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Characterization of Terebinth Fruit Oil and Optimization of Acidolysis Reaction with Caprylic and Stearic Acids

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Abstract Characterization of the fatty acid and triacylglycerol composition of terebinth fruit oil and the synthesis of structured lipids (SL) were performed in this study. Interesterification reaction of terebinth fruits oil (*Pistacia terebinthus* L.) with caprylic acid (CA) and stearic acid (SA) to produce a SL was performed in *n*-hexane using immobilized *sn*-1,3 specific lipase from *Mucor miehei*. The effect of reaction conditions and relationship among them were analyzed by response surface methodology (RSM) with a four-factors five-level central composite rotatable experimental design. The four major factors chosen were enzyme load (10–30 wt% based on substrates), reaction time (7–18 h), reaction temperature (40–60 °C) and substrate mole ratio (terebinth oil:SA:CA 1:1:1–1:1:3). The best fitting quadratic model was determined by regression and backward elimination. Based on the fitted model, the optimal reaction conditions for the incorporation of CA and SA were found to be temperature 50 °C; time 18 h; enzyme load 30 wt%; substrate ratio 1:1:3. Under these optimum conditions, the incorporation of SA and CA could be obtained as 19 and 14%, respectively.

Keywords Terebinth fruit oil · Enzymatic interesterification · Structured lipids

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Introduction

Fats and oils are complex mixtures of simple and mixed triacylglycerols (TAG), which function as energy reserves. Triacylglycerols (TAG) differ according to the type and composition of fatty acids and their distribution along the glycerol backbone. These characteristics play an important role in the physical, rheological and nutritional properties of fats and oils [1]. Based on this perspective, much attention has been directed to the synthesis of structured lipids (SL). SL are defined as triacylglycerols which are modified to change fatty acid composition and positional distribution in the glycerol backbone [2]. A variety of fatty acids are used in the synthesis of SL, taking advantage of the functions and properties of each to obtain maximum benefits from a given structured lipid. These fatty acids include short chain fatty acids (SCFA), medium chain fatty acids (MCFA), polyunsaturated fatty acids (PUFA), saturated long chain fatty acids (LCFA), and monounsaturated fatty acids (MUFA) [3]. Caprylic acid can be inserted to oil to reduce the caloric value and stearic acid can be inserted to increase the melting point of oils. These modifications can be done chemically or enzymatically. However, enzymatic interesterification is more preferable than chemical synthesis due to its milder reaction condition requirements. Moreover enzymatic modification is providing incorporation of specific fatty acid at specific positions of triacylglycerols in order to target specific diseases, metabolic conditions and for optimal nutrition for particular population groups [4, 5].

Pistacia terebinthus L. (Anacardiaceae) is 1 of the 20 Pistacia species widely distributed in the Mediterranean region and Asia possessing many biological activities [6]. This fruit is a product used for several purposes traditionally in various regions of the world and known as being

rich in aromatic components. As a folk medicine, the decoction of its leaves is used as a stomachic, while its fruits are used in the treatment of gastralgia (internally), rheumatism and coughs (externally) and as a stimulant, diuretic, antitussive and anti-rheumatic. It can be used as an ingredient in foods, in coffee making, as pulp or directly as an appetizer. *Pistacia terebinthus* fruit contains 58–60% oil and this oil is rich in fatty acids; oleic (52.3%), linoleic (19.7%) and palmitic acid (21.3%) [7]. Its oleic acid is mainly located in the *sn*-2 position. This composition makes it very similar to olive oil and possible to use in the production of structured lipids as a raw material with a lower cost. This fatty acid composition of terebinth fruit oil makes it a desirable oil.

However, no study has been found so far about the utilization of terebinth fruit oil (TO). The work presented in this paper focused on two aspects: the characterization of fatty acid and triacylglycerol composition of TO and the synthesis of a structured lipid from TO. Structured lipids were prepared by acidolysis reaction of terebinth fruit oil with CA and SA by using the *sn*-1,3 specific immobilized lipase from *Mucor miehei*. Response surface methodology (RSM) was used to evaluate the effect of several variables on the incorporation of CA and SA into the terebinth fruit oil. RSM is an effective statistical technique for the optimization of complicated systems, which enables the evaluation of effects of multiple parameters, alone or in combination, on response variables [5–8].

