Phosphatidylcholine Enrichment with Medium Chain Fatty Acids by Immobilized Phospholipase A_1-Catalyzed Acidolysis

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Phospholipids are a biologically and industrially important class of compounds whose physical properties can be improved for diverse applications by substitution of medium-chain fatty acids for their native fatty acid chains. In this study, phosphatidylcholine (PC) was enriched with medium-chain fatty acids (MCFAs) by acidolysis with phospholipase A_1 (PLA_1) immobilized on Duolite A568. Response surface methodology was employed to evaluate the effects of the molar ratio of substrates (PC to free MCFAs), enzyme loading, and reaction temperature on the incorporation of free MCFAs into PC and on PC recovery. Enzyme loading and molar ratio of substrates contributed positively, but temperature negatively, to the incorporation of free MCFAs into PC. Increases in enzyme loading and the molar ratio of PC to free MCFAs led to increased incorporation of the latter into the former, but increased temperature had the opposite effect. By contrast, an increase in enzyme loading led to decreased PC recovery. Increased temperature had also a negative effect on PC recovery. Optimal conditions for maximum incorporation and PC recovery were molar ratio of PC to free MCFAs of 1:16, enzyme loading of 16%, and 50°C. Under these conditions, the incorporation of free MCFAs was 41% and the PC recovery was 53%. © 2012 American Institute of Chemical Engineers *Biotechnol. Prog.*, 29: 230–236, 2013.
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Introduction

Phospholipids (PLs) comprise an important group of polar lipids that are part of all biological membranes. In addition to their structural role, PLs act as cofactors and activators for a variety of membrane-associated enzymes.[1] PLs have been widely used for their surfactant properties in the formulation of emulsions for foods, pharmaceuticals, and cosmetics.[2]

PLs can be obtained from crude soybean lecithin that is obtained as a by-product of soybean oil processing and typically contains 18% phosphatidylcholine (PC), 14% phosphatidylethanolamine (PE), 9% phosphatidylinositol (PI), 5% phosphatidyl acid (PA), 2% minor PLs, 11% glycolipids, 5% complex sugars, and 37% neutral oil. Deoiling of crude lecithin is currently used in industry for the separation of neutral oil and PLs; deoiled soybean lecithin contains mostly 23% PC, 20% PE, and 14% PI.[3]

PLs can be modified by physical, chemical, or enzyme-catalyzed methods to improve their emulsifying properties, viscosity, and dispersibility. These modified PL species exhibit a wider spectrum of hydrophile–lipophile balance than naturally occurring PL.[4] Compared to chemical methods, enzymatic modification of PLs has advantages that are attributed to the specificity of the enzymes. This approach is regarded as a safe technique for producing modified PLs.[5]

Replacement of existing fatty acids in native PL with desirable fatty acids can improve not only their physical and

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chemical properties, but also their nutritional, pharmaceutical, and medical functions.[6] Incorporation of saturated fatty acids with a chain length of 6–10 carbons (medium-chain fatty acid, MCFAs although sometimes dodecanoic acid is included) into PLs has led to improved heat and oxidation stability, as well as enhanced emulsifying properties.[7]

Recent studies have confirmed the potential of MCFAs to reduce the following aspects: body weight, particularly body fat[8–12]; lipoprotein secretion and attenuate postprandial triglyceride response[13,14]; fasting lipid levels in serum more quickly and efficiently than oils rich in mono- or polyunsaturated fatty acids[15,16]; intestinal injury,[17] and protect against alcohol-induced hepatotoxicity.[18] Since 1994, the use of MCFAs in food products is recognized as safe by the FDA.[19]

Several studies have been published concerning the modification of PLs using lipases,[2,20–27] phospholipase A_1 (PLA_1),[28–30] and PLA_2[31–35] by incorporation of saturated, mono- or polyunsaturated fatty acids. However, very few specific reports concerning the incorporation of MCFAs in soybean lecithin (PC) by lipase-mediated reactions have been published.[6,36–38] Only a single report of incorporation MCFAs into PC using PLA_2 has been published.[39]

The purpose of this study was to enrich PC with free MCFAs by acidolysis catalyzed by immobilized PLA_1. The effects of varying the molar ratio of substrates, enzyme loading, and reaction temperature on the incorporation of free MCFAs into PC and the percentage of recovery of PC were modeled using response surface methodology.

