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Study on response surface methodology (RSM) of lipase-catalyzed synthesis of palm-based wax ester

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Abstract

The synthesis of wax ester using refined, bleached and deodorized (RBD) palm oil and oleyl alcohol catalyzed by lipozyme IM was carried out. Response surface methodology (RSM) based on a five-level, four-variable central composite rotatable design (CCRD) was used to evaluate the interactive effects of synthesis, of reaction time (2.5–10 h), temperature (30–70 °C), amount of enzyme (0.1–0.2 g) and substrate molar ratio (palm oil to oleyl alcohol, 1:1–1:5) on the percentage yield of wax esters. The optimum conditions derived via RSM were: reaction time 7.38 h, temperature 53.9 °C, amount of enzyme 0.149 g, and substrate molar ratio 1:3.41. The actual experimental yield was 84.6% under optimum condition, which compared well to the maximum predicted value of 85.4%.

Keywords: Response surface methodology (RSM); Central composite rotatable design (CCRD); Palm oil; Lipozyme; Alcoholysis; Wax ester

1. Introduction

Wax esters are long chain esters that are derived from fatty acids and alcohols with chain lengths of 12 carbons or more. The compounds have many potential applications due to their excellent wetting behavior at interfaces [1] and a non-greasy feeling when applied on skin surfaces. Wax esters are important ingredients in cosmetic formulations (cleansers, conditioners and moisturizers), in pharmaceuticals (as an anti foaming agent in the production of penicillin), lubricants, plasticizers and polishes and the other chemical industries [2].

Natural waxes originate from animals, vegetables and minerals. Many of the important commercial waxes contain rather high percentages of saturated wax esters, such as beeswax. The raw materials for saturated and unsaturated wax ester are sperm whale and jojoba oil [2]. Since the naturally occurring wax esters are expensive and limited in access, the need to synthesize the compound has grown. Wax ester has been synthesized via chemical [3] and enzymatic reaction [4]. Enzymatic synthesis uses lower temperatures than chemical synthesis [2].

Wax esters can be produced by alcoholysis of vegetable oils such as palm oil. Palm oil consists of triacylglycerides, which are a combination of glycerol and different fatty acids. Enzymatic synthesis of wax esters from rapeseed fatty acid methyl ester [2] and lipase-catalyzed alcoholysis of crambe and camelina oil [5] have been reported.

Enzymatic synthesis of wax esters from palm oil and oleyl alcohol was studied using a commercial immobilized lipase. The classical method of optimization involves varying one parameter at a time and keeping the other constant. But the method is inefficient as it fails to understand relationships between the variables (reaction time, temperature, molar ratio and amount of enzyme) and the response (percentage yield) [6,7]. Response surface methodology (RSM) is an effective statistical technique for the investigation of complex processes. The main advantage of RSM is the reduced number

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of experimental runs needed to provide sufficient information for statistically acceptable result. It is a faster and less expensive method for gathering research result than the classical method [8]. RSM has successfully been applied to study and optimize the enzyme synthesis of flavor ester [6,9] and biodiesel (fatty acid alkyl ester) [10].

RSM comprising a five-level-four-factor central composite rotatable design (CCRD) was used in our work to evaluate the interactive effects and to obtain the optimum conditions for enzyme-catalyzed alcoholysis of palm-based wax ester. The substrates and parameters studies were selected based on previous study.

2. Materials and methods

2.1. Materials

Immobilized lipase from *Mucor miehei* (Lipozyme) was produced by Novo Nordisk (Denmark). Palm oil (MW = 3 × average of saponification equivalent of palm oil) was obtained from Southern Edible Oil Sdn. Bhd. (Malaysia). Fatty acid compositions of Malaysian palm oil are 0.1–0.3% of lauric acid, 0.9–1.5% of myristic acid, 39.2–45.2% of palmitic acid, 3.7–5.1% of stearic acid, 37.5–44.1% of oleic acid and 8.7–12.5% of linoleic acid [11]. Oleyl alcohol was obtained from Fluka Chemika (Switzerland). Ester standards, oleyl laurate, oleyl myristate, oleyl palmitate, oleyl stearate, oleyl oleate, oleyl linoleate and methyl linoleate were obtained from Sigma Aldrich (USA). Hexane was obtained from J.T. Baker (USA). All other chemicals were of analytical grade.

