Composition and Thermal Analysis of Lard Stearin and Lard Olein

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Abstract: Lard being an edible fat could be used in different forms in food systems. In this study, composition and thermal analysis of lard stearin (LS) and lard olein (LO) were undertaken to determine some common parameters which would enable their detection in food. A sample of native lard was partitioned into LS and LO using acetone as solvent and the fractions were compared to the original sample with respect to basic physico-chemical parameters, fatty acid and triacylglycerol (TAG) composition, and thermal characteristics. Although LS and LO displayed wider variations in basic physico-chemical parameters, thermal properties and solidification behavior, they do possess some common characteristic features with regard to composition. In spite of the proportional differences in the major fatty acids, both LS and LO are found to possess extremely high amount of palmitic (C16:0) acid at the *sn*-2 positions of their TAG molecules. Similar to native lard, both LS and LO contained approximately equal proportions of TAG molecules namely, linoleoyl-palmitoyl-oleoyl glycerol (LPO) and dioleoyl-palmitoyl glycerol (OPO). Hence, the calculated LPO/OPO ratio for LS and LO are comparably similar to that of native lard.

Key words: food adulteration, DSC, lard detection, lard stearin, lard olein, thermal analysis

1 INTRODUCTION

Animal fats such as lard and tallow have long been recognized as raw material for food^{1,2)} and industrial applications^{3,4)}. Lard may be used in its raw form as frying medium⁵⁾ or after modification of its physical properties as shortening for baking applications⁶⁾. Firmness of lard was considered as an important property as firm-lard could resist rancidity development⁷⁾ as well as meet a range of industrial requirements. Firmness of lard was improved through blending with hydrogenated stock²⁾ or hard component isolated from fractional crystallization. In the past, the separation of hard component of lard (stearin) has been accomplished through techniques such as short-path distillation⁸⁾, supercritical carbon dioxide extraction⁹⁾, or dry and solvent crystallization^{10,11)}. Although stearin is the mainly targeted product, a fair proportion of the liquid component is also recovered as the co-product of fractionation. While stearin has been used to improve the firmness of lard, olein isolated was found application as frying medium or bread pan lubricating agent. In fact, the use of lard or its fractionated products in food is prohibitive under halal and kosher food regulations¹²⁾. As a result, there has been a great deal of interest to develop analytical methodologies to detect

lard in food systems^{5,13,14)}. Most of the past researches, however, were mainly focused on the detection of genuine lard without considering lard occurring in the modified forms such as stearin and olein. Up to date, only a little information could be found on the composition and thermal curves of lard stearin and lard olein, though establishing their identity characteristics for detection purposes has become important. The information of this kind would be greatly helpful for food control authorities who are required to carry out routine test on commercial products that are suspected to contain lard. Hence, the objective of this study is to identify characteristic properties common to native lard and its fractions using gas liquid chromatography (GLC), high performance liquid chromatography (HPLC), differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR) spectroscopic techniques.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

Lard was extracted using adipose tissues of swine col-

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lected from local slaughter houses according to the method reported previously by Marikkar *et al.*¹³⁾. All chemicals used in this experiment were of analytical or HPLC grade.

2.2 Methods

2.2.1 Fractional crystallization of lard

Fractional crystallization was carried out using acetone as solvent medium. Lard was melted at 60°C and mixed with acetone in 1:2 (w/v) ratio. The solution was boiled at 60°C until become uniformly dissolved and left at 5 ± 1°C for 24 h to crystallize. The precipitated fat was filtered off to give a high melting fat fraction (LS). After removing the precipitate, the mother-liquor was evaporated under reduced pressure to yield a liquid called low-melting fraction (LO).

2.2.2 Determination of cloud point, slip melting point and iodine value

CP, SMP and IV of the fat samples were determined according to AOCS method Cc.6.25, AOCS method Cc.3.25, and AOCS method Cd Id-92, respectively¹⁵⁾.

