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Interesterification of Butter Fat by Lipase from *Rhizopus niveus* in Cosurfactant-Free Microemulsion System

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ABSTRACT

Butter fat was interesterified in a cosurfactant-free microemulsion system containing nonionic and ionic surfactants, using commercial lipase obtained from *Rhizopus niveus*, at different concentrations of surfactant mixtures and hydrophilic-lipophilic balance (HLB) values. The results indicated that the interesterification yield (IY) of lipase-catalyzed interesterified butter fat reached its maximum in the microemulsion system prepared with the surfactant mixture of HLB value of 9, followed by that of HLB value of 10. In addition, increasing concentrations of surfactant mixtures, from 3 to 6 m*M*, resulted by an increase in the IY. The interesterification of butter fat in the microemulsion prepared with 3 m*M* of surfactant mixture of HLB value of 10 showed a minimum hydrolytic activity. The results showed that the interesterified selected triacylglycerol molecules were enriched with the hypocholesterolemic fatty acid C18:1, originally located on *sn*-1,3 positions, on their *sn*-2 positions; this fatty acid was favorably interchanged with the hypercholesterolemic fatty acid C16:0, originally located on *sn*-2 position. The results also indicated that the use of 6 m*M* of surfactant mixtures increased the acyl exchange reaction toward the long-chain saturated fatty acid C16:0 on the *sn*-2 position of triacylglycerol molecules.

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Index Entries: Interesterification; lipase; butter fat; cosurfactant-free microemulsion system.

INTRODUCTION

Enzymes are used, generally, in an aqueous medium, but an organic solvent is more attractive when the reactants or products are hydrophilic compounds and a low-water environment is desired (*1*). The reverse micelles are structurally the reverse of normal micelles in that they have an external shell made up of the hydrocarbons chains of the amphiphilic molecules and the polar or charged head groups, with the counter ion being localized in the interior of the aggregate (*2*). Microemulsions possess special characteristics of relatively large interfacial area, ultra-low interfacial tension, and large capacity as compared to many other colloidal systems (*3*). It is well known that enzymes can be used in organic solvent media containing a surfactant without the loss of their catalytic activity (*4*). However, Martinek et al. (*5*) showed that the use of such media altered dramatically the specificity of alcohol dehydrogenase, a micelle-entrapped enzyme. Recent advances in micellar enzymology, however, provide good grounds to believe that a novel methodology has been developed for construction and practical use of complex model systems involving molecular components of the living matter, as well as for biomimetic design based on the use of synthetic building fragments (*6*).

Walde and Luisi (*7*) reported that the reverse micellar system, formed of anionic surfactant Aerosol-OT in isooctane, is an appropriate medium for lipase biocatalysis. The activities of lipase from *Candida cylindracea* and *Rhyzopus delemar* have also been investigated in water/AOT/isooctane reverse micellar media (*8*). Han et al. (*9*) studied the effects of water content and enzyme concentration on lipase activity in reverse micellar system. Friberg and Kayali (*10*) indicated that the addition of liquid triacylglycerols to the reverse micellar solutions resulted in the formation of lamellar liquid crystal phase, and thereby, an alcohol or acid cosurfactant was needed to form a microemulsion system (*3*). Friberg and Rydhag (*11*) suggested that the destabilization of the liquid crystal phase, containing triacylglycerols, could be performed by the addition of a hydrotrope, such as sodium xylenesulfonate, used as a potent cosurfactant. The common cosurfactants are, however, not useful because of their toxicity, taste, safety, and performance (*3,11*). The amphoteric lecithin is the only food approved surfactant containing a positive charge (*3*).

Luisi et al. (*12*) reported that thermodynamically stable and optically transparent reverse micellar aggregates formed spontaneously on dissolving certain surfactants in an apolar solvent containing small amounts of water; these authors also indicated that although a reverse micelle sys-

tem contains typically <5% (w/v) water, it was possible to solubilize aqueous-soluble substances at least as large as enzymes into the water pools of this system. Eicke and Rehak (*13*) showed, using a model of a water–oil (w/v) microemulsion (AOT/isooctane/water) that with increasing weighed-in water concentrations, the microemulsion is stabilized by aggregational processes of micelles containing water owing to a decrease of the free interfacial enthalpy; these authors suggested that this process conforms very satisfactorily to a model describing an adsorption of surfactants at the water–hydrocarbons interface resulting from dipole-image dipole interactions.

An enzyme molecule is entrapped into hydrated reverse micelles of a surfactant, where the dimensions of the internal hole of the micelles is dependent on the ratio of water to surfactant (*14*). However, the entrapped enzyme molecule is protected against denaturation (unfolding) in a fashion where the surface of the "interface" between the protein globule and the organic solvent is stabilized by molecules of the surfactant (*15*). As a result, the biocatalyst can avoid direct contact with the unfavorable organic medium, since the enzyme is enclosed in a sort of a microreactor containing a limited amount of water, i.e., dozens to several hundreds of water molecules/molecule of the enzyme (*15*). Walde and Luisi (*7*) showed that lipases are of particular interest, since they act on water-insoluble substrates that can be dissolved at a relatively high concentration in the bulk organic phase.

