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Lipase-Catalyzed Esterification of Ferulic Acid with Oleyl Alcohol in Ionic Liquid/Isooctane Binary Systems

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ABSTRACT: Lipase-catalyzed synthesis of ferulic acid oleyl alcohol ester in an ionic liquid (IL)/isooctane system was investigated. Considerable bioconversion and volumetric productivity were achieved in inexpensive 1-hexyl-3-methylimidazolium hexafluorophosphate ([Hmim][PF_6]) and 1-methyl-3-octylimidazolium hexafluorophosphate ([Omim][PF_6]) mediated systems, and thus, the two types of ILs were selected for further optimization of variables. The results showed that, before reaching a maximum, the increase of ferulic acid concentration, temperature, or enzyme dosage led to an increase in volumetric productivity. Variations of the ratios of IL/isooctane and concentrations of oleyl alcohol also profoundly affected the volumetric productivity. To a higher extent, [Hmim][PF_6]/isooctane and [Omim][PF_6]/isooctane show similar reaction behaviors. Under the optimized reaction conditions (60 °C, 150 mg of Novozym 435 and 100 mg of molecular sieves), up to 48.50 mg/mL productivity of oleyl feruleate could be achieved for the [Hmim][PF_6]/isooctane (0.5 mL/1.5 mL) system with a substrate concentration of ferulic acid of 0.08 mmol/mL and oleyl alcohol of 0.32 mmol; while an optimum volumetric productivity of 26.92 mg/mL was obtained for the [Omim][PF_6]/isooctane (0.5 mL/1.5 mL) system under a similar reaction condition other than the substrate concentrations of ferulic acid at 0.05 mmol/mL and oleyl alcohol at 0.20 mmol.

KEYWORDS: Ionic liquids, ferulic acid, lipase, volumetric productivity

INTRODUCTION

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) is a phenolic acid of low toxicity, and it can be absorbed and easily metabolized in the human body. It is widely used in the food and cosmetic industries as an antioxidant.[1] It has been reported to have many physiological functions, including antioxidative, antimicrobial, anti-inflammatory, and antithrombotic functions, display anticancer activities, protect against coronary disease, lower cholesterol, and increase sperm viability.[2] However, the solubility of ferulic acid in either a hydrophobic or hydrophilic solvent is low and thus limits its application. Incorporation of a hydrophobic moiety into ferulic acid such as aliphatic molecules represents a possible route to improve the solubility of ferulic acid in oil-based formulas and emulsions. In recent years, a number of studies reported the enzymatic synthesis of hydrophobic derivatives of ferulic acid to increase its oil-solubility.[3−5] However, most work focused on the enzymatic modification of ferulic acids with short- or medium-chain fatty alcohols in conventional solvents or solvent-free systems.[6−10] Only a few involved the use of ionic liquids as a medium for the enzymatic production of feruloylated acyl glycerols[11] or cinnamic acid derivatives,[10] which is different from the focus of this work.

Ionic liquids (ILs) are composed entirely of organic cations and organic or inorganic anions, displaying physicochemical properties, such as low melting point, negligible vapor pressure, low flammability, tunable polarity, and miscibility with other organic or inorganic compounds.[12] The advantages of ILs include adjustable solubility properties, protective effects or increased stability for enzymes, positive effects on the specificity of enzymes or on the shift of reaction equilibrium, as well as recoverability and recyclability.[13] All of these unique characteristics make ILs promising for biocatalytic as well as chemical processes. The application of ionic liquids in biotechnology has been well documented in recent reviews.[14−16] It has also been expanded for use in a biphasic system to enhance the 2-phenylethanol concentration by means of in situ product removal.[17] In our previous study, we demonstrated an efficient IL reaction system for the production of fatty acid ascorbyl esters with very high volumetric productivity (120−200 g L^{-1}).[18] The aim of this work was to investigate the potential of enzymatic esterification of ferulic acid with oleyl alcohol in ionic liquid−organic solvent binary systems for the highest volumetric productivity. The hypothesis is that the ionic liquids may help to dissolve ferulic acid at a higher concentration and that the hydrophobic organic solvent may create a favorable microenvironment for the enzyme to maximize the bioconversion, thus synergistically yield improved volumetric productivity. The promising systems were first determined by screening organic solvents and ionic liquids. The selected systems were optimized with respect to the ratios between the organic solvent and ILs, enzyme concentration, temperature, substrate molar ratio, and concentration.

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Table 1. Solvent Dependency of Lipase-Catalyzed Esterification of Ferulic Acid with Oleyl Alcohol[a]

solvent	*tert*-butanol	isooctane	toluene	2-butanone	cyclohexane	hexane
conversion of ferulic acid (mol %)	3.81 ± 0.43	97.68 ± 1.31	25.83 ± 0.65	0.00 ± 0.00	74.02 ± 0.84	99.17 ± 1.17

[a] Reaction conditions: 7.7 mg of ferulic acid and 86 mg of oleyl alcohol were dissolved in 10 mL of solvent. The reaction was conducted for 4 days at 60 °C and agitation at 200 rpm with an enzyme load of 60 mg of Novozym 435. The tabulated data are the means ± standard deviations of three replicates.