Materials and Methods

Materials

TAG and fatty acid standards were obtained from Sigma Chemical Co. (St. Louis, MO). Stearic acid and caprylic acid, porcine pancreatic lipase (EC 3.1.1.3, type II, crude), silica gel (SG 60, 70–230 mesh), thin layer chromatography (TLC) plates (Kieselgel G) were obtained from Merck (Darmstadt, Germany). Immobilized *sn*-1,3 specific lipase (Lipozyme IM, immobilized from *Mucor miehei*, 140 U/g) was purchased from Fluka Chemie GmbH. Acetone, acetonitrile and *n*-hexane were purchased from Sigma-Aldrich. All solvents used were of HPLC grade. All other reagents and solvents were of analytical or chromatographic grade.

Methods

Terebinth fruits were harvested from Yamaçoba village nearby Gaziantep, Turkey in September 2009. Fruits were transported to the laboratory and held at room temperature. They were cleaned in an air screen cleaner to remove all foreign matter such as dust, dirt, stones and chaff. After that they were washed and dried under sunlight and the fruit oil was extracted by cold pressing. The fine particles which were found in pressed oil were removed by centrifugation. This clear oil was used in experiments without any refining. Some chemical and physical properties (free fatty acid content, peroxide value, fatty acid composition, triacylglycerols composition and color of oil) were determined. The molecular weight of the oil was calculated from the percentage of TAG in the oil composition.

Free fatty acid content and peroxide value were analyzed according to official methods AOCS Ca 5a-40, and AOCS Cd 8-53, respectively [9]. The color of the extracted oil was measured by a Hunter-Lab ColorFlex, A60-1010-615 model colorimeter. The $L^*a^*b^*$ color space was used to express the color.

Fatty Acid Composition

The fatty acid composition of samples was determined after converting the fatty acids into corresponding fatty acid methyl esters (FAME). After methylation, the fatty acid composition was determined with a Shimadzu GC17A gas chromatograph equipped with a flame ionization detector and a BPX capillary column (30 m × 0.22 mm × 0.25 μm film thickness). The temperatures of the injector and detector were set at 225 and 250 °C, respectively. The oven was heated to 60 °C for 1 min, then the temperature was increased to 170 °C at a rate of 10 °C/min and then from 170 to 230 °C at a rate of 3 °C/min and held at this temperature for 15 min. Nitrogen was used as the carrier gas, flowing at a rate of 1 mL/min. FAME were identified by comparison with the relative retention times of standard mixtures. Fatty acid composition at the *sn*-2 position was determined using the method developed by Brockerhoff [10]. The oil was hydrolysed with porcine pancreatic lipase, a lipase selective for *sn*-1,3 positions of TAG. The products of lipolysis were separated by TLC plates that were developed with petroleum ether: diethyl ether: acetic acid (70:30:1, by volume) The band corresponding to *sn*-2 monoacylglycerol was scraped off and was extracted with diethyl ether and methylated for GC analysis as described above.

Triacylglycerol Composition

The TAG composition of oil was determined by reversed phase HPLC using the method proposed by Çiftçi et al. [11]. Oil was diluted in acetone, filtered and injected into an HPLC system consisting of a quadratic pump (model LC-10ADVP; Shimadzu, Japan) equipped with a column (Sphereclone 5 μm ODS (2), 250 × 4.6 mm; Phenomenex, USA) with an accompanying guard column (40 × 3 mm

id) of the same phase and an ultraviolet (UV) detector (Hewlett Packard Series 1100). Elution was monitored by UV absorbance at 215 nm. The mobile phase consisted of acetone and acetonitrile (50:50, v/v) with a flow rate of 1.0 mL/min. The column temperature was set at 50 °C with a column heater (Eppendorf CH-30 column heater). All triacylglycerol contents were given in percentage area.

Acidolysis Reaction

Firstly, the substrate was prepared in various substrate mole ratios by mixing terebinth fruit oil (864 mg, MW 864 g/mol) with SA and CA at corresponding ratios generated by RSM (Table 1). Then, the enzyme in varying amounts (based on weight of pooled substrates) was added and this reaction mixture was dissolved in 50 mL hexane. The reaction was performed in an orbital shaking water bath at 200 rpm at varying temperature and time ranges as indicated in Table 1. All reactions were performed duplicate. The efficiency of reactions was analyzed by a gas chromatography (GC17A Shimadzu, Japan).

