

Modification of Beef Tallow Stearin by Chemical and Enzymatic Interesterification with Rapeseed Oil

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Abstract: Beef tallow stearin was blended at various proportions with rapeseed oil and the blends were interesterified using sodium methoxide or immobilized lipases from *Rhizomucor miehei* (Lipozyme IM) and *Candida antarctica* (Novozym 435) as the catalysts. The starting blends and the products of interesterification were quantitatively separated into the triacylglycerols and non-triacylglycerol fractions containing free fatty acids and of mono- and diacylglycerols. It has been found that after interesterification the contents of free fatty acids and of mono- and diacylglycerols increased. For enzymatic interesterification these increases were strongly dependent on water content in enzymatic catalysts. On the other hand the slip melting temperatures and solid fat contents of triacylglycerols separated from interesterified samples were lower if compared with nonesterified blends. The total fatty acid composition of fats before and after interesterifications remained unchanged but their 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, Lipozyme IM, Novozym 435, rapeseed oil, sodium methoxide, 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 (Gunstone, 2000) with the annual production in Poland being about 0.12 million tons. Because of its high melting temperature and low level of unsaturated fatty acids (C18:2, C18:3) tallow is considered as a less valuable fat not suitable for direct human consumption. For edible purposes tallow should be modified before use. In the past it was fractionated to obtain liquid or plastic fractions suitable for edible purposes (Luddy *et al.*, 1976). Other ways of tallow modification is its intraesterification combined with fractionation (MacKenzie and Stevenson, 1995, 2000), or interesterification with vegetable oils. There are several papers on interesterification of beef tallow with vegetable oils (Lo and Handel, 1983; Forssell *et al.*, 1992; Foglia *et al.*, 1993; Ledochowska and Wilczynska, 1998; Rodriguez *et al.*, 2001; Gruczynska *et al.*, 2002; Kowalski *et al.*, 2004a-c). Substantially less information is available on the interesterification of beef tallow fractions with vegetable oils (Bhattacharyya *et al.*, 2000; Kowalska *et al.*, 2005a,b).

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The selection of vegetable oil depends on the regional production. In Europe rapeseed is cultivated on a large scale and the annual production of rapeseed oil is about 4.57 million tones (Gunstone, 2001). With the annual production of 0.35 million tons Poland belongs to the main European producers of rapeseed oil (Gunstone, 2001). As there is local deficiency of solid and semi-solid low trans fats their production is generally desired. Thus the methods that coupled beef tallow or its fraction and rapeseed oil can have some special meaning. Such interesterified fats can be used as low trans margarine oils and as the components of frying fats. The objective of this study was to investigate selected chemical and physical properties of beef tallow stearin and rapeseed oil blends modified by interesterification. Both chemical and enzymatic interesterifications were studied and the properties of interesterified fats were compared with those of the starting blends.

MATERIALS AND METHODS

Materials

Rapeseed oil was commercial product purchased on local market. Its main fatty acids composition was as follows: C16:0 (4.5%), C18:0 (1.8%), C18:1 cis9 (56.9%), C18:2 all cis (19.5%), C18:3 all cis (8.6%), C20:1 cis9 (1.7%), C22:1 cis13 (0.8%). Beef tallow was Laboratory refined, bleached and deodorised under vacuum at 105°C. Then it was fractionated from acetone and tallow stearin (20% yield, slip melting point 54±1°C) and tallow olein (80% yield, slip melting point 24±1°C) were obtained. The parameters of isolated stearin and its fatty acids composition were the same as given in our earlier publication (Kowalska *et al.*, 2005a).

Blends Preparation

Liquefied Stearin (S) was mixed at 70°C under nitrogen with Rapeseed Oil (RSO) in proportions ranging from 10 to 60% of RSO. Five blends containing rapeseed oil and stearin (10% RSO + 90% S, 25% RSO + 75% S, 40% RSO + 60% S, 50% RSO + 50% S and 60% RSO + 40% S) were prepared. The selected properties of starting mixtures are given in Table 1.

