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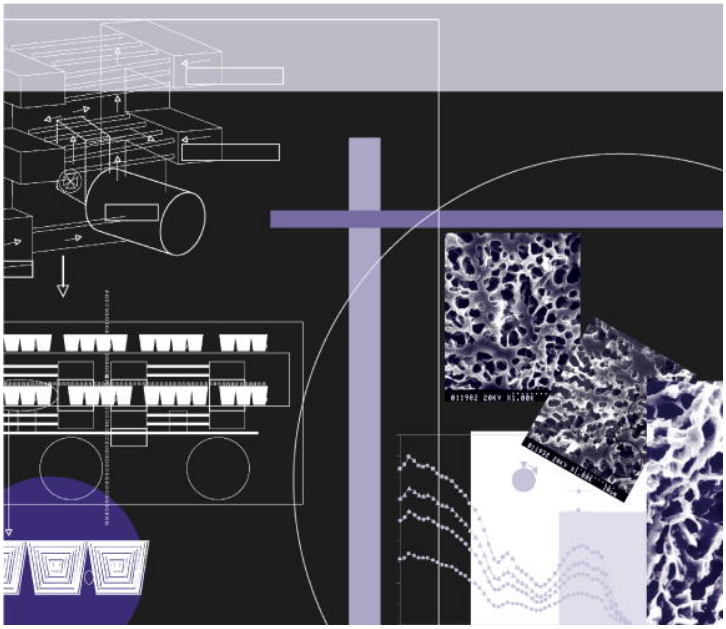

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Enzymatic acidolysis of sunflower oil with a palmitic–stearic acid mixture

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Abstract

Lipase-catalyzed acidolysis of sunflower oil (SO) with a mixture of palmitic–stearic acids (SPFA) was performed in a batch bioreactor to produce structured lipids (SLs). Fractional factorial methodology was used to screen between three immobilized lipases. Lipozyme RM IM resulted in the highest incorporation so it was used in further studied. Saturated fatty acid (SFA) incorporation was higher when reactions were carried out without added water, in the presence of solvent, working with Lipozyme RM IM at 8% (w/w) of total reactants with a substrate molar ratio (SPFA:SO) of 6:1. SLs yield was maximum at that conditions. Incorporation was higher at reaction temperature in the range of 50–60 °C and reaction time between 24 and 48 h. Melting profiles of SLs obtained at these conditions were different to that of SO. Acyl migration was positively influenced by substrate molar ratio, temperature and time of reaction, principally.

Keywords: Lipase-catalyzed acidolysis; Structured lipids; Sunflower oil

1. Introduction

For many food preparations the food industry needs plastic or solid fats (such as animal fats) with good stability. Bakery, pastry and, of course, margarine and spreads, require solid fat, while deep frying industry wants liquid oil resistant to thermo-oxidation. In the 80s, the food industry following nutrition expert recommendations and consumer demands switched from animal fats to vegetable oils. These oils do not have the appropriate properties to be used in these food preparations; they must be chemically modified through partial hydrogenation and/or transesterification. During hydrogenation the saturated fatty acids (SFAs) concentration increases, but at the same time the concentration of *trans* fatty acid isomers increases (Young et al., 1994). The *trans* isomers, although they are unsaturated fatty acids (UFAs), have physical properties similar to SFAs and have been shown to have adverse health implications inclusive more than SFAs (Ascherio, Katan, & Stampfer, 1999).

Such drawbacks have launched increased interest toward lipase-catalyzed reactions, because these enzymes, in addition to exhibiting high activity under mild environmental conditions, possess unusual selectivity and specificity toward their natural substrates (Jensen & Hamosh, 1995). Through enzymatic acidolysis, it is possible to incorporate a desired acyl group onto a specific position of the triacylglycerol (TAG) to produce structured lipids (SLs), whereas chemical catalysis does not possess this regiospecificity due to the random nature of the reaction. Lipase-catalyzed reactions, unlike chemical interesterification, do not produce by-products and give improved products with better quality. It is currently used for the biotechnological processing of fats and oils (Moussata & Akoh, 1998).