Analysis of Products

The TAG produced were isolated in two steps and then analysed with GC to determine the incorporation of CA and SA. First, the mixture obtained from the reaction was neutralized to remove free fatty acids, and then it was purified by silica gel column chromatography. Neutralization was done according to Çiftçi et al. [12].

Then, the TAG of the neutralized products were separated from monoacylglycerols and diacylglycerols by column chromatography on silica gel (SG 60, 70–230 mesh, Merck). Then, 1.6–2.0 g of the neutralized product was dissolved in 20 mL petroleum ether:diethylether (87:13, v/v) and eluted through the silica column with petroleum ether:diethylether (87:13, v/v). Then, the solvent was evaporated, and the purified reaction product (PRP) was obtained based on Polish Standard PN-ISO 8420, 1995 [13]. Then the fatty acid composition of PRP was determined by GC analysis as described above.

Experimental Design and Data Analysis

The design of the experiment was generated with four factors, five level central composite rotatable design using response surface methodology (RSM). The design was composed of 30 experiments with 6 center points, 6 star points and 18 axial points and two responses which are the percentage incorporation of SA and CA. The four major factors were chosen as reaction temperature (40–60 °C), reaction time (7–18 h), enzyme load (10–30 wt%), substrate mole ratio (terebinth oil:stearic acid:caprylic acid, 1:1:1–1:1:3).

The data were analyzed by means of RSM using commercial software (Stat-Ease, Design-Expert software, version 7). Second-order coefficients were generated by regression analysis with backward elimination. The goodness of fit of the model was evaluated by the coefficients of determination (R^2) and the analysis of variances (ANOVA). The quadratic response surface model was fitted to the following equation:

$$Y_i = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_f + \varepsilon$$

where Y_i ($i = 1$–2) are the predicted responses for percent caprylic acid (Y_1) and percent stearic acid (Y_2), β_0 intercept, β_i first-order model coefficients, β_{ii} quadratic coefficients for the ith variable, β_{ij} interaction coefficients for the interaction of variable i and j, and X_i are independent variables and ε is the random error.

Results and Discussion

Properties of Terebinth Fruit Oil

Terebinth fruit oil was composed of TAG (96%) in majority and free fatty acids, pigments, mono-diacylglcerols in minor amounts. The properties and TAG composition of terebinth fruit oil are tabulated in Table 2. The color parameters (L^*, a^*, b^*) of TO was measured as 80.04, 19.58 and 131.79, respectively. This means that TO exhibits a quite bright yellow color. The TAG composition is one of the most important chemical characteristics that determines the physical properties of fats and oils [14]. The predominant TAG were found as 1,2-dioleoyl-3-palmitoyl-glycerol (OOP), (23.30%); 1-palmitoyl-2 linoleyl-3 oleyl-glycerol + 1-stearoyl-2,3-dilinoleyl-glycerol (PLO + SLL), (15.03%); triolein (OOO), (13.88%).

The fatty acid compositions of terebinth fruit oil in TAG, at *sn*-2 and *sn*-1,3 positions were analysed by GC (Table 3). Oleic acid (18:1), palmitic acid (16:0) and linoleic acid (18:2) constituted the majority of the fatty acids with percentages of 54.50, 22.60 and 16.60%, respectively. Palmitoleic (16:1), stearic (18:0) and linolenic acids (18:3) were seen in minor amounts and myristic acid (14:0), margaric acid (17:0) and eicosenic acid (20:1) were present as traces with a percentage of 0.1%. These results were similar with the results of Özcan [7]. The major constituents of *sn*-2 were oleic and linoleic acids while *sn*-1,3 were oleic and palmitic acids.

