J Am Oil Chem Soc (2012) 89:103–110
DOI 10.1007/s11746-011-1895-8

ORIGINAL PAPER

Optimization of Lipase-Catalyzed Synthesis of Cetyl Octanoate in Supercritical Carbon Dioxide

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Received: 1 November 2010 / Revised: 8 February 2011 / Accepted: 23 March 2011 / Published online: 7 August 2011
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Abstract Cetyl octanoate, a wax ester of 24 carbons, is widely used in the cosmetic industry as a base oil. The current work focuses on lipase-catalyzed synthesis of cetyl octanoate in supercritical carbon dioxide (SC-CO_2) by esterification of cetyl alcohol and octanoic acid. Three immobilized lipases were screened, and 15 reaction conditions were tested in order to find the combination for maximal yield. The results showed that Novozym® 435 was the best catalyst for the synthesis, and a reaction time of 20 min was adequate for a maximal yield. Response surface methodology (RSM) with a 3-factor-3-level Box-Behnken design was employed to evaluate the effects of synthesis parameters, including reaction temperature (35–75 °C), pressure (8.27–12.41 MPa), and enzyme amount (5–15% wt of cetyl alcohol). A model for the synthesis was developed and the optimum conditions could be predicted to be reaction pressure of 10.22 MPa, reaction temperature of 63.70 °C, and enzyme amount of 11.20%. An experiment was performed under this optimum condition and a yield of 99.5% was obtained. This experimental yield correlated well with the predicted value of yield (97.6%). We concluded that, in a SC-CO_2 system, nearly 100% molar conversion of cetyl octanoate could be obtained by immobilized Novozym® 435 in a short reaction time (20 min) under the predicted optimal conditions.

Keywords Cetyl octanoate · Esterification · Lipase-catalyzed · Response surface methodology (RSM) · Supercritical carbon dioxide (SC-CO_2)

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Introduction

Wax esters are long chain esters obtained from natural sources (e.g. beeswax, sperm whale, skin lipids, jojoba seeds, carnauba) [1, 2]. Generally, they have carbon chain lengths of 12 or more. Because of their good emulsifying, stabilizing, and detergent-like properties, wax esters are frequently used in industry as carriers of water-insoluble materials for application onto various surfaces. Wax esters are biodegradable, biocompatible, nontoxic, and have excellent wetting capabilities [3]. Wax esters may also be very useful, particularly in cosmetic and pharmaceutical applications. Studies have shown that wax esters can be used as additives or auxiliaries in cleansers, conditioners, moisturizers, antifoaming agents, lubricants, plasticizers, polishers, and even anti-arthritis drugs [4, 5].

Cetyl octanoate, a wax ester of 24 carbons, is an analog of seafowl plumage oil and has an excellent moisture-retaining capability and non-greasy feeling on skin, making it an excellent base oil for the cosmetic industry. In

general, cetyl octanoate is synthesized by esterification of octanoic acid and cetyl alcohol. Synthesis of cetyl octanoate and other wax esters by chemical reaction requires high reaction temperatures (>100 °C), high pressure, long reaction time and catalyst (tin, titanium, ferric chloride, or sulphuric acid) [6–8]. The high temperature and long reaction time lead to degradation of the ester, and it also has a high energy cost. Compared with chemical synthesis, enzymatic synthesis usually takes place at atmospheric pressure, moderate pH, and moderate temperature [2, 9, 10]. In addition, the enzyme itself is environmentally friendly, non-toxic, and biodegradable. Wax esters produced by enzymatic reaction are considered identical to natural wax esters and therefore can be used safely in cosmetic and food industries. Lipase-catalyzed synthesis of wax esters, including oleyl oleate, cetyl myristate, cetyl palmitate, cetyl oleate, cetyl stearate, oleyl palmitate, and conjugated linoleic acid (CLA) C_6–C_{22} alkyl esters, have been reported [2, 5, 10, 11].

There is, however, still a major drawback with the lipase-catalyzed synthesis of wax esters [2, 5, 10, 11]. Most of the syntheses were carried out in an organic solvent system, causing problems of residual solvent, environmental pollution, and biohazards. The problem can be well solved by using supercritical carbon dioxide (SC-CO_2) as the solvent system for the synthesis. Several detailed review papers on enzymatic reactions in supercritical fluids (SCF) have been reported and published [12, 13]. Enzymatic synthesis of wax esters in SC-CO_2 is organic solvent-free, with the extra advantages of easy separation of unreacted substances, high diffusivity, low viscosity, and low surface tension [14, 15]. There have been a few studies regarding lipase-catalyzed synthesis of wax esters in SC-CO_2. This includes synthesis of lauryl oleate [14], *n*-octyl oleate [16], and citronellol laurate [17] in SC-CO_2. So far, there has been no reported study regarding lipase-catalyzed synthesis of cetyl octanoate in SC-CO_2.

