Enhancing Operational Stability and Exhibition of Enzyme Activity by Removing Water in the Immobilized Lipase-Catalyzed Production of Erythorbyl Laurate

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DOI 10.1002/btpr.1745
Published online May 9, 2013 in Wiley Online Library (wileyonlinelibrary.com)

Erythorbyl laurate was continuously synthesized by esterification in a packed-bed enzyme reactor with immobilized lipase from Candida antarctica. *Response surface methodology based on a five-level three-factor central composite design was adopted to optimize conditions for the enzymatic esterification. The reaction variables, such as reaction temperature (10–70°C), substrate molar ratio ([lauric acid]/[erythorbic acid], 5–15), and residence time (8–40 min) were evaluated and their optimum conditions were found to be 56.2°C, 14.3, and 24.2 min, respectively. Under the optimum conditions, the molar conversion yield was 83.4%, which was not significantly different ($P < 0.05$) from the value predicted (84.4%). Especially, continuous water removal by adsorption on an ion-exchange resin in a packed-bed enzyme reactor improved operational stability, resulting in prolongation of half-life (2.02 times longer compared to the control without water-removal system). Furthermore, in the case of batch-type reactor, it exhibited significant increase in initial velocity of molar conversion from 1.58% to 2.04%/min.* © 2013 American Institute of Chemical Engineers *Biotechnol. Prog.*, 29:882–889, 2013
Keywords: erythorbyl laurate, water removal, immobilized lipase-catalyzed esterification, response surface methodology, operational stability

Introduction

Ascorbyl fatty esters and erythorbyl fatty esters can be used as antioxidants with potential applications in the cosmetics and food industries. They are presently produced by chemical synthesis.[1] In the last decade, their enzymatic synthesis has been extensively developed at the laboratory scale due to their high selectivity and mild reaction conditions.[2–4] The lipase-catalyzed synthesis of erythorbyl fatty ester in an organic solvent using a batch reaction has been reported.[3,5] However, a continuous reaction would be preferable for large-scale production. The packed-bed enzyme reactor (PBER) has been extensively investigated by several researchers for use in industrial-scale applications.[6,7] The PBER is most commonly used for solid–fluid operations and is suitable for long-term and industrial-scale production. It differs from agitated bioreactors, in which enzyme granules are susceptible to breakdown because of the mechanical shear stress, and it is more cost-effective than a batch operation process.[8]

The water content in the reaction system is fundamentally important for lipase-catalyzed esterification.[9] Water accumulation throughout the reaction significantly affects the reaction equilibrium.[10,11] Therefore, the water content plays an important role in conversion efficiency.[12] Several approaches have been proposed to control water accumulation, including the use of salt hydrates,[13] pervaporation through water-selective membranes,[14,15] free and vacuum evaporation,[16] distillation,[17] and air sparging.[18] Adsorption has also been considered. Adsorbents such as alumina, silica gel, and zeolites are effective for removing water from organic solvents.[19] Ion-exchange resins are also capable of adsorbing water and can be regenerated simply by using water-miscible solvents. Sulfonated polystyrene-divinylbenzene resins in particular are relatively inert and do not react with the organic solvents and substrates typically used in

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enzymatic reactions. These resins are effective in removing water from various organic solvents.[20]

This study aimed to provide a better understanding regarding the effect of reaction variables on the continuous production of erythorbyl laurate catalyzed by immobilized lipase in acetonitrile using response surface methodology. The effect of adding an ion-exchange resin as a water absorbent to improve the operational stability and exhibition of enzyme activity was also studied in both continuous (PBER) and batch reactor, respectively.

Materials and Methods

Materials

Novozym 435 (the lipase from *Candida antarctica* immobilized onto macroporous acrylic resin, approximate density 0.40 g/mL) with a reported catalytic activity of 10,000 propyl laurate unit (PLU)/g was obtained from Novozymes (Bagsværd, Denmark). According to the manufacturer's specification, one unit (PLU) was defined as the amount of enzyme that synthesizes 1 μmol of propyl laurate per minute at 60°C. Erythorbic acid (≥99%) was purchased from Fluka Co. (Buchs, Switzerland), and lauric acid (≥99%) and Dowex-HCR W2 hydrogen form were obtained from Sigma-Aldrich Co. (St. Louis, MO). High performance liquid chromatography (HPLC)-grade acetonitrile (J.T. Backer Co., Phillipsburg, NJ) was dehydrated using a 4 Å molecular sieve (8–12 mesh, Sigma-Aldrich Co.) prior to use as a reaction medium. All other chemicals were analytical reagent grade.