The positional specificity of 1,3-specific lipases from *Mucor miehei, Aspergillus niger,* and *Pseudomonas fluorescens* in isooctane (*16*), in hexane (*17*), and without any organic solvent (*18*) was investigated. The optimum conditions for porcine pancreatic lipase-catalyzed acyl-exchange reactions among a free fatty acid, undecanoic acid, and butter oil in anhydrous media were established (*19*); these authors showed that no solvent was required for the enzymatic reaction, suggesting that the butter oil could act as dispersant in the reactive mixture. However, Bello et al. (*20*) investigated the effect of a microemulsion system composed of triolein, very low amount of water, a surfactant (Brij 35), and an alcoholic cosurfactant on the behavior of lipase from *C. cylindracea* during the interesterification reaction.

This study is part of ongoing research (*21–24*) aimed at the optimization of interesterification of selected fatty acids in butter fat using organic solvent media. As far as the authors are aware, there has been little information available regarding the effect of a cosurfactant-free microemulsion system on the positional distribution of fatty acids of lipase-catalyzed interesterified butter fat. The objective of this study was to interesterify the butter fat in a cosurfactant-free microemulsion, using commercial lipase from *Rhizopus niveus* to determine the interesterification yield and to monitor the changes in the positional distribution of fatty acids on selected triacylglycerol molecules.

MATERIALS AND METHODS

Materials

Commercial lipase (Lipase N), obtained by a unique fermentation process from a selected strain of *R. niveus*, was kindly supplied by Amano Pharmaceutical Co. LTD (Nagoya, Japan). Surfactants Span 60 (sorbitol monostearate) and Tween 60 (polyoxyethylene sorbitan monostearate) were obtained from ICI Specialty Chemicals, Alkemix Inc. (Brantford, Ontario). Phosphatidylcholine, a commercial soybean lecithin was purchased from Sigma Chemical Co. (St. Louis, MO). Butter fat used throughout this study was obtained from the local market. Fatty acids and mono-, di-, and triacylglycerols standards were purchased from Nu Check Prep. (Elysian, MN). Acetone, acetonitrile, chloroform, diethyl ether, and hexane (Omnisolv grade) were purchased from BDH Inc. (Toronto, Ontario). Commercial pancreatic lipase for deacylation of triacylglycerols was obtained from Solvay Enzyme Inc. (Elkhart, IN). Thin-layer chromatography (TLC) plates and silica gel GF 254 were purchased from Merck (Darmstadt, Germany).

Preparation of Surfactants

Span 60, Tween 60, and lecithin, approved by the Food and Drugs Act and Regulations of Canada as food surfactants, were used to prepare cosurfactant-free microemulsion systems. Stock solutions of 0.01*M* Span 60, 0.33*M* Tween 60, and 1.7*M* lecithin in hexane were prepared. Different hydrophilic–lipophilic balance (HLB) values were obtained by mixing different proportions of surfactants as described in Table 1.

Preparation of Microemulsion Systems

The preparation of the cosurfactant-free microemulsion systems was performed as a modification of the method described previously by El-Nokaly et al. (3). The enzyme stock suspension was prepared by the solubilization of 400 mg (80 mg protein) of lipase in 1 mL of sodium phosphate buffer solution (0.1*M*, pH 7.0). The selected concentrations of surfactant mixtures (3 and 6 m*M*) and surfactant HLB values were obtained by the solubilization of the appropriate proportions of surfactants in hexane as shown in Table 1. One hundred microliters of diluted lipase suspension (8 mg protein) were added to the surfactant mixture. The addition of the enzyme preparation to the surfactant mixtures, with HLB values of 8, 10, and 12, and lecithin produced spontaneously the microemulsion system.

Interesterification Reaction

Five grams of butter were melted at 37°C and mixed with 1 mL of 0.02 m*M* sodium xylenesulfonate solution in hexane; this solution was added

Table 1
Proportions of Surfactant Mixtures
Used for the Preparation of Cosurfactant-Free Microemulsion Systems

Mixture number	Relative percentage, %[a,b]				
	Span 60[c]	Tween 60[d]	Lecithin[e]	HLB value[f]	Mol wt[g]
1	100	0	0	5	430.0
2	87	13	0	6	541.8
3	68	32	0	8	705.2
4	0	0	100	9	511.6
5	48	52	0	10	877.2
6	28	72	0	12	1049.2
7	6	94	0	14	1238.4
8	0	100	0	15	1290.0