2.2. Experimental design

A five-level-four-factor CCRD was employed in this study, requiring 30 experiments [12]. The fractional factorial design consisted of 16 factorial points, 8 axial points and 6 center points. The variable and their levels selected for the wax esters synthesis were: time (2.5–10 h); temperature (30–70 °C); amount of enzyme (0.1–0.2 g); substrate molar ratio (palm oil to oleyl alcohol, 1:1–1:5). The data obtained were fitted to a second-order polynomial equation:

$$Y = b_o + \sum_{i=1}^{4} b_i x_i + \sum_{i=1}^{4} b_{ii} x_i^2 + \sum_{i=j}^{3} \sum_{j=i+1}^{4} b_{ij} x_{ij}$$

where Y is percentage of yield; b_o, b_i, b_{ii}, b_{ij} are constant coefficients and x_i the uncoded independent variables.

2.3. Synthesis and analysis

Different molar ratios of palm oil and oleyl alcohol were added to 10 mL *n*-hexane, followed by different amounts of enzyme. The mixture of palm oil, oleyl alcohol and lipozyme IM were incubated in a horizontal water bath shaker (150 rpm) at different reaction temperature and reaction times. The reactions were analyzed by a gas chromatograph (Hitachi model G-3000, Tokyo, Japan), using an Rtx-65TG capillary column (30 m × 0.25 mm). Helium was used as the carrier gas at a flow rate 30 mL/min. The temperature was programmed at 2 min at 150 °C, 20 °C/min to 300 °C and 10 min at 300 °C. The product composition was quantitated by an internal standard method with methyl linoleate as the internal standard. The concentration of esters were calculated by equation: $C_x = (A_x/A_{IS})(C_{IS}D_{Rf\,IS}/D_{Rf\,x})$, where C is the amount of component x or internal standard, A is area for component x or internal standard and D_{Rf} is detector response factor for component x or internal standard ($D_{Rfx} = A_x/C_x$ and $D_{Rf\,IS} = A_{IS}/C_{IS}$). The percentage yield of ester was calculated by equation:

$$\text{percentage yield (\%)} = (\text{mmol ester/mmol palm oil used}) \times 100\%$$

2.4. Data analysis

The data from the experiments performed are analyzed using design expert 6.06 version and then interpreted [13]. Three main analytical steps: analysis of variance (ANOVA), a regression analysis and the plotting of response surface were performed to establish an optimum condition for the alcoholysis.

3. Results and discussion

3.1. Model fitting and ANOVA

Experimental data for lipozyme-catalyzed synthesis of wax ester from palm oil and oleyl alcohol are given in Table 1. The predicted values were obtained from model fitting technique using the software design expert version 6.06 and were seen to be sufficiently correlated to the observed values. Fitting of the data to various models (linear, two factorial, quadratic and cubic) and their subsequent ANOVA showed that reactions of palm oil and oleyl alcohol were most suitably described with quadratic polynomial model. From the design expert, the quadratic polynomial is given below:

$$\begin{aligned}\text{yield (\%)} = {} & -334 + 9.05A + 31.8B + 32.4C + 508D \\ & - 0.0858A^2 - 1.29B^2 - 2.80C^2 - 655D^2 \\ & - 0.0725AB - 0.0471AC + 0.545AD \\ & - 1.49BC - 32.9BD - 32.4CD\end{aligned}$$

where A is the temperature; B the time; C the molar ratio; D the amount of enzyme.

The computed model F-value of 232 was higher then tabular value of $F_{0.01(14,15)} = 3.56$, implying the model are significant at 1% confidence level. The model also showed