Catalysts

Chemical interesterifications were catalyzed by powdered sodium metoxide (CH₃ONa, Merck, Darmstadt Germany) and it was used as supplied. As catalysts for enzymatic interesterification two commercial preparations Lipozyme IM and Novozym 435 (Novozymes A/S, Bagsvaerd, Denmark) were used. Lipozyme IM contains immobilized lipase from *Rhizomucor miehei* and Novozym 435 contains immobilised lipase from *Candida antarctica*. Commercial Lipozyme IM and Novozym 435 contained 4 and 2% of water, respectively.

Table 1: Free Fatty Acids (FFA), mono- and diacylglycerols (MAG + DAG), Triacylglycerols (TAG) and Slip Melting Points (SMP) of TAG for stearin (S) and its mixtures with rapeseed oil (RSO) before and after chemical interesterification

Fat sample	Before interesterification				After chemical interesterification			
	FFA (%)	MAG+ DAG (%)	TAG (%)	SMP of TAG (°C)	FFA (%)	MAG+ DAG (%)	TAG (%)	SMP of TAG (°C)
Stearin (S)	1.1	0.9	98.0	54.3	1.5	6.8	91.7	53.8
10% RSO + 90% S	0.9	1.1	98.0	53.0	1.4	5.6	93.0	48.5
25% RSO + 75% S	0.8	1.1	98.1	51.8	0.9	5.3	93.8	47.5
40% RSO + 60% S	0.5	1.3	98.2	50.5	0.6	6.3	93.1	45.5
50% RSO + 50% S	0.3	1.4	98.3	48.5	0.5	6.7	92.8	40.4
60% RSO + 40% S	0.2	1.2	98.6	46.8	0.5	7.0	92.5	30.5

Chemical Interesterification

Directly before interesterification fats were dried at 90°C under reduced pressure. Flasks containing fat blends were flushed with nitrogen, stoppered and positioned in thermostated mineral oil bath-shaker. After thermal equilibration of sample at 90°C the catalyst (0.6% CH₃ONa, Merck, Darmstadt, Germany) was added under nitrogen. The interesterification was carried out with continuous shaking for 2 h. The reaction was stopped by addition of hot water containing 5% H₃PO₄. Interesterified fats were extracted with hexane, washed with water and dried. Hexane was evaporated under reduced pressure and remaining interesterified fats were analysed.

Enzymatic Interesterification

After thermal equilibration of fat blend at desired temperature (80°C for Novozym 435 or 60°C for Lipozyme IM) 8% of enzymatic catalysts was added. The water contents in catalyst were adjusted directly before reactions. The interesterifications were performed with continuous shaking for 4 h (Novozym 435) or 8 h (Lipozyme IM). After predetermined time of inter-esterification filtering off the catalyst stopped the reaction. As filtering bed contained drying agent water was removed from interesterified fat.

Determination of Free Fatty Acids

Free Fatty Acids (FFA) were determined by titration of fat sample dissolved in a mixture of ethanol: Diethyl ether (1:1 vol/vol) with 0.1 M ethanolic potassium hydroxide solution. The molar masses of fatty acids from analyzed samples were calculated based on the results of gas-liquid chromatography analyses.

Gas-Liquid Chromatography Analyses

The fatty acid compositions of the fats were determined by Gas Liquid-Chromatography (GLC) after conversion of the fats to fatty acids methyl esters. The apparatus and procedure are reported elsewhere (Kowalski *et al.*, 2000).

Separation of Fat Samples into Pure Triacylglycerols and Non-Triacylglycerols Fractions

Fats before and after interesterifications were separated into Triacylglycerol (TAG) and non-TAG fraction, referred to as Polar Fraction (PF) by column chromatography on silica gel (SG 60, 70-230 mesh, Merck, Darmstadt, Germany). The TAG were eluted with the mixture of petroleum ether: Diethyl ether = 87:13 (vol/vol) and the PF fraction which contained FFA, Monoacylglycerols (MAG) and Diacylglycerols (DAG) was eluted with diethyl ether. The contents of the TAG and the PF were determined by weight, after evaporation of eluting solvent.

Slip Melting Point

The slip melting point (SMP, °C), the temperature at which the fat confined in open capillary immersed in water is moving upward, was determined in accordance with Polish Standard PN ISO 638, 1991.