Sunflower oil (SO) has traditionally been consumed in the world. It contains high levels of UFAs (Padley,

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Gunstone, & Harwood, 1994). Partially hydrogenated SO has been obtained over different catalysts with high levels of *trans*-isomers (molar fraction more than 36%) (Fernández, Piqueras, Tonetto, Crapiste, & Damiani, 2005). In contrast to the wealth of information pertaining to acidolysis reactions of single triglycerides or oils with UFAs, little has been reported on the lipase-catalyzed reactions between saturated long chain fatty acids and heterogeneous triglycerides, specially SO.

Taking into account the technological and nutritional data, in the present study sunflower oil was modified with palmitic–stearic acids using lipases as catalyst to obtain an alternative of hydrogenation products. The effect of reaction conditions on acidolysis were studied.

2. Materials and methods

2.1. Materials

Refined sunflower oil was purchased from a local grocery store. Immobilized lipases from *Rhizomucor miehei* (Lipozyme RM IM, immobilized on ion-exchange resin, sn-1,3 specific), from *Thermomyces lanuginosa* (Lipozyme TL IM, silica gel-granulated, sn-1,3 specific) and from *Candida antarctica* (Novozym 435, immobilized on macroporous polyacrylate resin beads, nonspecific lipase) were a generous gift of Novo Nordish (Bagsvaerd, Denmark). Stearic–Palmitic acid mixture (SPFA), porcine pancreatic lipase (type II, crude) (EC 3.1.1.3), sodium cholate (99%) and thin-layer chromatography (TLC) silica gel G plates were purchased Sigma–Aldrich (Germany). Fatty acid methyl esters (FAMEs) standards were purchased from Supelco (Bellefonte, USA). Solid phase extraction (SPE) cartouches were obtained from J.T. Baker (Philipsburg, NJ). All other reagents and solvents were of analytical or chromatography grade.

2.2. Experimental designs

2.2.1. Lipase screening

Three immobilized lipases were screened for their ability to incorporate SPFA into SO at different conditions through a fractional factorial experimental design of two levels, 2^5 1/2, constituted by 16 experiments (Peace, 1993) (Table 1). The comparison was not done between the 3 lipases all together, but 2 of them were compared with the third lipase used as reference. So, additional 8 experiments were done.

2.2.2. Variables and interactions effects

A completed experimental design was used to study the effect of solvent, water and reaction time on the incorporation of SPFA into SO (Table 2).

2.2.3. Response surface methodology (RSM)

RSM was used to study the effect of (i) substrate mole ratio and enzyme load, and (ii) reaction temperature and reaction time, on the incorporation of palmitic acid (PA) and stearic acid (SA) into SO. RSM enabled us to obtain sufficient information for statistically acceptable results and is an efficient method to evaluate the effects of multiple parameters, alone or in combination, on response variables (Kim & Akoh, 2005). The second-order polynomial model

Table 1
Design arrangement and response for the lipase-catalyzed SFA incorporation (SFAI) of sunflower oil

Assay No.	Factors					SFAI[f] (mol%)
	A[a]	B[b]	C[c]	D[d]	E[e]	
1	1	1	1	1	1	2.3 ± 0.4
2	1	1	1	2	2a	2.6 ± 0.8
					2b	1.2 ± 0.2
3	1	1	2	1	2a	15.2 ± 1.8
					2b	14.5 ± 0.1
4	1	1	2	2	1	20.8 ± 2.5
5	1	2	1	1	2a	3.7 ± 0.9
					2b	1.0 ± 0.4
6	1	2	1	2	1	5.8 ± 0.7
7	1	2	2	1	1	15.9 ± 1.4
8	1	2	2	2	2a	30.1 ± 2.6
					2b	0.9 ± 0.5
9	2	1	1	1	2a	2.4 ± 0.2
					2b	2.6 ± 0.1
10	2	1	1	2	1	3.4 ± 0.2
11	2	1	2	1	1	16.4 ± 1.3
12	2	1	2	2	2a	27.3 ± 0.8
					2b	15.6 ± 0.2
13	2	2	1	1	1	10.0 ± 1.1
14	2	2	1	2	2a	7.9 ± 0.7
					2b	2.4 ± 0.4
15	2	2	2	1	2a	17.2 ± 2.1
					2b	1.1 ± 0.4
16	2	2	2	2	1	41.9 ± 1.1

