Effect of Enzymatic Interesterification on the Melting Point of Tallow-Rapeseed Oil (LEAR) Mixture

P. Forssell[a,*], R. Kervinen[a], M. Lappi[a], P. Linko[b], T. Suortti[a] and K. Poutanen[a]
[a]VTT Food Research Laboratory, SF-02151 Espoo, Finland and [b]Helsinki University of Technology, Laboratory of Biotechnology and Food Engineering, Kemistintie 1, SF-02150 Espoo, Finland

To reduce the melting point of a tallow-rapeseed oil mixture, the triglyceride composition of the mixture was altered by enzymatic interesterification in a solvent-free system. The interesterification and hydrolysis were followed by melting point profiles and by free fatty acid determinations. The degree of hydrolysis was linearly related to the initial water content of the reaction mixture. The rate of the interesterification reaction was influenced by the amount of enzyme but not much by temperature between 50 and 70°C. The melting point reduction achieved by interesterification depended on the mass fractions of the substrates: the lower the mass fraction of tallow, the larger the reduction of the melting point.

KEY WORDS: Interesterification, lipase, melting point, rapeseed oil, tallow.

Interesterification reactions of lipids have traditionally been carried out with chemical catalysts. By replacing the catalysts with enzymes, interesterification reactions can be directed in controlled ways, and specific or totally new products can be produced. Since the study of Stevenson *et al.* (1) in 1979, many reports concerning enzymatic interesterification have been published. Much of this interest has focused on the production of cocoa-butter substitutes by means of 1,3-specific lipases (2). Reactions between solid fat and liquid oil have been studied to obtain fat mixtures with better melting behavior (3,4). Interesterification also has been proposed for enrichment of polyunsaturated fatty acids in fish oils (5), and for reduction of the linolenic acid content of soybean oil (6). Specialty lipids, which may have dietetic applications, are also potential products of enzymatic interesterification reactions (7).

Although considerable research has been conducted, the development of enzymatic lipid modification processes have not yet been adopted by industry. Despite the obvious advantages of enzymatic interesterification, the economics of this option still need to be improved. Of particular importance is the effect of water on reaction kinetics (8). The parameters influencing the reaction equilibrium are reasonably well understood, but knowledge on the control and kinetics of the reaction is still rather limited (9).

A few studies on enzymatic lipid modification without the use of organic solvents have been published (3,10–12). Typically, higher temperatures are required in the absence of solvents. Posorske, *et al.* (11) studied interesterification reactions of palm stearine and coconut oil in a continuous solvent-free reactor with the aim of changing the melting behavior of the fat mixture. Process configurations were studied by interesterification of olive oil or soybean oil with lauric acid. More recently, the solvent-free system was shown to be superior compared with organic-solvent systems (12). The 1,3-specificity of the lipase used was much more selective in the solvent-free environment.

In this work the enzymatic interesterification of tallow and LEAR (Low Erucic Acid Rapeseed) oil was studied in a solvent-free system to produce fat mixtures with improved melting properties.

MATERIALS

Enzyme. A commercial immobilized 1,3-specific *Mucor miehei* lipase preparation (Lipozyme, Novo Industry A/S, Bagsvaard, Denmark) was used as catalyst. The enzyme was immobilized on an anion exchange resin. The moisture content of the enzyme preparation used in interesterification reactions was 11%, which was determined as weight loss after drying the preparation at 105°C for 24 hr. In experiments in which dried lipase was used, the enzyme preparation was dried by lyophilization (Dr. Morand Minilyo I 3002, Zürich, Switzerland). The moisture content of the lyophilized enzyme was 1%. This was neglected when the initial water content of the system (weight percentage of water in the reaction mixture) was calculated. The declared activity of the enzyme was 25 BIU/g.

Substrates. The tallow and LEAR oil were refined, bleached and deodorized products donated by Raisio Group, Raisio, Finland. The moisture contents of the substrates were determined with a Karl Fischer titrator (DL 18, Mettler, Zürich, Switzerland).

METHODS

Interesterification reactions. They were carried out in 20-g batches in thermostated reactors with magnetic stirring. No solvent was used. The substrate mixture was incubated for five minutes at the reaction temperature before addition of the enzyme. The reaction was stopped by removing the enzyme from the reaction mixture after centrifugation.