Solid Fat Content by NMR Analysis

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

Positional Distribution of Fatty Acids among sn-2 and sn-1,3 Positions of TAG

The positional distributions of fatty acids between sn-2 and sn-1,3 positions of triacylglycerols were determined using the method developed by Brockerhoff (1965). The method is based on the

ability of an enzyme pancreatic lipase, to selectively hydrolyse ester bonds in the sn-1,3 positions of TAG. The products of lipolysis were separated by Thin Layer Chromatography (TLC) on plates covered with Kieselgel G (Merck) with a developing system petroleum ether: Diethyl ether: Acetic acid = 70:30: 1 (vol/vol/vol). The sn-2 MAG band was scraped off and its lipids were extracted into diethyl ether and subsequently used for fatty acid analysis by GLC. Next, the composition of fatty acids in the sn-1, 3 positions of TAG was computed from the GLC results for samples before and after enzymatic hydrolysis with pancreatic lipase (Kowalski *et al.*, 2004b).

RESULTS AND DISCUSSION

Chemical Interesterification

The chemical interesterifications of RSO + S 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 results are shown in Table 1. The FFA and MAG + DAG contents in interesterified samples increased compared to the starting blends. Consequently the contents of TAG in the post-reaction mixtures decreased. Similar trends have been observed in our earlier studies on interesterification of beef tallow blends with rapeseed oil (Kowalski *et al.*, 2004a,b, 2005). Studying interesterification of beef tallow stearin + soybean oil blends (Kowalska *et al.*, 2005a) we have observed that after chemical interesterification (90°C, 0.6% CH₃ONa) the crude post-reaction products contained 1.6-1.8% FFA, 6.8-11.3% MAG + DAG and 87.0-89.1% of TAG. In another study (Kowalska *et al.*, 2005b) we have found that systems consisted of beef tallow stearin and sunflower oil after chemical interesterification contained 1.2-1.7% FFA, 6.3-8.3% MAG + DAG and 90.0-92.5% TAG. In this research we have obtained slightly higher yields (92.5-93.8%) of TAG fractions in interesterified blends. The FFA and MAG + DAG of interesterified fats were in the range of 0.5-1.5 and 5.3-7.0%, respectively.

The slip melting points of TAG fractions were measured and the results are listed in Table 1. As seen from this Table the SMP values for TAG are lower (48.5-30.5°C) than for the starting blends (53.0-46.8°C). The dependencies of solid fat content on temperature for TAG fractions were determined by pulse-NMR and the results for selected systems (25% RSO + 75% S and 40% RSO and 60% S) are shown in Fig. 1 and 2. As seen from the plots the SFC

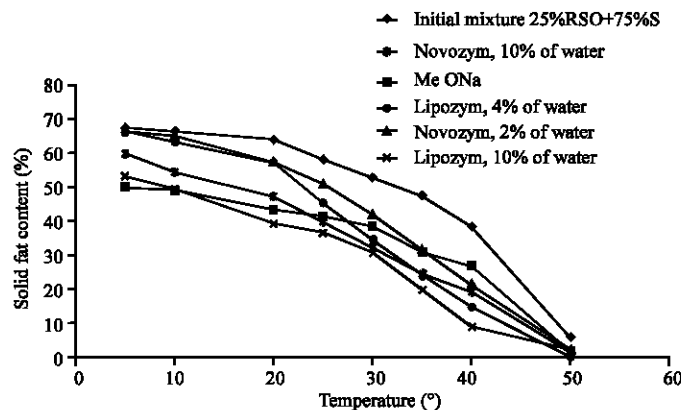


Fig. 1: The solid fat content versus temperature for initial and interesterified mixtures consisted of 25% RSO + 75% S

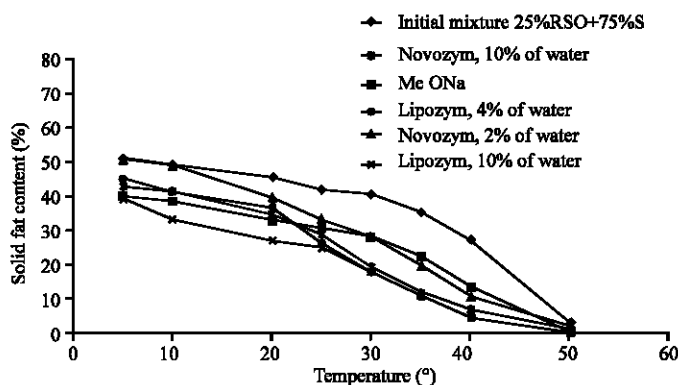


Fig. 2: The solid fat content versus temperature for initial and interesterified mixtures consisted of 40% RSO + 60% S