[a]Relative percent of concentration of each surfactant to the total weight of the mixture.
[b]Results were obtained from triplicate trials with a relative SD ≤ 0.05.
[c]Sorbitol monostearate surfactant.
[d]Polyoxyethylene sorbitan monostearate surfactant.
[e]Phosphatidylcholine.
[f]HLB of surfactants calculated according to the procedure described by Attwood and Florence (*31*).
[g]Molecular weight of the surfactant mixtures, which is the sum of the relative percentage mole for each component used in the preparation of the surfactant mixture.

to 9 mL of the microemulsion system. Interesterification reaction was performed on a reciprocal shaking water bath (37°C, 145 rpm; Model 25, Precision Scientific Inc., Chicago, IL) in a 50-mL vacuum-sealed flask for 48 h. Two milliliters of the reaction mixture were withdrawn, and the enzymatic action was halted by the addition of 4 mL chloroform. The solution was then filtered through a 0.45-μm membrane filter (*25*) to remove the residual lipase before HPLC analysis. The interesterification yield (IY) is defined (*22*) as:

$$\text{Interestification (\%)} = \{[(\%)\ \text{RPA of modified butter} - (\%)\ \text{RPA of untreated butter}] / [(\%)\ \text{RPA of untreated butter}]\} \times 100 \quad (1)$$

where RPA = relative peak area of selected triacylglycerol fractions, 8–16, obtained from high-performance liquid chromatography (HPLC) profiles (Fig. 1A–E).

Separation of Triacylglycerols

The triacylglycerols of interesterified butter fat (100 mg) were dissolved in 0.5 mL hexane and separated by their application on a Supelclean LC-SI column (Supelco Inc., Bellefonte, PA), pre-equilibrated with 5 mL of hexane. Triacylglycerols were eluted with 2 × 2.5 mL of a mixture of hexane/diethyl ester (93:7, v/v).

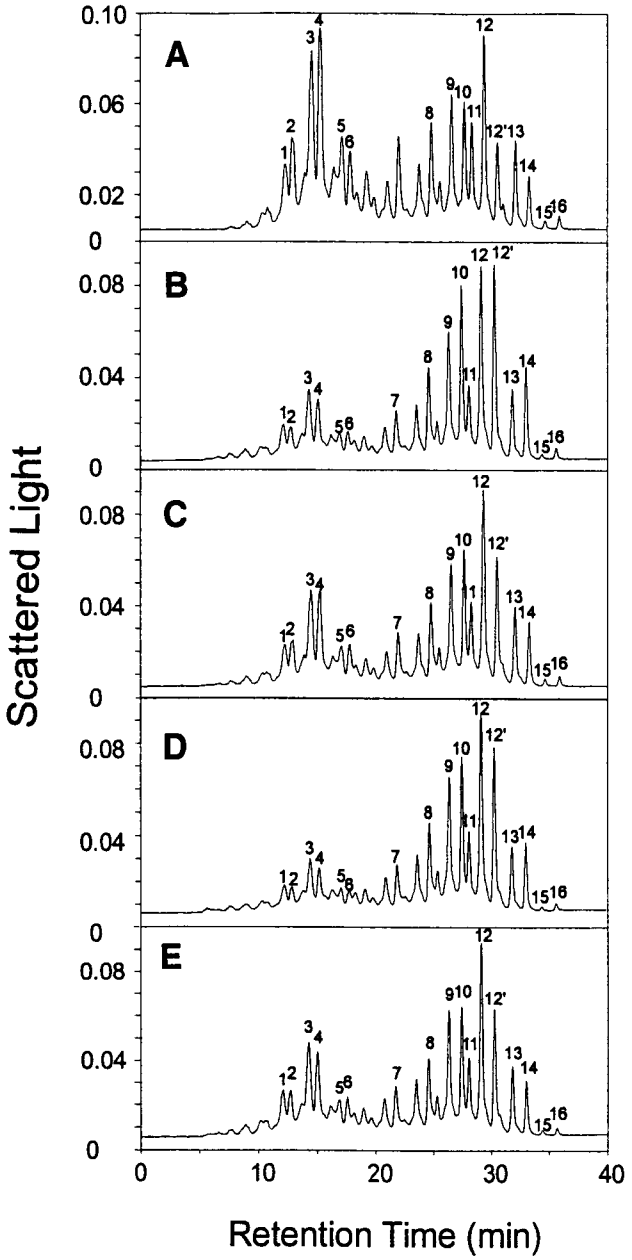

Fig. 1. Chromatograms of HPLC analysis of triacylglycerol fractions from (**A**) untreated butter fat and interesterified butter fats, using commercial lipase (lipase N, Amano), in cosurfactant-free microemulsion systems containing (**B**) 3 m*M* of surfactant mixture with HLB value of 8, (**C**) 6 m*M* of surfactant mixture with HLB value of 8, (**D**) 6 m*M* of surfactant mixture of HLB value of 9, and (**E**) 3 m*M* of surfactant mixture with HLB value of 10. Peak numbers correspond to triacylglycerol fractions separated on the basis of fatty-acid-length chain according to the method of Kermasha et al. (*21*).