2.2.3 Determination of fatty acid composition

FAME were prepared by dissolving 50 mg portion of oil in 0.8 mL of hexane and adding 0.2 mL portion of 1 M solution of sodium methoxide¹⁶⁾. The top hexane layer was injected on an Agilent 6890N gas chromatograph (Agilent Technologies, Singapore) equipped with a polar capillary column RTX-5 (0.32 mm internal diameter, 30 m length and 0.25 µm film thickness; Restex Corp., Bellefonte, PA) and a Flame Ionization Detector (FID). Split injection was conducted with a split ratio of 58:1, nitrogen was used as a carrier gas at a flow-rate of 1.00 mL/min. The temperature of the column was 50°C (for 1 min), and programmed to increase to 200°C at 8°C/min. The temperatures of the injector and detector were maintained at 200°C⁵⁾.

2.2.4 Analysis of fatty acids at the *sn*-2 position

The distribution of fatty acids at the *sn*-2 position of the TAG molecules was determined according to the modified method of Luddy *et al.*¹⁷⁾. Neutral TAG of oil samples were isolated using column chromatography and hydrolyzed with hog pancreatic lipase (Fluka Chemie, Buchs, Switzerland). The resulting 2-monoacylglycerols (2-MAG) were isolated by using TLC plates placed on tank containing mixture of hexane/diethyl ether/acetic acid (50:50:1, vol/vol/vol). FAME of isolated samples of 2-MAG were then determined as described previously. PAEF [PAEF (%): percent ratio of palmitic acid in 2-MAG to its overall percent in TAG] for each oil sample was calculated⁵⁾.

2.2.5 Determination of TAG composition

The TAG compositions of samples were determined by using Waters Model 510 liquid chromatography equipped with a differential refractometer Model 410 as the detector (Waters Associates, Milford, MA). The analysis of TAG was performed on a Merck Lichrosphere RP-18 column (5 µm) (12.5 cm × 4 mm i.d.; Merck, Darmstadt, Germany) which

was maintained at 30°. The mobile phase was a mixture of acetone:acetonitrile (63.5:36.5) and the flow rate was 1.5 mL/min. The injector volume was 10 µL of 5% (w/w) oil in chloroform. Each sample was chromatographed two times, and the data were reported as peak area percentages⁵⁾. The identification of the TAG peak of the samples was done in accordance with the TAG profiles of lard reported previously by Rashood *et al.*¹⁴⁾.

2.2.6 Thermal analysis

Thermal analysis was carried out on a Mettler Toledo differential scanning calorimeter (DSC 823 Model) equipped with a thermal analysis data station (STARe software, Version 9.0x, Schwerzenbach, Switzerland). Nitrogen (99.999% purity) was used as the purge gas at a rate of ~20 mL/min. Approximately 4-8 mg of melted sample was placed in a standard DSC aluminum pan and then hermetically sealed. An empty, hermetically-sealed DSC aluminum pan was used as the reference. The oil/fat samples were subjected to the following temperature program: 70°C isotherm for 1 min, cooled at 5°C/min to -70°C. The samples were held at -70°C isotherm for 1 min, and heated at 5°C/min to reach 70°C⁵⁾.

2.2.7 Determination of solid fat content

SFC was measured using a Bruker Minispec (Model Mq 20) pulse Nuclear Magnetic Resonance (pNMR) spectrometer (Karlsruhe, Germany), according to AOCS method Cd 16b-93¹⁵⁾. The sample in the NMR tube was melted at 70°C for 15 min, followed by chilling at 0°C for 60 min, and then held at each measuring temperature for 30 min prior to measurement. Melting, chilling and holding of the samples were carried out in pre-equilibrated thermostatic glycol containing baths, accurate to 0.1°C. SFC measurements were taken at 5°C intervals over the range of 0-60°C.

2.2.8 Statistical analysis

All analyses were carried out in duplicate and the results were expressed as mean value ± standard deviation. Data were statistically analyzed by one-way analysis of variance (ANOVA), by using Tukey's Test of MINITAB (version 15) statistical package at 0.05 probability level.