Response surface methodology (RSM) is a collection of mathematical and statistical techniques for designing experiments, building models, evaluating the relative significance of several independent variables, and determining the optimum conditions for desired responses. It is a useful tool for understanding the interactions among various parameters that affect the process and has been applied successfully for optimizing parameters in various processes [18, 19]. In this study, different reaction conditions for lipase-catalyzed synthesis of cetyl octanoate in SC-CO_2 are evaluated. RSM is employed to find out the relationships between condition factors (pressure, reaction temperature and enzyme amount) and the response results (molar conversion), in order to determine the optimal conditions for synthesis.

Materials and Methods

Materials

Immobilized lipase Novozym® 435 (10,000 PLU/g; Propyl laurate units) from *Candida antarctica* B (EC 3.1.1.3) supported on a macroporous acrylic resin, and Lipozyme® RMIM (5–6 BAUN/g; Batch acidolysis unit Novo) and Lipase IM-77 (7.7 BAUN/g) from *Rhizomucor miehei* (EC 3.1.1.3) supported on a macroporous weak anionic resin were purchased from Novo Nordisk Bioindustrials Inc. (Copenhagen, Denmark). Octanoic acid (99% purity), cetyl alcohol (99% purity) and *n*-hexane was purchased from Sigma Chemical Co. (St Louis, MO, USA). Molecular sieve 4 Å was purchased from Davison Chemical (Baltimore, MD, USA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) and of analytical reagent grade.

Esterification in SC-CO_2

Cetyl alcohol and octanoic acid were dehydrated by using a 4-Å molecular sieve for 24 h before use. Synthesis of cetyl octanoate in SC-CO_2 was carried out in a high pressure reactor as depicted in Fig. 1. Carbon dioxide was pressurized to the critical point (>7.38 MPa) by a compressor (Taiwan Supercritical Technology Co., Ltd., Taiwan) and the reaction temperature was controlled by a LE-529 incubator (Get Bio Co., Taiwan). As is typical for esterification reactions, the 0.86 ml of reaction mixture contained 30 mM cetyl alcohol, 60 mM octanoic acid, and various amounts of immobilized lipase (Novozym® 435,

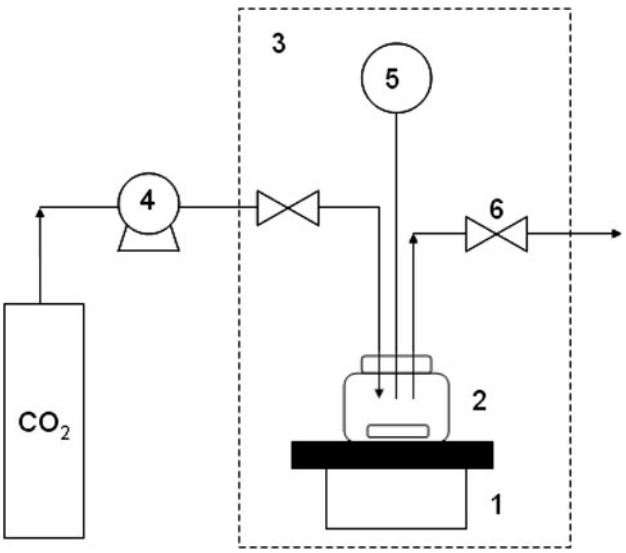

Fig. 1 Schematic diagram of the SC-CO_2 device. *1*, magnetic stirrer; *2*, reactor; *3*, incubator (temperature controlled); *4*, compressor; *5*, pressure gauge; *6*, decompression valve

Table 1 Box-Behnken design and observed experimental data for 3-level–3-factor response surface analysis

Treatment no.[a]	Factor			Experimental molar conversion (%)
	X_1 pressure (MPa)	X_2 temperature (°C)	X_3 enzyme amount (%)	
1	−1[b] (8.27)	−1 (35)	0 (10)	42.73 ± 0.67
2	1 (12.41)	−1 (35)	0 (10)	41.66 ± 2.05
3	−1 (8.27)	1 (75)	0 (10)	98.41 ± 1.01
4	1 (12.41)	1 (75)	0 (10)	97.29 ± 0.82
5	−1 (8.27)	0 (55)	−1 (5)	83.93 ± 1.69
6	1 (12.41)	0 (55)	−1 (5)	78.01 ± 1.46
7	−1 (8.27)	0 (55)	1 (15)	96.89 ± 1.69
8	1 (12.41)	0 (55)	1 (15)	93.82 ± 0.19
9	0 (10.34)	−1 (35)	−1 (5)	29.54 ± 1.37
10	0 (10.34)	1 (75)	−1 (5)	92.19 ± 0.63
11	0 (10.34)	−1 (35)	1 (15)	57.13 ± 0.59
12	0 (10.34)	1 (75)	1 (15)	98.20 ± 0.33
13	0 (10.34)	0 (55)	0 (10)	88.41 ± 1.25
14	0 (10.34)	0 (55)	0 (10)	86.25 ± 1.81
15	0 (10.34)	0 (55)	0 (10)	89.29 ± 0.51