[a] A: hexane (1 = 0, 2 = 3 mL/g substrate).
[b] B: water (1 = 0, 2 = 50 w% enzyme).
[c] C: enzyme load (1 = 1, 2 = 10 w% substrate).
[d] D: substrate molar ratio, SPFA:SO (1 = 1, 2 = 6).
[e] E: enzyme type (1 = RM IM, 2a = TL IM, 2b = Novozym 435).
[f] Mean ± SD; $n = 2$.

Table 2
Design arrangement and responses for the Lipozyme RM IM-catalyzed SFA incorporation (SFAI) of sunflower oil and product yield (Y)

Assay No.	Factors			SFAI[d] (mol%)	$Y^{e,d}$ (w%)
	A[a]	B[b]	C[c]		
1	2	2	1	51.0 ± 0.5	57.8 ± 2.7
2	2	2	2	51.3 ± 0.2	68.6 ± 2.5
3	2	1	1	46.0 ± 0.8	79.3 ± 2.8
4	2	1	2	53.5 ± 0.9	81.3 ± 1.9
5	1	2	1	43.0 ± 1.1	55.3 ± 2.1
6	1	2	2	52.8 ± 0.5	60.3 ± 1.9
7	1	1	1	49.8 ± 0.3	52.4 ± 1.9
8	1	1	2	54.6 ± 0.2	58.7 ± 2.0

[a] A: hexane (1 = 0, 2 = 3 mL/g substrate).
[b] B: water (1 = 0, 2 = 50 w% based on enzyme).
[c] C: reaction time (1 = 24, 2 = 48 h).
[d] Mean ± SD; $n = 2$.
[e] Y: SL/DRP.

used for the optimization of SFA incorporation into the oil (Y) was:

$$Y = b_0 + \sum_{i=1}^{2} b_i X_i + \sum_{i=1}^{2} b_{ii} X_i^2 + b_{12} X_1 X_2$$

where b_0, b_i, b_{ii} and b_{12} are the regression coefficients for the intercept, linear, quadratic, and interaction terms, respectively, and X_1 and X_2 are the independent variables. Multiple regression coefficients were obtained by employing a least-squares procedure implemented in the commercial software MatLab 5.3 (The MathWorks Inc., Massachusetts) to predict the second-order polynomial model for the incorporation of SFA into SO. The fits of the models were evaluated by coefficients of determination (R^2: fraction of the variation of the response explained by the model, and max: maximal variation of the response possible to explain) and a test for lack of fit from the analysis of variance (ANOVA). Regression coefficients were tested with the t-test.

2.3. Enzymatic acidolysis

Acidolysis reactions with lipases were carried out at different conditions. In general, substrates (410 mg SO + necessary quantity of SPFA), hexane and water were mixed and heated and reaction began when enzyme was added. Reactions were performed in a screw-capped test tube in a water bath with magnetic agitation at 150 rpm. Reactions were stopped filtering to remove enzymes. All reactions were performed in duplicate and mean values are reported.

2.4. Analysis of products

The modified TAGs (SLs) were isolated in two steps. First, a deacidification of the mixture removed free fatty acids and then, a purification by SPE was performed. Deacidification by alkaline extraction was done in a similar manner as Lee and Akoh (1998). Reaction mixture (400 mg) was mixed with hexane (6 mL), phenolphthalein solution, and 3.2 mL of 0.5 N KOH in 20% (v) ethanol (S1). The separatory funnel was shaken, and the upper phase was collected. Lower phase was washed again with 2 mL hexane and the upper phase was collected. Then, collected hexane phase was mixed with 1.2 mL of S1 solution and 2.4 mL of saturated NaCl solution. After shaken, the hexane phase was collected and hexane was evaporated to obtain the deacidified reaction product (DRP). DRP was dissolved in petroleum ether 40–60 obtaining a solution of 25 mg/mL; 2 mL of this solution were eluted through a silica SPE cartouche with 15 mL of petroleum ether/diethyl ether (90:10, v/v) obtaining the non polar compounds that were constituted by TAGs (SLs). To check the separation an aliquot of the mixture was spotted onto a silica gel G TLC plate and developed with petroleum ether/diethyl ether/acetic acid (80:20:0.5, v/v/v). The bands were visualized after been exposed to an iodine atmosphere and identified comparing their retardation factors with pure samples. DRP and SL were quantified by weight.