MATERIALS AND METHODS

Materials. Novozym 435 (from *Candida antarctica*) was a gift from Novozymes A/S (Bagsvaerd, Denmark). Oleyl alcohol was purchased from Sigma-Aldrich Co. (St. Louis, USA). Methanol, in HPLC grade, was obtained from Sinopham Chemical Reagent Co., Ltd. (Shanghai, China). Ferulic acid and molecular sieves (4 Å) were purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Ionic liquids including [Bmim][TF_2N] (1-butyl-3-methylimidazolium bis (trifluoromethylsulfonyl) mide), [Hmim][PF_6](1-hexyl-3-methylimidazolium hexafluorophosphate), [Omim][PF_6] (1-methyl-3-octylimidazolium hexafluorophosphate), [Bmim][PF_6] (1-butyl-3-methylimidazolium hexafluorophosphate), and [Bmim][BF_4] (1-butyl-3-methylimidazolium tetrafluoroborate) were procured from Shanghai Chengjie Chemical Co. Ltd. (Shanghai, China). All other regents were of analytical grade.

Enzymatic Esterification and Parameter Study. Lipase-catalyzed esterification of ferulic acid with oleyl alcohol in organic solvents or ionic liquids/organic solvent mixtures was carried out in Erlenmeyer flasks. In the organic solvent reaction system, 7.7 mg of ferulic acid and 86 mg of oleyl alcohol were dissolved in 10 mL of different organic solvents. The reaction was conducted for 4 days at 60 °C with agitation in an orbital air shaking bath (200 rpm) with 60 mg of Novozym 435 and 60 mg of molecular sieve. In the ionic liquids/organic solvent system, ferulic acid (11.6 mg) and oleyl alcohol (64.4 mg) were mixed with 2 mL of solvents (0.5 mL of ILs and 1.5 mL of isooctane). The reaction was incubated in an orbital air shaking bath (200 rpm) at 60 °C for 12 h until substrates were solubilized in the media. Novozym 435 (100 mg) and the molecular sieve (100 mg) were added to the mixture. Samples from the reaction mixture were taken at intervals of 2 days. After centrifugation, the supernatants were analyzed by high performance liquid chromatography.

Examinations on the effects of temperatures (50, 55, and 60 °C), substrate concentrations of ferulic acid (0.01−0.10 mmol/mL), substrate molar ratios of ferulic acid to oleyl alcohol (1:1, 1:4, 1:8, and 1:12), enzyme concentrations (10−100 mg/mL), and volume ratios of isooctane to ILs (3:1, 1:1, and 1:3) on the esterification volumetric productivity were performed.

Kinetic Study. The thermodynamic properties were acquired by investigating the plot of reaction rate against temperature and fitting the data to the Arrhenius equation, in which a 5-fold molar excess of oleyl alcohol to ferulic acid is applied to [Hmim][PF_6]/isooctane (1:3, v/v) and [Omim][PF_6]/isooctane (1:3, v/v) systems.

Esterification of ferulic acid with oleyl alcohol is a two-substrate reaction, and the reaction rate is largely dependent on the concentration of ferulic acid. Therefore, to simplify the kinetic model, an excess of oleyl alcohol was used for both systems, and the change of its concentration during the measurement of the reaction rate was neglected. Conversion of ferulic acid was used to determine the maximum reaction rate and Michaelis−Menten constants. All of the reactions were done in triplicate.

HPLC Analysis. Analysis of the mixture was performed by high performance liquid chromatography (Hewlett-Packard 1100 series, US) with a Hypersil ODS-C18 reversed-phase column (25 cm ×4.6 mm, 5 μm, Agilent) and a UV detector at 325 nm. A sample (10 μL) taken out from the reaction system was centrifuged, diluted with 990 μL of methanol to an appropriate concentration, and injected at a volume of 20 μL. Elution was conducted with methanol/water (95:5, vol/vol) at a flow rate of 1 mL/min and a column temperature at 35 °C.

The volumetric productivity of oleyl ferulate (mg/mL) is defined as the amount of the product generated per unit volume. This was estimated on the basis of the concentration of ferulic acid and its conversion.

RESULTS AND DISCUSSION

Dependency of Reaction Efficiency on Solvent Systems. Lipase catalyzed reactions are greatly influenced by the reaction media. It is well known that the nature of the reaction media has significant effect on the activity and selectivity of enzymes. Organic solvents, including 1-octane, isooctane, *n*-heptane, 2-butanone, and cyclohexane, were used to investigate the effect of reaction media on the esterification of ferulic acid with oleyl alcohol in this section. As presented in Table 1, the bioconversion yield reached 3.81%, 97.68%, 25.83%, 0%, 99.17%, and 74.02% for *tert*-butanol, isooctane, toluene, 2-butanone, hexane, and cyclohexane, respectively, after 4 days of reaction. Among them, isooctane and hexane gave higher conversions. The boiling point of isooctane (99.2 °C) is higher than hexane (68.7 °C). Therefore, isooctane was chosen as a model organic solvent to examine the enzymatic esterification of ferulic acid with oleyl alcohol in the mixture of isooctane with different ionic liquids.