Triglycerides. The reaction mixtures were analyzed by nonaqueous reverse-phase liquid chromatography on a Novapak C-18 column (Millipore/Waters, Milford, MA) with acetone-acetonitrile-tetrahydrofuran (75:25:10, v/v/v) as the eluent at 35°C, at a flow rate of 2 mL/min, and a refractive index detector (M-411, Millipore/Waters). The sample size was 25 μL and the sample concentration was 10 mg/mL. For identification of the triglycerides, triolein (T-9257), tristearin (T-1521) and tripalmitin (T-8127), all from Sigma, St. Louis, MO, and triglycerides made by interesterification of triolein with palmitic acid (P-5917), stearic acid (S-4751), linoleic acid (L-1268) or linolenic acid (L-2376) were used as standards.

Free fatty acids (FFA). FFA were quantitated with 0.1 M NaOH in an automatic titrator (Titroprocessor 686 and

*To whom correspondence should be addressed at VTT Food Research Laboratory, P.O. Box 203, SF-02151 Espoo, Finland.

Dosimat 665, Metrohm, Herisau, Switzerland), with acetone-ethanol-water mixture as solvent, by titrating the samples to the end point of pH = 12.

Percentage solid fat. This variable was measured in the fat mixture at a constant temperature by pulse nuclear magnetic resonance (NMR) (Minispec p-20, Bruker, Karlsruhe, Germany) after removal of free fatty acids by extraction. For the extraction, the fat mixture was dissolved in light petroleum ether, and free fatty acids were washed with ethanolic NaOH. After the extraction, the petroleum ether phase was dried with Na_2SO_4. Petroleum ether was evaporated from the triglyceride mixture by vacuum evaporator. Samples were prepared by tempering at 60°C for 10 min and by storing in an ice batch in a refrigerator for 30 min.

Melting points. They were measured by the closed-capillary technique (AOCS, Cc 1-25). Samples were prepared in the same way as in the determination of solid fat content except for the tempering procedure. Capillaries filled with fat mixture were stored in a refrigerator overnight before the measurements.

Fatty acid composition. Samples were analyzed by gas chromatography-flame ionization detection (Carlo Erba Fractovap 2300, Milano, Italy). A polar 25 m × 0.25 mm × 0.25 μm column (Scientific Glass Engineering, GE BP-21, Ringwood Victoria, Australia) was used for the separation. Elution was carried out with temperature programming from 150 to 190°C at 10°C/min.

RESULTS AND DISCUSSION

Interesterification of tallow. Pure tallow was used as substrate to study the effect of the water content of the system on the degree of hydrolysis, and also to investigate the changes in the melting behavior of the tallow caused by interesterification. At an initial water content of 0.5%, the reaction approached equilibrium within two hours. After 24 hr, the degree of hydrolysis was about 7% (Table 1). The relative amounts of free palmitic and stearic acids increased during the interesterification (Table 1). This can be explained by the 1,3-specificity of *Mucor miehei* lipase (3), because oleic acid is mainly located at the 2-position of the triglyceride backbone of the tallow (13).

The total degree of hydrolysis was linear with the initial water content of the reaction mixture in the range of 0 to 2% water. Thus, relatively small changes in the water content can markedly affect the degree of hydrolysis. The degree of hydrolysis did not depend on whether the water was added with the enzyme preparation or with the substrate. Even the low water content of tallow together with the lyophilized enzyme (≈ 0.05% water in the mixture) caused a small degree of hydrolysis (Fig. 1).

Changes in the triglyceride composition of tallow after the enzymatic treatment were minimal as analyzed by high-performance liquid chromatography (HPLC) (Fig. 2) and by solid fat content (Fig. 3). Although the enzyme was 1,3-specific, this result was similar to that obtained by interesterifying tallow with the aid of a chemical catalyst (14).

Interesterification of tallow and rapeseed oil. The lipase-catalyzed interesterification of tallow and rapeseed oil caused many changes in the triglyceride composition of the lipid mixture, especially among the stearic acid derivatives. Tristearin totally disappeared, and several new triglycerides were formed (Fig. 4). The identification of triglycerides was based on the concept of theoretical

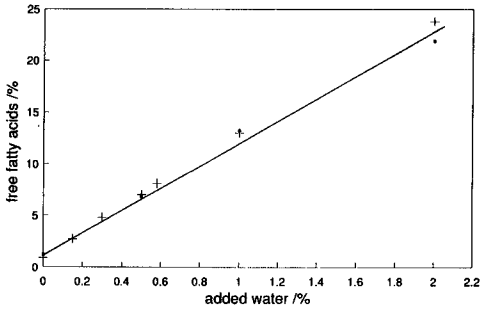

FIG. 1. Free fatty acids formed after the interesterification reaction of tallow at 60°C for 24 hr. The change of the initial water content of the system was made by adding water to the reaction mixture at an enzyme concentration of 50 mg/g substrate (■) or by varying the enzyme concentration (+).