2.5. Methylation and GLC analysis

FAMEs were prepared through cold transesterification with methanolic KOH according to the Official Method Ce 2-66 of AOCS (1997). The fraction corresponding to TAGs was dissolved in 2 mL n-hexane and methylated with 0.2 mL KOH 2 N in methanol 95%(v) at room temperature for 30 min. FAMEs were collected in the organic layer and analyzed by gas–liquid chromatography (GLC) with a 4890D series gas chromatograph (Agilent, Hewlett-Packard) and a fused-silica capillary column (SP-2380, 30 m × 0.25 mm × 0.2 μm film thickness; Supelco Inc.). The carrier gas was hydrogen with a linear velocity of 17 cm/s. The injector was used in split mode with a ratio of 1:100. The oven temperature was programmed to be at 170 °C for 15 min, further to increase to 210 °C at a rate of 4 °C/min, and held for 10 min. The injector and detector temperatures were 220 °C. FAMEs were identified by comparing their retention times with authentic standards.

2.6. Pancreatic lipase-catalyzed sn-2 positional analysis

About 0.1 g of purified TAG was dissolved in 0.2 mL of hexane with gentle warming and these mixture was treated with 20 mg of pancreatic lipase according to the Official Method Ch 3-91 of AOCS (1997). The mixture was centrifuged and the organic layer was removed and spotted onto a silica gel G TLC plate that was developed with hexane/diethyl ether/acetic acid (70:30:1, v/v/v). The 2-monoacylglycerol band was visualized after been exposed to an iodine atmosphere, scraped off and directly methylated as described before.

2.7. Melting profile

Differential scanning calorimetry (DSC) on a Perkin–Elmer (Norwalk, CT) Model DSC7 was used to determine melting profile according to the Official Method Cj 1-94 of AOCS (1997) of the SL from 10 °C until 80 °C.

2.8. Statistical analysis

Variables and their interactions effects were analyzed using ANOVA statistical analysis. Statistical differences between average values were evaluated with the t-test. A confidence value of 95% was used. Results are shown as average value of two results ± standard deviation.

3. Results and discussion

The predominant fatty acids (FA) in SO were oleic and linoleic acids, representing 36.7 ± 0.5 and 51.3 ± 0.7 mol% of the total FAs and having 48.1 ± 1.5 and 27.6 ± 1.8% of

them at the sn-2 position, respectively. SPFA had 40.1 ± 1.6 mol% of SA and 52.1 ± 2.1 mol% of PA.

3.1. Lipase screening

The three lipases catalyzed the incorporation of SPFA to varying degrees (Table 1) showing better results at higher substrate molar ratio and enzyme load in the following order and conditions: Lipozyme RM IM (with solvent and added water) > Lipozyme TL IM (without solvent and with added water, or with solvent and without added water) ≫ Novozym 435 (with solvent and without added water). Novozym 435 showed much lower activity than the others to catalyze the studied reaction in the analyzed range of parameters. Similar result was obtained by Haman and Shahidi (2005) studying the incorporation of long chain fatty acids into high laurate canola oil. Yang, Fruekilde, and Xu (2003) reported that Lipozyme TL IM had much lower activity than Lipozyme RM IM in the enzymatic acidolysis of sunflower oil with caprylic acid, in accordance with our results. On the other hand, Kim et al. (2001) found that Novozym 435 had more activity than Lipozyme RM IM when incorporation of conjugated linoleic acid into tricaprylin was studied and Kim, Kim, Lee, Chung, and Ko (2002) reported that caprylic acid incorporation in the lipase-catalyzed acidolysis of perilla oil showed no difference for Lipozyme RM IM and Lipozyme TL IM.