3 RESULTS AND DISCUSSION

3.1 Basic physico-chemical characteristics

Some basic physico-chemical characteristics of LS and LO are presented in Table 1. LS is characterized by a higher slip melting point (45.75°C), which is about 18 units higher than that of the native sample. As a natural outcome of the fractionation processes, the solid component is mainly consisted of the higher melting triacylglycerols of the native sample. Being a liquid at room temperature, the thermal characteristics of LO is described by cloud point, which is reasonably low-enough to resist against clouding phenomenon¹⁸⁾. In fact, the cloud point displayed by LO

(3.2°C) is comparable to the cloud point of super olein extracted from the second fractionation of crude palm olein¹⁹⁾. With respect to the native sample, iodine value of LS was decreased by 28 units, while that of LO was gone up by 29 units. As iodine value represents the degree of unsaturation of fatty matter, the values recorded for the fractions shows a reverse relationship with their slip melting points.

3.2 Fatty acid composition

The data presented in **Table 1** compares the fatty acid profiles of LS and LO with that of LD. The major fatty acids of LS are palmitic followed by stearic, oleic, and linoleic, comprising about 95% of the total. Unlike LD, palmitic is the most dominant fatty acid (31.68%) in LS followed by stearic (25.15%), and oleic (24.97%) acids. With respect to the native sample, tremendous increases in the proportions of stearic and palmitic could be noticed in LS, with concurrent reductions in oleic (34%) and linoleic (31%) acids. These changes in fatty acid distribution could be accounted for the lower iodine value and higher slip melting point of LS (**Table 1**). As a common feature, both LO and LD were found to possess higher percentage of unsaturated fatty acids than saturated fatty acids. In LO, oleic (42.76%) was the most dominant fatty acid followed by linoleic (23.62%) and palmitic (21.76%) acids. However, with respect to the native sample, there are slight increases in oleic and linoleic acids with the concurrent decreases in palmitic and stearic acids. Naturally, with the migration of more palmitic and stearic acids into the solid phase, the liquid phase becomes more enriched with oleic and linoleic acids. These changes in the fatty acid composition of LO are also found to tally with the change in the degree of unsaturation indicated by the iodine value (**Table 1**).

Lard having a higher proportion of C16:0 at the *sn*-2 position has been established in several previous studies^{6, 14)}. Based on the data in **Table 1**, total saturated and unsaturated fatty acid content of LS at the *sn*-2 position are 88.5% and 11.5%, respectively. Accordingly, the percentage of C16:0 content at the *sn*-2 position of LS is found to

be 76.57%. In LO, the total saturated and unsaturated fatty acid contents at the *sn*-2 position are 89.43% and 9.66%, respectively. In this fraction, the percentage of C16:0 content at the *sn*-2 position is found to be 79.18%. Once the fatty acid distribution of *sn*-2 position is known, the fatty acid enrichment factor (FAEF) corresponding to palmitic acid could be calculated. In comparison to the native sample, palmitic acid enrichment factor value of LS is lower (247%) while that of LO is found to be higher (363%). According to past studies, this feature has been effectively utilized to detect lard in food systems^{5, 14)}.

3.3 TAG composition

The TAG distributional profiles of LS and LO are compared with that of the native sample as shown in **Table 2**. The major TAG molecular species of LD are LPO, OPO, PPO and SPO comprising 61.5% of the total. This is in agreement with the previous findings of Rashood *et al.*¹⁴⁾. These four are also the most dominant TAG molecular species of LS, but PPO and SPO are found to increase tremendously with concurrent reductions in LPO and OPO. According to **Table 2**, the differences seen in the distribution of OOS, SPO, and PPS would be remarkable. Despite these differences, there exists some relationship between LS and LD with regard to TAG peak ratios of the most predominating TAG molecules. For instance, LPO/OPO of these two samples is comparably similar while SPO/LPO and SPO/OPO are distinctly different ($p < 0.05$) (**Table 2**). This could be due to the fact that the proportions of LPO and OPO are approximately similar in both LS and LD samples. Further, the higher proportions of disaturated and trisaturated TAGs in LS could have lead to the occurrence of increased amounts of palmitic and stearic acids in the overall fatty acid distribution. Consequently, this would have caused the increase in SMP and decrease in iodine value of LS as shown in **Table 1**. On the other hand, the TAG composition of LO is found to deviate considerably from that of the native sample as it is found to possess OPO, LPO, PLL and OOL as the major TAG molecules, but almost negligible amounts of the TAG molecules PPS, SSO,

Table 1 Basic physico-chemical characteristics and overall and *sn*-2 positional fatty acid compositions (% peak area) of lard stearin (LS) and lard olein (LO)¹