Table 1 Four factors, five level central composite rotatable experimental design for incorporation of caprylic acid and stearic acid

Experiment number	Reaction temperature (°C)	Time (h)	Enzyme load (%)	Substrate mole ratio[a]	% (wt) incorporation of caprylic acid		% (wt) incorporation of stearic acid	
					Experimental	Predicted	Experimental	Predicted
1	40	7	10	2	8.2 ± 0.01[b]	8.4	14.4 ± 0.01	14.2
2	60	7	10	2	9.3 ± 0.20	9.1	16.7 ± 0.10	17.4
3	40	18	10	2	19.0 ± 0.15	18.1	16.0 ± 0.04	16.4
4	60	18	10	2	7.9 ± 0.01	9.9	20.3 ± 0.65	19.5
5	40	7	30	2	5.0 ± 0.16	5.4	13.2 ± 0.01	12.9
6	60	7	30	2	9.6 ± 0.12	8.7	15.5 ± 0.01	16.1
7	40	18	30	2	14.4 ± 0.21	15.1	13.1 ± 0.40	17.0
8	60	18	30	2	12.6 ± 0.01	9.5	21.4 ± 0.01	20.1
9	40	7	10	4	13.5 ± 0.09	13.4	12.7 ± 0.20	13.0
10	60	7	10	4	15.0 ± 0.05	14.1	16.0 ± 1.90	16.1
11	40	18	10	4	16.7 ± 0.20	17.2	13.8 ± 0.01	13.9
12	60	18	10	4	10.3 ± 0.18	9.0	17.2 ± 0.01	17.0
13	40	7	30	4	11.1 ± 0.06	10.5	13.1 ± 0.01	14.0
14	60	7	30	4	12.0 ± 0.06	13.7	17.5 ± 0.01	17.1
15	40	18	30	4	15.5 ± 0.01	14.2	16.9 ± 0.01	16.8
16	60	18	30	4	7.2 ± 0.10	8.6	20.2 ± 0.01	19.9
17	30	13	20	3	10.6 ± 0.02	10.7	17.2 ± 0.01	16.2
18	70	13	20	3	5.3 ± 1.10	5.7	21.8 ± 2.2	22.5
19	50	2	20	3	12.0 ± 0.06	11.1	13.0 ± 0.01	13.1
20	50	24	20	3	15.7 ± 0.06	15.6	16.4 ± 0.01	17.1
21	50	13	0	3	0	0	0	0
22	50	13	40	3	18.0 ± 1.17	18.5	17.9 ± 0.40	18.0
23	50	13	20	1	1.9 ± 0.05	2.4	15.0 ± 2.10	14.9
24	50	13	20	5	6.7 ± 0.90	6.6	13.6 ± 1.80	13.5
25	50	13	20	3	12.5 ± 0.10	13.3	17.4 ± 0.03	17.2
26	50	13	20	3	14.3 ± 0.19	13.3	17.3 ± 0.80	17.2
27	50	13	20	3	13.9 ± 1.21	13.3	17.7 ± 0.30	17.2
28	50	13	20	3	11.7 ± 0.02	13.3	16.6 ± 1.10	17.2
29	50	13	20	3	13.1 ± 1.10	13.3	17.4 ± 0.75	17.2
30	50	13	20	3	13.1 ± 0.60	13.3	17.3 ± 0.10	17.2

[a] Substrate mole ratio refers to terebinth oil:stearic acid:caprylic acid, 1:1:1–1:1:5

[b] Standard deviations for two experiments

Experimental Design and Response Surface Methodology

Table 1 shows the experimental design, independent parameters and the incorporation percentages of CA and SA as responses. All experiments were carried out in duplicate and the average values were taken into account. In this design, the run with zero enzyme load was ignored during analysis since the RSM accepted the occurrence of an acidolysis reaction in this run although there was no medium for incorporation to occur.

The best fitting quadratic model was determined by regression and backward elimination. In Tables 4 and 5, the ANOVA tables for incorporation of CA and SA after interesterification reactions are presented, respectively. The models were significant at the 99% confidence level and the lack of fits were not significant ($P > 0.01$). Moreover, high coefficients of determination (R^2) values for CA and SA (0.92 and 0.94, respectively) and close agreement between adjusted and predicted R^2 indicates the goodness of model fit. The ANOVA tables showed that the main factors were all significant in the incorporation of CA and SA. However, even if the terms of Enz × S_r and T_e × Enz for CA model and Time × S_r for SA model are not statistically significant, they were not omitted by backward elimination to maintain the hierarchy of the models.