Another factor to have into account is that Lipozyme RM IM showed little attrition by agitation in the reaction conditions. On the other hand, Lipozyme TL IM and Novozym 435 showed some diminution in the support particle size although they were retained by the filter. This support attrition could be a problem to future studies at pilot plant or industrial scale. Because the acidolysis of SO with SPFA was best achieved with Lipozyme RM IM lipase and support attrition was not observed, this enzyme was selected for subsequent experiments.

3.2. Effect of reaction conditions

Table 2 shows the experimental design used to study the effect of solvent, water and reaction time on the incorporation of SPFA into SO and the product yield (w% of SL fraction respect to DRP) when Lipozyme RM IM was used as catalyst. Water and solvent content effects over incorporation were significant at a reaction time of 24 h only. It seems to be an equilibrium at 48 h of reaction provided for another more important parameter as could be substrate ratio or reaction temperature, that fix the level of incorporation. Kim and Akoh (2005) found that total incorporation of caprylic acid into sesame oil was positively affected by these parameters at long reaction time (30 h). We could not reproduce that result.

Better yields were obtained when water was not added. It is known that controlling water activity is very important in lipid modification. Monolayer of water on the surface of enzyme is required to maintain the three-dimensional structure of enzyme, but too much water can cause the hydrolysis of triglycerides. So the balance between the acidolysis and hydrolysis of the lipase activity is strongly dependent on the water content in the reaction media (Zhao, Lu, Bie, Lu, & Liu, 2007). Mu, Xu, and Høy (1998) presented different results obtained with different substrates and concluded that water content of the reaction substrate has to be carefully adjusted for the individual reaction systems to achieve both an acceptable reaction rate and product yield. In the range of our studied parameters, it seems to be a displacement of the acidolysis by the hydrolysis reaction when water is added to the system. The effect of the reaction time on the yield was not significant, which is in agreement with results presented for Xu, Mu, Skands, Høy, and Adler-Nissen (1999). On the other hand, the effect of solvent content when no water was added was highly significant. It could be explain by the lower viscosity of the solvent system and the fact that it helps substrates to move more freely being more accessible to the active site of enzymes. Zhao et al. (2007) showed that solvents with $\log P$ values >3 (hexane $\log P$ value = 3.5) were most suitable media for enzymatic acidolysis increasing the solubility of nonpolar substrates and shifting the reaction toward synthesis rather than hydrolysis. In our study, maximal yield and incorporation were obtained when acidolysis was carried out in presence of solvent, without added water and at a reaction time of 48 h. Zhao et al. (2007) and Tsuzuki (2005) found that lipase-catalyzed acidolysis in organic solvent without added water gave highest FA incorporation. Kim et al. (2002) obtained maximal incorporation of caprylic acid into perilla oil when solvent was used in the reaction media. Hexane will add to the cost of the industrial-scale production of SL. Therefore, the use of a solvent system would be analyzed economically taking into account the cost of production and the yield.

On the basis of these results, we elected to study the effects of the molar ratio of substrates, enzyme load, reaction time and temperature when solvent was incorporated to the reaction media and water was not added.

RSM was used to study the effect of (i) substrate mole ratio and enzyme load, and (ii) reaction temperature and reaction time, on the incorporation of PA and SA into SO. Tables 3 and 4 summarize the experimental data for response variable Y_1 (SFA incorporation into SO when substrate mole ratio and enzyme load are studied) and Y_2 (SFA incorporation into SO when reaction temperature and reaction time are studied) at each experimental set, respectively. Multiple regression coefficients are summarized in Table 5. Testing of regression coefficients with the t-test indicated that independent, linear and quadratic terms were significant and interaction was not significant (95% confidence) when Y_1 was analyzed. The bigger contribution was done by substrate mole ratio. Kim and Akoh (2005) reported similar conclusion studying the incorporation el caprylic acid into sesame oil. On the other hand, Y_2 was positively correlated with independent and linear terms

Table 3
Experimental data for response variables Y_1 (SFA incorporation into SO) and Y_3 (SFA at *sn*-2 position related to total SFA) at each experimental set using normalized variables X_1 and X_2[a]

