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OPTIMIZATION OF *Nigella sativa* OIL-BASED FERULATE ESTER SYNTHESIS USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Nigella sativa L. seed, also recognized as black cumin is a medicinal plant that possesses miraculous power of healing due to the abilities to cure various ailments. Owing to the oily properties of plant oil, the oil ester is more preferable exhibiting special features such as non-toxic, outstanding moisturizing action and most importantly the absence of oily texture. In this study, *Nigella sativa* oil-based ferulate ester was synthesized via transesterification of *Nigella sativa* oil (NSO) and ethyl ferulate (EF) in the presence of immobilized lipase, Lipozyme RM IM from *Rhizomucor miehei*. A five-level-four-factor central composite rotatable design (CCRD) from response surface methodology (RSM) was applied to study the influence of synthesis parameters: lipase dosage (50-80 mg), temperature (40-60°C), substrate ratio of NSO:EF (2.5:1-5.5:1 w/w) and time (3-7 hr) aiming for the maximum percentage yield of ester. The optimized synthesis conditions were lipase dosage of 67 mg, temperature of 56°C, substrate ratio (NSO:EF) of 4.4:1 w/w and time of 4 hr. The maximum production of ester obtained was 49.87% which is acceptable with the predicted value of 48.82%.

Key words: *Nigella sativa* oil, enzymatic transesterification, plant oil-based ferulate ester, optimization, response surface methodology (RSM)

INTRODUCTION

Nigella sativa L. or often called as black cumin is an annual medicinal herb owned by the *Ranunculaceae* family. It is vastly cultivated in the Middle East, Mediterranean countries and Western Asia. The hot-peppery taste of the black cumin causes its usage as a spice in bread, salads and other foods (Venkatachallam *et al.*, 2010). It is an important functional food that is applied as remedies for many diseases including asthma, bronchitis and influenza (Khattak & Simpson, 2008). Based on current studies on black cumin, it consists of several properties such as antioxidant (Thippeswamy & Naidu, 2005), antifungal (Khan *et al.*, 2003) and anticarcinogenic (Rooney & Ryan, 2005) activities.

However, black cumin oil has an oily feeling and is susceptible to oxidation. Black cumin oil is easily oxidized because it contains a high amount of unsaturated double bonds which could develop unwanted off-flavors and destruction of endogenous antioxidants leading to reduced nutritional value and the existence of harmful oxidation products (Reische *et al.*, 2008). According to Barone *et al.* (2009) oxidation of plant oil can be prevented by adding antioxidants such as ethyl ferulate. On the other hand, oil ester is broadly utilized as plasticizers, lubricants and as a natural substance in pharmaceuticals and cosmetics products due to its non-toxic, excellent moisturizing action and the most significant feature that is non-greasy (Abd Gani *et al.*, 2011).

Oil ester can be synthesized by chemical or enzymatic methods. The conventional chemical process is affiliated with many drawbacks including

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danger in managing hazardous chemicals, high-energy usage and deterioration of ester (Petersson *et al.*, 2005; Antczak *et al.*, 2009). The enzymatic process is employed to combat the disadvantages of chemical approach by utilizing enzyme as a biocatalyst in the formation of ester. This 'greener' process delivers various benefits such as the need for mild surrounding to function, low-energy consumption and very low thermal destruction of ester. Moreover, the enzyme has its own distinct selectivity and specificity which offers the alteration of a particular area on a molecule and the creation of required product only (Sharma *et al.*, 2011; Zaidan *et al.*, 2015).

A greater understanding of important parameters affecting the synthesis of oil ester ought to be researched. Response Surface Methodology (RSM) is an effective program for analyzing the impact of multiple factors affecting the responses by reducing the number of experimental run and cost (Gunawan *et al.*, 2005). Several studies had employed RSM in the lipase-catalyzed synthesis of oil esters (Rahman *et al.*, 2011; Yang *et al.*, 2012; Radzi *et al.*, 2014). To the best of our knowledge, no research has ever been conducted on the optimization of the formation of novel oil ester produced by combining NSO and EF endowed with valuable properties. In the present study, RSM was applied in the optimization of transesterification of NSO with EF catalyzed by *Rhizomucor miehei* immobilized lipase (Lipozyme RM IM). The influences of four synthesis factors (lipase dosage, temperature, substrate ratio and time) on the response yield were examined.