	Iodine value (g I ₂ /100 g)	Slip melting point (°C)	Cloud point (°C)		C 14:0	C 16:0	C 16:1	C 18:0	C 18:1	C 18:2	Others
LS	45.98 ± 0.02	45.75 ± 0.35	—	Overall	1.23 ± 0.15 ^A	31.68 ± 0.81 ^B	0.72 ± 0.05 ^A	25.15 ± 0.11 ^C	24.97 ± 1.00 ^A	14.04 ± 0.06 ^A	2.21 ± 0.02
				<i>sn</i> -2	3.03 ± 0.64 ^a	76.57 ± 3.41 ^{c,b}	—	8.87 ± 5.34 ^a	5.47 ± 2.28 ^a	5.09 ± 3.21 ^a	0.97 ± 0.45
LO	103 ± 0.06	—	3.25 ± 0.35	Overall	1.46 ± 0.15 ^A	21.76 ± 0.01 ^A	2.30 ± 0.01 ^C	6.38 ± 0.03 ^A	42.76 ± 0.18 ^C	23.62 ± 0.03 ^C	1.72 ± 0.2
				<i>sn</i> -2	4.43 ± 1.20 ^a	79.18 ± 0.28 ^b	0.26 ± 0.37 ^a	5.82 ± 1.47 ^a	5.37 ± 0.18 ^a	4.03 ± 2.84 ^a	0.91 ± 0.07
LD	73.80 ± 0.34	27.50 ± 0.71	—	Overall	1.24 ± 0.01 ^A	22.68 ± 0.48 ^A	1.42 ± 0.05 ^B	12.70 ± 0.28 ^B	38.24 ± 0.13 ^B	20.39 ± 0.04 ^B	3.33 ± 0.12
				<i>sn</i> -2	2.72 ± 0.20 ^a	67.54 ± 1.80 ^a	1.16 ± 1.63 ^a	8.23 ± 5.71 ^a	9.88 ± 5.30 ^a	7.41 ± 5.30 ^a	3.06 ± 0.48

¹Each fatty acid value in the table represents the mean ± standard deviation of two replicates. Means within column with different superscripts are significantly ($p < 0.05$) different.

Table 2 Triacylglycerol (TAG) compositions (% peak area) of lard stearin (LS) and lard olein (LO)¹

TAG	LS	LO	LD
LLLn	0.22 ± 0.00 ^c	2.29 ± 0.01 ^a	1.54 ± 0.21 ^b
LLL	0.23 ± 0.00 ^b	1.43 ± 0.01 ^a	0.68 ± 0.21 ^b
OLL	2.11 ± 0.01 ^c	6.01 ± 0.01 ^a	4.68 ± 0.08 ^b
PLL	3.26 ± 0.01 ^c	9.33 ± 0.04 ^a	7.05 ± 0.06 ^b
OOL	3.40 ± 0.02 ^c	8.48 ± 0.01 ^a	6.93 ± 0.04 ^b
LPO	9.32 ± 0.00 ^c	24.52 ± 0.11 ^a	20.0 ± 0.30 ^b
PPL	3.96 ± 0.01 ^a	2.63 ± 0.03 ^b	2.62 ± 0.04 ^b
OOO	2.46 ± 0.04 ^c	5.61 ± 0.07 ^a	4.33 ± 0.21 ^b
OPO	9.48 ± 0.03 ^c	26.11 ± 0.01 ^a	20.67 ± 0.11 ^b
PPO	22.87 ± 0.03 ^a	3.05 ± 0.05 ^c	10.63 ± 0.01 ^b
OOS	1.79 ± 0.01 ^c	4.30 ± 0.02 ^a	3.62 ± 0.04 ^b
SPO	30.19 ± 0.01 ^a	2.16 ± 0.00 ^c	12.52 ± 0.12 ^b
PPS	2.53 ± 0.04 ^a	ND	0.81 ± 0.00 ^b
SSO	2.29 ± 0.01 ^a	ND	0.83 ± 0.01 ^b
SSS	4.14 ± 0.01 ^a	ND	1.31 ± 0.01 ^b
Unknown	1.78 ± 0.02 ^b	4.12 ± 0.35 ^a	1.84 ± 0.09 ^b
SPO/LPO	3.24 ^a	0.09 ^b	0.63 ^c
LPO/OPO	0.99 ^a	0.94 ^a	0.97 ^a
SPO/OPO	3.19 ^a	0.08 ^c	0.61 ^b