Figure 1 depicted the time course of enzymatic synthesis of oleyl ferulate in isooctane mixed with different ionic liquids. The results demonstrated that the volumetric productivity varied significantly with the anions and substitiuents in the imidazolium ring, even though all belong to the same IL analogue imidazolium type. [Bmim][TF_2N]/isooctane gave the highest volumetric productivity of oleyl ferulate (16.51 mg/mL), followed by [Hmim][PF_6]/isooctane (14.67 mg/mL) and[Omim][PF_6]/isooctane (12.53 mg/mL). However, [Bmim][PF_6]/isooctane and [Bmim][BF_4]/isooctane yielded remarkably low productivity(4.03 and 2.37 mg/mL) (Figure 1). Hu et al. investigated the multiple interactions in IL-organic solvent systems as well as their effects on enzyme activity and reaction performance. For the three chosen ionic liquids anions, namely, $[PF_6]^-$, $[BF_4]^-$, and $[TF_2N]^-$, their thermal stability, viscosity, and strength of H-bonding between anion and water are different. The reaction in an IL with the $[TF_2N]^-$ had higher enzyme activity than $[PF_6]^-$ and $[BF_4]^-$.[19] It is clear that the observation in this work agreed with their results. However, considering that the productivities of [Hmim][PF_6]/isooctane and[Omim][PF_6]/isooctane were not largely lower than that of [Bmim][TF_2N]/isooctane and that [Bmim][TF_2N] is much more expensive, [Hmim][PF_6] and [Omim][PF_6] mediated systems were selected for further parameter optimization.

A drawback of the reaction mediated by ILs is the high viscosity of the system. Adding organic cosolvent to lower the viscosity of ionic liquids may offer another advantage besides increasing the solubility of the substrate that has low solubility in conventional solvents. This effect has been quantitatively illustrated by Chen et al.[18,20]

Effects of Reaction Temperatures. Reaction temperature has an influence on the kinetic activity and stability of biocatalysts. It also affects the viscosity of ILs and the thermodynamic

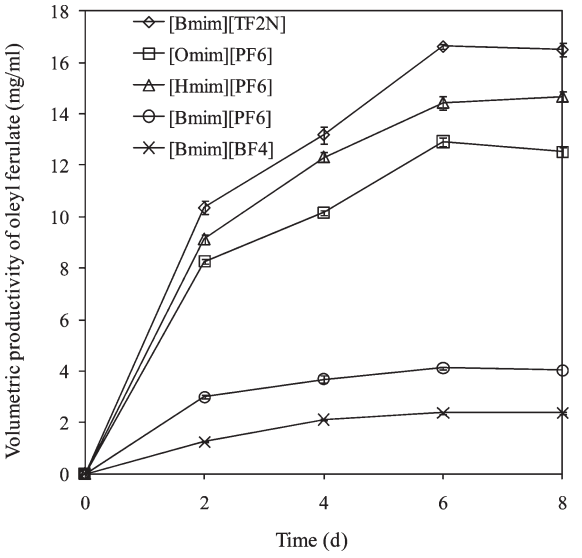

Figure 1. Effects of ionic liquid/isooctane on the volumetric productivity of lipase-catalyzed esterification of ferulic acid with oleyl alcohol. Reaction conditions: ferulic acid, 11.6 mg; oleyl alcohol, 64.4 mg; Novozym 435, 100 mg; molecular sieves, 100 mg; isooctane, 1.5 mL; ILs, 0.5 mL; 8 days; and 60 °C. The plotted data are the means ± standard deviations of 3 replicates.

equilibrium of enzyme-catalyzed reactions. The volumetric productivity in the esterification of ferulic acid with oleyl alcohol catalyzed by Novozym 435 was investigated at temperatures ranging from 50 to 60 °C over an 8 day reaction time period. Low solubility of ferulic acid and high viscosity of ILs at temperatures <50 °C and the volatility of organic solvent and the thermodeactivation effect on lipase at temperatures >60 °C represent the leading reason why the reactions at temperature <50 °C or >60 °C were not intensively investigated in this work.[21] As can be seen in Figure 2A, there was an increase in volumetric productivity from 11 mg/mL at 50 °C to 14.7 mg/mL at 60 °C after 8 days of incubation. The time to reach equilibrium at higher temperatures was faster than that at lower temperatures, for example, at 60 °C the volumetric productivity at 4 days was almost the same as that at 8 days, while at 50 °C, 4 day productivity was remarkably lower than that of 8 days. However, the difference is not very significant. In principal, the reaction time to reach equilibrium depends largely on mass transfer, temperature (kinetics) parameters, and so forth. In this work, temperature-dependent solubility is also a crucial factor. The similar patterns in terms of volumetric productivity at different temperatures might be the consequence from all these important variables (Figure 2). The result in this work was in accordance with the previous study.[22] Figure 2B presented the effect of temperature on volumetric productivity in the [Omim][PF_6]/isooctane reaction system. The data indicated that volumetric productivity increased with temperature. The volumetric productivity reached 9.27 mg/mL and 13.75 mg/mL at temperatures of 50 and 60 °C after 8 days, respectively. The optimum reaction temperature in both systems was 60 °C, taking the deactivation effect of higher temperature on the enzyme also into consideration so that further increases in temperature were not examined. Similar observations have been reported in other IL-mediated systems.[23,24] In enzymatic production of diglycerids, with increasing temperatures, both triacylglycerol conversion and diacylglyerol yield were improved, which was considered to be a result of the decrease in the viscosity of the *n*-hexane−IL systems induced by the increased temperatures.[23] Ganske and Bornscheuer showed that only 4% lauric acid vinyl ester was converted at 40 °C, while up to 59% conversion could be obtained at 60 °C in the [Bmim][BF_4]/*t*-BuOH system.[24]