[a] The treatments were run as a random order

[b] The values −1, 0, and 1 are coded levels

Lipozyme® RMIM, or Lipase IM-77). Experiments were conducted at different temperatures and pressures in the 50 ml reactor for 20 min. All reactions were carried out in duplicate.

Experimental Design and Statistical Analysis

For synthesis of cetyl octanoate in SC-CO_2, a 3-level-3-factor Box-Behnken design with three replicates at the center was employed, which required 15 experiments in total. The variables included pressure (8.27, 10.34, and 12.41 MPa), reaction temperature (35, 55, and 75 °C), and amount of Novozym® 435 (5, 10, and 15% wt of cetyl alcohol). The experimental data (Table 1) was analyzed by SAS software. Response surface regression (RSREG) was used in order to fit the following second-order polynomial equation [20]:

$$Y = \beta_{k0} + \sum_{i=1}^{3} \beta_{ki} X_i + \sum_{i=1}^{3} \beta_{kii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{kij} X_i X_j \qquad (1)$$

where Y is response (production rate), β_{k0}, β_{ki}, β_{kii} and β_{kij} are constant coefficients, and X_i and X_j the uncoded independent variables. The ridge max option was employed to compute the estimated ridge of maximum response for increasing the radius from the center of the original design.

Quantitation of Cetyl Octanoate

After the reaction over the desired time, the reaction mixture was depressurized and eluted with hexane. The enzyme and any residual water were removed by passing the reaction mixture through glass wool and an anhydrous sodium-sulfate column. Cetyl octanoate in the reaction mixture was quantitated by injecting a 1-μL aliquot of the reaction mixture into a gas chromatograph (GC) (Acme 6100, Young Ling Instrument, Korea) equipped with a flame-ionization detector (FID) and MTX-65TG fused-silica capillary column (30 m × 0.25 mm i.d.; film thickness 0.1 μm; RESTEK, USA) in splitless mode. Injector and FID temperatures were set at 240 and 250 °C, respectively, and the oven temperature was maintained at 125 °C for 5 min followed by elevation to 230 °C at a rate of 50 °C min^{-1}, and then at 230 °C for 12.9 min. Nitrogen was used as the carrier gas. The molar conversion was defined as (mmol cetyl octanoate/mmol of initial cetyl alcohol) × 100%. Calibration curves were prepared from cetyl octanoate (chemical synthesis) and cetyl alcohol standards and samples diluted to the appropriate level to fall within the calibration curve.

Results and Discussion

Efficacy of Different Catalysts

Three different lipases, Novozym® 435, Lipozyme® RMIM, and Lipase IM-77, were screened initially for their efficacy on the synthesis of cetyl octanoate. The reaction was performed at 55 °C with a substrate molar ratio of 1:2 (cetyl alcohol: octanoic acid) and 10% of enzyme amount (by weight of cetyl alcohol) in SC-CO_2 for 20 min. Fig. 2 shows results of conversion of cetyl octanoate for the three immobilized lipase at the different pressure. Of the three lipases

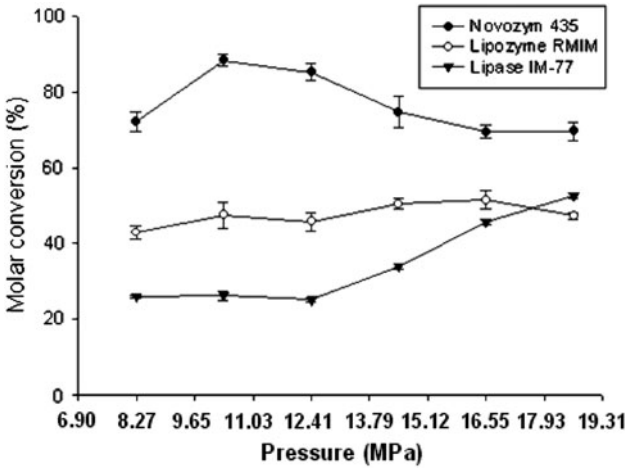

Fig. 2 Screening of different catalysts for synthesis of cetyl octanoate in SC-CO_2. The reaction conditions were set as: substrate molar ratio, 1:2 (alcohol:fatty acid); enzyme amount, 10%; agitation speed, 100 rpm; reaction temperature, 55 °C; reaction time, 20 min; and pressure, 8.27–19.31 MPa