Lipase-catalyzed esterification on a packed-bed enzyme reactor

A substrate solution that dissolved erythorbic acid and lauric acid in acetonitrile was subjected to enzymatic esterification in a PBER. The PBER was based on an 18.0-cm-long and 2.0-cm-internal diameter glass column with an inlet for substrate solution and an outlet for product solution containing erythorbyl laurate. The PBER was filled with 50,000 PLU of Novozym 435, and the reactor was controlled at a desired reaction temperature by water circulation through the water jacket surrounding the glass column. Experiments on lipase-catalyzed esterification were performed with erythorbic acid and lauric acid in various proportions. The product solution was continuously pumped out from the outlet of the reactor into the storage vial at various flow rates (0.44–2.21 mL/min). The flow rate of the substrate solution was regulated with a peristaltic pump (SMP-21; EYELA, Tokyo, Japan) considering the residence time between the substrate solution and immobilized enzyme in the reactor. During a continuous run, periodic samples were taken from the outlet of the reactor for further analysis. A schematic representation of the PBER system is shown in Figure 1.

Determination of the bed void fraction and residence time

An enzyme bed has a void fraction and a residence time.[21] To determine the void fraction, the volume of the substrates (V_s) was determined using a measuring cylinder in which immobilized enzyme was contained. After Novozym 435 was poured into the measuring cylinder, the substrate solution was filled to the total volume of the sum of the substrate solution and immobilized enzyme. The volume of the measuring cylinder (V_c), as a substitute enzyme bed, was calculated from the diameter of the column and length of the measuring cylinder. The void fraction (ε) was then calculated as $\varepsilon = V_s/V_c$ and its value was determined to be 0.75. The density of Novozym 435 was almost unaffected by changes in temperature. Thus, the calculated ε at room temperature could be used for other temperatures without introducing large errors.

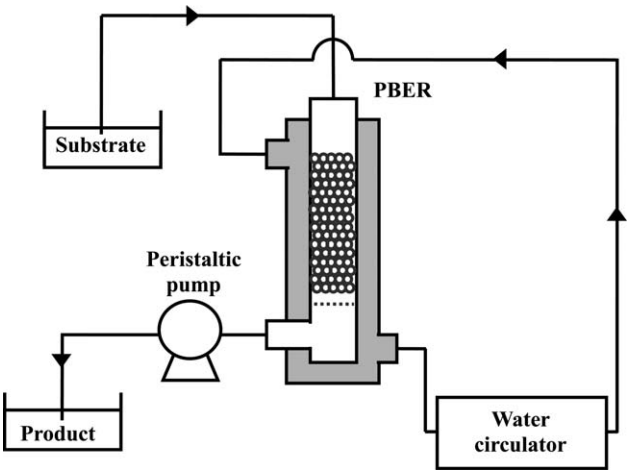

Figure 1. The PBER system for the production of erythorbyl laurate.

The residence time was calculated as $V \times \varepsilon/V_f$, where V is the enzyme bed volume, ε is the void fraction, and V_f is the flow rate. The volume of the enzyme bed was calculated to be 23.55 mL based on the diameter of the column and length of the enzyme bed.

Quantitative analysis of esterification product

Esterification products were periodically analyzed using an HPLC instrument (LC-2002; JASCO, Inc., Tokyo, Japan) equipped with a Spherisorb-ODS column (5 μm, 100 Å, I.D. 4.6 mm × 250 mm; Waters, Milford, MA), a refractive index (RI) detector (RI-2031; JASCO, Inc.), and a UV detector (UV-2075; JASCO, Inc.). The mobile phase was acetonitrile/water/acetic acid (90:5:5, v/v/v) at a 1.0 mL/min flow rate. Samples collected at various time intervals were filtered through a 0.45-μm membrane filter, and 20 μL of each was analyzed. Lauric acid was detected using an RI detector, and erythorbic acid and erythorbyl laurate were detected using a UV detector at 265 nm. The retention times were 2.51 ± 0.01, 3.39 ± 0.03, and 4.63 ± 0.03 min for the erythorbic acid, erythorbyl laurate, and lauric acid, respectively. The molar conversion yield (%) was calculated using peak area, integrated by the online package Borwin (Ver. 1.21; JASCO, Inc.) as defined by the following equation:

$$\text{Molar conversion}\,(\%) = \frac{C_{\text{erythorbyl laurate}}}{C_{\text{erythorbic acid}} + C_{\text{erythorbyl laurate}}} \times 100$$

where $C_{\text{erythorbyl laurate}}$ is the molar concentration of erythorbyl laurate and $C_{\text{erythorbic acid}}$ is the molar concentration of erythorbic acid.