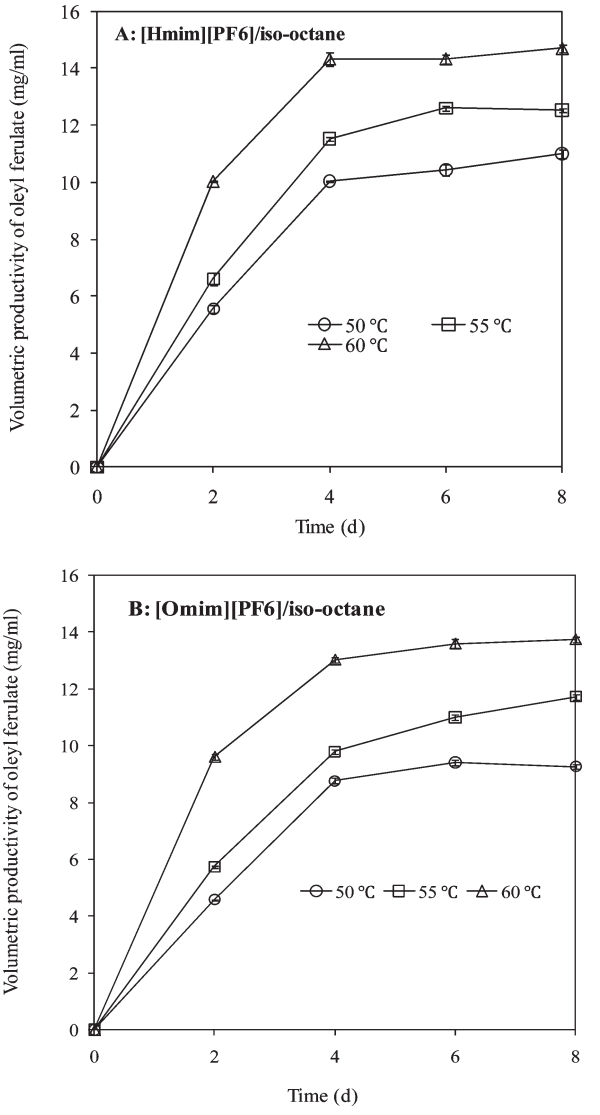

Figure 2. Effect of reaction temperature on the volumetric productivity of lipase-catalyzed esterification of ferulic acid with oleyl alcohol. The reactions were performed at 50, 55, or 60 °C, with 11.6 mg of ferulic acid, 64.4 mg of oleyl alcohol, 100 mg of Novozym 435, 100 mg of molecular sieves, 0.5 mL of IL, and 1.5 mL of isooctane for 8 days. The plotted data are the means ± standard deviations of 3 replicates.

Effects of Substrate Concentrations. Figure 3 showed the volumetric productivity of the ferulic acid ester at different ferulic acid concentrations, monitored over 10 days. In the [Hmim]-[PF_6]/isooctane reaction system, the highest volumetric productivity of 39.62 mg/mL was obtained with a ferulic acid concentration of 0.08 mmol/mL after 10 days of reaction (Figure 3A). However, further increasing the ferulic acid concentration from 0.08 to 0.1 mmol/mL led to a slight decrease in the volumetric productivity of ferulic acid ester from 39.62 to 38.52 mg/mL. In the [Omim][PF_6]/isooctane system, the highest volumetric productivity of 19.32 mg/mL at a ferulic acid concentration of 0.1 mmol/mL was obtained (Figure 3B), but it was very close to

