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Modification of Beef Tallow Fractions by Chemical and Enzymatic Interesterification with Sunflower Oil

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Abstract: Mixtures of beef tallow stearin and beef tallow olein were interesterified with sunflower oil using sodium methoxide or immobilized lipases from *Rhisomucor miehei* (Lipozyme IM) and *Candida antarctica* (Novozym 435). The starting fats and the interesterified products were separated into the triacylglycerol and non-triacylglycerol fractions, which contained free fatty acids and mono- and diacylglycerols. After interesterification, the contents of free fatty acids and of mono- and diacyloglycerols increased. The slip melting point and solid fat content of the triacylglycerol fractions of the interesterified samples containing stearin and sunflower oil were lower compared with the nonesterified blends. For the interesterified mixtures containing olein and sunflower oil, the opposite dependencies for slip melting point and solid fat content were observed. The total fatty acid composition of fats before and after interesterifications remained unchanged but their distributions between sn-1, 3 and sn-2 positions were modified depending on the catalysts used. These distributions were random after chemical interesterification and close to random when Novozym 435 was used. When Lipozyme IM was used the fatty acid composition at sn-2 position remained practically unchanged compared with the starting blend.

Key words: Interesterification, lipases, sodium methoxide, sunflower oil, tallow olein, tallow stearin

INTRODUCTION

Beef tallow is one of the most important by-products of meat industry. European annual production of tallow is about 1.42 million tones[1]. Because of its high melting temperature and low level of unsaturated fatty acids (C18: 2, C18: 3) tallow practically cannot be directly used for edible purposes. For edible use it has to be fractionated and /or modified by interesterification with edible oils[2,3]. The selection of vegetable oil depends on the purpose of tallow modification. When the fat produced is intended to be used as a component of a frying fat with a low content of linolenic acid, sunflower oil can be used. It also can be used for production of some confectionery fats, shortenings, "low trans" margarine oils and plastic fats. There are several papers on interesterification of beef tallow with sunflower oil[4-6]. Substantially less information is available on theinteresterification of tallow fractions with vegetable oils[7-9]. This study deals with the modification of beef tallow by acetone fractionation followed by blending or interesterification of beef tallow fractions with sunflower oil in different proportions. The objective of this study was to investigate selected chemical and physical properties of beef tallow stearin or olein and sunflower oil blends modified by interesterification. Both chemical and enzymatic interesterifications were studied and the properties of final fats were compared with those of the starting blends.

MATERIALS AND METHOD

Materials: Sunflower oil was purchased on a local market. Its main fatty acid composition was as follows: C16:0 (6.0%), C18:0 (3.3%), C18:1cis 9 (27.8%), C18:2 all cis (60.1%). Beef tallow (acid value < 1.0 mg KOH/g) was laboratory refined, bleached and deodorised under vacuum at 105 °C and subsequently fractionated using acetone. Beef tallow (100g) was dissolved in acetone (600g) and boiled under reflux for 1 hour. The solution was then left for 15 hours at 20 °C. Precipitated white

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Table 1: Free fatty acids (FFA), mono- and diacylglycerols (MAG + DAG), triacylglicerols (TAG) contents and slip melting points (SMP) of TAGs for stearin (S) and olein (O) and their mixtures with sunflower oil (SFO) before and after chemical interesterification.

	Before interesterification				After chemical interesterification			
Fat sample	FFA [%]	MAG+ DAG [%]	TAG [%]	SMP of TAG [°C]	MAG+ FFA [%]	DAG [%]	TAG [%]	SMP ofTAG [°C]
Stearin (S)	1.1	0.9	98.0	54.3	1.5	6.8	91.7	53.8
10% SFO + 90% S	0.8	2.2	97.0	51.8	1.6	8.4	90.0	48.3
25% SFO + 75% S	0.6	2.0	97.4	50.2	1.2	6.3	92.5	41.4
40% SFO + 60% S	0.5	1.8	97.7	48.5	1.4	7.2	91.4	38.8
50% SFO + 50% S	0.4	1.6	98.0	47.2	1.6	8.1	90.3	33.3
60% SFO + 40% S	0.2	1.7	98.1	46.0	1.7	8.3	90.0	30.1
Olein (O)	1.0	2.1	96.9	22.8	1.5	7.2	90.3	30.6
10% SFO + 90% O	0.8	2.2	97.0	22.1	2.4	10.6	87.0	28.1
25% SFO + 75% O	0.7	2.3	97.0	21.1	1.9	9.5	88.6	26.7
40% SFO + 60% O	0.6	2.2	97.2	18.2	2.0	9.9	88.1	24.5
50% SFO + 50% O	0.5	2.0	97.5	9.9	2.0	9.8	88.2	17.2
60% SFO + 40% O	0.5	1.9	97.6	5.9	1.9	10.2	87.9	16.4

