Effect of Enzymatic Transesterification with Flaxseed Oil on the High-Melting Glycerides of Palm Stearin and Palm Olein

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ABSTRACT: The effects of enzymatic transesterification on the melting behavior of palm stearin and palm olein, each blended separately with flaxseed oil in the ratio of 90:10 and catalyzed by various types of lipases, were studied. The commercial lipases used were Lipozyme IM, Novozyme 435, and mycelium-bound lipases of *Aspergillus flavus* and *A. oryzae*. The slip melting point (SMP) of the palm stearin/flaxseed oil (PS/FS) mixture transesterified with lipases decreased, with the highest drop noted for the mixture transesterified with Lipozyme IM. However, when palm stearin was replaced with palm olein, the SMP of the palm olein/flaxseed oil (PO/FS) mixture increased, with the commercial lipases causing an increase of 41 to 48% compared to the nontransesterified material. As expected, the solid fat content (SFC) of the transesterified PS/FS was lower at all temperatures than that of the nontransesterified PS/FS sample. In contrast, all transesterified PO/FS increased in SFC, particularly at 10°C. Results from DSC and HPLC analyses showed that the high-melting glycerides, especially the tripalmitin of palm stearin, were hydrolyzed. Consequently, 1,3-dipalmitoylglycerol was found to accumulate in the mixture. There was no difference in the FA compositions between the transesterified and nontransesterified mixtures.

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KEY WORDS: DSC analysis, high-melting glyceride, palm olein, palm stearin, slip melting points, solid fat content.

Varying fat properties tailored to suit the requirements of a product can expand the application of vegetable oils. These changes can be brought about either through simple blending or by enzymatic transesterification. Palm olein or palm stearin, when blended with flaxseed oil, is a unique mixture because it contains the EFA α-linolenic acid, an n-3 FA, and linoleic acid, an n-6 FA, in appreciable amounts. Palm olein and palm stearin blends are used in numerous food and nonfood applications. Graille *et al.* (1) reported that the transesterification of a palm stearin/palm kernel oil (30:70) mixture for 30 min produced a firm margarine, whereas a soft margarine was produced after 3.5 h of treatment. Transesterification with fluid oils could also give fats that are virtually fluid at 20°C and that could be used as salad oils in tropical countries.

By utilizing the specific properties of lipolytic enzymes, one can effect a selective transesterification reaction that is not possible in transesterification using traditional chemical catalysts. Under the right conditions, "tailor-made" glycerides with desired configurations and characteristics can be obtained. For example, lipase from *Rhizomucor miehei* was used by Zainal and Yusoff (2) as a catalyst in the enzymatic interesterification of palm stearin and palm kernel olein to achieve the physical properties of margarine fats. A commercial immobilized 1,3-specific *R. miehei* lipase also has been reported to reduce the m.p. of a tallow/rapeseed oil mixture (3). Lai *et al*. (4) reported using nonspecific (*Pseudomonas* sp.) and 1,3-specific (*R. miehei*) lipases for the transesterification of palm stearin and sunflower oil, resulting in melting properties appropriate for use as table margarine. The present study deals with the solid fat content (SFC) and the melting and crystallization behavior of palm stearin/flaxseed oil (PS/FS) and palm olein/flaxseed oil (PO/FS) mixtures, at a 90:10 (w/w) mass ratio, following tranesterification using commercial and mycelium-bound lipases.

MATERIALS AND METHODS

Materials. Refined, bleached, and deodorized palm stearin and palm olein were obtained from Golden Jomalina Industries Sdn. Bhd. (Kuala Langat, Selangor, Malaysia), and flaxseed oil was obtained from Waihi Bush Ltd. (Woodbury, Geraldine, New Zealand). Commercial lipases, Lipozyme IM, and Novozyme 435 were obtained from Novo Nordisk Industry A/S (Copenhagen, Denmark), and mycelium-bound lipases of *Aspergillus flavus* and *A. oryzae* were prepared according to the method of Long *et al*. (5). All other chemicals and solvents used were of the highest purity available.