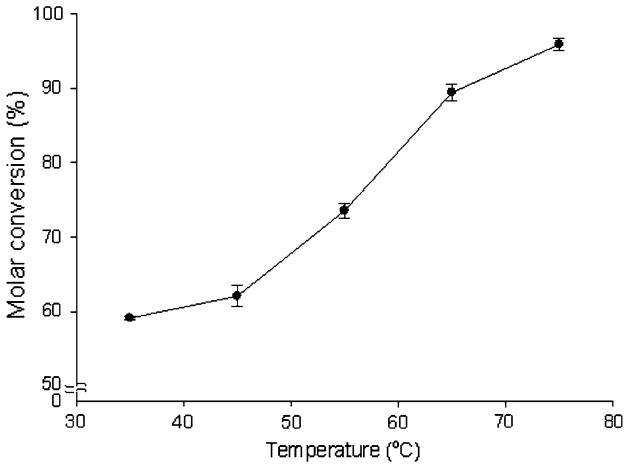

Fig. 3 The effect of temperature on synthesis of cetyl octanoate in SC-CO_2. The reaction conditions were set as: substrate molar ratio, 1:2 (alcohol:fatty acid); Novozym® 435 amount, 10%; agitation speed, 100 rpm; reaction time, 20 min; pressure, 10.34 MPa; and reaction temperature, 35–75 °C

tested, Novozym® 435 showed the best activity with maximum conversion of cetyl octanoate and Lipase IM77 the least. At a reaction pressure of 10.34 MPa, approximately 90% molar conversion of cetyl octanoate was achieved with Novozym® 435. In other words, this experiment demonstrated a high yield of cetyl octanoate in SC-CO_2 could be achieved within a short time (20 min) via Novozym® 435 catalysis. In other studies, Novozym 435 has also been shown to be very active and useful in catalyzing the synthesis of esters [21–24]. For this reason, Novozym® 435 was employed for the following experiments in this study.

Effect of Reaction Temperature

The effect of temperature on cetyl octanoate synthesis in SC-CO_2 was tested in the range of 35–75 °C and the results are shown in Fig. 3. The conversion increased with increasing temperature. The highest conversion was found from 65 to 75 °C where at least 90% conversion was achieved. All further experiments using Novozym® 435 were performed below 75 °C. According to its product sheet, Novozym® 435 is a heat-tolerant immobilized enzyme with maximum activity at 70–80 °C. However, on the product sheet, it is suggested that the enzyme be used at 40–60 °C for stability purposes. It was previously reported that enzymatic synthesis of sugar fatty acid esters in 2-methyl-2-butanol solvent at temperatures higher than 60 °C would deform the enzyme with a decrease in activity, but in SC-CO_2 the optimal reaction temperature was shifted to 80 °C. The increased stability of lipase in SC-CO_2 could be due to the rigidity of the immobilized lipase in SC-CO_2 at high temperatures [25]. Our results also clearly indicated that Novozym® 435 was active in SC-CO_2 from 65 to 75 °C and that activity increased as the temperature increased (Fig. 3). From these results, it appeared that deformation of Novozym® 435 at temperatures higher than 60 °C probably would not occur in SC-CO_2.

Model Fitting

One aim of this study was to develop a statistical model for understanding the relationships between the manipulated variables and the response (molar conversion of cetyl octanoate) in the Novozym® 435-catalyzed synthesis of cetyl octanoate in SC-CO_2. Compared with one-factor-at-a-time design used in most of the studies found in the literature, RSM is less frequently used and yet is very efficient in reducing the number of experimental treatments and times to obtain optimal reaction conditions. The experimental conditions and the response values from the experimental Box-Behnken design are listed in Table 1, which shows that the highest molar conversion (98.20 ± 0.33%) was obtained at 10.34 MPa, 75 °C, and 15% enzyme (treatment no. 12), and the lowest (29.54 ± 1.37%) was achieved at 10.34 MPa, 35 °C, and 5% enzyme (treatment no. 9). From the SAS output of RSREG, the second-order polynomial Eq. (2) was given as below:

$$\begin{aligned} Y(\%) = & -146.112593 - 3.607214x_1 + 6.954580x_2 \\ & + 4.046022x_3 + 0.109299x_1x_1 - 0.000302x_2x_1 \\ & - 0.046073x_2x_2 + 0.068841x_3x_1 - 0.053950x_3x_2 \\ & - 0.011567x_3x_3 \end{aligned} \quad (2)$$