Determination of Fatty Acid Composition

Qualitative and quantitative analyses of free fatty acids (FFA) were performed by gas-liquid chromatography (GLC). The preparation of methyl esters from free and bound fatty acids was carried out according to the procedure described by Badings and De Jong (*26*). The GLC analyses of free and bound fatty acids of interesterified butter fat were carried out according to the procedure described previously (*22*).

Determination of Positional Distribution of Fatty Acids

The determination of fatty acids at *sn*-2-position was performed according to the procedure described by Mattson and Volpenhein (*27*). The untreated and interesterified butter fat samples were treated according to the procedure developed in our laboratory (*23*). The determination of fatty acids at *sn*-1,3-positions was carried out by the formation of *rac*-1,2- and 1,3-diacylglycerols, generated by Grignard reaction, according to the procedure described by Myher and Kuksis (*28*). After washing the mixture with water and sodium hydrogen carbonate, the aqueous phase was removed and the *rac*-1,2- and 1,3-diacylglycerols were recovered by TLC coated with borate-impregnated silica gel GF-254 using chloroform-acetone (97:3, v/v) as the mobile phase. The *sn*-1,3-diacylglycerols were separated, recovered, and subjected to fatty acid analyses.

HPLC Separation of Triacylglycerols

HPLC analyses of triacylglycerols were performed according to the procedure described by Kermasha et al. (*21*). The separation of triacylglycerol fractions were carried out with a gradient elution and demonstrated with a laser light-scattering detector (Varex Corporation, Burtonsville, MD).

RESULTS AND DISCUSSION

Effect of Surfactant HLB Values on the IY

The HPLC analyses of untreated buffer fat (Fig. 1A) show the presence of 16 major fractions (*21*). The preliminary GLC analyses of fatty acid composition (data not shown) of triacylglycerol fractions (peaks 1–7, Fig. 1) indicated the presence of lauric (C12:0) and myristic (C14:0) acids, as well as the absence of longer-chain saturated, such as palmitic (C16:0) and stearic (C18:0) acids, and unsaturated fatty acids, such as oleic acid (C18:1). Since the objective of this work, in the long term, was to interchange the hypercholesterolemic fatty acids, such as myristic and palmitic acids at *sn*-2 position by hypocholesterolemic fatty acids, such as

Table 2
IY and Degree of Hydrolysis of Lipase-Catalyzed Interesterified Butter Fat Catalyzed by Lipase B (Amano) in Cosurfactant-Free Microemulsion System

Parameter	Untreated butter	Interesterified butter fat							
		HLB value[a,b]							
		8		9		10		12	
		3[c]	6[c]	3[c]	6[c]	3[c]	6[c]	3[c]	6[c]
IY[d]	100[e]	47.0	82.7	86.3	110.0	70.4	101.0	86.0	88.4
FFA[f]	0.41	1.52	1.81	1.53	1.24	0.91	0.90	1.12	1.28

[a]HLB values for surfactants were calculated according to the procedure described by Attwood and Florence (*31*).

[b]Results were obtained from triplicate trials with a relative SD $\leq$ 0.05.

[c]Concentrations (m*M*) of surfactant mixtures, used for the preparation of cosurfactant-free microemulsion systems.

[d]Percent of IY of lipase-catalyzed interesterification of selected triacylglycerol molecules, using lipase N (Amano) in the cosurfactant-free microemulsion system. The IY was calculated according to the formula described by Safari et al. (*22*).

[e]Total relative peak area percentages of fractions 8–16 of untreated butter fat (Fig. 1A), used as a basis for calculation of IY.

[f]Total FFA (mmol) produced in 1 g of untreated and lipase-catalyzed interesterified butter fat, using lipase N (Amano) in the cosurfactant-free microemulsion system.

stearic and oleic acids, it was, therefore, important to demonstrate the changes in fatty acid distribution in the second half of the HPLC elution profile. The changes in the relative peak area percentages, from peak numbers 8–16, were measured to estimate the IY.

The HPLC analyses of triacylglycerols of lipase-catalyzed interesterified butter fat, in microemulsion systems with HLB values of 8, 9, and 10, are shown in Figs. 1B, 1D, and IE, respectively. The results (Fig. 1) demonstrate that the lipase-catalyzed interesterification of butter fat in cosurfactant-free microemulsion systems resulted in an increase in the triacylglycerol fraction 12′, which indicates that the selected triacylglycerol fractions were enriched with those of high molecular weight.

The results (Table 2), obtained from the changes in relative peak area percentages of selected triacylglycerol fractions, show that increasing the surfactant HLB values from 8 to 10 resulted in an increase in the IY, from 64.8 to 85.7%. However, further increase in the surfactant HLB value suppressed the IY. The results also demonstrate that the maximum IY was obtained in microemulsion system media containing lecithin (HLB value of 9) followed by that containing a surfactant of HLB value of 10.

