitic acid in 2-MG to its overall percent in TG] of genuine lard (280%) and randomized lard (110%) are quite different. This finding is in good agreement with the published PAEF(%) of both lard (275–289) (19) and randomized lard (101) (26). In addition, the percent myristic acid enrichment factor [MAEF(%)] of genuine lard (270%) is of the same magnitude as its PAEF(%), and is higher than that of other fatty acids. Moreover, the ratio of fatty acid distribution in the C-2 position (2-MG) and in TG of all fatty acids, expressed in percentage enrichment factor (EF%), were calculated and are shown in Figure 2. The calculated EF% of fatty acids of randomized lard showed that interesterification of lard by 1% sodium methoxide normalized the fatty acid distribution in the C-2 position to give an overall value of EF% = 96.3 for all major fatty acids. The EF% of $C_{16:1}$ of lard (177) and randomized lard (87) showed wide variability.

TG compositional analyses. Table 2 shows the TG composition (%) as obtained by GC analysis. The separated TG peaks were recognized by their carbon numbers relative to those of standard TG (Fig. 3) and palm oil. (A short-hand nomenclature is used to designate TG. The total number of carbon atoms in the aliphatic chains of the TG, excluding the carbon number of the glycerol moiety, are calculated, and this number is used to denote the compound. For example, palmitostearinolein, palmitodiolein, and palmitoleiolinolein are referred to as C_{52} TG or having a carbon number of 52.) Figure 3 also displays comparative TG profiling of both genuine and randomized lard by GC. Six designated TG were identified (C_{46}, C_{48}, C_{50}, C_{52}, C_{54}, and C_{56}), and their concentrations are quite different. However, TG with C_{52} represent the major constituents of both genuine lard (61.1%) and randomized lard (34.2). Randomization reduced C_{52} by 47% and increased remaining TG. The GC chromatogram of lard has a similar pattern and peak area percentages as reported by Precht (23).

TG profiling of genuine and randomized lard was also carried out by RP-HPLC with an RI detector. The identification of sequential separated TG was done with standard TG and

TABLE 2
Triacylglycerol Composition (%) of Genuine and Randomized Lard by Gas Chromatography

	Triacylglycerol concentration (%)[a]					
Fat sample	C_{46}	C_{48}	C_{50}	C_{52}	C_{54}	C_{56}
Genuine	0.13	1.79	15.17	61.08	21.56	0.27
Randomized	1.67	8.02	25.53	34.16	25.37	5.25

[a]Average of 2–3 determinations.

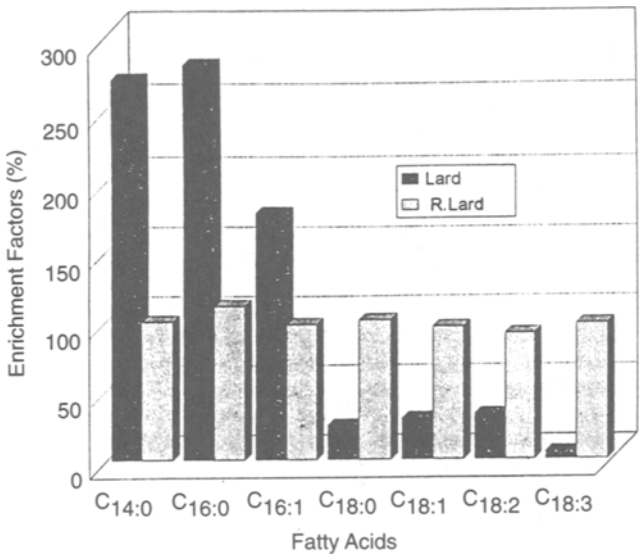

FIG. 2. Comparative fatty acid enrichment factors (%) of both genuine and randomized lard.