Materials and Methods

Materials and reagents

PLA_1 (Lecitase® Ultra) was provided by NOVO (Salem, VA). Duolite A568, polymerized phenol–formaldehyde anionic exchange resin of particle size 0.15–0.85 mm, donated by Rohm & Haas (Philadelphia, PA), was used as the support for immobilizing PLA_1. Soybean lecithin (PC, 95%) was purchased from Avanti Polar Lipids (Alabaster, AL). Original Thin Oil® (OTO, Sound Nutrition, Dover, Idaho) was used to prepare a mixture of free MCFAs by saponification as explained below. High-performance liquid chromatography (HPLC)-grade solvents were purchased from Tecsiquim (Mexico City) and all other reagents were purchased from SIGMA (Mexico City).

Immobilization of PLA_1

PLA_1 was immobilized onto Duolite polymeric resin via a direct adsorption mechanism as reported by García et al.[28] Briefly, 100 mL of undiluted enzyme concentrate containing about 1.5% protein was mixed with 100 mL of 0.1 N Tris-HCl buffer (pH 8) and the enzyme suspension was mixed with 20 g of support. The mixture was placed in an orbital shaker operating at 300 rpm and 50°C. Samples (50 μL) of the supernatant were withdrawn and mixed with 450 μL of buffer for the analysis of protein. After 12 h, the suspension of the immobilized enzyme was separated by vacuum filtration. The solids were rinsed with 500 mL of buffer and then dried overnight in a vacuum oven at 30°C. The dried preparation was employed to catalyze the acidolysis reactions.

Preparation of free MCFAs

Free MCFAs were obtained by saponifying the OTO according to the method reported by Kim and Hill[40]: 100 g of OTO was added to a solution of sodium hydroxide (40 g) in distilled water (100 mL) and ethanol (99%, 300 mL). The mixture was refluxed with stirring at 500 rpm for 30 min. After cooling, the mixture was transferred to a 2-L separation funnel and water (200 mL) was added to the saponified mixture; the unsaponifiable matter was extracted with 300 mL of hexane and discarded. The aqueous layer containing the saponified matter was acidified by adding concentrated HCl to reach a pH of 1.0. The resulting lower layer was removed using a separation funnel and discarded. The upper layer containing the fatty acids was mixed with 400 mL of hexane and washed twice with 200 mL of distilled water. The hexane layer containing the mixture of free MCFAs was then dried over anhydrous sodium sulfate. The solvent was then removed in a rotary evaporator at 40°C. The mixture of free MCFAs was stored at −18°C until used for acidolysis reactions. The average molecular weight of the mixture of free MCFAs was calculated on the basis of its fatty acid composition and used to determine the amount of this mixture that should be added to the PC to obtain the desired ratio of substrates.

Acidolysis reactions

Acidolysis reactions were conducted in solvent-free systems. Substrates (PC and free MCFAs) were placed in Erlenmeyer flasks and mixed in a heating plate with magnetic stirring at 300 rpm and 40°C until complete dissolution. Three grams of substrates was placed in 25-mL Erlenmeyer flasks and mixed with the corresponding amount of immobilized enzyme, according to the treatment to be evaluated. The reaction was carried out in an orbital shaker operating at 300 rpm and the desired temperature for a period of 72 h. Samples were withdrawn periodically (1, 2, 3, 6, 9, 12, 24, 36, 48, and 72 h) to determine the extent of incorporation of free MCFAs into PC. All experiments were performed in duplicate.

Analyses

Protein was determined by the method of Bradford[41] using an Agilent photodiode array spectrophotometer to measure the absorbance at 595 nm. Bovine serum albumin was employed as a standard.

Total fatty acids in the saponified OTO and PC were determined by direct methylation with 1 N HCl–methanol at 60°C for 30 min. The fatty acid composition in the modified PC was determined as follows: 100 μL of reaction mixture was mixed with 1 mL of 0.5 N sodium methoxide in methanol and held at room temperature for 5 min; then, 100 μL of distilled water was added to stop the reaction. The methyl esters were extracted with 2 mL of hexane. One microliter was injected into an HP Model 6890 gas chromatograph fitted with a flame ionization detector and a HP-INNOWAX (60 m × 0.25 mm × 0.25 mm) capillary column. The temperature program consisted of initial temperature of 50°C for 1 min followed by heating to 200°C at 15°C/min. Then, this temperature was maintained for 24 min. Injector and FID temperatures were set at 200 and 230°C, respectively.