Product identification

The isolated erythorbyl laurate was identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The MALDI-TOF MS measurements were performed using an Auto Flex II from Bruker

Table 1. Experimental Results Based on Central Composite Design

Run No.	Temperature, X_1 (°C)	Substrate Ratio, X_2	Residence Time, X_3 (min)	Molar Conversion Yield (%)	
				Observed	Predicted
1	25 (−1)	7.5 (−1)	16 (−1)	61.0 ± 0.1	59.8
2	55 (1)	7.5 (−1)	16 (−1)	68.6 ± 2.4	68.0
3	25 (−1)	12.5 (1)	16 (−1)	70.8 ± 2.2	69.5
4	55 (1)	12.5 (1)	16 (−1)	79.5 ± 2.0	79.1
5	25 (−1)	7.5 (−1)	32 (1)	65.9 ± 2.7	64.5
6	55 (1)	7.5 (−1)	32 (1)	73.6 ± 1.2	73.0
7	25 (−1)	12.5 (1)	32 (1)	71.8 ± 2.9	70.5
8	55 (1)	12.5 (1)	32 (1)	81.1 ± 0.3	80.4
9	10 (−2)	10.0 (0)	24 (0)	54.6 ± 2.3	56.4
10	70 (2)	10.0 (0)	24 (0)	74.3 ± 2.9	74.5
11	40 (0)	5.0 (−2)	24 (0)	63.3 ± 1.8	64.3
12	40 (0)	15.0 (2)	24 (0)	80.5 ± 4.9	81.5
13	40 (0)	10.0 (0)	8 (−2)	65.4 ± 5.0	66.2
14	40 (0)	10.0 (0)	40 (2)	71.1 ± 2.4	72.2
15	40 (0)	10.0 (0)	24 (0)	75.0 ± 0.1	74.8
16	40 (0)	10.0 (0)	24 (0)	75.4 ± 0.8	74.8
17	40 (0)	10.0 (0)	24 (0)	74.5 ± 0.4	74.8
18	40 (0)	10.0 (0)	24 (0)	75.1 ± 0.3	74.8
19	40 (0)	10.0 (0)	24 (0)	73.5 ± 4.1	74.8
20	40 (0)	10.0 (0)	24 (0)	73.5 ± 0.8	74.8

Daltonics (Bremen, Germany) in reflectron mode and negative polarity. Desorption/ionization was accomplished using a 337-nm N_2 laser. The matrix used was a solution containing 2 mg CHCA (α-cyano-4-hydroxy-cinnamic acid) and 0.1% TFA (trifluoroacetic acid) in 1 mL of acetonitrile/deionized water (7:3, v/v).

Experimental design and statistical analysis

A central composite experimental design with three independent variables and five levels was used to optimize the continuous production of erythorbyl laurate (Table 1). The 20 experiments consisted of 2^3 factorial points, six axial points, and a center point that was replicated six times. To avoid bias, the 20 experiments were performed in a totally random order (Table 1). Temperature (X_1), substrate molar ratio ([lauric acid]/[erythorbic acid], X_2), and residence time (X_3) were selected as the independent variables in this design. X_1 (10–70°C), X_2 (5–15), and X_3 (8–40 min) were used as the critical ranges for significant effects on the production of erythorbyl laurate. The quadratic polynomial regression model was assumed for predicting the response (Y, molar conversion yield) using the following equation:

$$Y=\beta_0+\sum_{i=1}^{3}\beta_i X_i+\sum_{i=1}^{3}\beta_{ii}X_i^2+\sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij}X_iX_j$$

where Y is response (molar conversion yield, %) and β_0, β_i, β_{ii}, and β_{ij} are regression coefficients for the intercept, linear, quadratic, and interaction terms, respectively. X_i and X_j are independent variables. The experimental data were analyzed using the statistical software package Design-Expert 8 (Stat-Ease, Minneapolis, MN) for regression analysis and to evaluate the statistical significance of the equation. The quality of fit of the polynomial model equation was expressed by the coefficient of determination (R^2).