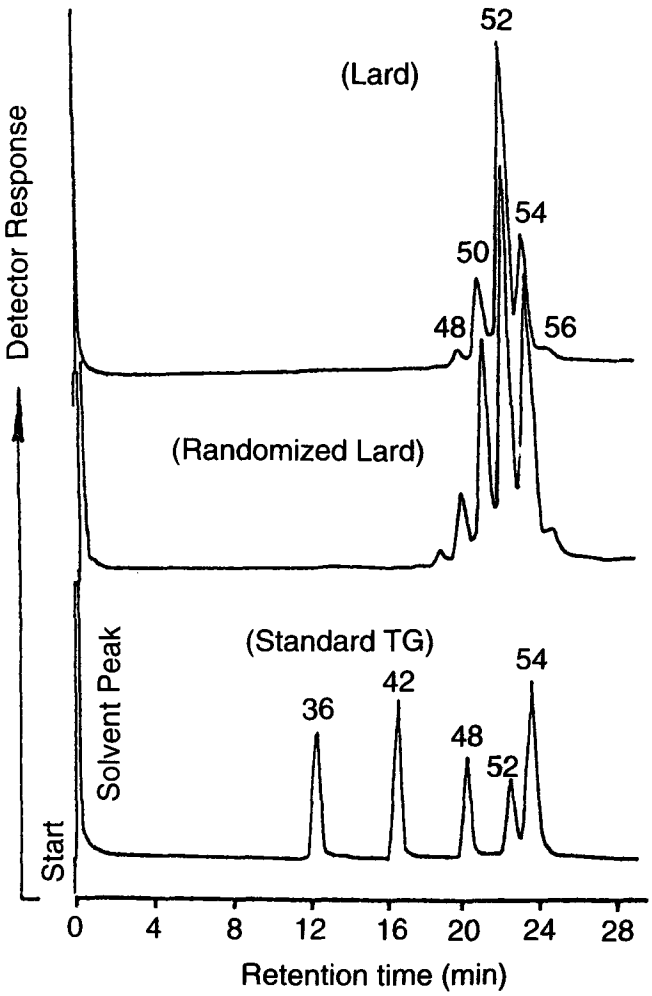

FIG. 3. Gas chromatographic separation of standard mixed triacylglycerols (TG) of genuine and randomized lard on glass column [number used to denote TG is the total number of carbon atoms in the aliphatic chains of the TG (excluding the carbon number of glycerol moiety); for example, palmitostearolein (PSO), palmitodiolein (POO), and palmitooleiolinolein (POL) are referred to as C_{52} TG or having a carbon number of 52].

palm oil. The elution order of some TG was predicted on the basis of theoretical carbon number (27) and the standard TG. Fourteen peaks were eluted in both samples, but the area percentages of the majority of these peaks were changed due to randomization of the lard. Comparative TG profiles of the two sample are given in Figure 4 and the peaks area % are tabulated in Table 3. Comparing the TG profiles of lard, randomized lard, and other animal fats (unpublished data) shows that peak #12 [2-palmitooleostearin (SPO)] is present in a higher proportion in lard (13.17%) than in randomized lard (8.58%), chicken (3.89%), and turkey (2.75%). Filer *et al.* (15) and Hawley and Holman (28) reported that lard contains a higher proportion of SPO.

The ratio of peak #12 (SPO) and peak #7 [tentatively: 2-palmitooleolinolein (LPO)] for lard, randomized lard, beef, mutton, chicken, and turkey are 0.66, 0.56, 4.24, 6.17, 0.21, and 0.14, respectively. This reveals that the ratio of SPO/LPO of both genuine lard and randomized lard are close (0.61 ± 0.05) and significantly distinguishable from other common animal fats. Table 3 discloses that the concentration of 2-palmitodiolein (18.9) and LPO (13.4) are almost identical for both genuine and randomized lard, but cannot be used as distinguishable merits from other common fats.