Friberg and Kayali (*10*) suggested that the optimum adsorption of a surfactant at the water-in-oil (w/o) interface could be achieved by choosing a surfactant mixture with the appropriate HLB value. In addition, El-Nokaly et al. (*3*) reported that triacylglycerol are semipolar compounds compared to hydrocarbons; therefore, a surfactant of higher HLB value is

needed to favor the formation of a w/o system with a low solubility in the bulk phase, thereby increasing the adsorption at the interface. Friberg and Kayali (*10*) showed that the oil-soluble surfactants adsorb strongly at the oil–water interface, so that they could form a reverse micelle system; however, the emulsfier efficiency will decrease if it is lost to the bulk organic phase and, therefore, become unavailable to the interface.

Effect of Surfactant Concentrations on the IY

The HPLC analyses of triacylglycerols from butter fat interesterified in a microemulsion system containing different concentrations of surfactants, 3 and 6 m*M*, at HLB value of 8, are shown in Fig. 1B and C, respectively. The results (Table 2) indicate that increasing the concentrations of surfactants of HLB values of 8–10 from 3 to 6 m*M* resulted in a 49.0% increase in the IY. However, the increase of the concentration of the surfactant of HLB value of 12 had only a slight effect on the IY. These findings indicate that the effect of concentration of surfactants on the IY may be the result of the fact that the internal structure and other characteristics of micelles are altered by varying the concentration of surfactants and maintaining that of the water (*15*). The increase in IY could also be the result of the changes in the hydration degree (w) or the molar ratio of water to surfactant for which the catalytic activity of the soluble enzyme is maximum (*29*). Martinek et al. (*29*) suggested that the superactivity of enzyme in microemulsion system could be owing to the relatively high rigidity of the surfactant shell surrounding the molecule of the solubilized enzyme.

Effect of Concentrations and HLB Values of Surfactant Mixtures on Hydrolysis Reaction

The quantitative GLC analyses of FFA, released by the lipase-catalyzed interesterification of butter fat (Table 2), demonstrate that increasing the surfactant HLB values from 8 to 10 resulted in a decrease in the amounts of FFA from 1.7 to 0.9 mmol/g of treated butter fats. However, the use of a microemulsion system containing a surfactant of higher HLB value, 12, increased the level of hydrolysis. The results also demonstrate that the increase in the concentrations of surfactant mixtures from 3 to 6 m*M* had little effect on the degree of hydrolysis.

The results (Table 2) indicate that the changes in the hydrolytic activity of lipase under experimental conditions were independent of surfactant concentrations, but instead were influenced by the surfactant HLB values. These findings also suggest that the optimum curvature of reverse micelles, which occurred at the HLB value of 10, could prevent the enzyme from leaking from the reverse micellar core to the bulk phase of organic solvent, and thereby shifted the equilibrium constant to the interesterification reaction.

Since throughout this study the use of a wide range of surfactant mixtures and HLB values was intended to determine the most appropriate HLB value and to obtain spontaneous curvature, these objectives were achieved by changing surfactant-partitioning characteristics, and thereby decreasing the sensitivity of the interface to the composition fluctuations, and by providing formulations to their optimum state (*11*). El-Nokaly et al. (*3*) indicated that the optimum curvature of reverse micelles could also be achieved throughout the selection of an appropriate surfactant HLB value and the fluidity of the interface. Indeed, the nature and the concentration of the surfactant became of utmost importance to obtain a microemulsion system with the maximum solubilization of the aqueous enzyme solution in a given w/o microemulsion (*30*).

FFA Content of Reaction Products

The GLC analyses of FFA composition of lipase-catalyzed interesterified butter fats show (Fig. 2A and B) that the use of different HLB values resulted in an increase in the total proportions of C4-C12:0 and C18:0 fatty acids with a concomitant decrease in the total amounts of C14:0 and C18:1, respectively, from 12.6 to 35.4%. These findings indicate that the change in the FFA profiles of interesterified butter fats were HLB-value-dependent. However, the results (Fig. 2A and B) demonstrate also that the changes in concentrations of surfactants from 3 to 6 m*M* had a negligible effect on the FFA profiles of treated butter fats; these findings could be the result of a reduction in the interface sensitivity to the composition fluctuations, thereby bringing formulation to their optimum state, or the equal partitioning of surfactant between the liquid phases (*3*).

sn-2- Fatty Acid Compositions of Interesterified Butter Fats in Microemulsion Systems