PC recovery was determined by HPLC. Samples were prepared by diluting the reaction mixture with ethanol to a concentration of about 10 mg/mL PC and 10 μL was injected into a Waters HPLC System fitted with a Partisil silica

Table 1. Fatty Acid Residue Composition (mol %) of the Starting Mixture of MCFAs, and the Original Unmodified PC

Fatty acid	Fatty acid from mixture of MCFAs	Unmodified PC
C6:0	1.6	
C8:0	67.1	
C10:0	30.6	
C12:0	0.6	
C16:0		13.3
C18:0		3.6
C18:1(9)		10.7
C18:1(7)		1.6
C18:2(6)		64.4
C18:3(3)		6.3

Tabular entries are the average of duplicate determinations from different experimental trials. The mixture of MCFAs used as the acyl donor was obtained by saponification of commercial oil product rich in MCFAs (Original Thin Oil®).

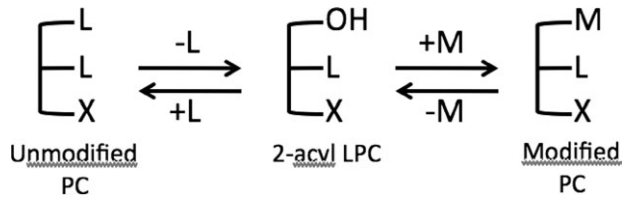

Figure 1. Scheme of the reaction of the PLA_1-catalyzed acidolysis for the production of modified PC (L, LCFAs; M, MCFAs; X, Polar head).

column 5 μm (4.6 × 250 mm). PC was detected at 205 nm with a UV–visible detector (Waters model 2487). The mobile phase consisted of acetonitrile/methanol/phosphoric acid (130:5:1.5 vol/vol/vol) run isocratically at a flow rate of 1.5 mL/min. Retention time for PC was 16.1 min. This peak was identified using an external standard.

Experimental design and data analyses

A central composite design with three factors at three levels and two axial points was used to assess the effects of the molar ratio of substrates, enzyme loading, and reaction temperature on both the incorporation of free MCFAs into PC and PC recovery.

Data from the experiments were analyzed by response surface methodology using the statistical software package MINITAB v. 14.0. The quadratic response surface model for the course of these reactions was fitted using the following model:

$$Y = \beta_0 + \sum_{i-1}^{3} \beta_i X_i + \sum_{i-1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{J=i+1}^{3} \beta_{ij} X_i X_j$$

where Y is the response variable (extent of incorporation of MCFAs), X_i is the i^{th} independent variable, β_0 the intercept, β_i the coefficients of the first-order model, β_{ii} the quadratic coefficients for the variable i, and β_{ij} is coefficient of the linear model for the interaction between factors i and j.

Results and Discussion

A 61% immobilization of PLA_1 on Duolite (46 mg protein/g support was achieved from a suspension initially containing 75 mg protein/g support). This result is consistent with that reported by García et al.[28]

Saponification of OTO yielded a mixture of free MCFAs composed of caproic acid-C6:0 (1.65 mol %), caprylic acid-C8:0 (67.14 mol %), C10:0-capric acid (30.57 mol %), and lauric acid-C12:0 (0.64 mol %) (Table 1). Prior to acidolysis, the primary fatty acid residues in the starting PC were primarily palmitic acid-16:0 (13.29 mol %), oleic acid-18:1 *n*-9 (10.72 mol %), and linoleic acid-18:2 *n*-6 (64.42 mol %).