Table 2: Fatty acid composition (TAG Total) and its content in the (sn-2) position for triacylglycerols isolated from the mixture of rapeseed oil (25%) and stearin (75%) before and after chemical interesterification

Fatty acid	TAG total (%)	Before interesterification		Chemically interesterified	
		% in sn-2*	% of a given fatty acid in sn-2	% in sn-2*	% of a given fatty acid in sn-2
14:0	3.1	4.7	50.5	3.3	35.5
16:0	28.8	22.4	25.9	26.8	31.0
16:1 (9 c)	1.3	0.9	23.1	1.3	33.3
17:0	1.3	1.7	43.5	1.3	33.3
18:0	26.0	21.9	28.1	25.7	32.9
18:1 (9 t)	1.5	0.7	15.6	1.3	28.9
18:1 (9 c)	24.6	27.0	36.5	23.6	32.0
18:2 (9, 12 c)	5.5	8.7	52.7	6.1	36.9
18:3 (9,12,15c)	2.2	3.9	59.0	2.2	33.3

*: % sn-1,3 = (3 x (% TAG total) - (% in sn-2)) : 2

values for TAG isolated from chemically interesterified blends are lower than for starting blends. For other compositions of blends their SFC patterns were similar and there were only quantitative differences.

As expected the positional distribution of fatty acids between the sn-2 and sn-1,3 positions in TAG of chemically interesterified mixtures was near random and different from the starting blends. The data showed in Table 2 for blend containing 25% of RSO and 75% of stearin before and after chemical interesterification serve as the example.

Enzymatic Interesterification

For enzymatic interesterification, the blends studied, the time and temperature of reaction and catalyst dose were established in our earlier experiments (Kowalski *et al.*, 2004a,b; Kowalska *et al.*, 2005a,b). 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 shown in Table 3 and 4. Comparing the results for initial blends (Table 1) and enzymatically interesterified blends (Table 3 and 4) a sharp increase in the FFA and MAG + DAG contents is observed, especially at 10% water content in biocatalyst used. The TAG fractions were isolated from crude interesterified fats and their slip melting points were measured (Table 3 and 4). As seen from these Tables the SMP of enzymatically

Table 3: Free Fatty Acids (FFA), mono-and diacylglycerols (MAG + DAG), Triacylglycerols (TAG) and Slip Melting Points (SMP) of TAG isolated from Stearin (S) and from its mixtures with rapeseed oil (RSO) after enzymatic interesterification catalyzed by Novozym 435

Fat sample	After interesterification catalyzed by Novozym 435 containing 2% of water				After interesterification catalyzed by Novozym 435 containing 10% of water			
	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% RSO + 90% S	2.4	10.6	87.0	49.3	9.6	13.4	87.0	43.2
25% RSO + 75% S	2.0	7.6	90.4	47.2	9.9	13.0	77.1	40.8
40% RSO + 60% S	1.8	7.2	91.0	44.0	9.8	15.7	74.5	37.8
50% RSO + 50% S	2.3	6.7	91.0	40.7	9.7	25.8	64.5	29.2
60% RSO + 40% S	2.0	7.0	91.0	34.3	9.7	21.9	68.4	24.9

Table 4: Free Fatty Acids (FFA), mono-and diacylglycerols (MAG + DAG), Triacylglycerols (TAG) contents and Slip Melting Points (SMP) of TAG isolated from stearin (S) and from its mixtures with rapeseed oil (RSO) after enzymatic interesterification catalyzed by Lipozyme IM