Transesterification reaction. PS/FS and PO/FS mixtures were formulated in the mass ratio of 90:10 (w/w). The enzymatic reaction was carried out by mixing 50 g of oil blends with 1.5 g of lipase and agitating in an orbital shaker at 45°C, 200 rpm. After a 2-h reaction, 6 g of molecular sieve was added (to adsorb water) and the reaction was allowed to proceed for another 4 h. Sample was then filtered to separate the enzyme and

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molecular sieve from the oil mixture, and 100 mL of *n*-hexane and 300 mL of acetone/ethanol (1:1, vol/vol) were added. Titration with 0.05 M NaOH to a phenolphthalein end point was conducted. The sample was then transferred into a 1000-mL separating funnel. After being shaken and allowed to stand for several minutes, the bottom layer (aqueous phase containing FA) was discarded. The upper layer (organic phase) was then transferred into a 1000-mL beaker and dried overnight at 80°C.

Hydrolytic activity. The amount of FFA present was determined according to the method of Cocks and van Rede (6). At the end of the reaction period, 5 mL of acetone/ethanol mixture (1:1) was added to 2 mL of the sample and the mixture was titrated with 0.05 N NaOH to a phenolphthalein end point. Duplicate runs were carried out for each sample.

Slip melting point (SMP). The SMP was determined by AOCS method Cc 4-25 (7).

SFC. The SFC was determined according to AOCS method Cd 16-81 (8). Prior to analysis, the fat was melted and tempered at 70°C for 30 min, then chilled at 0°C for 90 min. The SFC was measured within the temperature ranges of 10, 20, 30, 40, and 50°C.

Thermal analysis by DSC. Thermal analysis by DSC was determined according to Lai *et al.* (9). The sample was cooled at −80°C and held for 5 min before being melted to 60°C at the rate of 5°C/min and held at this temperature for 5 min for the cooling thermograms.

FA analysis by GLC. The FA composition of the oil samples were analyzed by GLC after removal of FFA of the transesterified oil. FAME were prepared by dissolving 0.05 g of the sample in 0.95 mL hexane to which 0.05 mL of 1 M sodium methoxide was added, and the samples were analyzed on a gas chromatograph. A polar capillary column, BPX 70 (0.25 mm i.d., 30 m length, and 0.25 μm film thickness; SGE Australia Pty. Ltd., Ringwood, Victoria, Australia) was used to separate the esters.

TAG profile by HPLC. TAG composition was determined by HPLC with a commercially packed RP-18 column (250 × 4 mm) with 5-μm particle size (Merck, Darmstadt, Germany). TAG were eluted with acetone/acetonitrile (60:40) at a 1 mL/min flow rate. TAG in the oil were identified using the method of Ghazali *et al.* (10), whereas the 1,3-dipalmitoyl glycerol peak was identified according to the profile and retention time given by Swe *et al.* (11).

Quantification of DAG and TAG by TLC scanner. The analysis was carried out using TLC on glass plates (20 × 20 cm) coated with silica gel 60G (Merck). Oil samples were dissolved in hexane at a concentration of 0.1 g/mL. A 2-μL dissolved sample was applied to the plate, which was then placed in a chamber containing glacial acetic acid/petroleum ether/diethyl ether (0.4:210:90, vol/vol). Quantitative analysis was carried out using a TLC scanning instrument (Camag, Muttenz, Switzerland).

RESULTS AND DISCUSSION

Table 1 shows the amount of FFA released, the DG and TG left after the reaction, and the SMP of the PS/FS and PO/FS mixtures following transesterification by different types of lipases. Enzymatic transesterification of the PS/FS mixture with all the lipases used in this study produced softer fats, as reflected by their SMP (Table 1). Lipozyme caused a greater drop in SMP (16%) as compared to only a 3% reduction in SMP when *A. oryzae* lipase was used. Conversely, when palm stearin was replaced with palm olein, the SMP of the transesterified PO/FS mixture increased (Table 1). The PO/FS mixture transesterified with the commercial lipases Lipozyme IM and Novozyme 435 gave about a 41 and 48% increase in SMP, respectively. Marangoni *et al.* (12) reported that transesterification of triolein with tripalmitin using lipase at 47°C for 48 h caused the m.p. of the product to drop from 61 to 57°C. Lai *et al.* (9) studied the effect of enzymatic transesterification using 1,3-specific and -nonspecific lipase on the m.p. of palm stearin/sunflower oil mixtures. Their results showed that SMP of the transesterified mixtures were generally lower than that of the nontransesterified control.