MATERIALS AND METHODS

Materials

NSO was obtained from Institute of Bioscience, Universiti Putra Malaysia. EF and Lipozyme RM IM were purchased from Sigma-Aldrich (St. Louis, USA). n-Hexane, ethanol, acetone and potassium hydroxide were obtained from R&M, UK. All chemicals were of analytical grade.

Enzymatic transesterification and analysis of reaction product

Transesterification procedure was altered from Compton *et al.* (2000). Different amounts of Lipozyme RM IM were placed in screw-capped vials consisting various ratio of NSO to EF in 2 mL n-hexane. The vials were put in a water-bath shaker at 150 rpm at different temperature and time. The reaction was ended with 2 mL of ethanol: acetone (1:1 v/v) and the immobilized lipase was filtered. The ester conversion (%) was determined by titration method with 0.1 N NaOH by identifying the remaining unreacted fatty acids in the operation (Equation (1)).

$$\text{Ester conversion (\%)} = \frac{\text{Volume of NaOH (control)} - \text{Volume of NaOH (sample)}}{\text{Volume of NaOH (control)}} \times 100 \quad (1)$$

Response surface design and statistical analysis

A four-factor-five-level central composite rotatable design (CCRD) was used, needing thirty experiments comprising sixteen factorial points, six axial points and six center points. The parameters and levels chosen for ester synthesis were: lipase dosage (50-80 mg), temperature (40-60°C), substrate ratio of NSO:EF (2.5:1-5.5:1 w/w) and time (3-7 hr). The value acquired was fitted to a second-order polynomial equation (Equation (2)) produced by RSM software using Design Expert version 10.0 (Stat-Ease, Minneapolis, MN, USA).

$$Y = b_0 + \Sigma_{i=1}^{4} b_i x_i + \Sigma_{i=1}^{4} b_{ii} x_i^2 + \Sigma_{i=j}^{3} \Sigma_{j=i+1}^{4} b_{ij} x_{ij} \quad (2)$$

where Y is the dependent variable (percentage ester conversion) while x_i and x_j are the independent variables. In addition, b_0, b_i, b_{ii} and b_{ij} are the constant coefficients of the model.

RESULTS AND DISCUSSION

Mathematical model and Analysis of Variance (ANOVA)

CCRD was used to evaluate the coefficient of the empirical model and statistical data in optimizing the enzymatic transesterification of *Nigella sativa* oil-based ferulate ester. Table 1 shows the observed and predicted value for ester conversion. The predicted data were attained by a model fitting approach and were adequately correlated to the experimental data. Fitting of the value to several models (linear, two factorial, quadratic and cubic) and their resultant ANOVA proved that *Nigella sativa* oil-based ferulate ester synthesis was well properly explained with a quadratic polynomial model (Equation (3)):

$$\begin{aligned} Y_{\text{Ester Conversion}}\,(\%) = {} & 46.90 + 1.10A + 2.87B + 1.91C \\ & - 3.55D + 0.42AB - 0.37AC \\ & - 0.11AD + 1.23BC + 0.56BD \\ & + 0.75CD - 3.81A^2 - 2.71B^2 \\ & - 3.70C^2 - 3.64D^2 \end{aligned} \quad (3)$$

where A is lipase dosage; B is temperature; C is substrate ratio; and D is time. The model *F*-value (104.74) with *p*-value < 0.0001 showed the model was significant. The coefficient of determination (R^2) of the model was 0.9899 that designated the

Table 1. CCRD layout and data for the model of *Nigella sativa* oil-based ferulate ester synthesis