Fat sample	After interesterification catalyzed by lipozyme IM containing 4% of water				After interesterification catalyzed by lipozyme IM containing 10% of water			
	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% RSO + 90% S	4.0	10.0	86.0	45.2	14.0	13.2	72.8	41.9
25% RSO + 75% S	3.3	7.7	89.0	43.8	14.2	13.8	72.0	38.9
40% RSO + 60% S	3.5	11.5	85.0	41.3	14.6	13.0	72.4	36.6
50% RSO + 50% S	3.5	9.5	87.0	35.2	14.7	13.6	71.7	26.2
60% RSO + 40% S	3.5	9.5	87.0	29.6	14.2	13.5	72.3	21.3

Table 5: Fatty acids composition (TAG total) and its content in the (sn-2) position for triacylglycerols isolated from the mixture of rapeseed oil (25%) and stearin (75%) after enzymatic interesterification catalyzed by Novozym 435 and Lipozyme IM

Fatty acid	TAG total (%)	Interesterified/Novozym 435		Interesterified/Lipozyme IM	
		% in sn-2*	% of a given fatty acid in in sn-2	% in sn-2*	% of a given fatty acid in sn-2
14:0	3.1	3.5	37.6	4.4	47.3
16:0	28.8	25.1	29.1	22.9	26.5
16:1 (9 c)	1.3	1.1	28.2	1.0	25.6
17:0	1.3	1.4	35.9	1.6	41.0
18:0	26.0	25.4	32.6	22.0	28.2
18:1 (9 t)	1.5	1.3	28.9	0.9	20.0
18:1 (9 c)	24.6	23.1	31.3	26.4	35.7
18:2 (9, 12 c)	5.5	6.0	36.4	8.4	50.9
18:3 (9,12,15c)	2.2	2.5	37.9	4.0	60.6

*% sn-1,3 = (3 x (% TAG total) - (% in sn-2)): 2

interesterified TAG are lower than for starting blends. The reduction in SMP was proportional to the water content in the enzyme catalysts. It appeared that TAG from the reactions catalyzed by Novozym 435 or Lipozyme IM showed similar SMP reductions. The reduction of SMP of interesterified stearin + rapeseed oil blends is caused by alteration of lipids structure on molecular level. Due to exchange of fatty acids within and between TAGs new acylglycerols are formed and new interrelations among them can appear. The distribution of fatty acids between the sn-2 and sn-1,3 positions of TAG after enzymatic interesterification were determined for selected (25% RSO + 75% S) systems (Table 5). When Novozym 435 was used as catalyst we have observed that some positional randomization of fatty acids in TAGs has occurred. On the other hand, the sn-2 percentage data (Table 5) showed that the distribution of fatty acids is still far from statistical (33.3%) and even from this one obtained for chemical interesterification (Table 2).

A different situation was observed for TAG obtained from enzymatic interesterification catalyzed by Lipozyme IM. Due to positional (sn-1,3) specificity of the lipase, the interesterifications in these cases occurred mainly at the sn-1,3 positions of TAGs. Comparison of the data for starting and interesterified in presence of Lipozyme IM blends indicated that the distribution of fatty acids at the sn-2 and sn-1,3 positions are similar. As the enzyme reacts on the sn-1,3 ester linkages the percentage of particular fatty acids in the sn-2 positions of TAG in comparison with their counterparts in the starting blends remain nearly unchanged. The small changes in fatty acids at the sn-2 positions may result from acyl migration as reported by Xu *et al.* (1998).

The altered fatty acid composition of the interesterified stearin-rapeseed oil mixtures also was reflected in the solid fat content over the temperature range of 5-50°C. Significant reductions in the solid fat contents were detected for the TAG fractions isolated from blends after interesterification. The experimental data of SFC dependency versus temperature for selected systems (25% RSO + 75% S and 40% RSO + 60% S) are shown in Fig. 1 and 2.

Beef tallow stearin contained 1.9% of C18:1 trans acid (Kowalska *et al.*, 2005a,b). Rapeseed oil contained 0.1% C18:2 and 0.8% C18:3 trans fatty acids. Blending reduced the concentrations of trans isomers and after interesterifications, their content remained at the same level, independent of the catalyst used. The results obtained in this work show that interesterifications of stearin + RSO blends produce new fats that, when purified, are suitable for use in various food application, thus widening the utilization of beef tallow.

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