TABLE 1
The Amounts of FFA, DAG, and TAG and the Slip Melting Point (SMP) in Transesterified and Nontransesterified Palm Stearin/Flaxseed Oil (PS/FS) and Palm Olein/Flaxseed Oil (PO/FS) Mixtures

Types of samples	FFA (μmol/mL)	DAG (%)	TAG (%)	SMP (°C)
Nontransesterified PS/FS	11.3	2.4	97.6	48.3
PS/FS transesterified with *Aspergillus flavus* lipase	68.8	7.7	92.3	42.1
PS/FS transesterified with *A. oryzae* lipase	46.3	5.0	95.0	46.9
PS/FS transesterified with Lipozyme IM	93.8	10.6	89.4	40.7
PS/FS transesterified with Novozyme 435	91.3	13.9	86.1	43.5
Nontransesterified PO/FS	10.0	5.2	94.8	14.1
PO/FS transesterified with *A. flavus* lipase	67.5	6.2	93.8	16.2
PO/FS transesterified with *A. oryzae* lipase	48.8	3.4	96.6	15.4
PO/FS transesterified with Lipozyme IM	75.0	12.7	87.4	19.9
PO/FS transesterified with Novozyme 435	77.5	8.7	91.3	20.9

Figures 1A and 1B show the SFC values of the transesterified PS/FS and PO/FS, respectively. The largest decline in SFC occurred in the 20 to 30°C range in the nontransesterified PS/FS. However, in the Lipozyme IM and Novozyme 435 lipase-transesterified PS/FS, the sharp decline in SFC at 20 to 30°C in nontransesterified PS/FS shifted to 10 to 20°C. This could be due to the presence of a larger proportion of TG that liquefy in this temperature range. The shift to a lower temperature range could also explain the lower SMP obtained after transesterification as compared to nontransesterified PS/FS. No solid fat was detected at 50°C except in the nontransesterified mixtures. Enzymatic transesterification of rapeseed oil with palm oil was done by Kurashige *et al.* (13). The SFC of the transesterified oil was reduced relative to the nontransesterified material, and the modified palm olein in 20% rapeseed

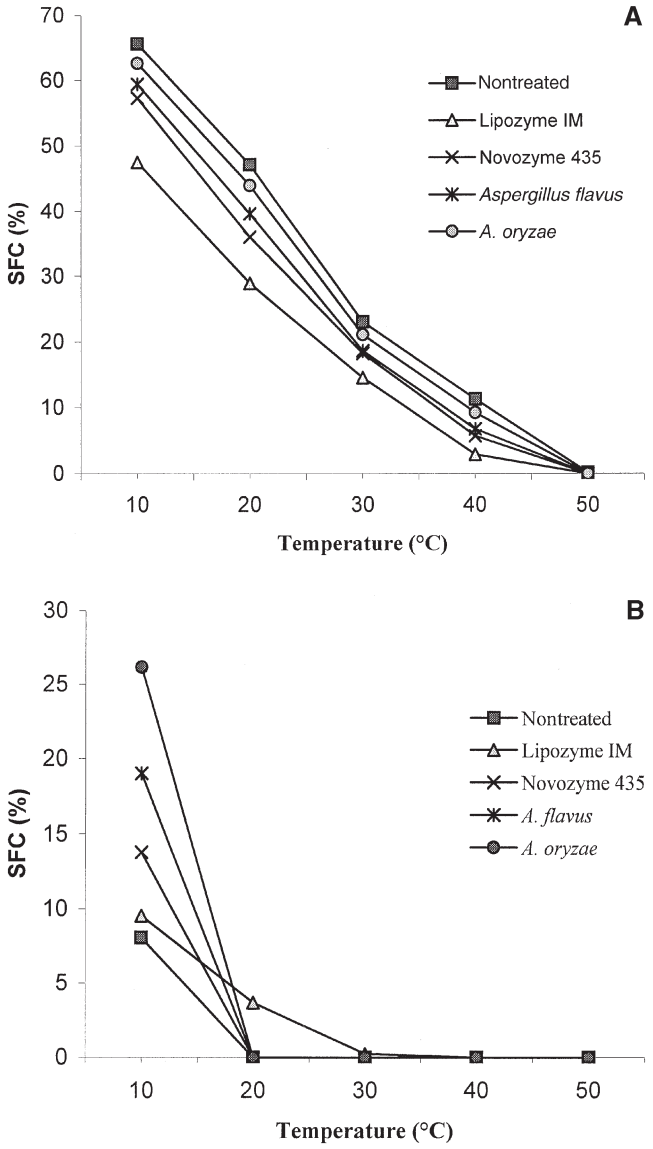

FIG. 1. (A) Solid fat content (SFC) of transesterified and nontransesterified palm stearin/flaxseed oil mixtures. (B) SFC of transesterified and nontransesterified palm olein/flaxseed oil mixtures.