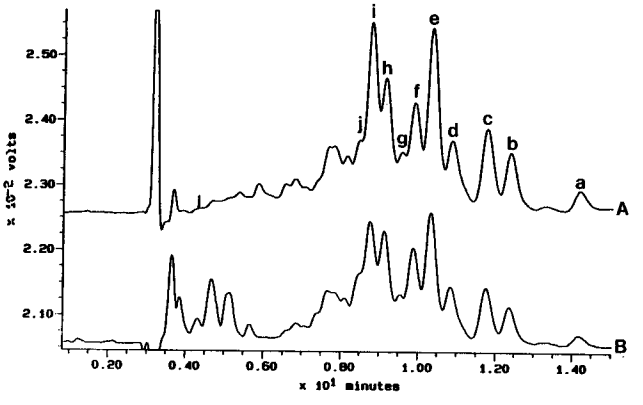

FIG. 2. HPLC chromatograms of the triglyceride composition of tallow before (A) and after (B) interesterification for 24 hr at 60°C with an enzyme dosage of 50 mg/g substrate. a = SSS, b = SSP, c = SSO, d = SPP, e = PSO, f = SOO (or SSLi), g = PPP, h = PPO, i = POO (or LiSP) and j = OOO, where S = stearic acid, P = palmitic acid, O = oleic acid, Li = linoleic acid.

TABLE 1

The Formation of Free Fatty Acids (FFA) and Composition of the FFA-Fraction During the Interesterification of Tallow at 60°C with Enzyme Dosage of 50 mg/g Substrate. For Comparison, the Fatty Acid Composition of Tallow Is Also Shown.

Reaction time (hr)	FFA (%)	Fatty acid (%)			
		16:0	18:0	18:1	18:2
1	6.0	33	35	24	3
6	5.7	30	34	27	3
24	6.8	29	34	29	4
Tallow		24	25	33	5

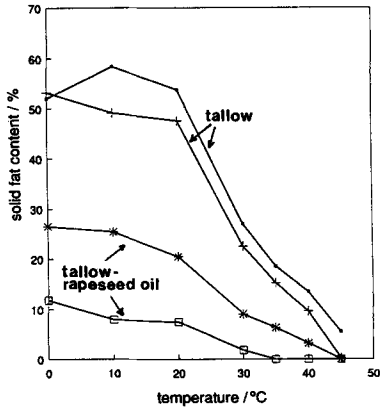

FIG. 3. The effect of interesterification on the solid fat content of tallow and tallow-rapeseed oil mixture before (■,*) and after (+,□) interesterification for 24 hr at 60°C.

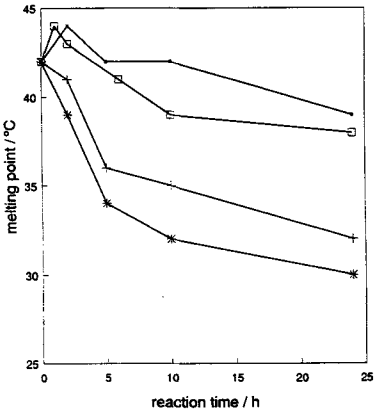

FIG. 5. The melting point of tallow-rapeseed oil mixture during the interesterification reaction. Reaction conditions: mass fraction of tallow 0.5, temperature 60°C and wet lipase dosage 5 mg (■), 25 mg (+), 50 mg (*) per gram of substrate, and dried lipase dosage 50 mg (□) per gram of substrate.

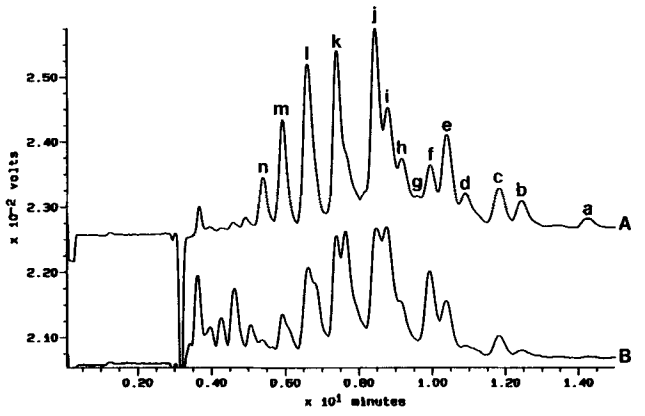

FIG. 4. HPLC chromatograms of tallow-rapeseed oil mixture before (A) and after (B) interesterification for 24 hr at 60°C with an enzyme dosage of 50 mg/g substrate. k = LiOO, l = LnOO and LiLiO, m = LiOLn (or LnLnLn) and n = LnLnO, where Ln = linolenic acid. Other notations as for Figure 2.