Assay No.	Factors		Responses	
	X_1	X_2 (%)	Y_1 (mol%)	Y_3 (mol%)
1	2	4	31.8 ± 0.5[b]	
2	3	4	34.8 ± 0.7	
3	4	4	39.0 ± 0.5	
4	5	4	42.8 ± 0.5	
5	6	4	48.6 ± 1.1	25.7 ± 2.0
6	2	6	31.1 ± 0.1	
7	3	6	37.3 ± 0.8	
8	4	6	42.6 ± 0.5	
9	5	6	47.1 ± 0.4	
10	6	6	50.1 ± 0.7	29.6 ± 1.6
11	2	8	32.7 ± 0.4	
12	3	8	40.0 ± 1.1	
13	4	8	45.8 ± 1.2	
14	5	8	50.2 ± 0.9	
15	6	8	53.1 ± 0.4	30.5 ± 1.4
16	2	10	32.5 ± 0.2	
17	3	10	40.6 ± 1.6	
18	4	10	46.1 ± 2.3	31.2 ± 3.2
19	5	10	48.4 ± 1.9	
20	6	10	50.6 ± 1.4	32.1 ± 2.4

[a] X_1 = substrate molar ratio (SPFA:SO); X_2 = enzyme load (w% based on total substrate).
[b] Mean ± SD; $n = 2$.

Table 4
Experimental data for response variable Y_2 (SFA incorporation into SO) and Y_3 (SFA at *sn*-2 position related to total SFA) at each experimental set using normalized variables X_1 and X_2[a]

Assay No.	Factors		Responses	
	X_1 (h)	X_2 (°C)	Y_2 (mol%)	Y_3 (mol%)
1	16	40	29.3 ± 0.8[b]	10.1 ± 1.7
2	24	40	39.6 ± 1.1	12.3 ± 1.9
3	48	40	45.5 ± 0.6	15.8 ± 1.5
4	16	50	36.5 ± 0.5	12.3 ± 1.4
5	24	50	44.2 ± 1.3	17.7 ± 1.8
6	48	50	48.7 ± 0.6	23.9 ± 0.9
7	16	60	37.5 ± 0.9	20.9 ± 1.8
8	24	60	55.8 ± 0.7	27.3 ± 1.7
9	48	60	62.4 ± 1.1	30.5 ± 2.0

[a] X_1 = reaction time; X_2 = reaction temperature; Y_2 = incorporation of PA+SA into SO varying reaction time and reaction temperature; Y_3 = SFA at *sn*-2 related to total SFA.
[b] Mean ± SD; $n = 2$.

but quadratic and interaction did not show any significant effect. Reaction time showed the greatest effect. ANOVA also showed that the models were statistically good, and they had no lack of fit at a 95% level of significance ($F_{0.05,14,20} = 2.225$ and $F_{0.05,3,9} = 3.86$ for Y_1 and Y_2 model, respectively). In the range of the studied independent variables, the models found maximal SFA incorporation at a substrate molar ratio of 6:1 (SPFA:SO) and an amount of enzyme of 8% (w/w, relative to total substrate) when other reaction conditions were 60 °C (reaction temperature) and 48 h (reaction time).

Table 5
Regression coefficients of predicted quadratic polynomial model for response Y[a]

Coefficients[b]	Y_1 (mol%)	Y_2 (mol%)
b_0	44.11 ± 1.31[c]	45.28 ± 3.97
Linear		
b_1	4.60 ± 0.46	8.88 ± 2.18
b_2	1.14 ± 0.44	6.88 ± 2.18
Quadratic		
b_{11}	−0.47 ± 0.39	−3.22 ± 3.77
b_{22}	−0.40 ± 0.37	1.88 ± 3.77
Interaction		
b_{12}	−0.10 ± 0.31	2.18 ± 2.67
R^2	0.971	0.964
Max[d]	0.979	0.970
Lack of fit ratio[e]	1.990	3.635

[a] see Tables 3 and 4 for description of abbreviations.
[b] Coefficients refer to the general model.
[c] Value ± confidence interval.
[d] Max: maximal variation of the response possible to explain.
[e] Ratio between lack of fit and pure error variances.