The plot of experimental values of the conversion (%) versus those calculated from Eq. (2) indicates a good fit, as shown in Fig. 4. The analysis of variance (ANOVA),

summarized in Table 2, reveals that this second-order polynomial model, having a very small P-value (<0.0001) and high coefficient of determination ($R^2 = 0.9973$), was highly significant and adequate to represent the actual relationship between the response (molar conversion) and the independent variables. Furthermore, the overall effects of the three synthesis variables of the molar conversion were analyzed and reported in Table 3. The P-values mark the significance of coefficients and are also important for understanding the pattern of the mutual interactions between the parameters. A value of Prob. $> F < 0.05$ indicates that the model terms are significant. Table 3 demonstrates that the linear term of the temperature (x_2; $P < 0.0001$) and the enzyme amounts (x_3; $P = 0.0001$) are significant parameters for cetyl octanoate production. The quadratic ($x_2 \times x_2$; $P < 0.0001$) and interactions ($x_2 \times x_3$; $P = 0.0032$) terms are also statistically significant. However, the variable pressure and its quadratic or interactions terms did not display any significant effect. In this study, where pressures from 8.27 to 12.41 MPa were studied, pressure was found to not strongly influence the product yield. This is somewhat unexpected since pressures of 9–14 MPa correspond to log P (index of the polarity of solvent) of 1.536–2.74 in this system, which is postulated to be the best region for the esterification reaction. However, a similar result was also reported in a study of lavandulol esterification [26]. On the other hand, Knez et al. and Laudani et al. [14, 16] demonstrated the highest degree of wax ester synthesis is at 10 MPa, and that if the pressure was out of the range 8.27–12.41 MPa the degree of esterification significantly decreased.

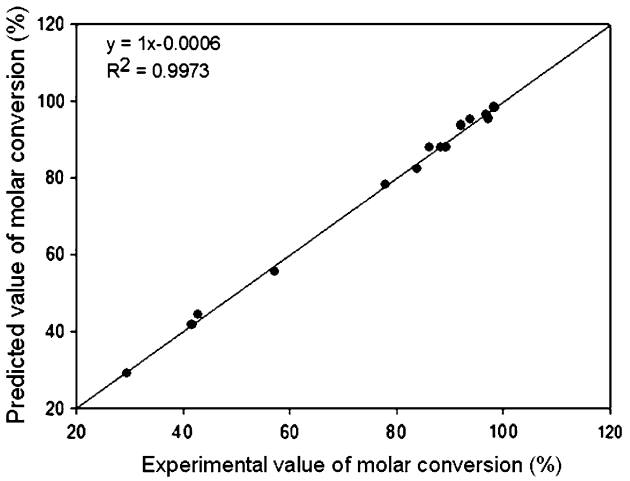

Fig. 4 The relationship between predicted and experimental molar conversion of cetyl octanoate

Mutual Effect of Parameters

The relationships between reaction factors and responses can be better understood by examining the series of response surface plots generated by holding constant the enzyme amount (Fig. 5), reaction temperature (Fig. 6), or pressure (Fig. 7). Reaction temperature and pressure were investigated in the range of 35–75 °C and 8.27–12.41 MPa, respectively. Fig. 5 shows the effect of varying temperature and pressure on esterification with 5% enzyme loading. At any given pressure from 8.27–12.41 MPa, an increase in temperature to 75 °C led to a curvilinear increase in molar conversion to 95%, indicating that the temperature was one of the most important factors in the synthesis of cetyl octanoate. However, the reaction pressure had no significant effect on the conversion. This result was consistent with that shown in Table 3, which indicated that reaction pressure (x_1) did not have a significant effect ($P > 0.05$) on the synthesis of cetyl octanoate. However, increasing or decrease the pressure out of this study range (8.27–12.41 MPa) significant decreases cetyl octanoate production as shown in Fig. 2. The effect of varying enzyme amounts and pressure on molar conversion at constant reaction temperature (55 °C) is shown in Fig. 6. At any given pressure, an increasing enzyme amount seemed to have higher molar conversion. At the reaction condition of lowest pressure (8.27 MPa) and highest enzyme amount (15%), the maximal molar conversion (96.6%) would be reached. However, increasing pressure resulted in only a slight decrease in esterification efficiency at any given enzyme amount. This indicates that Novozym® 435 is stable in SC-CO_2 within the experimental pressure range. Fig. 7 shows the effect of

Table 2 ANOVA for synthesis variables pertaining to the response percent molar conversion