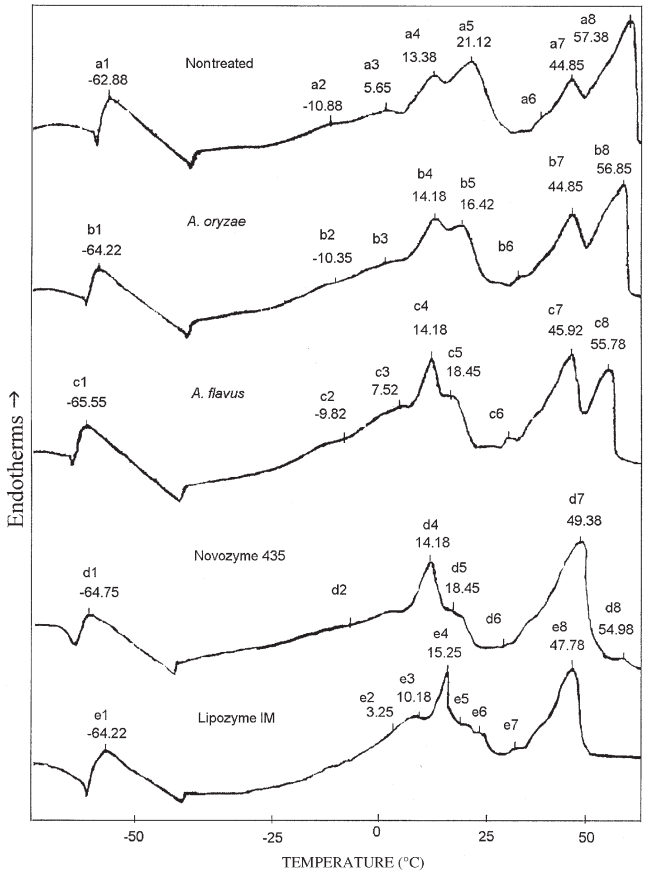

FIG. 2. Heating thermograms of transesterified and nontransesterified palm stearin/flaxseed oil mixtures.

oil had a 0% SFC at 10°C. Results in Figure 1B show that in transesterified PO/FS mixtures the percentage of solid fat at 10°C increased. At 10°C, samples transesterified by *A. oryzae* lipase had the highest percentage of solid fat, followed by samples transesterified by *A. flavus*, Novozyme 435, and Lipozyme IM lipases. No solid fat was detected at 20 and 30°C except in mixtures transesterified with Lipozyme IM. This indicated that enzymatic treatment with Lipozyme IM caused the formation of high-melting glycerides.

The heating and cooling thermograms of the lipase-transesterified and nontransesterified PS/FS are given in Figures 2 and 3, respectively. Enzymatic transesterification produced prominent changes in the heating profile. Peak a8 (Fig. 2) of the heating thermogram of the nontreated material, which contained high-melting glycerides (57°C), disappeared in the PS/FS mixture transesterified with Lipozyme IM. The disappearance of peak a8 was followed by an increase in the a7 peak. The increase in peak a7 and the decrease in peak a8 were noticed in all the transesterified samples. This could explain why the SMP of the transesterified PS/FS was lower than in the untreated starting materials. This indicates that the lipases were active on the high-melting trisaturated TAG of the palm stearin, which is known to contain about 13% tripalmitin. Our HPLC results support this conclusion, with the amount of tripalmitin in the PS/FS mixture being reduced after the treatment Table 2. However, when palm olein was replaced with palm stearin, the amount of tripalmitin increased slightly during transesterification. In both types of transesterified oils, the reaction caused an increase in the amount of 1,3-dipalmitoyl glycerol, which was identified by Swe *et al.* (11) as a high-melting glyceride. However, in transesterified PS/FS the increase in the amount of 1,3-dipalmitoyl glycerol did not affect its melting properties as much as the decrease in tripalmitin. The medium-melting glycerides, which melted between 10 and 25°C, also showed some changes. Thus, peak a5 was reduced or formed a shoulder peak for samples transesterified with Lipozyme IM, Novozyme 435, and *A. flavus* lipases. Few changes occurred in the region of low-melting glycerides (−75 to −40°C). The