Fig. 1 shows the incorporation of PA and SA into SO at various substrate mole ratios. The incorporation increased as the mole ratio increased until the incorporation reached a maximal level beyond a mole ratio of SPFA to SO of 5. As it can be seen, differences between both incorporations can be directly related to the difference in concentration in the original substrate of free FAs (higher level marked with dashed line in the figure) and not to any specificity of the enzyme toward an specific FA.

The effect of enzyme load on the incorporation of PA and SA into SO are shown in Fig. 2. The extents of PA and SA incorporations were increased by increasing the amount of enzyme in the reaction mixtures, but a significant increase was not observed when immobilized enzyme was present at greater than 8% by weigh of substrates. Kim et al. (2001) found similar results when conjugated linoleic acid was incorporated to tricaprylin. The PA incorporation ratio seems to show a preference of the enzyme by PA instead of SA until an excess of enzyme occurs in the

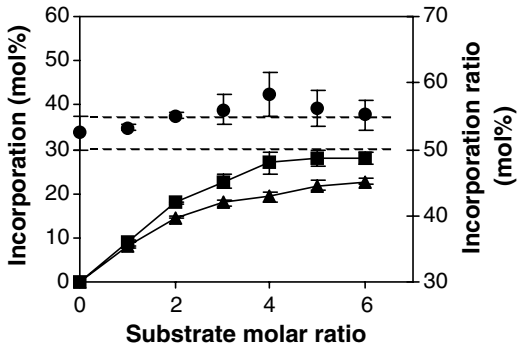

Fig. 1. Effect of substrate molar ratio on the incorporation of palmitic (■) and stearic (▲) acids into sunflower oil and on the incorporation ratio (% PA/(PA + SA), (●)). Dashed lines: standard deviation around average % PA/(PA + SA) in SPFA. Reaction conditions: 3 mL hexane/g substrate, no added water, enzyme: 10% by weight of reactants, 60 °C, 48 h.

reaction system. This behavior did not appear when substrate ratio was studied (Fig. 1) probably because in that cases not only substrate ratio changed but also enzyme load did (this is defined as a percentage of total substrate) so the relation between reactants and catalyst is kept. Sellapan and Akoh (2000) reported a higher reactivity and/or selectivity of oleic acid over lauric acid by Lipozyme IM60 (*sn*-1,3 specific lipase from *Rhizomucor miehei*) showing the high specificity of the enzyme toward UFAs rather than saturated ones.

Time course is important in monitoring enzymatic reactions in order to determine the optimal rate necessary to obtain good yields and to minimize the overall production cost for the process. Reaction temperature can affect parameters such as enzyme stability, affinity of enzyme for substrate, and preponderance of competing reactions. Thermostability of enzymes is a major factor considered in their industrial use, mostly because of the potential for minimizing thermal degradation. The effects of temperature and time on the acidolysis of SO with SPFA as acyl donor are shown Fig. 3. The incorporation of SFAs was faster in the first 4 h of reaction reaching between 40% and 60% of maximal incorporation (obtained at 48 h of reaction) depending on reaction temperature. Results with reaction temperature between 40 and 60 °C showed significant differences at the first 16 h of reaction. A slight decrease of SFA incorporation was observed below 50 °C confirming a decrease in the initial reaction rate. Similar results were obtained by Kim et al. (2001) to the conjugated linoleic acid incorporation into tricaprylin.

Tables 3 and 4 summarizes the results of studies on the positional distribution of FAs in the modified sunflower oil. SFAs esterified to the *sn*-2 position of the unmodified oil represented the 7.9% of the total SFA composition, changing until 32.1% in modified oil. Enzyme and substrate relationships had little significant effect over the composition of the *sn*-2 position at the studied range. On the other hand, SFA composition at *sn*-2 position increased significantly when temperature or time increased. So, although