Source	Degrees of freedom	Sum of squares	F value	Prob. $> F$[a]
Linear	3	6,281.613775	503.55	<0.0001
Quadratic	3	1,270.931883	101.88	<0.0001
Cross product	3	118.455350	9.50	0.0166
Total model	9	7,671.001008	204.98	<0.0001
Lack of fit	3	15.897125	2.17	0.3314
Pure error	2	4.893867		
Total error	5	20.790992		
$R^2 = 0.9973$				

[a] (Prob. $> F$) = level of significance

Table 3 ANOVA for the fitted quadratic polynomial model of all independent variables

Factor	Degrees of freedom	Sum of squares	Prob. > F[a]
Pressure (x_1)	1	15.624	0.1103
Temperature (x_2)	1	5,779.737	<0.0001
Enzyme amount (x_3)	1	486.252	0.0001
$X_1 \times X_2$	1	0.001	0.9907
$X_1 \times X_3$	1	2.031	0.5158
$X_2 \times X_3$	1	116.424	0.0032
$X_1 \times X_1$	1	0.810	0.6774
$X_2 \times X_2$	1	1,254.0339	<0.0001
$X_3 \times X_3$	1	0.309	0.7961

Underlined model terms are significant ones

[a] (Prob. > F) = level of significance

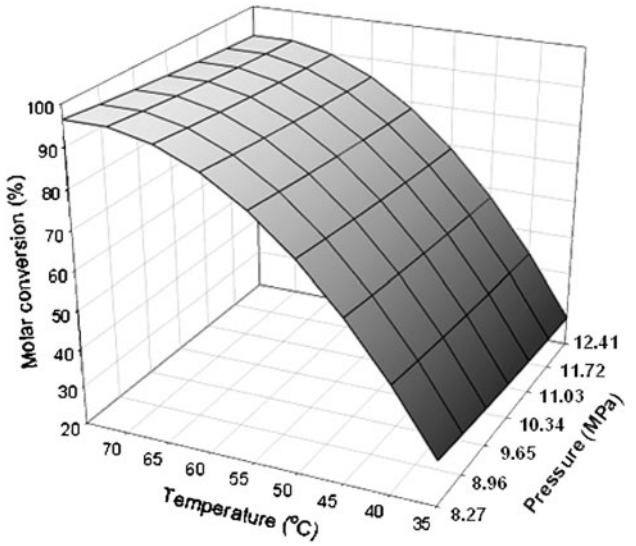

Fig. 5 Response surface plot showing the mutual effect of reaction temperature and pressure on molar conversion of cetyl octanoate. The Novozym® 435 amount was kept constant (5%) throughout the experiment

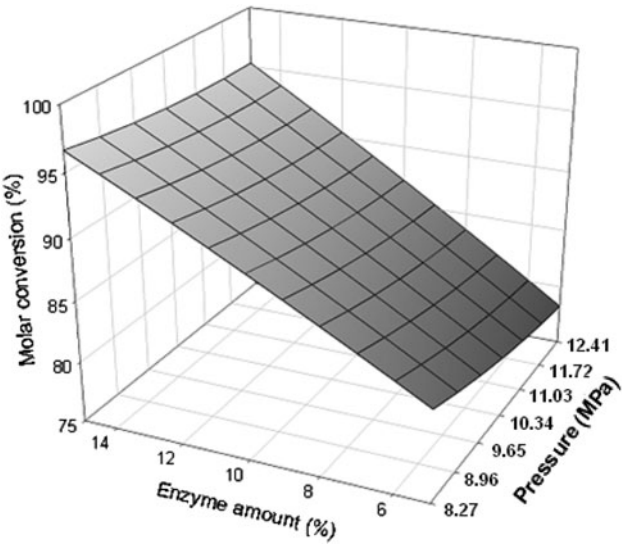

Fig. 6 Response surface plot showing the mutual effect of Novozym® 435 amount and reaction pressure on molar conversion of cetyl octanoate. The reaction temperature was kept constant (55 °C) throughout the experiment

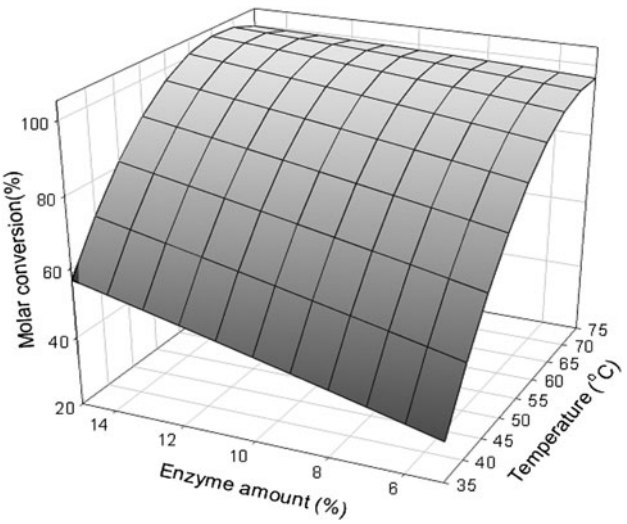

Fig. 7 Response surface plot showing the mutual effect of Novozym® 435 amount and reaction temperature on molar conversion of cetyl octanoate. The reaction pressure was kept constant (8.27 MPa) throughout the experiment