TABLE 2
Tripalmitin and 1,3-Dipalmitoyl Glycerol (area % by HPLC) of Transesterified and Nontransesterified Oil Mixtures[a]

Treatment	1,3-Dipalmitoyl glycerol (%)	Tripalmitin (%)
Nontransesterified PS/FS	4.3	12.8
PS/FS transesterified with Lipozyme IM	5.7	9.1
PS/FS transesterified with Novozyme 435	7.9	9.6
PO/FS nontransesterified	0.9	0
PO/FS transesterified with Lipozyme IM	6.3	1.3
PO/FS transesterified with Novozyme 435	6.1	0.2

[a]For abbreviations see Table 2.

cooling thermogram of transesterified PS/FS showed that the onset temperature and the peak temperature of peak 2 were shifted to a higher temperature by transesterification (Fig. 3), consistent with a decrease in the amount of diunsaturated TAG during transesterification.

Figures 4 and 5 show the heating and cooling thermograms, respectively, of transesterified and nontransesterified PO/FS mixtures. In all the transesterified PO/FS mixtures, new high-melting shoulder peaks were formed, especially in samples transesterified with Lipozyme IM (the e5 peak) and *A. flavus* (the c7 peak) lipases (Fig. 4). There was a new high-melting peak (e6) in samples transesterified with Lipozyme IM lipase (Fig. 4).

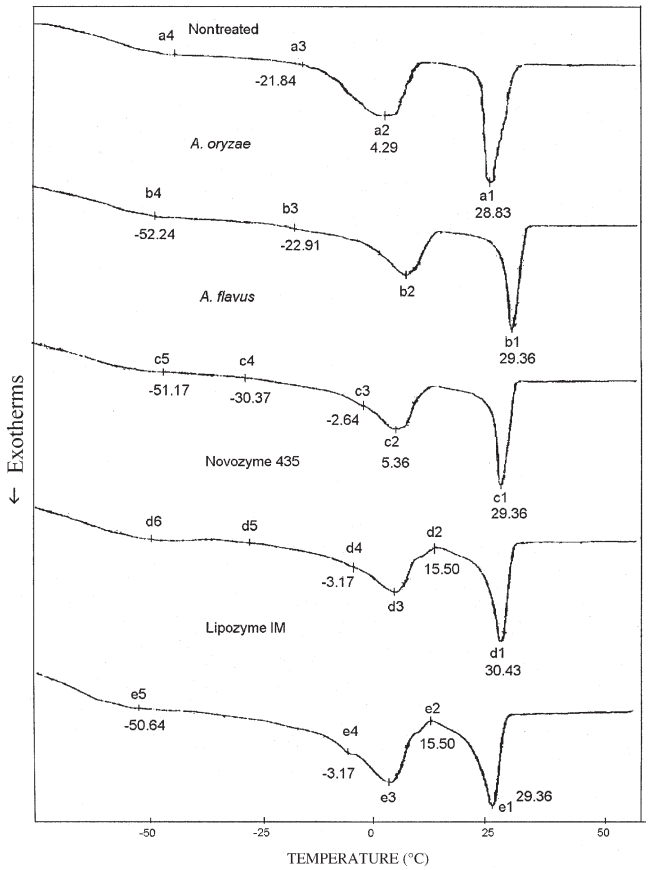

FIG. 3. Cooling thermograms of transesterified and nontransesterified palm stearin/flaxseed oil mixtures.