Table 2 Properties and TAG composition of terebinth fruit oil

Property	Value
Free fatty acid (%)	0.59 (oleic acid)
Peroxide value (meq O_2/kg)	1.81
Color	L^*: 80.04, a^*: 19.58, b^*: 131.79
Monoacylglycerol + diacylglycerol (%)	3.39
Molecular weight	864 g/mol
TAG (%)	96
TAG content (%)	
OOP	23.30
PLO + SLL	15.03
OOO	13.88
OOL + PPLn	12.52
POP	9.64
OLL + OLP_O	6.76
PPL	4.34
P_OOP	3.77
PLL	2.68
LLL	1.98
SOO	1.70
POS	1.08
OOP_O	1.80

Ln linolenic acid, *L* linoleic acid, *S* stearic acid, *P* palmitic acid, *O* oleic acid, P_o palmitoleic acid

Table 3 Fatty acid composition of terebinth fruit oil

Fatty acid	FA % in TAG	FA % in *sn*-2	FA % in *sn*-1,3
14:0	0.10	0.30	–
16:0	22.60	4.50	31.65
16:1	3.30	2.50	3.70
17:0	0.10	–	0.15
17:1	0.10	0.10	0.10
18:0	2.00	1.20	2.40
18:1	54.50	67.00	48.25
18:2	16.60	23.60	13.10
18:3	0.60	0.70	0.55
20:1	0.10	0.10	0.10

Effects of Reaction Parameters

The effects of the reaction parameters on the incorporation of CA and SA mixtures into terebinth fruit oil were investigated. Temperature was found to be the most effective factor on the percent incorporation of CA ($P < 0.05$) and it was followed by time, substrate mole ratio and enzyme load in decreasing effectiveness order as it was seen on Table 4. Time and substrate mole ratio had positive coefficients although enzyme load and temperature had negative coefficients for caprylic acid. The coefficients found clearly represent that the incorporation of CA had a synergistic relationship with time and substrate mole ratio while had a contrary relationship with temperature and enzyme load. In addition, the quadratic terms of substrate mole ratio and temperature had negative effects on incorporation of CA while Enz × Enz had positive effect ($P < 0.05$).

The effects of the main parameters showed some differences for the percentage incorporation of SA (Table 5). Temperature and time had the most significant effects and they were followed by enzyme load and substrate mole ratio. Temperature, enzyme load and time had synergistic effects on the incorporation of SA but the substrate mole ratio had an inverse effect. The second-order parameters for temperature and time had positive effects but had a negative effect for the substrate molar ratio on stearic acid incorporation ($P < 0.05$).

The interaction terms of T_e × Time and Time × S_r had negative effects on the incorporation of caprylic acid. Although, the main effects of time and substrate mole ratio separately had positive effects on caprylic acid incorporation, their quadratic interactions had a negative effect. Time × S_r had also negative effect and Time × Enz and Enz × S_r had positive effects on the incorporation of stearic acid.

Figure 1 shows the response surface plots of incorporation of both SA and CA for various interaction parameters. Figure 1a represents the enzyme load against time response surface plot. It is clearly seen that the percent incorporation of SA increased up to 15 h with increasing time. However it almost reached an equilibrium after 15 h at each particular enzyme load. This is probably because of the inhibition of any further reaction by the limited amount of substrate. In the incorporation of CA, the plot of the enzyme load shows that the highest incorporation was obtained at a 10% enzyme load in the studied range. The incorporation of CA decreased at higher enzyme loads. It could result from the negative effect of increased incorporation of SA on the incorporation of CA at increased times. These results were in agreement with the findings of Akoh [15].

Percentage incorporation of CA as a function of the substrate mole ratio and enzyme load is given in Fig. 1b. An increasing substrate mole ratio caused a decrease in the incorporation of SA at low enzyme loads while the incorporation of CA increases. The possible reason of this is the increasing mole ratio of caprylic acid, while the mole ratio of stearic acid is kept constant. The incorporation SA and CA showed a maximum at around a substrate mole ratio of approximately 3.0 and then decreased. As reported by Akoh [15] and Koh et al. [16], this could be related to the acidification of the lipase environment by increasing CA.