varying enzyme amount and temperature on esterification at constant pressure (8.27 MPa). With the highest enzyme amount (15%) and an appropriate reaction temperature (65 °C) condition, the maximum percent molar conversion of cetyl octanoate was obtained. However, a lower reaction temperature and enzyme amount significantly decreased the molar conversion to 30%, indicating that both the enzyme amount and reaction temperature were the two most important parameters for the synthesis of cetyl octanoate.

Attaining Optimum Conditions

The optimum point was determined by ridge max analysis [20]. The ridge analysis uses a quadratic regression model to estimate optimal experimental-variable values at fixed distances from a defined center of an experimental region [27]. The radius is the given distance from the ridge starting point at which to compute the optima. The ridge analysis method computes the estimated ridge of maximum response for increasing radius from the center of original design. The ridge max analysis (Table 4) predicted maximum conversion to be 97.55 ± 1.11% at 10.22 MPa, 63.70 °C, and 11.20% enzyme. The validity of the predicted model was examined by experiments at the

Table 4 Estimated ridge of maximum response for variable percent molar conversion

Coded radius	Pressure (MPa)	Temperature (°C)	Enzyme amount (%)	Estimated response (%)	Standard error
0	10.34	55.00	10.00	87.98	1.18
0.1	10.33	56.91	10.15	90.60	1.17
0.2	10.32	58.77	10.32	92.86	1.16
0.3	10.29	60.58	10.54	94.75	1.15
0.4	10.26	62.25	10.82	96.31	1.13
0.5	10.22	63.70	11.20	97.55	1.11

predicted optimum conditions. The molar conversion of the actual experiment was 99.47 ± 0.05%. The result indicated that observed value was almost the same as that predicted from the equation (Eq. 1). The model generated adequately predicted the percent molar conversion.

Conclusions

Cetyl octanoate, a wax ester of 24 carbons, was synthesized enzymatically within a short time (20 min) in the green solvent "SC-CO_2". Three enzymes were examined; Novozyme 435 was chosen for further study since it demonstrated the highest activity. The optimum reaction condition for high conversion of cetyl octanoate was achieved by using Box-Behnken design and response surface methodology. Three parameters, i.e., reaction temperature, pressure, and enzyme amount, were evaluated by joint test. Based on the results of the evaluation, it appears that reaction temperature and enzyme amount significantly affected the molar conversion, but reaction pressure did not affect yield within the range of pressures studied here, 8.27–12.41 MPa. A model for the synthesis was built and the optimal synthesis condition was derived as follows: reaction pressure, 10.22 MPa; reaction temperature, 63.7 °C; and enzyme amount, 11.20%. Under this optimal condition, a conversion value of 99.5% could be expected. Synthesis of cetyl octanoate under this optimal condition was performed experimentally and 97.6% conversion was obtained. Thus, optimization of the synthesis of cetyl octanoate catalyzed by Novozym® 435 was successfully developed by Box-Behnken design and RSM.