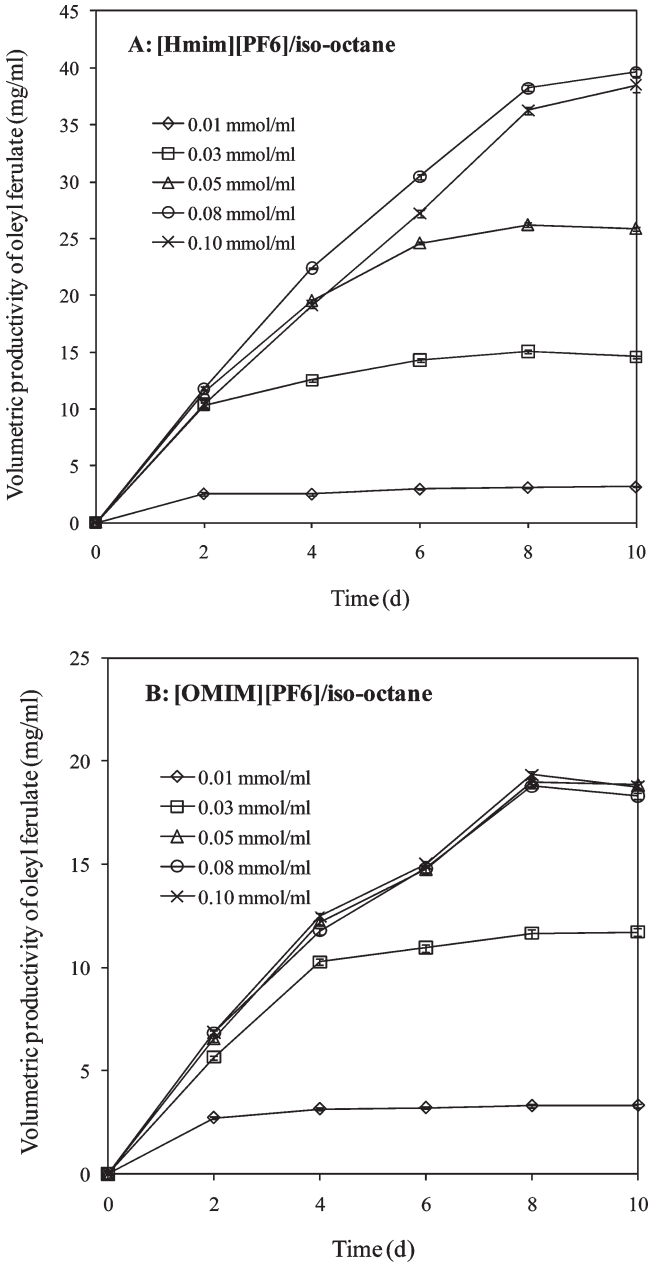

Figure 3. Effect of substrate concentrations on the volumetric productivity of lipase-catalyzed esterification of ferulic acid with oleyl alcohol. Reactions conditions: ferulic acid concentration, 0.01, 0.03, 0.05, 0.08, or 0.1 mmol/mL; oleyl alcohol, 64.4 mg; Novozym 435, 100 mg; molecular sieves, 100 mg; IL, 0.5 mL; isooctane, 1.5 mL; 10 days; and 60 °C. The plotted data are the means ± standard deviations of 3 replicates.

the volumetric productivity at 0.05 mmol/mL (18.95 mg/mL). These results suggested that, for a set system, there is a capacity limitation where maximum yield could be reached, in which solubility limitation of the substrate might be a key factor. For a specific system only the solubilized substrate is available for reaction. Thus, a further increase of substrate load higher than the solubility limitation may not increase the substrate concentration available for reaction, instead increase viscosity and decrease mass transfer, and thus might result in a slight decrease of volumetric productivity. Therefore, 0.08 and 0.05 mmol/mL were treated as optimum ferulic acid concentrations for [Hmim]-[PF_6]/isooctane and [Omim][PF_6]/isooctane systems, respectively. These experimental findings were in agreement with those obtained previously by Katsoura et al., where increasing the substrate concentration of naringin up to a specific value led to an increase in the conversion of naringin, while a further increase of naringin concentration had a negative effect on the conversion yield on the enzymatic acylation of plant polyhydroxylated compounds, including phenolic and flavonoid glucosides in imidazolium-based ionic liquids such as [Bmim][BF_4] and [Bmim]-[PF_6].[21] Another example is the lipase-catalyzed esterification of ascorbyl benzoate in organic media, where when the substrate concentration was increased, the conversion rate first increased and reached a maximum, then decreased, while the output increased to the highest level and was unchanged afterward. The reason is similar to the aforementioned one: that the increase of concentration and the decrease of conversion rate result in almost the same productivity as the maximum value.[25]

In order to maximize the enzymatic synthesis of the ferulic acid ester, the effect of substrate molar ratio on the lipase-catalyzed esterification was also investigated by varying ferulic acid to oleyl alcohol ratios from 1:1 to 1:12 (Figure 4). Among the tested substrate molar ratios, the maximum volumetric productivity of 14.59 mg/mL in [Omim][PF_6]/isooctane and 13.85 mg/mL in [Hmim][PF_6]/isooctane were obtained at ferulic acid to oleyl alcohol molar ratios of 1:4 and 1:8, respectively. As shown in Figure 4B, when the ferulic acid to oleyl alcohol molar ratio was 1:4, a volumetric productivity of 13.79 mg/mL was achieved. However, the volumetric productivity decreased when the molar ratios of ferulic acid to oleyl alcohol were 1:8 and 1:12 (Figure 4A). The possible explanation might be that too much oleyl alcohol introduction may change the properties of the system and lead to decrease in solubility of ferulic acid. Thus, the substrate molar ratio of 1:4 was selected as the optimized condition in terms of achieving a higher volumetric productivity of the ferulic acid ester. The results in this work were in accordance with the previous study. Lue et al.'s results showed that a decrease in the molar ratio of cinnamic acid to oleyl alcohol through an incremental increase in the concentration of oleyl alcohol resulted in an increase in the bioconversion yield. The maximum bioconversion yield of 100% was obtained with a ratio of cinnamic acid to oleyl alcohol of 1:6 after 12 days of reaction. The lowest bioconversion yield of 38.4% was obtained with a cinnamic acid to oleyl alcohol ratio of 1:0.5 after 9 days of reaction in organic solvent media.[26] Lee et al. demonstrated that a ferulic acid to ethanol molar ratio of 1:5 gave the highest conversion, when the synthesis of ethyl ferulate from ferulic acid and ethanol was carried out in Novozym 435 and isooctane.[8]

Effects of ILs/Isooctane Volume Ratios. The effects of different isooctane/IL volume ratios on the lipase-catalyzed esterification were investigated with varied ratios at 1:3, 1:1, and 3:1. Figure 5 shows the reaction time course, where increasing the volume ratio of isooctane in the mixture resulted in a higher volumetric productivity. The maximum volumetric productivity of 15.44 mg/mL was obtained with an isooctane/[Hmim][PF_6] volume ratio of 3:1 (Figure 5A). For the [Omim][PF_6]-based system, the volumetric productivity increased from 3.77 to 13.77 mg/mL when the volume ratio of [Omim]-[PF_6]/isooctane was increased from 1:3 to 3:1(Figure 5B). The decrease of volumetric productivity with higher IL content is more likely due to the deleterious effect on enzyme activity from the coordinating property of ions in ILs.[27] These results were not surprising but rather support our initial hypothesis that a binary