TABLE 2

Dependence of the Rate of the Melting Point Reduction (ΔMP) of Interesterified Tallow-Rapeseed Oil Mixture (1:1) on Enzyme Concentration, Water Content and Temperature. ΔMP Corresponds to the Rate During the First Two Hours of the Reaction.

Enzyme dosage, mg/g substrate	Temperature °C	ΔMP °C/hr
5	60	0.1
25	60	0.5
50	60	2.0
50[a]	60	0.2
50	50	1.5
50	70	3.0

[a]Enzyme was dried before the reaction.

carbon number (TCN) (15). Diglycerides and monoglycerides are products of hydrolysis of the interesterification reaction of tallow and rapeseed oil. These products were also shown in the chromatograms as a cluster of peaks, which were not well separated from each other under the conditions used. Because the substrate mixture contained several triglycerides, it was impossible to study the kinetics of the interesterification by analyzing the concentration changes of certain specified triglyceride components by HPLC. As the technical aim of the work was to reduce the melting point of tallow-rapeseed oil mixtures, the time course of the reaction was followed by melting point determinations only.

The melting point reduction obtained by interesterification increased with time and enzyme dosage (Fig. 5). The initial extent of the melting point reduction increased 20-fold when the enzyme concentration was increased from 5 to 50 mg/g substrate (Table 2). Under the same conditions, the initial extent of hydrolysis increased only fivefold (results not shown). The total degree of hydrolysis increased with increasing enzyme dosage because, at the same time, the water content of the system became higher (Fig. 6). The degree of hydrolysis achieved was in good agreement with the results obtained with pure tallow (Fig. 1). Increase of temperature from 50 to 70°C doubled the degree of melting point reduction (Table 2).

The effect of water content on the extent of the interesterification reaction also was of importance. The melting point reduction achieved with a dosage of dried lipase of 50 mg/g substrate was similar to that observed with 5 mg/g substrate of wet lipase (Figs. 5 and 6). The optimum moisture content of the lipase is known to be 10% (3). The result obtained may be explained by assuming that only about 10% of the dried catalyst was in active form.

The altered triglyceride composition of the interesterified tallow-rapeseed oil mixture was also reflected in the solid fat content in the temperature range of 0 to 45°C. Significant changes in the solid fat content were detected after interesterification (Fig. 3). The reduction of melting point achieved by interesterification of tallow and rapeseed oil depended on the mass fractions of the substrates (Table 3). An increase in the mass fraction of the tallow decreased the overall change in melting point in the mass fraction range of 0.5 to 0.8. With a fat

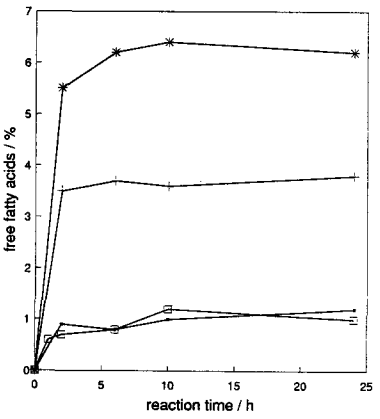

FIG. 6. Formation of free fatty acids in tallow-rapeseed oil mixture during the interesterification reaction. Reaction conditions as for Figure 5.

TABLE 3

Melting Points (°C) of the Fat Mixture During the Interesterification of Tallow and Rapeseed Oil at Different Mass Fraction of the Substrates. Enzyme Dosage Was 50 mg/g Substrate and the Temperature was 60°C.

Reaction time (hr)	Mass fraction of tallow		
	0.50	0.67	0.80
0	42	45	46
2	38	41	41
6	34	38	40
10	32	37	38
24	30	35	39

mixture consisting mostly of tallow (mass fraction 0.8), the melting point reduction was only 6°C. The highest reduction of the melting point, 12°C, was obtained when the mass fraction of tallow was 0.5.

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