Improving the operational stability and exhibition of enzyme activity using ion-exchange resin

The resin Dowex HCR-W2 was used as a water adsorbent. This is a sulfonated, gel-type polystyrene-divinylbenzene resin with a nominal 8% degree of crosslinking. Prior to use, 30 g of the resin was converted to the potassium form with 100 mL of 1 N KOH solution, rinsed with deionized distilled water, and dried in an oven at 120°C.[11] In a continuous reactor, 5 g (50,000 PLU) of immobilized lipase was well mixed with 5 g of ion-exchange resin and the mixture was then packed by pouring into the glass column. The weight ratio of 1:1 immobilized lipase/ion-exchange resin (Dowex HCR-W2 of potassium form) was selected (among five kinds of resins: molecular sieve 3Å, methyl tertiary-butyl ether form of Dowex M-31, phenol alkylated form of Dowex DR-2030, sodium form of Dowex HCR-W2, and potassium form of Dowex HCR-W2) based on a preliminary experiment (data not shown). An experiment was carried out under the optimum conditions determined in this study. To investigate the water removal effect of the ion-exchange resin on the operational stability and expression ability of enzyme activity, the half-life based on molar conversion in the continuous reactor (PBER) and initial velocity of molar conversion in the batch-type reactor. The batch-type reaction has been accomplished under the optimum conditions previously determined from response surface methodology (RSM) as the values of 2,994 PLU (enzyme content), 24.23 (molar ratio of lauric acid to erythorbic acid), and 53.03°C (reaction temperature). More precise conditions are described in the previous report.[5]

Storage stability of erythorbyl laurate

An appropriate amount of erythorbyl laurate was weighed into micro test tubes. The micro test tubes were stored in a refrigerator, incubator, or dry oven controlled at a specified temperature of −20, 4, 30, 50, 70, and 90°C in the dark for 30 days. At appropriate intervals, 2 mL of acetonitrile was added to the micro test tube to dissolve erythorbyl laurate, and 20 μL of each aliquot was analyzed to determine the remaining erythorbyl laurate using HPLC. The storage stability (%) was defined by the following equation:

$$\text{Storage stability}\,(\%)=\frac{C_s}{C_i}\times 100$$

where C_s is the molar concentration of erythorbyl laurate after the storage and C_i is the initial molar concentration of erythorbyl laurate.

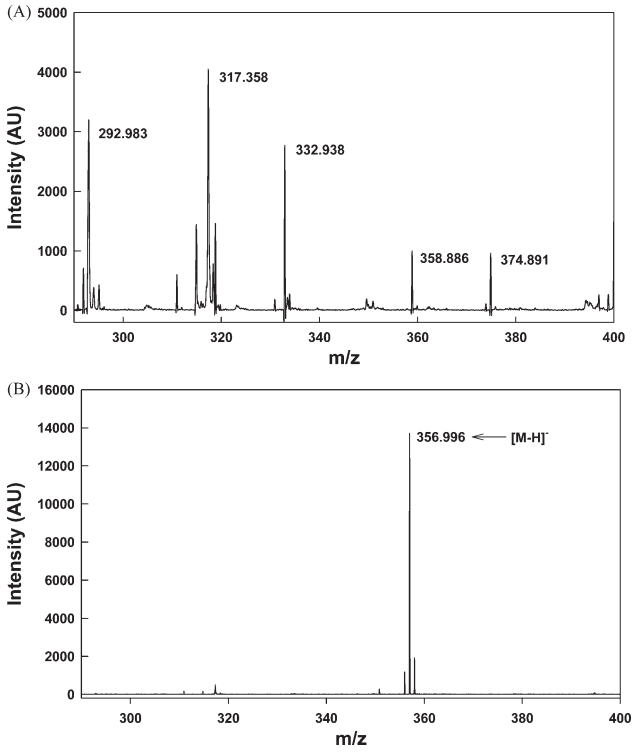

Figure 2. MALDI spectra of CHCA (A) and erythorbyl laurate with CHCA (B) over mass range *m*/*z* 300–400 in negative ion.

Results and Discussion

Identification of the reaction product

The isolated erythorbyl laurate was analyzed by MALDI-TOF MS with CHCA as the matrix in the negative ion mode. The resulting MALDI spectra for erythorbyl laurate and a matrix blank are shown in Figure 2. Ions originating from CHCA and/or the solvent are clearly visible in Figure 2A. Under the same instrument setting and laser intensity, the signals from CHCA are clearly repressed, but not eliminated, in the presence of erythorbyl laurate (Figure. 2B). The major molecular ion was $[M–H]^-$ at *m*/*z* 356.996, corresponding to the predicted molecular mass (358.1994) of deprotonated erythorbyl laurate. Our finding agrees well with previous studies regarding the structural analysis of erythorbyl laurate, identified by liquid chromatography-electrospray ionization-mass spectrometry and ^1H and ^{13}C nuclear magnetic resonance.[3,5]

Analysis of the central composite experimental design for the continuous production of erythorbyl laurate