crystals of stearin (~20g, slip melting point 54 ± 1 °C) were filtered out and stored in a vacuum dessicator to remove acetone. From the filtrate, acetone was evaporated at reduced pressure and the remaining fat(~80g of olein, slip melting point 24 ± 1 °C) was obtained. The fatty acid composition of the stearin and olein were reported in our recent publication in this journal [9].

Blends preparation: Stearin (S) or olein (O) were mixed at 70 °C under nitrogen with sunflower oil (SFO) in proportions ranging from 10 to 60 wt % of SFO. Ten blends, five containing sunflower oil and stearin (10% SFO + 90% S, 25% SFO + 75% S, 40% SFO + 60% S, 50% SFO + 50% S, 60% SFO + 40% S) and five containing sunflower oil and olein (10% SFO + 90% O, 25% SFO + 75% O, 40% SFO + 60% O, 50% SFO + 50% O, 60% SFO + 40% O) were prepared. The selected properties of stearin and olein and of starting mixtures are given in Table I.

Catalysts: Chemical interesterifications were catalyzed by powdered sodium methoxide (CH_3ONa, Merck, Germany). For the enzymatic interesterifications two commercial preparations Lipozyme IM and Novozym 435 (Novozymes, Bagsvaerd, Denmark) were used. Lipozyme IM contains immobilised lipase from *Rhizomucor miehei* and Novozym 435 from *Candida antarctica*. Lipozyme IM and Novozym 435 contained 4 % and 2 % of water, respectively.

Methods:

Chemical interesterification: Directly before interesterifications the fats were dried at 90 °C under reduced pressure. Flasks containing the fat blends were flushed with nitrogen, stoppered and positioned in a the rmostated mineral oil shaker bath. After thermal equilibration at 90 °C, the catalyst (0.6 wt-% sodium methoxide) was added under nitrogen. The interesterification was carried out with continuous shaking for 2 hours. The reaction was stopped by the addition of hot water containing 5% H_3PO_4. Interesterified fats were extracted with hexane, washed with water, dried with magnesium sulphate and filtered. Hexane was evaporated under reduced pressure and the interesterified fats were analysed.

Enzymatic interesterifications: After the rmal equilibration of the fat blends at the desired temperature (80 °C for Novozym 435 or 60 °C for Lipozyme IM) 8 wt-% of catalyst was added. Water content in the biocatalyst was adjusted by addition of water directly before the reaction. The interesterifications were performed with continuous shaking. After a predetermined time (Novozym 4 h, Lipozyme 8 h), the samples were filtered to stop the interesterification reactions. As the filtering bed contained a drying agent, water was also removed from fat.

Determinations and analyses: Free fatty acids (FFA) were determined by titration of the fat sample dissolved in a mixture of ethanol : diethyl ether (1:1 vol/vol) with 0.1-M ethanolic potassium hydroxide solution. The fatty acid composition of the fats was determined by gas liquid chromatography (GLC) after conversion of the fats to fatty acid methyl esters (Polish Standard PN-ISO 5509).

Fats before and after interesterification were separated into triacylglycerols (TAG) and non-TAG fraction, referred to as polar fraction (PF), by column chromatography on silica gel (SG 60, 70-230 mesh, Merck), and then the weight percents of TAG and PF were determined in accordance with the Polish Standard PN-ISO 8420, 1995). The polar fraction consists of FFA,

monoacylglycerols (MAG) and diacylglycerols (DAG).The slip melting point, (SMP, °C), a temperature at which the fat confined in open capillary immersed in water moves upward was determined in accordance with Polish Standard (PN ISO 638, 1991).