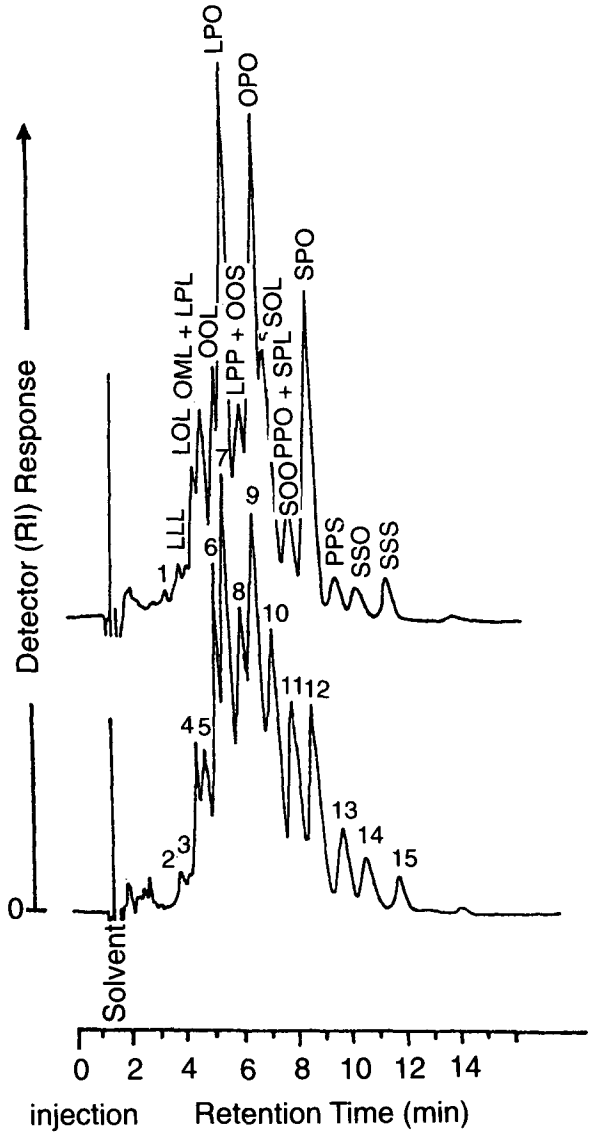

FIG. 4. Triacylglycerol profiles of genuine and randomized lard by reversed-phase high-performance liquid chromatography/refractive index detection. Peak numbers and their tentative identification are given as in Table 3. Abbreviation as in Figure 3.

TABLE 3
Triacylglycerol (TG) Composition of Genuine and Randomized Lard by High-Performance Liquid Chromatography

Peak no.[a] (TG)	Retention time (min)	Concentration (%)[b]	
		Genuine	Randomized
1	3.43	0.11	—
2 (LLL)	3.91	1.03	0.68
3	4.21	0.89	0.54
4	4.50	3.72	3.75
5	4.79	6.92	5.09
6	5.26	5.40	7.88
7	5.59	19.99	15.30
8	6.26	8.08	11.76
9 (OPO)	6.70	19.31	18.50
10	7.11	13.46	13.32
11	8.02	4.19	8.88
12 (SPO)	8.66	13.17	8.58
13	9.71	1.00	2.64
14	10.49	0.99	1.77
15	11.61	1.74	1.31

[a]Tentative identification of peaks numbers 1, 4, 5, 6, 7, 8, 10, 11, 13, 14, and 15 are LLLe, LOL, OML + LPL, OOL, LPO, LPP + OOS, SOL + PPO + SPL, SOO, PPS, SSO, and SSS, respectively. Each TG represents all possible isomers of constituent fatty acids, where L = $C_{18:2}$; Le = $C_{18:3}$; O = $C_{18:1}$; P = $C_{16:0}$; M = $C_{14:0}$; and S = $C_{18:0}$.
[b]Average of three determinations.

Thermal characterization. The measuring principle in DSC is to compare the rate of heat flow to the sample and to inert material that are heated or cooled at the same rate. Changes in the sample that are associated with absorption or evolution of heat cause a change in the differential heat flow, which is then recorded as a peak. The area under the peak is directly proportional to the enthalpic change and is representative of composition. Therefore, thermal properties of fats and oil are closely related to those of TG that constitute the major part of the lipids. In addition, thermal behavior of a TG admixture is described by phase diagrams with phase temperature boundaries (29,30) and thermodynamic parameters (31). Figure 5 shows the comparative DSC thermograms of both genuine and randomized lard under a constant stream of N_2 (150 mL/min) and heating rate (10°C/min). The thermogram of genuine lard is quite different from that of randomized lard, and these differences could be attributed to the different TG composition (Tables 2 and 3) that resulted from interesterification. Thermodynamic parameters of phase transitions of both genuine and randomized lard are shown in Table 4. Thermal transitions of genuine lard occurred in three steps with peak maxima at –0.35, 30.25, and 44.28°C. The total heat of transition is equal to 107.14 J/g with an overall peak maximum of 30.25°C. On the other hand, thermal transitions of