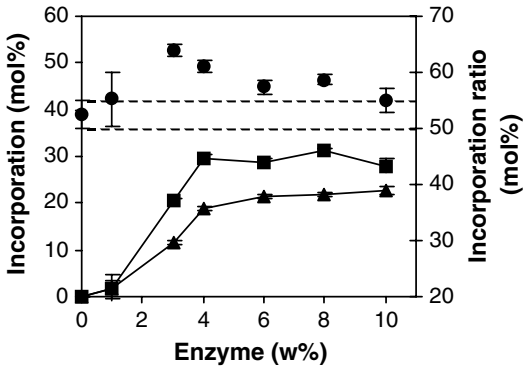

Fig. 2. Effect of enzyme load on the incorporation of palmitic (■) and stearic (▲) acids into sunflower oil (SO) and on the incorporation ratio (% PA/(PA + SA), (●)). Dashed lines: standard deviation around average % PA/(PA + SA) in SPFA. Reaction conditions: 3 mL hexane/g substrate, no added water, 6:1 SPFA:SO, 60 °C, 48 h.

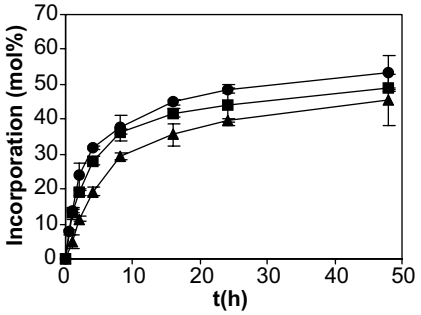

Fig. 3. Effect of temperature and time on the incorporation of SFA into SO (▲: 40 °C, ■: 50 °C, ●: 60 °C). Reaction conditions: 3 mL hexane/g substrate, no added water, 6:1 SPFA:SO, enzyme: 10% by weight of substrate.

SFAs were mostly incorporated into the *sn*-1,3 positions of the glycerol backbone of the modified oil, migration of SFAs to the *sn*-2 position occurred. Kim and Akoh (2005) found that temperature and time had positive effect on acyl migration being temperature effect the greatest. Ortega, López-Hernandez, García, and Hill (2003) reported that the distribution of fatty acid residues in the TAG in terms of saturated and unsaturated fatty acid is not the main factor affecting the melting points of lipase-mediated acidolysis products. The physicochemical properties and nutritional value of fats and oils are determined not only by the particular composition of their fatty acid residues, but also by the positional distribution of the acyl groups in the TAGs (Rao, Udaya, Sambaiah, & Lokesh, 2001).

Fig. 4 shows the melting profiles of SO and products synthesized at 6:1 substrate molar ratio (SPFA:SO) and 8%w enzyme, at the ranges of 50–60 °C and 24–48 h. It seems to be little differences in the behavior of SO and the product obtained at 24 h and 50 °C. The peaks from the lower-melting polymorphs seem to shift gradually toward higher melting ranges as the reaction temperature increased from 50 to 60 °C (curves 1 and 2) and more drastically from 24 to 48 h of reaction time (curves 2 and 3).

With these results, we can conclude that a compromise relation exists between incorporation, melting profile and

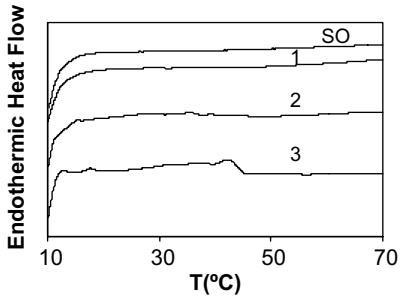

Fig. 4. Differential scanning calorimetry (DSC) of sunflower oil (curve SO) and reaction products obtained at: 24 h and 50 °C (curve 1), 24 h and 60 °C (curve 2), and 48 h and 60 °C (curve 3). Other reaction conditions: 6:1 substrate molar ratio, 8%w RM IM enzyme load, 3 ml hexane/g substrate, no added water.

migration so, depending on the expected use to the final product it would be the better reaction conditions to choose. In the particular case of margarine substitute where a good plasticity is expected, SFAs should be at least at a level of 25–30% (Petrauskaite, De Greyt, Kellens, & Huyghebaert, 1998). So, the better conditions are 50–60 °C of reaction temperature and 24–48 h of reaction time, working to a substrate molar ratio of 5–6 and with an enzyme load of 8%w.

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