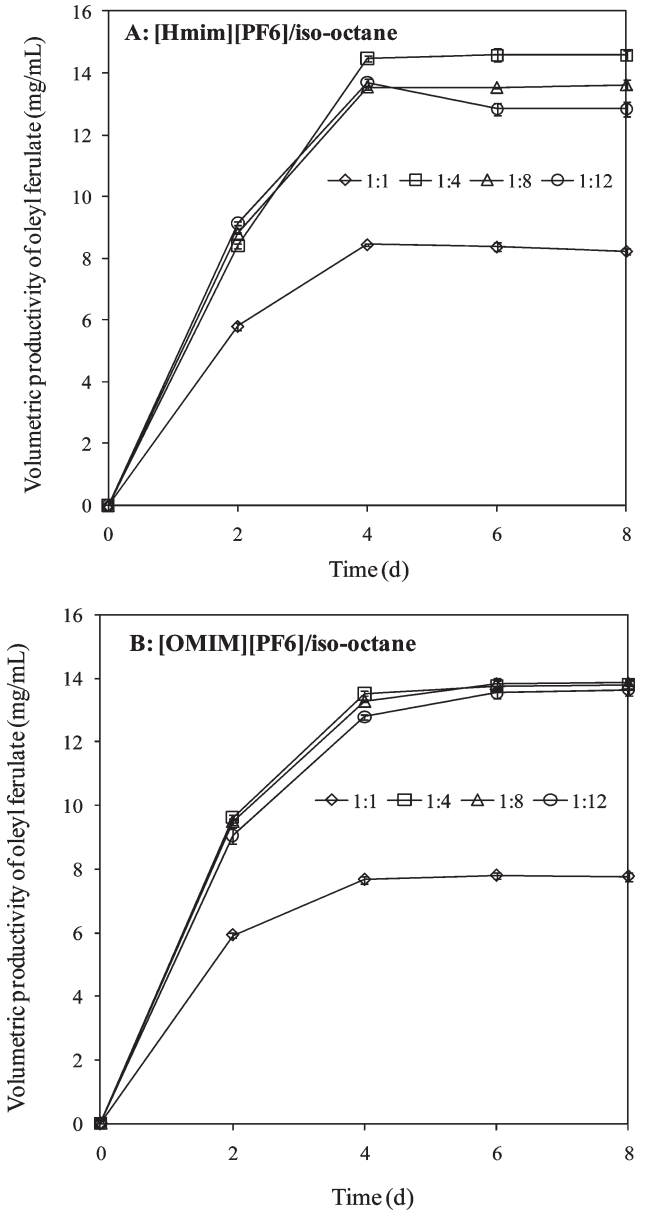

Figure 4. Effect of substrate molar ratio on the volumetric productivity of lipase-catalyzed esterification of ferulic acid with oleyl alcohol. Reaction conditions: ferulic acid to oleyl alcohol molar ratio, 1:1, 1:4, 1:8, or 1:12; ferulic acid, 11.6 mg; Novozym 435, 100 mg; molecular sieves, 100 mg; IL, 0.5 mL; isooctane, 1.5 mL; 8 days; and 60 °C. The plotted data are the means ± standard deviations of 3 replicates.

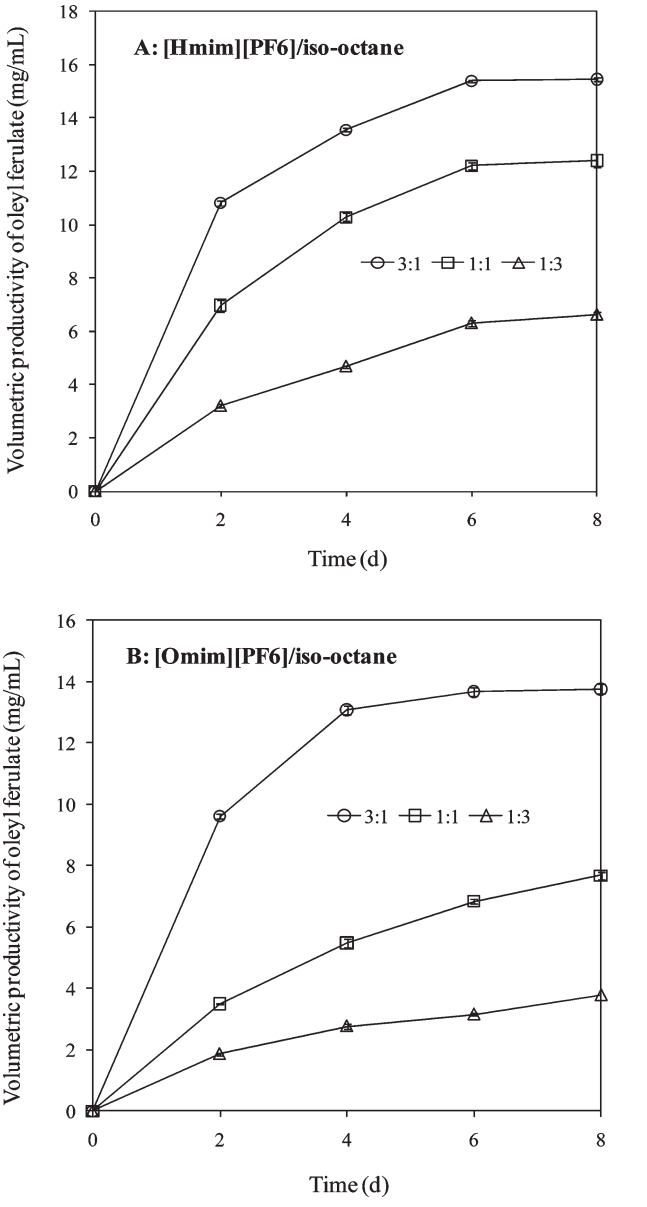

Figure 5. Effect of isooctane to ionic liquid on the volumetric productivity of the lipase-catalyzed esterification of ferulic acid with oleyl alcohol. Volume ratio of isooctane to ionic liquids: 1:3, 1:1, and 3:1. Reaction conditions: ferulic acid, 11.6 mg; oleyl alcohol, 64.4 mg; Novozym 435, 100 mg; molecular sieves, 100 mg; media volume, 2 mL; 8 days; and 60 °C. The plotted data are the means ± standard deviations of 3 replicates.