No.	A (mg)	B (°C)	C (w/w)	D (h)	Ester Conversion (%)	
					Actual Value	Predicted Value
1	65	50	4:1	5	48.72	46.90
2	95	50	4:1	5	33.18	33.86
3	80	60	5.5:1	7	37.88	37.86
4	50	60	2.5:1	7	26.49	27.27
5	65	30	4:1	5	31.18	30.34
6	65	50	1:1	5	29.20	28.28
7	80	60	5.5:1	3	43.45	42.55
8	50	60	2.5:1	3	34.63	34.52
9	65	50	4:1	5	45.08	46.90
10	65	50	4:1	5	46.90	46.90
11	65	50	4:1	9	25.96	25.27
12	65	50	4:1	5	46.62	46.90
13	50	60	5.5:1	3	39.70	40.06
14	50	40	2.5:1	7	22.69	23.70
15	50	60	5.5:1	7	36.58	35.79
16	35	50	4:1	5	30.35	29.47
17	65	70	4:1	5	41.18	41.82
18	80	40	5.5:1	3	35.32	34.63
19	80	60	2.5:1	7	30.75	30.84
20	50	40	5.5:1	3	33.79	33.81
21	80	40	5.5:1	7	27.49	27.70
22	50	40	2.5:1	3	33.07	33.19
23	80	40	2.5:1	7	25.86	25.60
24	65	50	4:1	5	46.90	46.90
25	80	60	2.5:1	3	38.99	38.51
26	65	50	7:1	5	35.20	35.92
27	50	40	5.5:1	7	26.73	27.30
28	65	50	4:1	5	47.20	46.90
29	65	50	4:1	1	38.96	39.45
30	80	40	2.5:1	3	34.61	35.51

appropriateness of the model to address the actual interactions among the factors studied. The lack of fit (0.7000) was insignificant which proved that the model describes well the actual value in the selected intervals.

Mutual effects of process parameters

The optimum level of parameters was identified by creating response surface plots based on Equation (1). Figure 1(a) shows the relationship between lipase dosage and temperature on the ester conversion. An increased in lipase dosage at higher temperature lead to greater ester conversion. The presence of more lipases lead to their high interaction with acyl donor molecules and yield many acyl-enzyme complexes, simultaneously improving the product yield (Ashari *et al.*, 2009). Furthermore, as the temperature was raised, the percentage of *Nigella sativa* oil-based ferulate ester conversion correspondingly elevated (Krishna *et al.*, 2001; Radzi *et al.*, 2005).

Figure 1(b) illustrates the interaction between temperature and substrate ratio on the ester conversion. Basically, at a lower temperature, the ester conversion was relatively decreased due to mass transfer constraint. Poor substrate concentration causes fewer availability of substrate for reaction leading to a rather low yield although at high temperature (Bouaid *et al.*, 2007; Shekarchizadeh *et al.*, 2009). High temperature and substrate ratio are recommended because they would enhance the interaction between substrate and lipase, therefore raising the percentage of *Nigella sativa* oil-based ferulate ester conversion (Rahman *et al.*, 2008).

Figure 1(c) describes the impact of substrate ratio and time on the ester conversion while the other parameters were kept constant. It can be seen that higher ester conversion was obtained when the substrate ratio was increased while the time was decreased. It was discovered that the percentage of *Nigella sativa* oil-based ferulate ester conversion increased until the time reached 4 hr while longer than 4 hr of reaction time lead to the decrease of ester conversion. This finding was also alike to the data acquired by Radzi *et al.* (2014) who researched the optimized enzymatic synthesis of olive-based ferulate ester using RSM. The high amount of substrates have greater interaction with enzymes but the substrates move away from the active areas of the enzymes when additional incubation time was employed.