The fatty acid compositions of 2-monoacylglycerols, obtained from untreated and lipase-catalyzed interesterified butter fats, are shown in Fig. 3A and B. The results (Fig. 3A) demonstrate that using 3 m*M* surfactant at an HLB value of 9, the total proportions of C4-C10:0 fatty acids decreased by 45.5%, with a concomitant increase of 30.1% in the amount of C16:0. In addition, the results (Fig. 3A) show that the use of surfactants with HLB values of 10 and 12 resulted in an increase of 53.3 and 40.0%, respectively, in the total proportions of C18:0 and C18:1 with a concomitant decrease in C14:0 of 33.3 and 22.2% respectively. These findings (Fig. 3A) indicate that the lipase-catalyzed interesterification of butter fat in a microemulsion system containing 3 m*M* surfactant of an HLB value of 10 resulted in an exchange, at the *sn*-2 position of triacylglycerol molecules, of hypocholesterolemic fatty acids (C18:0 and C18:1) with the hypercholesterolemic fatty acid C14:0; in addition, the results (Fig. 1, Table 3) also show that the use of this media resulted in a greater relative ratio of peak

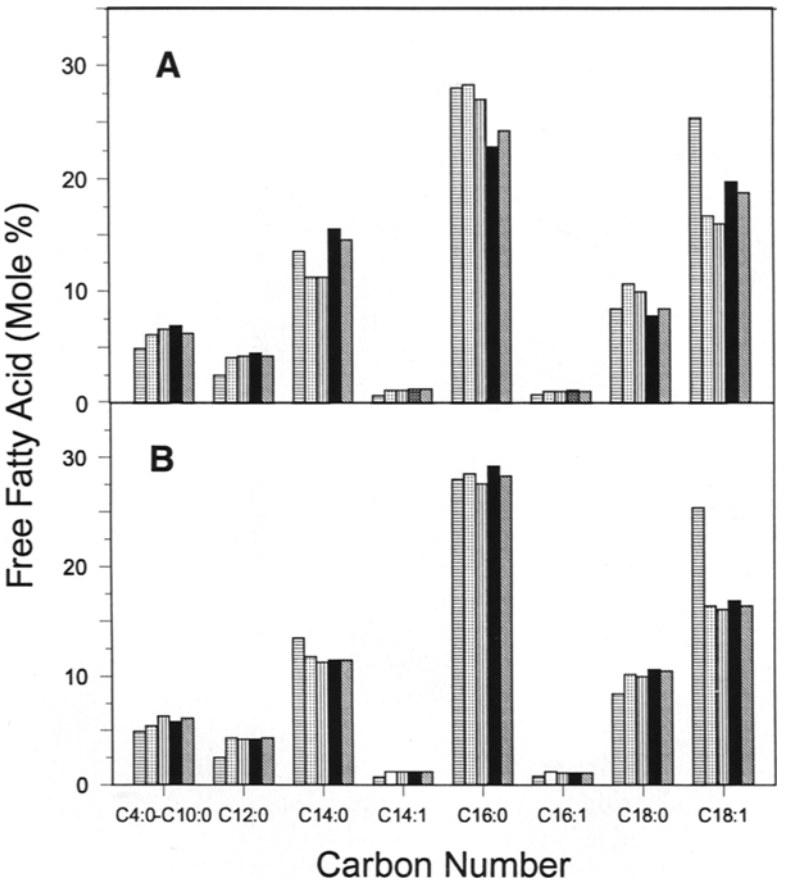

Fig. 2. Changes in FFA compositions of lipase-catalyzed interesterified butter fats by lipase N (Amano) in cosurfactant-free microemulsion systems containing (A) 3-m*M* and (B) 6-m*M* surfactant mixtures at different values of HLB: Untreated butter (≣ --- ≣), 8 (⁘ --- ⁘), 9 (||||| --- |||||), 10 (■ --- ■), and 12 (▨ --- ▨).

area of fraction 11 compared to that of fraction 12. Kermasha et al. (*21*) reported that the fatty acid analyses of fraction 12 of untreated butter fat contains a relatively high amount of the hypocholesterolemic fatty acid C18:1 at the *sn*-2position of triacylglycerol molecules. The overall results shown in Table 3 indicate generally that there are important quantitative changes in the profile of triacylglycerol fractions at the concentrations and the HLB values of surfactants change.

The results (Fig. 3B) also demonstrate that the use of microemulsions containing 6 m*M* surfactants of HLB values of 8–9 in the lipase-catalyzed interesterification of butter fats resulted in a 50.2% decrease in the total amounts of short-chain fatty acids (C4-C10:0). However, further increase in the surfactant HLB values from 10 to 12 resulted in a 28.6% increase in