system of IL/hydrophobic solvent could reach a promising effect between the contribution from IL for higher substrate solubility and the contribution from the hydrophobic solvent for buffering the negative effect on enzyme activity from IL. The result was also similar to a previous study on enzymatic synthesis of esculin ester, where the esterification rate and esculin conversion increased as the acetone ratios increased in IL−acetone mixtures.[19]

Effects of Enzyme Concentrations. The effect of Novozym 435 dosage on the volumetric productivity of oleyl ferulate is shown in Figure 6. The volumetric productivity was enhanced by increasing lipase dosage. The highest volumetric productivity for the [Hmim][PF_6]/isooctane system was 15.49 mg/mL with 75 mg/mL (150 mg) lipase used (Figure 6A), while the maximum volumetric productivity of 14.65 mg/mL for the [Hmim][PF_6]/isooctane was achieved at the same enzyme dosage (Figure 6B). However, for both systems, a further increase in the enzyme concentration to 100 mg/mL (200 mg) resulted in decreasing volumetric productivity. In this study, an enzyme concentration of 75 mg/mL was employed. The presence of the higher amount of enzyme provides more active sites for acyl−enzyme complex formation and also increases the probability of enzyme−substrate collision and subsequent reaction.[28] The negative effect of further increase in enzyme concentration may be due to mass transfer limitation and poor dispersion of enzyme particles as experimentally observed. These results were in agreement with those

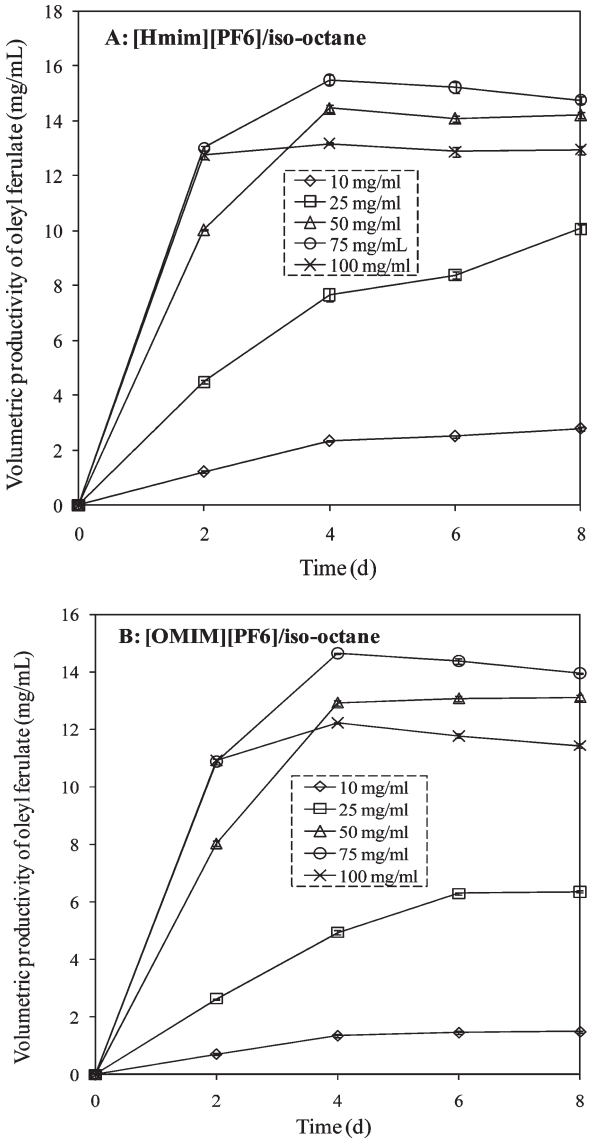

Figure 6. Effect of enzyme dosage on the volumetric productivity of the lipase-catalyzed esterification of ferulic acid with oleyl alcohol. Enzyme dosages: Novozym 435, 10, 25, 50, 75, or 100 mg/mL. Reactions conditions: ferulic acid, 11.6 mg; oleyl alcohol, 64.4 mg; molecular sieves, 100 mg; isooctane, 1.5 mL; IL, 0.5 mL; 8 days; and 60 °C. The plotted data are the means ± standard deviations of 3 replicates.

obtained by Zhang et al.[22] and Li et al.[29] They indicated that as the enzyme loading increased, the conversion of pyridoxine also increased; when the enzyme loading was raised to 10 mg/mL acetonitrile, a further increase in the enzyme loading was not capable of further enhancing the conversion.[22]

Volumetric Productivity under the Optimized Conditions. The time course of Novozym 435-catalyzed esterification of ferulic acid with oleyl alcohol under the optimum conditions was depicted in Figure 7. The volumetric productivity of ferulic acid ester in [Hmim][PF_6]/isooctane increased rapidly in the first 8 days and reached equilibrium at 12 days with productivity up to 48.12 mg/mL (Figure 7). Similarly in the [Omim][PF_6]/isooctane system, the reaction equilibrium was attained after 12 days with the highest volumetric productivity at 26.92 mg/mL. The volumetric productivity of the [Hmim][PF_6]/isooctane system was around 45% higher than that of the [Omim][PF_6]/isooctane system.