Table 2. Conditions Utilized in the Experimental Design for the PLA_1-Catalyzed Acidolysis and the Corresponding Responses

	Variables			Incorporation of free MCFAs	PC recovery
Experiment	S	E	*T*	(mol %)	(%)
1	1:8	16	50	41	33
2	1:16	16	50	51	34
3	1:8	32	50	56	18
4	1:16	32	50	70	14
5	1:8	16	60	30	38
6	1:16	16	60	28	53
7	1:8	32	60	45	23
8	1:16	32	60	50	27
9	1:4	24	55	29	26
10	1:20	24	55	51	31
11	1:12	8	55	36	57
12	1:12	40	55	60	13
13	1:12	24	45	52	28
14	1:12	24	65	31	44
15	1:12	24	55	51	22
16	1:12	24	55	51	24
17	1:12	24	55	51	24

Abbreviations: S, molar ratio of substrates (PC/free-MCFAs); E, enzyme loading (wt % based on total amount of substrates); T, reaction temperature (°C). Tabular entries are the average of duplicate determinations from different experimental trials corresponding to 72 h of reaction.

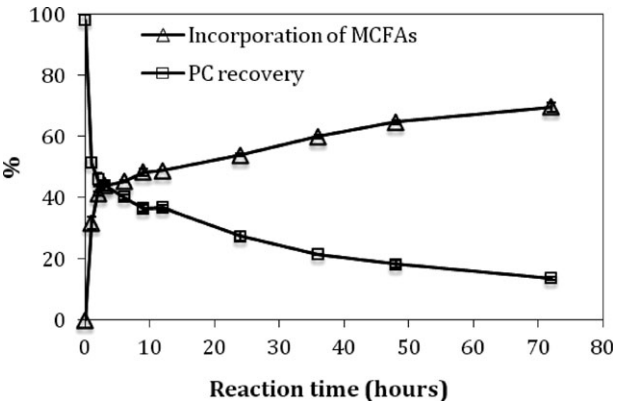

Figure 2. Molar incorporation of free MCFAs in PC and recovery of PC as a function of time of acidolysis mediated by PLA_1 Lecitase® Ultra. Reaction conditions: molar ratio of substrates (PC/MCFAs) (*S*), 1:16; enzyme loading (wt % based on total amount of substrates) (*E*), 32%; temperature (*T*), 50°C.

Figure 1 shows schematically the synthesis of modified PC at the sn-1 position by PLA_1, which catalyzes the acidolysis between PC and free MCFAs, so that the content of modified PC (ML-type PC) increased with reaction time, whereas that of unmodified PC (LL-type PC) decreased.

Results for incorporation of free MCFAs into PC and recovery of PC after 72 h of reaction are summarized in Table 2 for the experimental conditions employed in the experimental design. Inspection of Table 2 indicates that the experimental conditions leading to higher incorporation of free MCFAs into PC also produced lower recovery of PC. Figure 2 shows the percentage incorporation of free MCFAs and the percent recovery of PC as a function of reaction time for the

Table 3. Regression Coefficients Describing the Influence of Different Variables on the Incorporation of MCFAs into PC for each of the Sampling Times

Time (h)	C	S	E	T	SS	EE	TT	SE	ST	ET	R^2
1	32.54	4.75	13.88	−3.14	−19.44	−7.87	−12.79	−0.09	0.67	2.51	0.93
2	35.94	5.63	15.54	−5.65	−18.60	−7.22	−10.80	4.19	−6.08	0.53	0.95
3	37.34	5.82	14.63	−5.99	−18.61	−6.71	−10.34	0.27	−7.59	6.49	0.94
6	41.03	7.48	12.13	−7.48	−16.26	−7.94	−11.74	4.52	−3.86	7.98	0.95
9	42.68	7.62	12.36	−6.65	−14.34	−7.82	−11.46	2.62	−8.39	10.36	0.94
12	43.11	7.61	12.68	−7.19	−12.73	−4.68	−10.78	−0.09	−5.14	10.22	0.97
24	46.04	7.99	11.97	−8.49	−9.93	−5.18	−10.14	3.84	−5.93	6.66	0.96
36	48.48	8.38	12.96	−10.46	−10.54	−4.90	−9.99	3.63	−7.13	5.74	0.95
48	49.82	8.82	13.82	−11.92	−9.97	−4.49	−9.96	3.58	−7.17	4.24	0.94
72	51.73	8.93	14.98	−13.25	−11.00	−3.05	−9.25	5.13	−10.93	2.05	0.96

Abbreviations: C, constant; S, molar ratio of substrates; E, enzyme loading; T, reaction temperature; R^2, coefficient of determination of the models. The factor is significant at the $\leq$0.05 level.