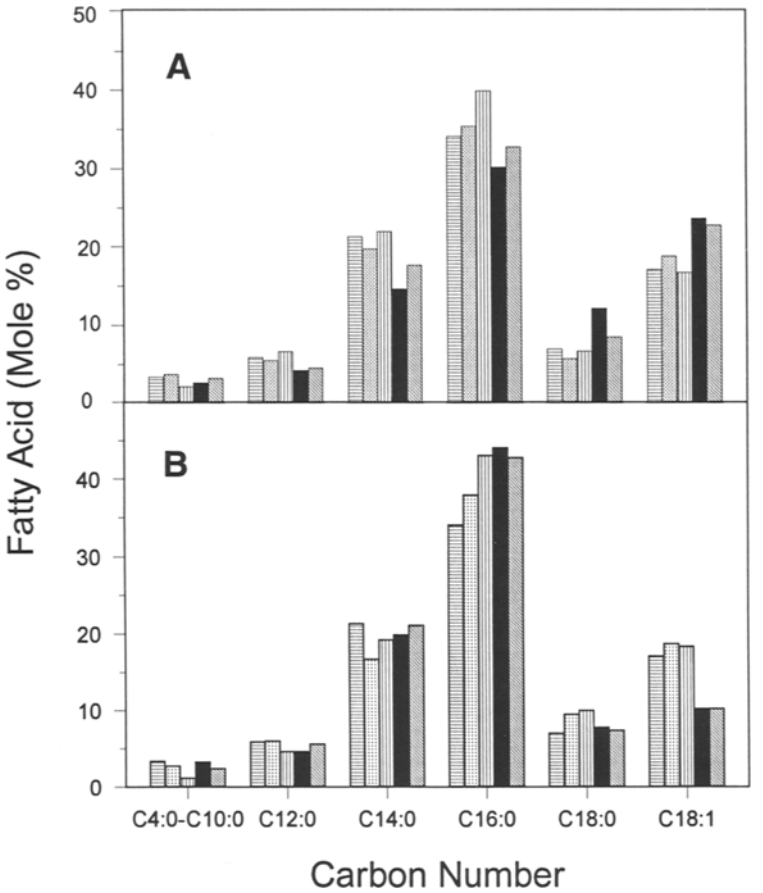

Fig. 3. Changes in *sn*-2-fatty acid compositions of selected triacylglycerols of interesterified butter fats by lipase N (Amano) in cosurfactant-free microemulsion systems containing (A) 3-m*M* and (B) 6-m*M* surfactant mixtures at different values of HLB: Untreated butter (▤ --- ▤), 8 (---), 9 (▥ --- ▥), 10 (■ --- ■), and 12 (▨ --- ▨).

the amount of C16:0, with a concomitant 47.4% decrease in that of C18:1, at the *sn*-2 position of triacylglycerols. These findings (Fig. 3) suggest that the use of a wide range of surfactant concentrations and HLB values could affect the positional changes in fatty acid distribution on triacylglycerol molecules of interesterified butter fat.

sn-1,3- Fatty Acid Compositions of Lipase-Catalyzed Interesterified Butter Fats

The differences in fatty acid distributions on *sn*-1 and *sn*-3 positions of triacylglycerols between untreated and interesterified butter fats (Table 4) show that the use of 3 m*M* of surfactants of HLB values 8 and 9 decreased

Table 3
Changes in Relative Peak Area Percentage of Selected Interested Triacylglycerol Fractions of Lipase-Catalyzed Interesterified Butter Fat by Lipase B (Amano) in Cosurfactant-Free Microemulsion System

Fraction number[c]	Untreated butter	Interesterified butter fat							
		HLB value[a,b]							
		8		9		10		12	
		3[d]	6[d]	3[d]	6[d]	3[d]	6[d]	3[d]	6[d]
8	4.0	5.5	5.6	3.1	3.3	2.9	4.1	4.4	2.5
9	6.0	6.7	9.6	15.7	15.5	13.0	13.2	13.6	15.6
10	3.1	10.2	8.3	4.4	5.3	2.4	4.6	5.2	2.6
11	4.2	2.9	4.1	6.6	8.4	11.4	8.0	6.8	10.9
12	7.2	13.1	16.3	24.2	25.7	18.1	24.9	21.6	22.7
13	2.6	4.2	8.1	7.0	9.7	8.1	10.7	9.0	8.3
14	1.6	5.9	7.9	1.8	2.8	1.0	2.2	1.6	1.0
15	0.1	0.4	0.8	0.5	0.6	1.0	0.7	0.7	0.5
16	0.4	0.9	1.5	0.01	0.04	0.01	0.0	0.3	0.01
11/12[e]	0.6	0.2	0.3	0.3	0.3	0.6	0.3	0.3	0.5

[a]HLB values for surfactants were calculated according to the procedure described by Attwood and Florence (*31*).
[b]Results were obtained from triplicate trials with a relative SD ≤ 0.05.
[c]Fraction numbers of untreated and interesterified butter fat obtained from HPLC chromatograms (Fig. 1).
[d]Concentrations (m*M*) of surfactant mixtures used to prepare cosurfactant-free microemulsion systems.
[e]The ratio of relative peak area percentages of selected fractions (11 and 12) obtained from HPLC chromatograms (Fig. 1).

the amounts of C16:0 by 48.3 and 60.9%, respectively, with a concomitant increase in those of C4:0–C10:0. However, the results demonstrate also that the use of surfactants of higher values of HLB, 10 and 12, resulted in small changes in the amounts of C16:0.