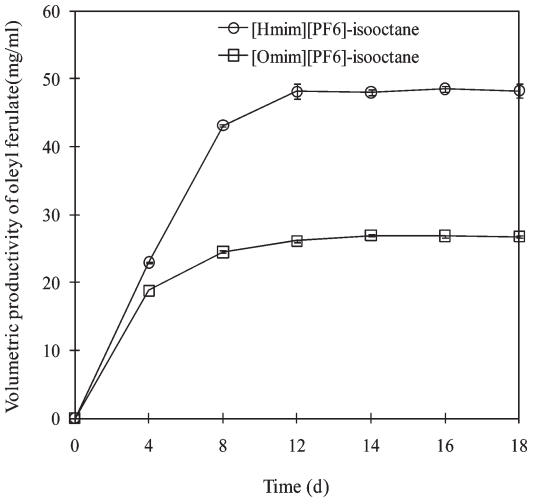

Figure 7. Time course of lipase-catalyzed esterification of ferulic acid and oleyl alcohol in the optimized reaction conditions. The reactions were carried out at (A) 1.5 mL of isooctane/0.5 mL of [Hmim][PF_6], 0.08 mmol/mL (15.5 mg/mL) ferulic acid, 86 mg/mL oleyl alcohol, 150 mg (75 mg/mL) of Novozym 435, and 100 mg (50 mg/mL) molecular sieves for 18 days at 60 °C; (B) 1.5 mL isooctane/0.5 mL [Omim][PF_6], 0.05 mmol/mL (9.7 mg/mL) ferulic acid, 53.7 mg/mL of oleyl alcohol, 150 mg (75 mg/mL) of Novozym 435, and 100 mg (50 mg/mL) molecular sieves for 18 days at 60 °C. The plotted data are the means ± standard deviations of 3 replicates.

Kinetic Properties. In order to rationalize the lipase performance in the IL/solvent systems and the effects of media, we have studied the kinetic behaviors of Novozym 435-catalyzed esterification of ferulic acid with oleyl alcohol in the two selected systems and compared their performances with that in the 2-butanone/isooctane system, which has been optimized in our previous work [9] (Table 2).

One of the most striking differences between the three systems is that the maximum reaction rates in IL-based binary systems are much higher than those in the binary solvent system (4.78 and 2.67 times, respectively). Different ILs also results in different V_{max} values, namely, the [Hmim][PF_6]/isooctane system achieved 1.78 times of the maximum reaction rate compared to that of the [Omim][PF_6]/isooctane system. This indicates that the [Hmim][PF_6] system has a higher volumetric productivity, which is consistent with the previous observations in Figures 3 and 7. However, the similar apparent K_m values of the three systems seem to indicate that the medium property does not change the affinity of lipase to substrate significantly (Table 2). Therefore, the higher solubility of ferulic acid in IL-mediated systems most likely represents the leading reason for higher productivity in [Hmim][PF_6]/isooctane and [Omim][PF_6]/isooctane systems than in organic solvents.

In terms of E_a values, both IL-based binary systems are over 2 times higher than those of the 2-butanone/isooctane system, which suggests that the increase of temperature in IL-based systems will lead to a bigger effect on the change of reaction rate. This is a very characteristic thermodynamic behavior pertaining to an IL-mediated system, in which an increase in temperature will dramatically decrease the viscosity of the system and thus facilitate mass transfer enhancement.[13] The result also suggested that in order to achieve an optimal reaction rate for the IL-mediated system, it is necessary to conduct the reaction at a higher temperature to overcome the elevated energy barrier.

Table 2. Some Kinetic Properties of the Enzymatic Esterification of Ferulic Acid in [Hmim][PF_6]/Isooctane and [Omim][PF_6]/Isooctane Systems[a]

	V_{max} (mM h^{-1} (g enzyme)$^{-1}$)	K_m (mM)	E_a (kJ·mol^{-1})
[Hmim][PF_6]/isooctane[a]	7.75 ± 0.38	28.06 ± 1.41	52.72 ± 3.26
[Omim][PF_6]/isooctane[a]	4.33 ± 0.21	23.51 ± 1.18	66.15 ± 3.64
2-butanone/isooctane[b]	1.62 ± 0.10	24.06 ± 0.95	24.99 ± 1.29

[a] All kinetic assays were done at 60 °C with an excessive and constant concentration of oleyl alcohol. For both IL-binary systems, the variation of the concentration of ferulic acid is in the range of 0.01−0.10 mol L^{-1}. [b] For 2-butanone/isooctane, the variation of the concentration of ferulic acid is in the range of 0.001−0.02 mol L^{-1}. E_a was measured at 40−60 °C for all three systems.

In conclusion, this work examined lipase-catalyzed esterification of ferulic acid with oleyl alcohol in an ionic liquid/isooctane binary system. [Hmim][PF_6]/isooctane and [Omim][PF_6]/isooctane systems are found to be capable of yielding high volumetric productivities of the ferulic acid oleyl ester. Substrate concentration, IL/isooctane volume ratio, and temperature are found to be crucial parameters governing the volumetric productivity and the reaction conditions of both systems, and have been optimized individually. The optimum volumetric productivity of 48.12 mg/mL for [Hmim][PF_6]/isooctane and 26.92 mg/mL for [Omim][PF_6]/ isooctane were obtained under their optimized conditions.

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