To maximize erythorbyl laurate production, the enzyme reaction conditions including temperature, substrate molar ratio, and residence time were optimized independently. The central composite design is useful for determining the optimal values of independent variables and of any resulting interaction effects. The regression equation obtained after analysis of variance (ANOVA) indicated the molar conversion yield as a function of the levels of the three independent variables. The observed and predicted values of molar conversion yield values of the 20 experiments carried out are shown in Table 1. These were fitted into a quadratic polynomial equation, as follows:

$$Y = -2.51489 + 1.02661X_1 + 3.97998X_2 + 1.66831X_3 - 0.104160X_1^2 - 0.763756X_2^2 - 0.0218346X_3^2 + 0.00914005X_1X_2 + 0.000715675X_1X_3 - 0.0460557X_2X_3,$$

where Y is the response variable (molar conversion yield, %) and X_1, X_2, and X_3 are temperature, substrate molar ratio, and residence time, respectively.

The statistical significance of the equation obtained was determined by ANOVA (Table 2). The effects of the independent variables (linear, quadratic, or interaction) on the response were tested for adequacy. The R^2 value, the coefficient of multiple determination of the polynomial model, was 0.9768, indicating that the fitted model could explain 97.68% of the variability in the response. A precision ratio of 25.334 indicated an adequate signal, given that a ratio greater than 4 is desirable. A relatively low coefficient of variation (CV = 1.96%) indicated good precision and reliability. From Table 2, the P value (<0.0001) implies that the quadratic model was highly significant ($P < 0.01$), although the lack-of-fit P value of 0.0583 was not significant. Temperature demonstrated very significant linear and quadratic effects ($P < 0.01$). The substrate molar ratio had a significant linear effect ($P < 0.01$) but had no significant quadratic effect ($P > 0.05$). For residence time, significant linear and quadratic effects were observed ($P < 0.01$). However, the interaction effects among the independent variables did not show a significant linear effect ($P > 0.05$).

Effects of independent variables on the continuous production of erythorbyl laurate

The response surface and contour plots represented in Figure 3 show the main, interaction, and quadratic effects of two independent variables on the molar conversion yield. Figure 3A shows the effect of varying temperature (10–70°C) and substrate molar ratio (5–15) on the synthesis of erythorbyl laurate for a residence time of 24 min. The increase in both reaction temperature and substrate molar ratio elevated the molar conversion yield. However, increasing the reaction temperature and substrate molar ratio above their optimum values (~50°C and 12.5, respectively) did not further increase the molar conversion yield. Even though there is well accumulated information about the kinetics of lipase-catalyzed hydrolysis, there is little information on the

Table 2. ANOVA of the Response Surface Quadratic Model

Source	Sum of Squares	Degree of Freedom	Mean Square	*F*-value	*P*-value
Model	825.70	9	91.74	46.79	<0.0001
X_1	328.12	1	328.12	167.33	<0.0001
X_2	293.35	1	293.55	149.59	<0.0001
X_3	36.32	1	36.32	18.52	0.0016
X_1^2	0.94	1	0.94	0.48	0.5045
X_2^2	0.059	1	0.059	0.030	0.8657
X_3^2	6.79	1	6.79	3.46	0.0924
X_1X_2	138.10	1	138.10	70.42	<0.0001
X_1X_3	5.73	1	5.73	2.92	0.1182
X_2X_3	49.10	1	49.10	25.04	0.0005
Residual	19.61	10	1.96		
Lack of fit	16.14	5	3.23	4.66	0.0583
Pure error	3.47	5	0.69		
Corrected total	845.31	19			

$R^2 = 0.9768$; adj $R^2 = 0.9559$; CV = 1.96%; adequate precision ratio = 25.334. For the identification of X_1, X_2, and X_3, refer to Table 1.