Table 4. Regression Coefficients Describing the Influence of Different Variables on PC Recovery for Each of the Sampling Times

Time (h)	C	S	E	T	SS	EE	TT	SE	ST	ET	R^2
1	48.96	2.22	−25.35	4.90	21.42	14.58	18.66	4.80	0.98	−3.92	0.94
2	44.69	1.38	−24.62	5.25	22.40	16.10	17.50	3.32	5.60	−5.66	0.96
3	44.01	3.16	−23.99	6.04	17.34	16.60	18.38	0.10	5.63	−8.94	0.97
6	39.86	3.25	−22.54	4.41	13.24	15.16	16.97	−0.06	6.29	−10.83	0.96
9	35.93	4.24	−21.88	5.97	15.01	14.88	17.81	−6.67	7.50	−16.21	0.93
12	35.33	1.38	−21.09	2.91	13.43	15.32	19.19	−0.90	6.02	−10.20	0.95
24	28.46	2.33	−23.42	4.51	10.95	13.96	19.99	−1.20	5.92	−9.30	0.95
36	25.64	2.46	−22.43	6.03	9.42	11.87	18.46	−2.52	6.78	−6.61	0.95
48	23.53	3.92	−23.96	8.07	7.64	13.68	19.84	−1.74	8.24	−7.34	0.96
72	22.62	3.36	−20.75	9.24	5.82	11.75	13.21	−8.22	11.37	−2.94	0.93

Abbreviations: C, constant; S, molar ratio of substrates; E, enzyme loading; T, reaction temperature; R^2, coefficient of determination of the models. The factor is significant at the $\leq$0.05 level.

experimental conditions consisting of *S* (1 mol PC to 16 mol free MCFAs), *E* (32 wt % immobilized PLA_1), and *T* (50°C) for which the maximum incorporation of free MCFAs into PC occurred after 72 h of reaction. The data clearly indicate that the extent of incorporation of free MCFAs into PC and percentage of recovery of PC display opposite trends. This behavior was similar for all the experimental conditions and trends for the trials in Table 2.

Thus, a compromise between these opposing effects should be sought out to achieve both a relatively large incorporation of free MCFAs into PC and a relatively high PC recovery %. The regression coefficients of the model and probability values for the response variables for the incorporation of free MCFAs into PC are listed in Table 3 and those for recovery of PC in Table 4. *S* and *E* were the variables that had significant positive effects. However, the crossinteractions *EE*, *TT*, and *ST*, and *T* had a significant negative effect on the incorporation of free MCFAs into PC (Table 3). By contrast, *S*, *T*, *EE*, *TT*, and *ST* had positive significant effect, whereas *E* and *ET* had a negative significant effect on PC recovery (Table 4). The response surfaces and contour plots associated with the model parameters and interactions of Tables 3 and 4 are shown in Figures 3 and 4, respectively.

Analysis of Figure 3A reveals that increased enzyme loading increased the incorporation of free MCFAs into PC, but it had the opposite effect on PC recovery (Figure 4A). The enzyme loading had the most significant effect on both incorporation of free MCFAs into PC and on PC recovery. These results are consistent with those reported by Vikbjerg et al.,[6,38] Reddy et al.,[2] Mutua and Akoh,[25] and Vijeeta et al.,[30] who reported that greater enzyme loading (10–20%) and increased substrate molar ratio (1:3.5–1:5) had a positive effect on the incorporation of fatty acids into PC.

Substrates molar ratio had a positive and significant effect on the incorporation of free MCFAs into PC and on PC recovery. These results are consistent with those reported by Svensson et al.[26] who reported good incorporation of heptadecanoic acid (C17:0) for a substrate molar ratio of 1:40 (PC:free MCFAs), whereas Vikbjerg et al.[6] observed a similar trend for the incorporation of caprylic acid (C8:0) into PC at a substrate molar ratio of 1:15. Reddy et al.[2] and Mutua and Akoh[25] found that substrates molar ratios of 1:2 and 1:5 had positive effect on the incorporation of stearic acid and eicosapentaenoic acid into PC.