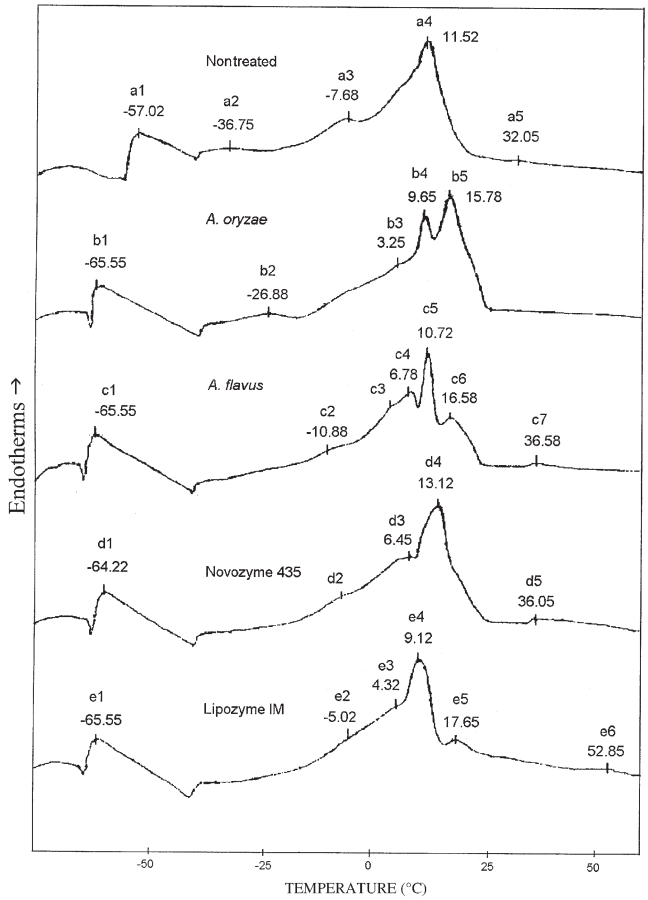

FIG. 4. Heating thermograms of transesterified and nontransesterified palm olein/flaxseed oil mixtures.

From the cooling thermograms (Fig. 5), it was noted that samples treated with Lipozyme IM or Novozyme 435 started to solidify earlier than nontransesterified samples. Samples transesterified with Lipozyme IM and Novozyme 435 started to solidify at 23.5 and 17.6°C, respectively, compared with nontransesterified samples, which began solidifying at 8°C (Fig. 5). This probably explains the increased SMP of transesterified mixtures (Table 1).

Enzymatic transesterification was observed to be effective in modifying the SMP, SFC content, and thermal behavior of PS/FS and PO/FS mixtures. The drop in SMP in the PS/FS mixtures is probably due to hydrolysis of the trisaturated TAG tripalmitin which is a known high-melting glyceride. On the other hand, the increase in SMP in transesterified PO/FS mixtures could be due to the increase in the amount of high-melting glycerides from the synthesis of tripalmitin and 1,3-dipalmitoyl glycerol formed during the transesterification of palm olein.

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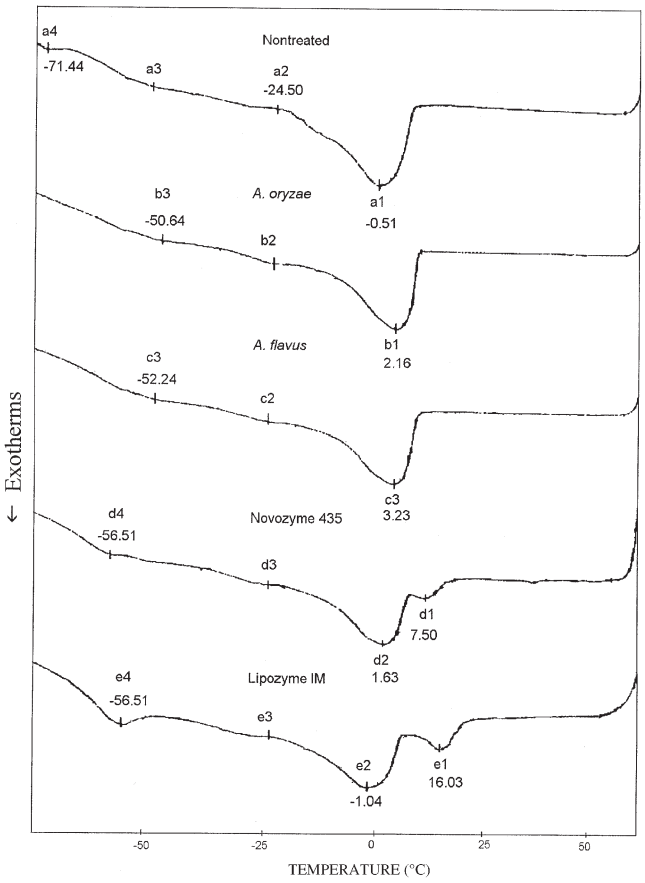

FIG. 5. Cooling thermograms of transesterified and nontransesterified palm olein/flaxseed oil mixtures.

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