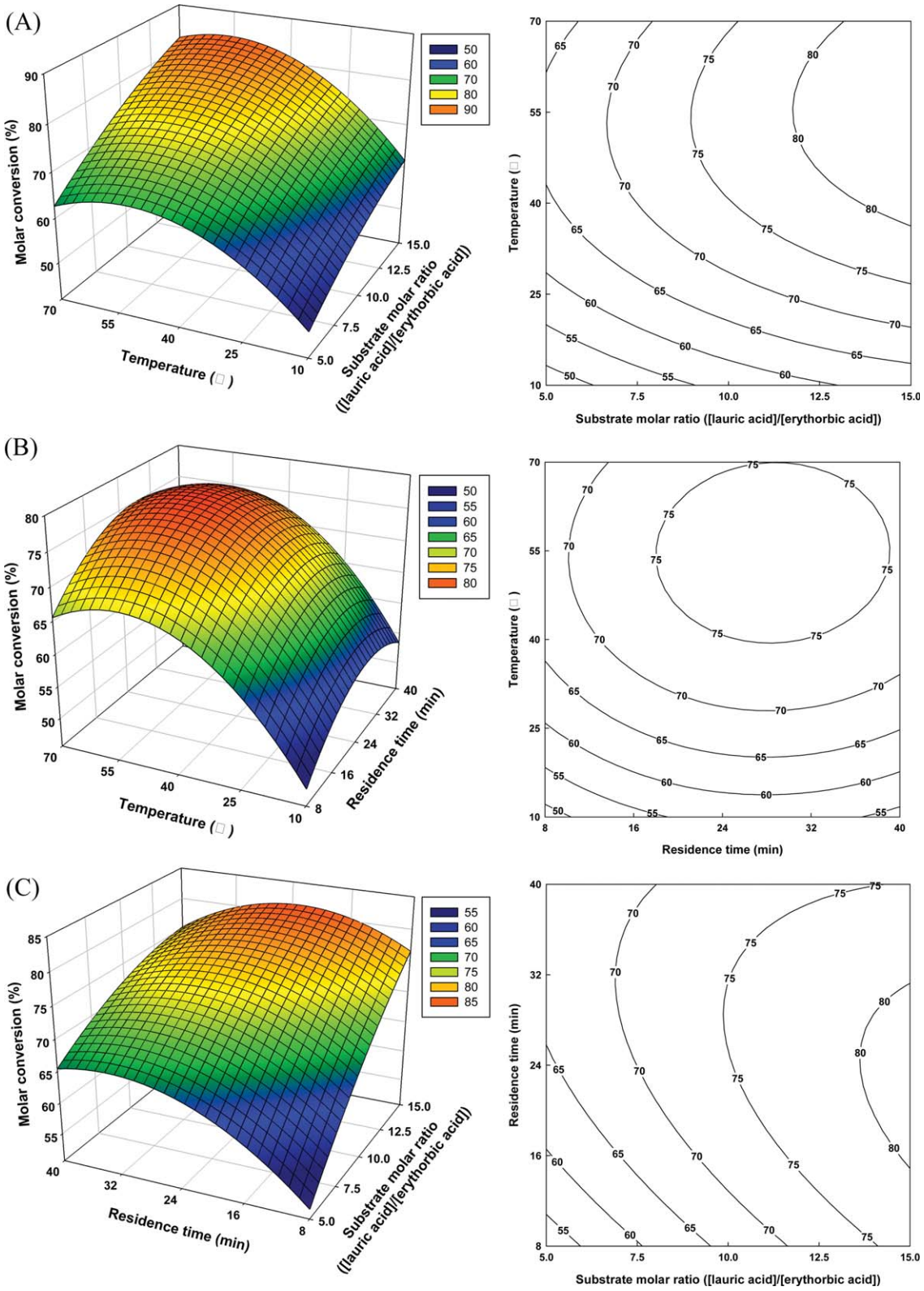

Figure 3. Response surface and contour plots showing the effects of variables on the molar conversion yield. (A): Effect of temperature and substrate molar ratio on the molar conversion yield at 24 min of residence time. (B): Effect of temperature and residence time on the molar conversion yield at 10 of substrate molar ratio. (C): Effect of residence time and substrate molar ratio on the molar conversion yield at 40°C of temperature.

kinetics of esterification, especially the effect of initial substrate ratio on the reaction rate. Previous studies have proposed that kinetic model for lipase-catalyzed esterification could follow Ping-Pong mechanism,[22,23] which occurs via the initial complex formation between an acyl group and a lipase's active site.[24,25] Phenomenon about the increased conversion yield at high substrate molar ratio is not well explained. However, there are possible explanations as follows: (a) because of very high content of lauric acid over erythorbic acid, acyl-enzyme complex could be easily

formed at high substrate molar ratio, (b) very high initial contents of both substrates could lead lipase to escape product inhibition. Figure 3B shows the effect of varying temperature (10–70°C) and residence time (8–40 min) on the synthesis of erythorbyl laurate at substrate molar ratio of 10.0. For a fixed residence time, the molar conversion yield increased rapidly when temperature reached a certain value (~50°C), and then leveled off. For a fixed temperature, the molar conversion yield varied only slightly when the residence time was increased, especially when temperature exceeded 50°C. According to the manufacturer's specification about Novozyme 435, the ideal range of temperature for esterification is 40–60°C. Therefore, in Figures 3A,B, the immobilized lipase did not esterify at relatively high (above 70°C) and low (below 30°C) temperature and had optimum temperature for esterification around 50°C. Figure 3C shows the effect of substrate molar ratio on the molar conversion yield at varying residence times, when the temperature was fixed at 40°C. The molar conversion was enhanced essentially by increasing the substrate molar ratio. At a fixed substrate molar ratio, the molar conversion yield did not significantly vary with changes in residence time. Figure 3C shows that a high molar conversion yield (>70%) could be obtained using a substrate molar ratio (>10.0) at all residence times tested. This observation suggests that all independent variables were the important controlling factors on the molar conversion yield but residence time did not affect erythorbyl laurate synthesis as much as reaction temperature and substrate molar ratio.