Egger et al.[32] studied the effect of the type of fatty acid substrate on acyl exchange, and suggested that reaction rate decreased with increasing concentration of free fatty acids as a consequence of either an increased viscosity, a decreased solubility, or owing to the changes in the polarity of the reaction medium. These authors found that the reaction rate also depended on the chain length and the degree of unsaturation of the fatty acid to be incorporated into PC molecules. Thus, the reaction rate decreased sharply when the length of the fatty acids increased from C6 to C12, C14 (myristic acid) and C16 (palmitic acid). This result was attributed to the lower solubility of these fatty acids in the reaction mixture. Moreover, C18 (stearic acid) was not soluble in the reaction mixture. Oleic acid (C18:1) exhibited the highest reaction rate, but fatty acids with a higher degree of unsaturation (C18:2 and C18:3) were associated with lower reaction rates. Yankah and Akoh[42] assessed the incorporation of oleic acid and caprylic acid into tristearin and found that the incorporation of oleic acid was greater than for caprylic acid for all the variables studied. They also observed that incorporation of oleic acid increased by increasing the substrates molar ratio from 1:1 to 1:8, whereas maximum incorporation of caprylic acid was found at a substrate molar ratio of 1:6. These authors also noted substrate inhibition, attributing the

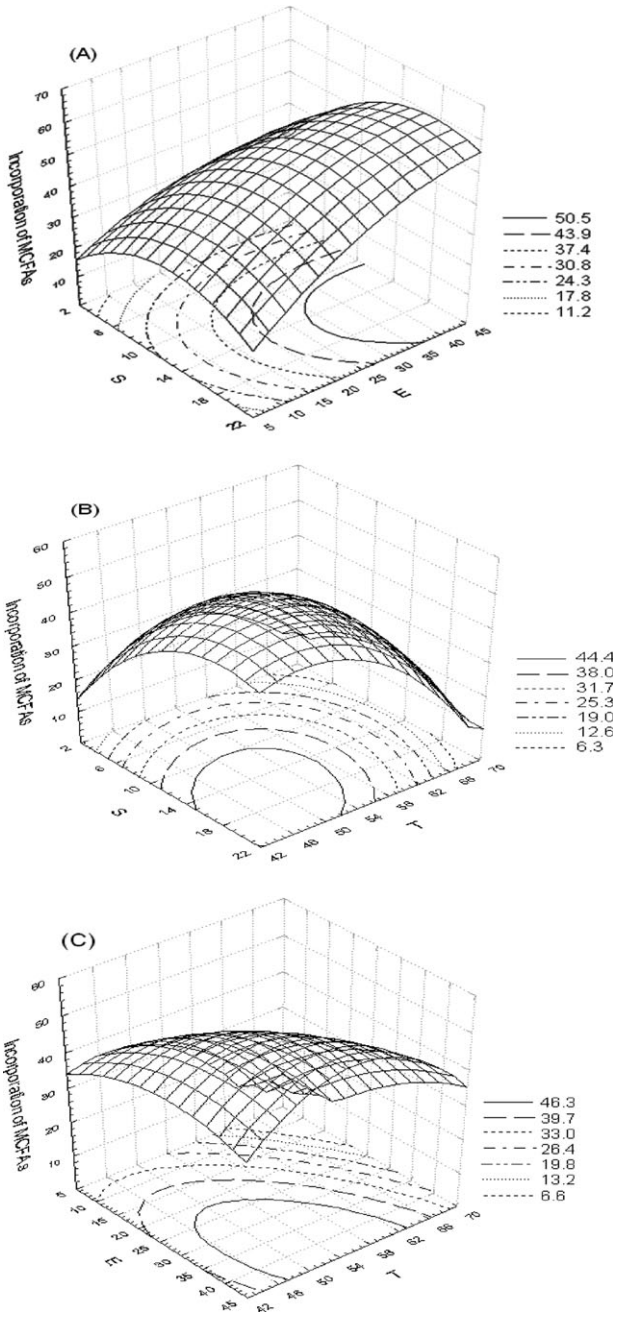

Figure 3. Response surface and contour plots for incorporation of free MCFAs (mol %) into PC, after 24 h of reaction mediated by PLA_1 Lecitase® Ultra at: Reaction temperature (*T*) of 55°C, (A); enzyme loading (*E*) of 24%, (B); and molar ratio of substrates (*S*) of 1:12, (C).