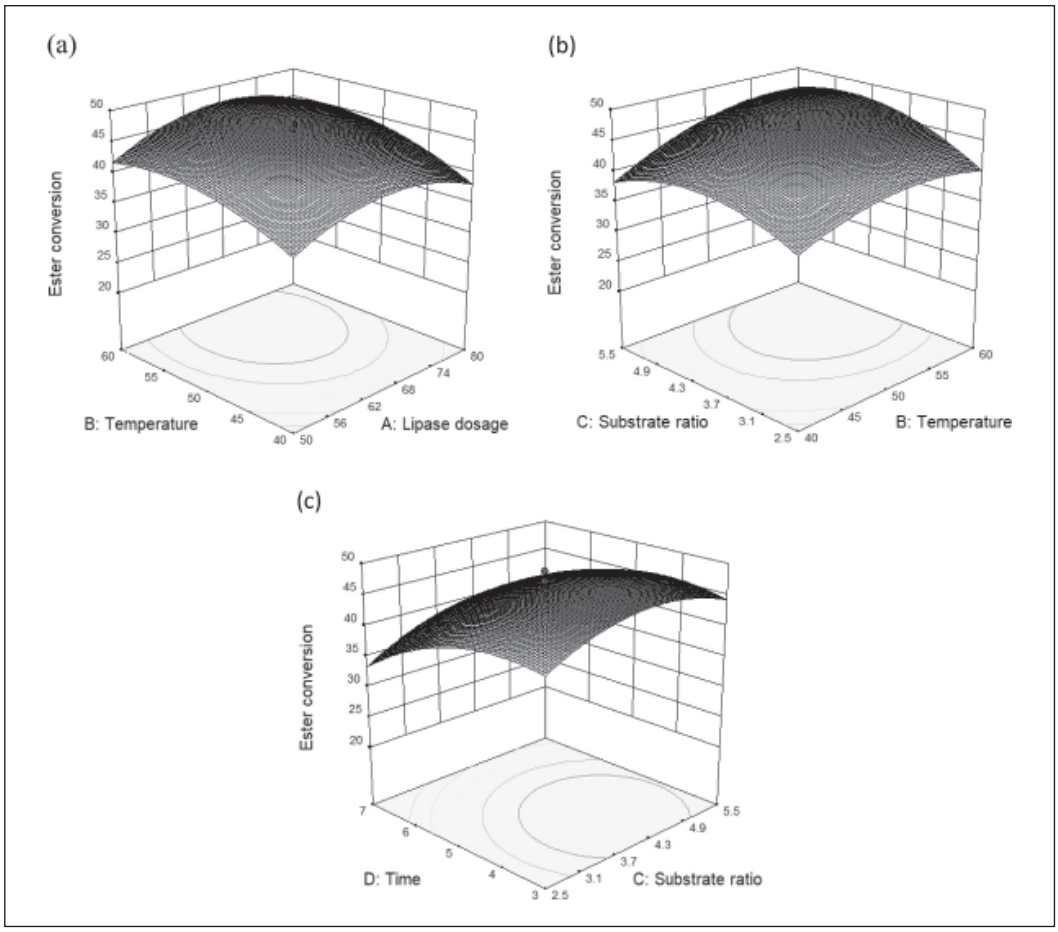

Fig. 1. Response surface plots: (a) lipase dosage versus temperature; (b) temperature versus substrate ratio; (c) substrate ratio versus time on the ester conversion as a response.

Reaction optimization and model validation

The percentage of ester conversion obtained under the optimum criteria was 49.87% which displayed great correspondence with the predicted data (48.82%), indicating that empirical model created from RSM can be utilized to sufficiently explain the correlation between the parameters and response of *Nigella sativa* oil-based ferulate ester synthesis. The optimum test parameters were: 67 mg of lipase dosage, 56°C of temperature, 4.4:1 w/w of substrate ratio (NSO:EF) and 4 hr of time.

CONCLUSION

The optimization of enzymatic transesterification process to generate *Nigella sativa* oil-based ferulate ester using RSM was successfully done. The influences of four synthesis parameters (lipase dosage, temperature, substrate ratio and time) were assessed using an empirical model. The R^2 data of 0.9899 was acceptable and proven an excellent fit with the experimental result. This fitted model could be used to obtain the response of *Nigella sativa* oil-based ferulate ester conversion under any specified conditions within the test range. Research was conducted and maximum ester conversion of 49.87% was obtained, which was very close to the predicted value (48.82%). Thus, the scaled-up formation of *Nigella sativa* oil-based ferulate ester catalyzed by lipase can be performed in the future by using RSM approach bringing into account the financial and surrounding elements.

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