The results (Fig. 2, Table 5) indicate that the interesterification of butter fat in microemulsion, prepared with 3 m*M* surfactant of HLB value of 10, resulted in an acyl exchange between C18:1, originally located on the *sn*-1,3 positions, with those of C14:0 and C16:0, originally located on the *sn*-2 position, of triacylglycerol molecules.

The results (Table 4) also show that the use of 6 m*M* of the surfactant mixtures of HLB values of 8, 9, 10, and 12 for the interesterification of butter fat decreased the proportions of C16:0 by 24.0, 55.7, 65.7, and 42.8%, respectively, with concomitant slight increases in C4:0 and C18:1. The overall result shown in Table 4 indicate generally that there are important changes in fatty acid composition as the concentrations and the HLB values of surfactant change.

Table 4
sn-1,3 Fatty Acid Composition of Interesterified Butter Fat
Using Lipase B (Amano) in Cosurfactant-Free Microemulsion System

Carbon number[c]	Untreated butter	Interesterified butter fat							
		HLB value[a,b]							
		8		9		10		12	
		3[d]	6[d]	3[d]	6[d]	3[d]	6[d]	3[d]	6[d]
C4:0	20.5	27.5	26.5	28.5	28.0	24.0	28.5	20.5	26.5
C6:0	5.0	6.5	4.8	5.6	3.0	5.5	4.3	4.5	4.9
C8:0	2.4	4.3	3.0	4.3	3.0	2.5	3.1	2.8	3.2
C10:0	4.0	7.1	4.1	6.5	5.7	4.4	4.0	5.0	5.0
C12:0	3.7	7.0	4.0	5.7	6.7	4.4	4.2	4.7	4.9
C14:0	10.5	5.2	12.5	11.1	14.0	13.6	8.6	13.3	10.8
C16:0	27.1	14.0	20.6	10.6	12.0	29.1	9.3	26.0	15.5
C18:0	8.9	11.6	9.0	11.51	8.2	5.0	9.0	6.5	9.7
C18:1	17.8	16.8	15.5	16.2	19.4	11.5	29.0	16.7	18.5

[a]HLB values for surfactants were calculated according to the procedure described by Attwood and Florence (*31*).
[b]Results were obtained from triplicate trials with a relative SD ≤ 0.05.
[c]Carbon number of fatty acid chain at *sn*-1,3 positions of triacylglycerol molecules of untreated and lipase-catalyzed interesterified butter fats.
[d]Concentration in m*M* of surfactant mixtures used for the preparation of cosurfactant-free microemulsion systems.

Table 5
Changes in Positional Distributions
of Selected Fatty Acids in Lipase-Catalyzed
Interesterified Butter Fat in Cosurfactant-Free Microemulsion System

Carbon number	*sn*-2[a]		*sn*-1,3[a]	
	Untreated	Interesterified[b]	Untreated	Interesterified[b]
C12:0	5.9	3.8	3.7	4.4
C14:0	21.5	15.0	11.5	13.6
C16:0	34.1	34.9	27.1	29.1
C18:0	6.9	12.6	8.9	5.0
C18:1	16.9	23.9	17.8	11.5
C12:0–C16:0[c]		12.7[d]		14.1[e]
C18:0–C18:1[f]		53.3[e]		38.2[d]

[a]Results were obtained from triplicate trials with a relative SD ≤ 0.05.
[b]Interesterified butter fat in microemulsion system containing 3 m*M* of surfactant of HLB value of 10.
[c]The changes in percentage of hypercholesterolemic fatty acids C12:0, C14:0, and C16:0 on *sn*-2 and *sn*-1,3-positions of lipase-catalyzed interesterified butter fat triacylglycerols.
[d]The relative percentage decrease in the proportion of the selected fatty acids.
[e]The relative percentage increase in the proportion of the selected fatty acids.
[f]The changes in percentage of hypercholesterolemic fatty acids C18:0 and C18:1 on *sn*-2 and *sn*-1,3-positions of lipase-catalyzed interesterified butter fat triacylglycerols.

SUMMARY

The results gathered in this study indicated that the use of different concentrations of surfactant mixtures with a narrow range of HLB values in the lipase-catalyzed interesterification of butter fat, using a cosurfactant-free microemulsion system, could affect the positional distribution of fatty acids within triacylglycerol molecules. The addition of limited amounts of surfactant mixtures enhanced the proportion of the hypocholesterolemic fatty acid C18:1 on *sn*-2 position, whereas the use of higher amounts of surfactant mixtures increased the hypercholesterolemic long-chain saturated fatty acid C16:0 on the same position.

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