Optimization of the continuous production of erythorbyl laurate and verification of the model

The conditions that resulted in the highest molar conversion yield were as follows: temperature of 56.6°C, substrate molar ratio of 15, and a reaction residence time of 23.3 min. Under these conditions, the molar conversion yield was $80.8 \pm 3.9\%$, which was not significantly different ($P < 0.05$) from the value predicted (84.4%) by the quadratic model. This good agreement between the observed and predicted values verified the validity of the model designed in this study.

Improvement of operational stability and exhibition of enzyme activity using an ion-exchange resin

As described in Introduction section, the esterification activity of lipase in an organic medium is strongly influenced by the content of water absorbed on lipase.[11,26–28]. If a large amount of water is bound to lipase or accumulates around lipase, the equilibrium of the reaction can shift from an esterification reaction toward a hydrolysis reaction.[29,30] Therefore, to maintain the reaction equilibrium for an esterification reaction, the amount of water in the reaction system should be limited to maintain the active conformation of lipase. The operational stability of esterification during continuous production could be achieved by maintaining the amount of water in the PBER below a certain level. However, water is continuously produced by esterification in an organic medium and then accumulates in this reaction system. To improve the operational stability of esterification during continuous production, an ion-exchange resin in potassium form was used. As shown in Figure 4A, the presence of an ion-exchange resin produced a positive effect on

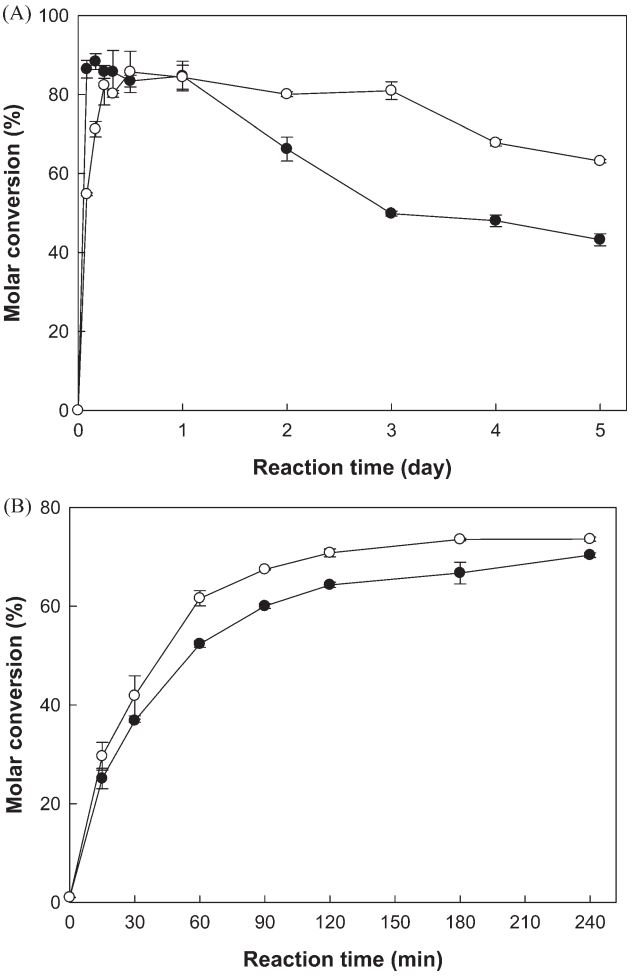

Figure 4. Effect of ion-exchange resin in potassium form on the synthesis of erythorbyl laurate in continuous (A) and batch type reactor (B). (●), Without ion exchange resin; (○), with ion exchange resin.