The solid fat content (SFC, %) of TAG as a function of temperature (5 – 50 °C) was determined by a pulse nuclear magnetic resonance in a Brucker Minispec 120 NMR Analyzer. Samples for SFC determinations were prepared according to the Polish Standard (PN ISO 8292, 1991).

The positional distributions of fatty acids between sn-2 and sn-1,3 positions of triacylglycerols were determined using the method developed by Brockerhoff [10]. The method is based on the ability of pancreatic lipase, to selectively hydrolyze ester bonds in the sn-1,3 positions of TAG.

The detailed procedures for all determinations and analyses were reported in our erlier publications [9,11,12].

RESULTS AND DISCUSSION

The chemical interesterifications of SFO + S and SFO + O blends were performed at 90 °C for 2 h using 0.6 % of sodium metoxide. During interesterifications of fats, apart from new triacylglycerols, free fatty acids, mono- and diacylglycerols are also formed. These products were determined in the post-reaction mixtures and the results are listed in Table 1. For comparative purposes the compositions of the blends before interesterification are also given. As seen from the Table 1 with interesterification there was an increase in FFA and MAG + DAG contents.

Similar trends have been observed in our earlier studies on interesterification of beef tallow blends with rapeseed oil [11,12]. Studying interesterification beef tallow stearin + soybean oil and beef tallow olein + soybean oil blends containing from 10 to 60 wt-% of soybean oil Kowalska *et al.* (2005) have observed that after chemical interesterification (90 °C, 0.6 wt-% CH_3ONa) of mixtures containing stearin the crude post-reaction products contained 1.5 – 1.8 % FFA, 6.8 – 11.3 % MAG + DAG and 87.0 – 89.1 % TAG. For mixtures containing olein they have observed 2.4 – 2.6 % FFA, 9.6 – 9.8 % MAG + DAG and 87.6 – 87.9 % of TAG. In this work we have obtained similar yields of TAG (91.3 ± 1.3 % for blends containing stearin and 87.8 ± 0.8 % for blends containing olein). The slip melting points of the TAGs isolated from post-reaction mixtures were measured and the results are also listed in Table 1. As seen from Table 1 the SMP values for TAGs of interesterified blends containing stearin are lower (48.3 – 30.1 °C) than for starting blends (51.8 – 46.0 °C). On the contrary, for TAGs of interesterified blends containing olein, increases of the SMP (28.1 – 16.4 °C) were observed. The dependencies of solid fat content against temperature for TAG fractions were determined by pulse-NMR and the results for selected systems containing 10 and 60 wt-% of SFO are illustrated in Figures 1 and 2. As seen from the plots the SFC values for TAG isolated from chemically interesterified blends containing stearin are lower than for starting blend. For other compositions of blends their SFC patterns were similar and there were only quantitative differences. An opposite relationships were obtained for blends consisted of olein and sunflower oil. For such blends the increase of SFC values were observed after interesterification.

As expected, the positional distribution of fatty acids between the sn-2 and sn-1,3 positions in TAG of chemically interesterified blends was near random and different from the starting blends.

The data showed in Tables 2 and 3 for equal-weight blends containing stearin or olein and SFO before and after interesterification serve as the examples.

For enzymatic interesterification, the blends studied and the time and temperature of reaction and catalyst dose were established in our earlier experiments [11,12], and these parameters were kept constant as specified in Materials and Methods section.. Only the water contents in catalysts were fixed at two levels (2 and 10 wt-% for Lipozyme IM and 4 and 10 wt-% for Novozym 435).

The crude post-reaction mixtures were characterized by determinations of FFA, MAG + DAG and TAG percentages and the results are listed in Tables 4 and 5 .Comparing the results for initial blends (Table 1) and enzymatically interesterified blends (Tables 4 and 5) a sharp increase in the FFA and MAG + DAG concentrations is observed, especially at 10 % water content in biocatalyst used. These increases are in agreement with the findings reported in literature [5,9,11,12]. Consequently, the concentration of TAG fractions in interesterified fats decreased compared with starting blends. The TAG fractions were isolated from crude interesterified fats and their slip melting points were measured. The results are also listed in Tables 4 and 5. As seen from Table1 (initial blends) and Tables 4 and 5, the SMP of TAG from enzymatically interesterified blends containing stearin are lower than for the starting blends.