¹ Each value in the table represents the mean ± standard deviation of two replicates. Means within each row having different superscripts are significantly ($p < 0.05$) different. Abbreviations: O, oleic; P, palmitic; L, linoleic; Ln, linolenic; S, stearic; ND, not detected.

and SSS. The proportions of LPO and OPO in LO are remarkably higher than those of the same TAG molecules found in either LD or LS. The other TAG molecular species of LO showing significant increases ($p < 0.05$) are OLL, PLL, and OOL. In comparison to the native sample, the peak ratio values SPO/LPO, and SPO/OPO has decreased significantly ($p < 0.05$) (Table 2). However, the LPO/OPO ratio of LO is comparably similar value to that of LD. The higher proportions of diunsaturated and triunsaturated TAGs in LO could have lead to the occurrence of increased amounts of oleic and linoleic acids in the overall fatty acid distribution. As a consequence, LO displays an increased value of IV as shown in Table 1.

3.4 Thermal characteristics by cooling curve

Thermal characteristic of LS and LO by DSC cooling curves are compared with those of LD as shown in Fig. 1. The cooling profile of the native sample is represented by curve (B), while those of LS and LO are represented by the curves (A) and (C), respectively. It is clear that both LS and LO possessed cooling profiles, which are distinctly different from that of the native sample. In the cooling curve of LD, the occurrence of two major exothermic thermal transitions at widely separated temperature regions indicated the

Fig. 1 DSC cooling curves of lard stearin (curve-A), lard (curve-B), and lard olein (curve-C).

presence of two distinguishable TAG groups with differing melting ranges. This is in agreement with the previous findings of Marikkar *et al.*¹³⁾. The major exothermic peaks at 10.3°C (b_2) with a shoulder peak at 16.8°C (b_1) could be considered to represent the high-melting TAG group, which appear to crystallize first while the other sharp peak at -18.7°C (b_3) should be corresponding to the low-melting TAG group, which undergo crystallization later.

Curve (A), representing LS displays a major and a minor transitions at 26.1°C (a_2) and 30.0°C (a_1), respectively, but no other significant transition below 30.0°C. In comparison to the native sample, the thermal transitions of LS were also found to shift to the higher temperature region due to increases in the proportions of disaturated (PPL, PPO, SPO and SSO) (59.51%) and trisaturated (PPS and SSS) (5.14%) TAG molecules as given in Table 2. Concurrently, the amount of diunsaturated (PLL, LPO, OPO, and OOS) (23.7%) and triunsaturated (LLL, OLL, OOL, and OOO) (8.17%) TAG species of LS were found to decline remarkably with respect to proportions found in the native sample. The reductions in both diunsaturated and triunsaturated TAG molecules would have lead to the disappearance of the low melting transition (below 20°C) in LS.

The cooling curve of LO as shown in Fig. 1 (curve C), displayed a profile completely different from those of LS and LD. It had its major exothermic transitions at -36.1°C (c_3) and two minor peaks at -2.5°C (c_1) and -28.3°C (c_2). In comparison to the native sample, the thermal transitions of LO were found to have shifted into the low-temperature region of the DSC curve. The absence of any thermal transition above 0°C would be an indicative feature to establish its identity as the liquid fraction of LD. Based on the HPLC analysis, LO was found to have 67.28% of diunsaturated (PLL, OOS, OPO, and LPO) and 20.82% triunsaturated (LLL, OLL, OOL, and OOO) TAG molecules (Table 2). Clearly, the amounts of disaturated (PPL, PPO, SPO and SSO) (9.06%) and trisaturated (PPS and SSS) (0%) TAG species possessed by LO were lower than those of the native sample. It could be assumed that the drastic reductions in both disaturated and trisaturated TAG molecules

would have lead to the disappearance of the high melting transition (above 0°C) in LO.