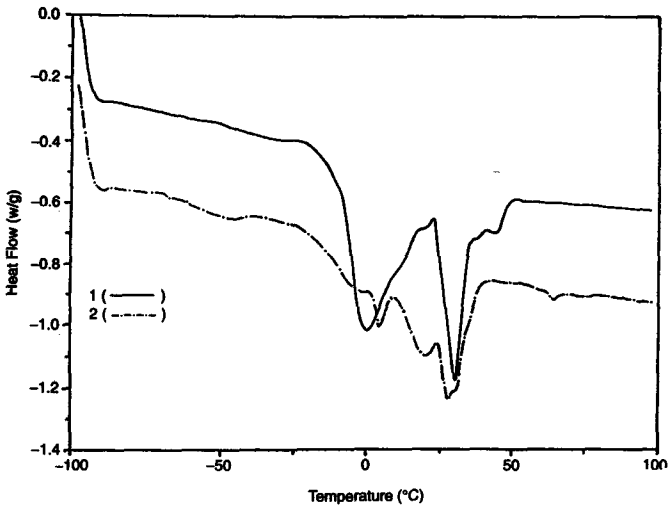

FIG. 5. Comparative differential scanning calorimetry thermograms of genuine (1) and randomized (2) lard. Sample size is 4–5 mg, heated at 10°C/min under constant flow of N_2 gas (150 mL/min).

randomized lard occurred in five steps with peak maxima at −51.26, −1.44, 4.3, 19.04, and 27.8°C. The total heat of transition is reduced to 62.73 J/g with an overall peak maximum of 27.8°C. The percentage of peak area of the thermal transitions that occurred below 0.0°C is 62.9 and 19% for genuine and randomized lard, respectively. This finding explains the improved texture of randomized lard, but does not reveal common characteristics that could be used for immediate detection of lard in fat admixtures.

In conclusion, the fatty acid composition in the total fat of both genuine and randomized lard was almost identical in both individual and total saturated and unsaturated fatty acids. Thus, the application of PAEF(%) of randomized lard is of no value for detection. However, normalization of the fatty acid distribution in 2-MG by randomization made the individual and total saturated and unsaturated fatty acids almost identical to that of the total fat and neutral TG of lard.

TG compositional analysis of both genuine and randomized lard by GC and RP-HPLC/RI methods revealed that randomization of lard kept the number of dominant TG, and their isomers, constant, but changed their concentrations. The RP-HPLC/RI method disclosed that SPO is present in high proportion in lard, and its peak could be used for visual detection of lard in fat specimens. In addition, the ratio of SPO to LPO of both genuine and randomized lard was found to be 0.61 ± 0.05 and quite different from that of beef, mutton, chicken, and turkey fats.

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TABLE 4
Thermodynamic Parameters of Phase Transitions of Neutral Triacylglycerols of the Genuine and Randomized Lard from Differential Scanning Calorimetry

		Temperature of peak(s) transition(s)						
Name	No. of peaks	Start (°C)	Onset (°C)	Max (°C)	End (°C)	Peak height (W/g)	Peak area ΔH (J/g)	Percentage of peak area
Genuine	1	−22.75	−9.61	−0.35	22.09	−0.55	67.40	62.90
	2	22.09	21.73	30.25	41.14	−0.63	35.30	33.00
	3	41.14	—	44.28	50.19	−0.13	4.40	4.10
	Total	−22.75	21.73	30.25	50.19	−0.63	107.10	100.00
Randomized	1	−69.41	—	−51.26	−41.63	−0.03	2.68	4.80
	2	−41.63	−16.99	−1.44	1.62	−0.14	9.26	14.80
	3	1.62	—	4.30	9.16	−0.24	8.13	13.00
	4	9.16	—	19.04	23.84	−0.29	19.91	31.70
	5	23.84	20.34	27.80	40.89	−0.41	22.73	36.20
	Total	−69.41	20.34	27.80	40.89	−0.41	62.71	100.00

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