Table 4 Analysis of variance table and coefficients for caprylic acid

Source	Coefficients	Sum of squares	*df*	Mean square	*F* value	*P* value Prob > *F*
Model		421.99	10	42.20	23.47	<0.0001[1]
Intercept	**13.39**	–	–	–	–	–
Linear						
T_e	−1.25	37.35	1	37.35	20.77	0.0002[1]
Time	1.14	31.33	1	31.33	17.42	0.0006[1]
Enz	−0.84	12.26	1	12.26	6.82	0.0177[1]
S_r	1.04	25.83	1	25.83	14.37	0.0013[1]
Interactive						
T_e × Time	−2.23	79.39	1	79.39	44.15	<0.0001[1]
T_e × Enz	0.64	6.50	1	6.50	3.62	0.0734[2]
Enz × S_r	−0.43	2.92	1	2.92	1.61	0.2255[2]
Time × S_r	−1.48	35.05	1	35.05	19.49	0.0003[1]
Quadratic						
T_e × T_e	−1.29	45.41	1	45.41	25.25	<0.0001[1]
Enz × Enz	1.70	46.26	1	46.26	25.73	<0.0001[1]
S_r × S_r	−2.21	133.87	1	133.87	74.45	<0.0001[1]
Residual		32.37	18	1.80		
Lack of fit		27.65	13	2.13	2.25	0.1896[2]
Pure error			4.72	5	0.94	
Cor total			454.36	28		

R^2 0.92, adj R^2 0.89, pred R^2 0.81

T_e Temperature, *Enz* enzyme load, S_r substrate mole ratio

[1] Significant at 'Prob > *F*' <0.05; [2] not significant at 'Prob > *F*' higher than 0.05

Table 5 Analysis of variance table and coefficients for stearic acid

Source	Coefficients	Sum of squares	*df*	Mean square	*F* value	*P* value Prob > *F*
Model		152.69	10	15.27	31.25	<0.0001[1]
Intercept	**17.26**	–	–	–	–	–
Linear						
T_e	1.57	59.13	1	59.13	121.03	<0.0001[1]
Time	1.24	36.93	1	36.93	75.59	<0.0001[1]
Enz	0.41	3.26	1	3.26	6.67	0.0187[1]
S_r	−0.36	3.09	1	3.09	6.32	0.0216[2]
Interactive						
Time × Enz	0.48	3.68	1	3.68	7.53	0.0134[2]
Time × S_r	−0.32	1.63	1	1.63	3.34	0.0842[2]
Enz × S_r	0.57	5.23	1	5.23	10.71	0.0042[1]
Quadratic						
T_e × T_e	0.54	7.80	1	7.80	15.97	0.0008[1]
Time × Time	0.66	11.45	1	11.45	23.45	0.0001[1]
S_r × S_r	−0.76	15.27	1	15.27	31.25	<0.0001[1]
Residual		8.79	18	0.49		
Lack of fit		8.11	13	0.62	4.60	0.0513[2]
Pure error			0.68	5	0.14	
Cor total			161.48	28		

R^2 0.95, adj R^2 0.91, pred R^2 0.80

T_e temperature, *Enz* enzyme load, S_r substrate mole ratio

[1] Significant at 'Prob > *F*' <0.05; [2] not significant at 'Prob > *F*' higher than 0.05

Figure 1c shows the response surface plot as a function of time and temperature. Although, Table 4 shows that the temperature had a negative effect on CA incorporation, indeed incorporation of CA increases and reaches equilibrium with increasing temperature in short reaction times. However, incorporation of CA decreased and the incorporation of SA was increased with increasing temperature at longer reaction times. This could be related to