As the enzyme operated on the external ester

Table 2: Fatty acid composition (TAG total) and distribution between the (sn-2) and(sn-1,3)[a] positions for triacylglycerols obtained from the mixture of sunflower oil (50%) and stearin (50%) before and after chemical interesterification.

		Before interesterification		Chemically interesterified	
Fatty acid	TAG total [%]	% in sn-2	% of a given fatty acid in sn-2	% in sn-2	% of a given fatty acid in sn-2
14:0	1.9	3.1	54.4	2.1	36.8
16:0	20.2	14.6	24.1	19.9	32.8
16:1 (9 c)	0.7	0.6	28.6	0.7	33.3
17:0	1.3	1.1	28.2	1.3	33.3
18:0	18.7	14.7	26.2	17.9	31.9
18:1 (9 t)	1.0	0.5	16.7	0.9	30.0
18:1 (9 c)	21.7	24.8	38.1	21.8	33.5
18:2 (9, 12 c)	30.4	36.6	40.1	30.1	34.0

[a] sn-1,3 = [3 TAG total – (sn-2)] : 2

Table3: Fatty acid composition (TAG total) and distribution between the (sn-2) and (sn-1, 3)[a] positions for triacylglycerols obtained from the mixture of sunflower oil (50%) and olein (50%) before and after chemical interesterification.

		Before interesterification		Chemically interesterified	
Fatty acid	TAG total [%]	% in sn-2	% of a given fatty acid in sn-2	% in sn-2	% of a given fatty acid in sn-2
14:0	1.6	2.0	41.7	1.7	35.4
16:0	16.2	10.1	20.8	15.8	32.5
16:1 (9 c)	1.3	1.3	33.3	1.3	33.3
17:0	0.9	0.5	18.5	0.8	29.6
18:0	11.4	7.2	21.1	10.9	31.9
18:1 (9 t)	1.2	1.5	16.7	1.1	30.6
18:1 (9 c)	31.4	29.1	38.2	31.6	33.5
18:2 (9, 12 c)	31.0	27.9	40.0	31.5	33.9

[a] sn-1,3 = [3 TAG total – (sn-2)] : 2

linkages the percentages of particular fatty acids in the sn-2 positions of interesterified TAGs in comparison with their counterparts for initial blends remain nearly unchanged. The small changes in sn-2 percentages can be caused by possible acyl migration in TAG molecules during a prolonged time of interesterification, as reported by Xu at al. [3].

The altered triacylglycerol compositions of the interesterified mixtures of sunflower oil with stearin and olein catalyzed by enzymatic catalysts were reflected in the solid fat content over the temperature range of 5 – 50 °C. Significant reductions in the solid fat content were detected for the TAG fraction isolated from blends containing stearin and sunflower oil after interesterification. On the contrary, the blends containing sunflower oil and olein after interesterifications displayed increases in solid fat contents of their TAG fractions. Typical dependencies for SFC versus temperature for . in Figs. 1 and 2.

Beef tallow stearin and olein contained 1.9% and 2.3 % of C18:1trans acid, respectively [9]. Blending with

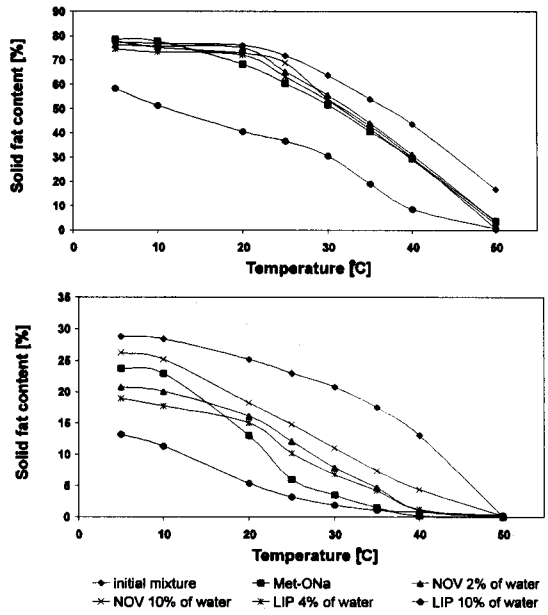

Fig.1: The solid fat content (SFC) versus temperature for initial and interesterified mixtures consisted of 10 % SFO + 90 % S (A) and 60 % SFO + 40 % S (B).