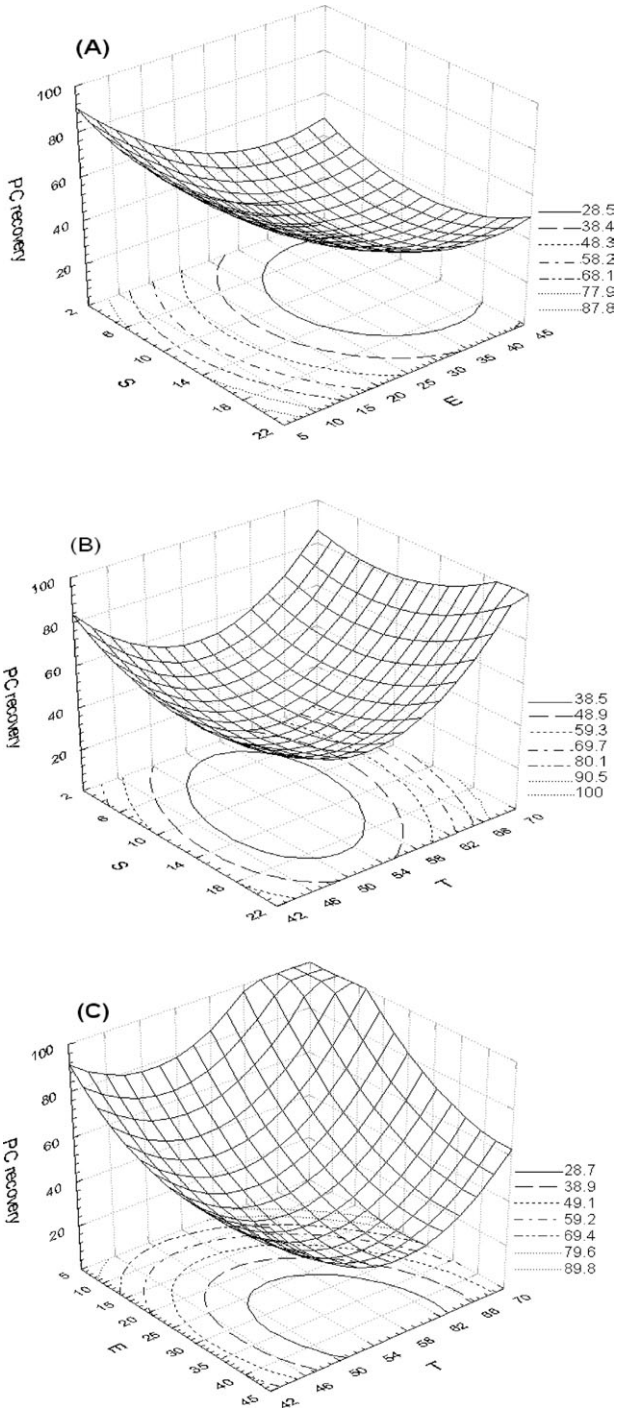

Figure 4. Response surface and contour plots for recovery of PC (%) for acidolysis, after 24 h of reaction catalyzed by PLA_1 Lecitase® Ultra at: Reaction temperature (*T*) of 55°C, (A); enzyme loading (*E*) of 24%, (B); and molar ratio of substrates (*S*) of 1:12, (C).

loss of enzyme activity to the presence of high concentrations of free MCFAs, which could acidify the microaqueous phase surrounding the enzyme or cause desorption of water from the interface. There was less inhibition when longer-chain fatty acids were used.