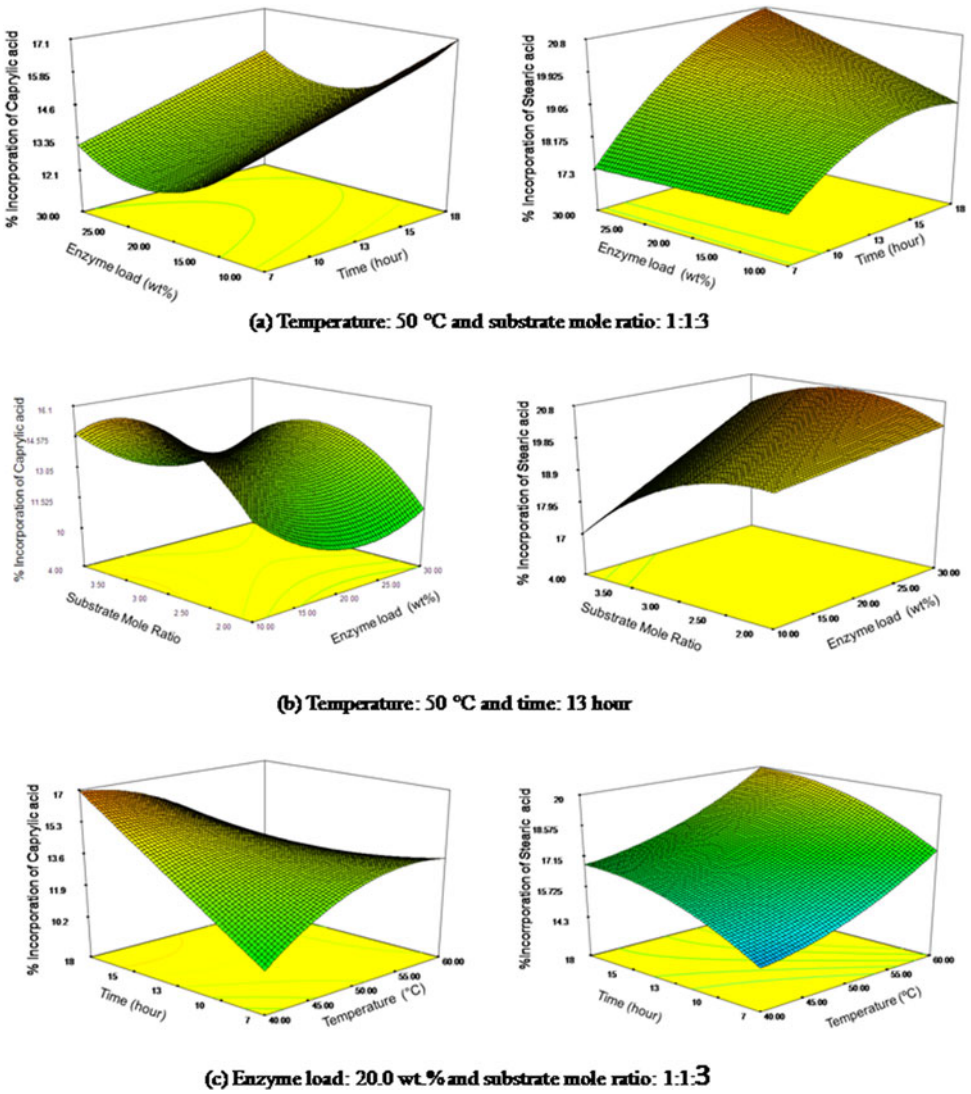

Fig. 1 Response surface plots for the incorporation of CA and SA: **a** enzyme load versus time, **b** substrate mole ratio (terebinth fruit oil:SA:CA) versus enzyme load and **c** time versus temperature

the substrate specificity of lipase changing with incubation temperature. High temperatures would be more convenient for the incorporation of stearic acid due to its high melting point (69–72 °C). The reduction of CA at high temperatures could be due to a temperature effect on the equilibrium rather than enzyme instability because Lipozyme IM60 was able to incorporate the SA at these temperatures [15]. Substrate specificity of lipase and the increase in the incorporation of SA could decrease the percentage incorporation of CA at higher temperatures.

Optimization

The optimization of lipase-catalyzed acidolysis reactions was performed by Design Expert Software. The reaction conditions were optimized for the maximal incorporation of CA and SA. At selected ranges for two responses optimum conditions were found as being 50 °C temperature, 18 h time, 30% enzyme load, 1:1:3 substrate ratio. Under these conditions, the percentage incorporations of SA and CA were 19.28 and 14.39%, respectively.

Conclusion

The characterization of the fatty acid and triacylglycerol composition of terebinth fruit oil was carried out and optimization of the acidolysis reaction of terebinth fruit oil with SA and CA, catalyzed by *sn*-1,3 specific *Mucor miehei* lipase, was performed by RSM. The best fitting quadratic model was determined by regression and backward elimination (modified model) and this model was used to analyze the effects of the main parameters, their relationships, and to optimize the reaction conditions for maximum incorporation of CA and SA. The combined highest percentage for SA (19%) and CA (14%) was obtained at temperature 50 °C; time 18 h; enzyme load 30 wt%; substrate mole ratio 1:1:3.

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