References

1. Patel S, Nelson DR, Gibbs AG (2001) Chemical and physical analyses of wax ester properties. J Insect Sci 1:1–7
2. Salis A, Solinas V, Monduzzi M (2003) Wax esters synthesis from heavy fraction of sheep milk fat and cetyl alcohol by immobilised lipases. J Mol Catal B Enzym 21:167–174
3. Petersson AEV, Gustafsson LM, Nordblad M, Börjesson P, Mattiasson B, Adlercreutz P (2005) Wax esters produced by solvent-free energy-efficient enzymatic synthesis and their applicability as wood coatings. Green Chem 7:837–843
4. Hunter KW Jr, Gault RA, Stehouwer JS, Tam-Chang SW (2003) Synthesis of cetyl myristoleate and evaluation of its therapeutic efficacy in a murine model of collagen-induced arthritis. Pharmacol Res 47:43–47
5. Gunawan ER, Basri M, Rahman MBA, Salleh AB, Rahman RNZA (2005) Study on response surface methodology (RSM) of lipase-catalyzed synthesis of palm-based wax ester. Enzyme Microb Technol 37:739–744
6. Kawabata T, Mizugaki T, Ebitani K, Kaneda K (2003) Highly efficient esterification of carboxylic acids with alcohols by montmorillonite-enwrapped titanium as a heterogeneous acid catalyst. Tetrahedron Lett 44:137–140
7. Sunitha S, Kanjilal S, Reddy PS, Prasad RBN (2007) Liquid-liquid biphasic synthesis of long chain wax esters using the Lewis acidic ionic liquid choline chloride·2$ZnCl_2$. Tetrahedron Lett 48:6962–6965
8. Ieda N, Mantri K, Miyata Y, Ozaki A, Komura K, Sugi Y (2008) Esterification of long-chain acids and alcohols catalyzed by ferric chloride hexahydrate. Ind Eng Chem Res 47:8631–8638
9. Isono Y, Nabetani H, Nakajima M (1995) Wax ester synthesis in a membrane reactor with lipase-surfactant complex in hexane. J Am Oil Chem Soc 72:887–890
10. Hadzir NM, Basri M, Rahman MBA, Razak CNA, Rahman RNZA, Salleh AB (2001) Enzymatic alcoholysis of triolein to produce wax ester. J Chem Technol Biotechnol 76:511–515
11. Dianóczki C, Recseg K, Kovári K, Poppe L (2007) Convenient enzymatic preparation of conjugated linoleic acid alkyl esters with C6–C22 alcohols. J Mol Catal B Enzym 45:45–49
12. Hobbs HR, Thomas NR (2007) Biocatalysis in supercritical fluids, in fluorous solvents, and under solvent-free conditions. Chem Rev 107:2786–2820
13. Wimmer Z, Zarevúcka M (2010) A review on the effects of supercritical carbon dioxide on enzyme activity. Int J Mol Sci 11:233–253
14. Knez Z, Laudani CG, Habulin M, Reverchon E (2007) Exploiting the pressure effect on lipase-catalyzed wax ester synthesis in dense carbon dioxide. Biotechnol Bioeng 97:1366–1375
15. Leitner W (2002) Supercritical carbon dioxide as a green reaction medium for catalysis. Acc Chem Res 35:746–756
16. Laudani CG, Habulin M, Knez Z, Porta GD, Reverchon E (2007) Immobilized lipase-mediated long-chain fatty acid esterification in dense carbon dioxide: bench-scale packed-bed reactor study. J Supercrit Fluid 41:74–81
17. Habulin M, Šabeder S, Sampedro MA, Knez Z (2008) Enzymatic synthesis of citronellol laurate in organic media and in supercritical carbon dioxide. Biochem Eng J 42:6–12
18. Chen HC, Ju HY, Twu YK, Chen JH, Chang CM, Liu YC, Chang C, Shieh CJ (2010) Optimized enzymatic synthesis of caffeic acid phenethyl ester by RSM. N Biotechnol 27:89–93
19. Ju HY, Huang KC, Chen JH, Liu YC, Chang CMJ, Lee CC, Chang C, Shieh CJ (2010) Optimization of the extraction of *Alpinia oxyphylla* essence oil in supercritical carbon dioxide. J Am Oil Chem Soc 87:1063–1070

20. AS S (1990) SAS user guide. SAS Institute, Cary
21. Sun S, Shan L, Liu Y, Jin Q, Song Y, Wang X (2009) Solvent-free enzymatic synthesis of feruloylated diacylglycerols and kinetic study. J Mol Catal B Enzym 57:104–108
22. Yadav GD, Dhoot SB (2009) Immobilized lipase-catalysed synthesis of cinnamyl laurate in non-aqueous media. J Mol Catal B Enzym 57:34–39
23. Lystvet SM, Hoff BH, Anthonsen T, Jacobsen EE (2010) Chemoenzymatic synthesis of enantiopure 1-phenyl-2-haloethanols and their esters. Biocatal Biotransform 28:272–278
24. Varma MN, Madras G (2010) Effect of chain length of alcohol on the lipase-catalyzed esterification of propionic acid in supercritical carbon dioxide. Appl Biochem Biotechnol 160:2342–2354
25. Habulin M, Šabeder S, Knez Z (2008) Enzymatic synthesis of sugar fatty acid esters in organic solvent and in supercritical carbon dioxide and their antimicrobial activity. J Supercrit Fluid 45:338–345
26. Olsen T, Kerton F, Marriott R, Grogan G (2006) Biocatalytic esterification of lavandulol in supercritical carbon dioxide using acetic acid as the acyl donor. Enzyme Microb Technol 39: 621–625
27. Peterson J (1993) A general approach to ridge analysis with confidence intervals. Technometrics 35:204–214