The results of Figure 3B indicate that increasing the reaction temperature decreased the extent incorporation of free MCFAs into PC, but improved PC recovery (Figure 4B). By contrast, Kim et al.[29] observed an increased incorporation of *n-3* polyunsaturated fatty acids into PC by acidolysis mediated by free PLA_1 as the temperature was increased from 25 to 55°C, but as temperature was further increased from 55 to 65°C the incorporation remained constant. Vikbjerg et al.[39] obtained maximum incorporation of caprilyc acid into PC during PA_2-catalyzed acidolysis at 45°C. At higher temperatures, there was a decrease in the extent of incorporation of fatty acids. Vikbjerg et al.[6,38] and Egger et al.[32] reported a positive effect on the incorporation of fatty acids into PC during Lipozyme TL IM, Lipozyme RM IM, and PLA_2-catalyzed acidolysis at temperatures below 55°C. This positive

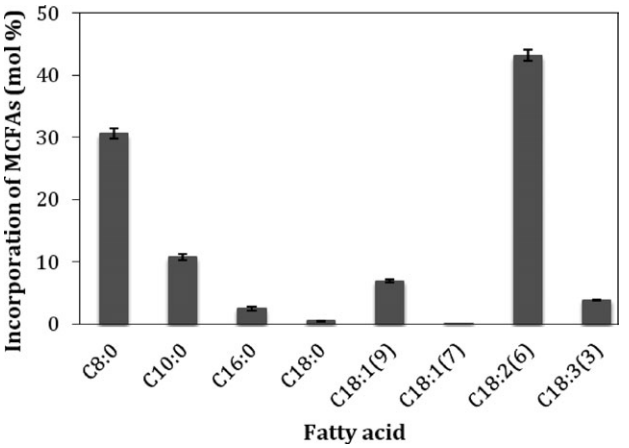

Figure 5. Composition (mol %) of the free MCFAs incorporated into PC using the optimum predicted conditions for the maximum incorporation of MCFAs and maximum PC recovery (molar ratio of substrates 1:16, enzyme loading of 16%, and 50°C).

effect was attributed to an increased reaction rate driven by increased solubility of free fatty acids and decreased viscosity of the reaction medium as the temperature increased.[31]

In this study, the maximum incorporation of free MCFAs into PC was obtained at a molar ratio of PC to free MCFAs of 1:16, an enzyme loading of 32%, and 50°C. Under these conditions, an incorporation of 49% was achieved after 12 h of reaction and of 70% after 72 h of reaction. However, the PC recoveries corresponding to these reaction times were 37 and 14%, respectively. According to the predictions of the model, optimum conditions for the maximum incorporation of free MCFAs and maximum PC recovery are molar ratio of substrates 1:16, enzyme loading of 16%, and 50°C. Under these conditions, up to 41% incorporation of free MCFAs and up to 53% recovery of PC occurred after 12 h of reaction. Figure 5 shows the composition of the free MCFAs found using these experimental conditions, and it is evident that a successful incorporation of capric (10.84 mol %) and caprylic (30.58 mol %) acids into PC as esterified residues was achieved, whereas the mole percentages of palmitic, oleic, and linoleic fatty acid residues were substantially reduced. Vikbjerg et al.[6,38] reported incorporation of 46% caprylic acid into PC after 50 h of acidolysis catalyzed by the lipases Lipozyme TL IM and Lipozyme RM IM. Peng et al.[37] described the incorporation of 39% caprylic acid into PC by acidolysis after 70 h of reaction in the presence of Lipozyme TL IM. PLA_2 immobilized on amberlite was employed by Vikbjerg et al.[39] to incorporate caprylic acid into PC. These investigators attained an incorporation of 36% after 48 h. The results obtained in this study reveal that we achieved greater incorporation of free MCFAs into PC and substantial PC recovery at shorter reaction times.

Conclusions

The incorporation of free MCFAs into PC and PC recovery via the PLA_1-catalyzed acidolysis reaction was significantly affected by the molar ratio of substrates, enzyme loading, and reaction temperature. Esterification of free MCFAs into PC was favored by increasing the enzyme loading and the molar ratio of MCFAs to PC. By contrast, increasing temperature and enzyme loading affected negatively the incorporation of free MCFAs into PC. The use of central composite design and response surface methodology allowed to obtain a predictive model, establishing the relationships between the molar ratio of substrates, enzyme loading, and reaction temperature allowed for a balanced relatively high incorporation of free MCFAs and PC recovery. These conditions were a molar ratio of PC to free MCFAs of 1:16, an enzyme loading of 16%, and 50°C. Under these conditions, the incorporation of MCFAs was 41% and PC recovery was 53%.

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