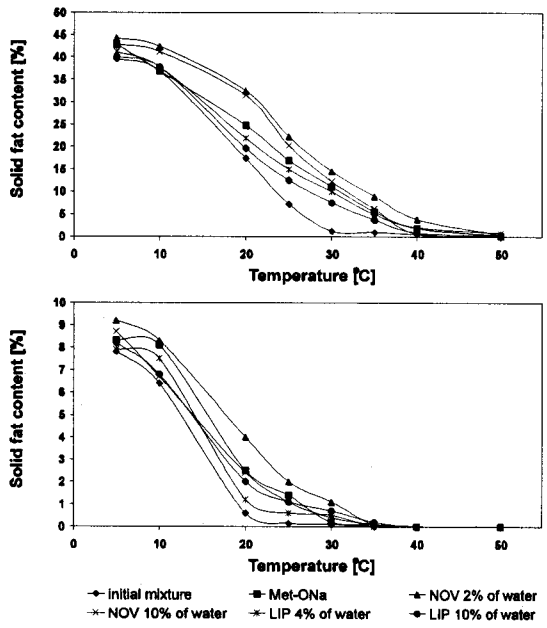

Fig.2: The solid fat content (SFC) versus temperature for initial and interesterified mixtures consisted of 10 % SFO + 90 % O (A) and 60 % SFO + 40 % O (B).

Table 4: Free fatty acids (FFA), mono- and diacylglycerols (MAG + DAG), triacylglicerols (TAG) contents and slip melting points (SMP) of TAGs isolated from stearin (S) and olein (O) and from their mixtures with sunflower oil (SFO) after enzymatic interesterification catalyzed by Novozym 435.

	After interesterification catalyzed by Novozym 435 containing 2% of water				After interesterification catalyzed by Novozym 435 containing 10% of water			
Fat sample	FFA [%]	MAG+ DAG [%]	TAG [%]	SMP of TAG [°C]	FFA [%]	MAG+ DAG [%]	TAG [%]	SMP of TAG [°C]
Stearin (S)	2.0	13.0	85.0	53.1	11.2	18.0	70.8	49.3
10% SFO + 90% S	2.2	9.8	88.0	50.3	9.7	18.1	72.2	46.5
25% SFO + 75% S	2.1	9.5	88.4	47.9	9.7	20.4	69.9	41.4
40% SFO + 60% S	1.8	6.4	91.8	45.5	9.5	22.0	68.5	39.0
50% SFO + 50% S	1.7	6.3	92.0	42.5	9.9	23.0	67.1	34.2
60% SFO + 40% S	1.5	5.5	93.0	40.2	10.6	22.9	66.5	28.5
Olein (O)	3.1	5.8	91.1	41.7	9.0	18.8	72.2	40.1
10% SFO + 90% O	2.4	7.6	90.0	37.9	10.1	16.1	73.8	36.3
25% SFO + 75% O	3.0	7.7	89.3	34.6	10.1	17.2	72.7	31.5
40% SFO + 60% O	2.8	8.0	89.2	33.3	11.2	18.3	70.5	28.4
50% SFO + 50% O	2.6	8.2	89.2	32.1	10.7	19.3	70.0	20.2
60% SFO + 40% O	2.7	11.2	86.1	28.0	11.2	21.5	67.3	17.8

Table 5: Free fatty acids FFA, mono- and diacylglycerols (MAG + DAG), triacylglycerols (TAG) contents and slip melting points (SMP) of TAGs isolated from stearin (S) and olein (O) and from their mixtures with sunflower oil (SFO) after enzymatic interesterification catalyzed by Lipozyme IM.