esterification. At the early stage of esterification (up to 12 h), no significant difference was observed in the molar conversion between the control and the system containing an ion-exchange resin. However, after 1 day of operation, the molar conversion of the PBER system containing the ion-exchange resin was much higher than that of the control. The half-life of the immobilized enzyme with an ion-exchange resin for water removal was approximately 11.3 days as estimated by extrapolating the time course of the molecular conversion, whereas the half-life of the control was only approximately 5.6 days. The prolonged high operational stability (~2 times) during the continuous esterification with water removal system might be attributed to the preventive effect from product (water) inhibition and this result was similar to previous studies whereby ion-exchange resins have been used as absorbents for the removal of water in organic mediums.[11,26,31,32] The ionogenic groups on an ion-exchange resin allow it to selectively adsorb water molecules from its environment. This continuous removal of water molecules produced during esterification should allow the enzyme activity to remain high for long durations.[33,34] In our previous report, the synthesis of erythorbyl laurate was undertaken in a batch-type process.[5] The molar conversion yield at 240 min and the initial velocity of molar conversion also increased from 68.39% to 73.64% (~1.08 times) and from 1.58% to 2.04%/min (~1.29 times), respectively,

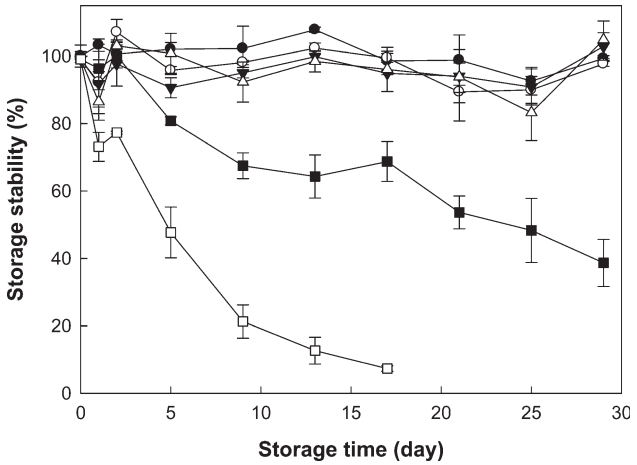

Figure 5. Effect of storage temperature on the storage stability of erythorbyl laurate at −20 (●), 4 (○), 30 (▼), 50 (△), 70 (■), and 90°C (□), respectively.

following the incorporation of an ion-exchange resin in the batch-type reactor (Figure 4B). These results indicate that the addition of ion-exchange resin improves not only the operational stability in a continuous-type reaction but also the production yield in a batch-type reaction.

Storage stability of erythorbyl laurate

The thermal stability of the newly synthesized erythorbyl laurate in this study is important for its wide application into various foods because many sectors of the food industry require heat treatment for food production and safety. Therefore, the effect of storage temperature on the thermal stability of erythorbyl laurate was examined by measuring its residual content during storage at various temperatures. As shown in Figure 5, erythorbyl laurate stored below 50°C was unlikely to be degraded over a 30-day storage period. However, when erythorbyl laurate was stored over 70°C, the storage stability dramatically decreased. Two possible explanations exist for this observation. One is degradation of the erythorbyl moiety of the erythorbyl laurate, and the other possibility is hydrolysis of the ester bond in the erythorbyl laurate.[35] Therefore, to investigate the instability of erythorbyl laurate at a high temperature, the degradation of the compound was monitored during storage at 70°C and 90°C using HPLC. A peak of released erythorbic acid or lauric acid from erythorbyl laurate was rarely detected during the storage (data not shown), indicating the low storage stability of erythorbyl laurate at such high temperatures, which was likely due to thermal degradation, rather than hydrolysis of the ester bond.

Conclusions

The continuous production of erythorbyl laurate using immobilized lipase in acetonitrile was studied using a PBER system, and the optimum conditions for erythorbyl laurate production were determined using response surface methodology. The maximal molar conversion yield of 83.4% was achieved using a reaction temperature of 56.6°C, a substrate molar ratio of 15, and a residence time of 23.3 min. An ion-exchange resin in the form of potassium was useful for improving the operational stability (∼2 times of half-life compared to without water removal system) of esterification during continuous reaction by removing the water formed as a by-product. In addition, by the incorporation of an ion-exchange resin in the batch-type reactor, it could be found out the molar conversion yield at 240 min and the initial velocity of molar conversion also increased from 68.39% to 73.64% (∼1.08 times) and from 1.58% to 2.04%/min (∼1.29 times), respectively. Erythorbyl laurate powder was stable at room temperature but was thermally degraded at high temperatures (>70°C). From these results, we could obtain the useful information to develop an economical method to produce erythorbyl laurate as a new kind of emulsifier with antioxidant properties in the fields of food and cosmetic industries.

Acknowledgments

This research was financially supported in part by a grant (10162KFDA995) from Korea Food & Drug Administration in 2012 and by R&D Convergence Center Support Program, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

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Manuscript received Dec. 14, 2012, and revision received Feb. 18, 2013.