3.5 Thermal characteristics by heating curve

In Fig 2, DSC melting curve of LD is represented by curve(B), while those of LS and LO are represented by curve(A) and curve(C), respectively. In curve(B), two distinguishable regions could be found out for LD by taking 10°C as the point of reference. The region below 10°C is found to have two low-melting transitions b_1 (−21.4°C) and b_2 (−3.59°C), which can be designated as Low-melting TAG group. Meanwhile, the three transitions b_3 (−26.79°C), b_4 (−29.01°C) and b_5 (−32.46°C) found in the region above 10°C can be termed as high-melting TAG group. The melting profile of LS represented by curve(A) is found to display four transitions, namely a_1 (−32.05°C), a_2 (−35.49°C), a_3 (−44.61°C) and a_4 (−49.06°C). LS not having any significant thermal transition in the temperature region below 10°C is a notable feature. It can be assumed that the four transitions displayed by LS would have emerged from the high-melting TAG group of the native sample, while the contribution of low-melting TAG group in it was significantly lower. Moreover, the shifting trend in thermal transitions of LS toward the higher temperature region would be accounted to the increases in the proportions of disaturated (PPL, PPO, SPO and SSO) (59.51%) and trisaturated (PPS and SSS) (5.14%) TAG molecules as shown in Table 2. On the other hand, the heating curve of LO is characterized by three thermal transitions, a major peak c_2 (−3.38°C) with two shoulder peaks c_1 (−19.58°C) and c_3 (13.83°C). This cluster of thermal transitions would have emerged from the low-melting TAG group of the native sample, while the contribution of high-melting TAG group in it was significantly low. Also, in comparison to those of the native sample, the transitions of LO have become wide and broad. This could be probably due to the polymorphism phenomenon taking place during the process of heating. As the samples are heated, some of the less thermally stable polymorphs could melt early; the re-

maining TAG rearranges and re-crystallizes into more stable polymorphs, which may melt at a higher temperature. Because of this, melting of two or more TAG structures might take place simultaneously, leading to the overlapping of two successive thermal transitions, which might end up in peak broadening.

3.6 Solid fat content

The solid fat content (SFC) profiles of LS and LO are compared with that of LD as shown in Fig. 3. At 0°C, LD is found to possess 30.8% solid content, which is in accordance with the previous findings of Kamel²⁰ and Ospina-E *et al.*²¹. The solidification behaviors of LS and LO are quite different from that of the native sample in greater part of the temperature region. LO could be distinguishable easily from the native sample as it remains as a liquid throughout the temperatures. Since the SFC value of LO at 0°C was 0.15%, it displayed a fairly lower cloud point (3.2°C). This could be probably due to enhanced proportions of di and triunsaturated TAG molecules possessed by LO with respect to the native sample (Table 2). In major part of the temperature region, the SFC values of LS are also quite different from those of LD. For instance, the SFC value at 0°C is almost two times higher than that of LD. Although the changing SFC values of LS are represented by a smooth curve from 0 to 35°C, its shape is found to change remarkably in the temperature region extending from 35 to 55°C. With the slop of the SFC curve going down, SFC values of LS at 50 and 55 °C were 0.1% and 0%, respectively. This clearly shows that the plastic range of LS would be wider in comparison to that of the native sample, whose SFC value tended to become zero just at 40°C. This difference in solidification behavior could be accounted for the enhanced proportions of di and trisaturated TAG molecules present in LS as noticed in Table 2.

Fig. 2 DSC heating curves of lard stearin (curve-A), lard (curve-B), and lard olein (curve-C).

Fig. 3 Solid fat content profiles of lard stearin (LS), lard (LD) and lard olein (LO)

4 CONCLUSION

SMP and IV of LS, and LO are largely different from those of LD. Based on the overall fatty acids, palmitic was the most dominant fatty acid of LS while oleic was the major fatty acid of LO. Similar to LD, majority of the *sn*-2 positions in LS and LO are occupied by saturated fatty acids, of which palmitic was the most dominant. TAG distributional analysis showed that both LS and LO displayed some characteristic differences from native lard based on the proportions of TAG molecules such as OOS, SPO, and PPS. Because of this, LS and LO also differed from the native sample on the basis of peak ratios SPO/LPO and SPO/OPO. The DSC thermal profiles of both LS and LO were significantly different from those of the native sample on the basis of the position as well as the number of thermal transitions.

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