	After interesterification catalyzed by Lipozyme IM containing 4% of water				After interesterification catalyzed by Lipozyme IM containing 10% of water			
Fat sample	FFA [%]	MAG+ DAG [%]	TAG [%]	SMP of TAG [°C]	FFA [%]	MAG+ DAG [%]	TAG [%]	SMP of TAG [°C]
Stearin (S)	2.7	15.3	82.0	51.0	14.1	9.8	76.1	44.9
10% SFO + 90% S	2.8	10.2	87.0	49.5	11.5	12.5	76.0	39.9
25% SFO + 75% S	2.5	10.0	87.5	47.6	11.0	17.0	72.0	34.6
40% SFO + 60% S	2.6	9.4	88.0	44.0	11.4	15.6	73.0	33.2
50% SFO + 50% S	2.7	8.4	88.9	40.7	11.3	23.7	75.0	26.7
60% SFO + 40% S	2.7	7.9	89.4	39.6	11.9	25.1	73.0	20.8
Olein (O) 4.5	9.7	85.8	36.5	10.6	14.1	75.3	36.3	
10% SFO + 90% O	5.4	9.6	85.0	32.6	11.0	14.0	75.0	34.0
25% SFO + 75% O	6.6	11.1	81.8	23.8	10.5	16.5	73.0	31.2
40% SFO + 60% O	5.4	9.9	84.7	17.1	11.0	18.0	71.0	25.7
50% SFO + 50% O	5.3	11.8	82.9	12.1	11.4	16.6	72.0	19.3
60% SFO + 40% O	5.8	12.9	81.3	11.9	11.6	14.4	74.0	11.2

Table 6: Fatty acids composition (TAG total) and distribution between the (sn-2) and(sn-1,3)* positions for triacylglycerols obtained from the mixture of sunflower oil (50%) and stearin (50%) after enzymatic interesterification.

		Interesterified / Novozym 435		Interesterified / Lipozyme IM	
Fatty acid	TAG total [%]	% in sn-2	% of a given fatty acid in sn-2	% in sn-2	% of a given fatty acid in sn-2
14:0	1.9	2.2	38.5	2.9	50.9
16:0	20.2	17.9	29.5	14.9	24.6
16:1 (9 c)	0.7	0.7	33.3	0.6	28.6
17:0	1.3	1.2	30.8	1.2	30.8
18:0	18.7	16.7	29.8	15.0	26.7
18:1 (9 t)	1.0	0.7	23.3	0.6	20.0
18:1 (9 c)	21.7	23.2	35.6	24.5	37.6
18:2 (9, 12 c)	30.4	33.4	36.6	36.8	40.4

* sn-1,3 = [3 TAG total – (sn-2)] : 2

Table 7: Fatty acids composition (TAG total) and distribution between the (sn-2) and (sn-1, 3)* positions for triacylglycerols obtained from the mixture of sunflower oil (50%) and olein (50%) after enzymatic interesterification.

		Interesterified / Novozym 435		Interesterified / Lipozyme IM	
Fatty acid	TAG total [%]	% in sn-2	% of a given fatty acid in sn-2	% in sn-2	% of a given fatty acid in sn-2
14:0	1.6	1.8	37.5	1.9	39.6
16:0	16.2	15.4	31.7	10.2	21.0
16:1 (9 c)	1.3	1.3	33.3	1.3	33.3
17:0	0.9	0.7	25.9	0.4	14.8
18:0	11.4	10.4	30.3	7.5	21.9
18:1 (9 t)	1.2	1.0	27.8	0.5	13.9
18:1 (9 c)	31.4	31.9	33.9	38.3	40.7
18:2 (9, 12 c)	31.0	31.8	34.2	36.3	39.0

* sn-1,3 = [3 TAG total – (sn-2)] : 2

sunflower oil reduced its concentration. After interesterifications the content of trans C18:1 isomer retained on the same level as for initial blends, independent on catalyst used.

The results obtained in this work showed that interesterifications of sunflower oil and stearin or olein blends produce new fats that, when purified, are suitable for use in various applications, thus widening the utilization of beef tallow.

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