



Effect of ionic liquids on enzymatic preparation of lipophilic feruloylated structured lipids using distearin as feruloylated acceptor and kinetic analysis



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ABSTRACT

Feruloylated acylglycerols (FAGs) are lipophilic structured lipids of ferulic acid (FA), which have antioxidant activities and UV-absorbing properties. In the paper, FAGs were prepared by the enzymatic transesterification of ethyl ferulate (EF) with distearin using ionic liquids (ILs) as the reaction media. The effects of ILs on the enzymatic transesterification and the selective formation of lipophilic FAGs or hydrophilic glyceryl ferulates (FGs) were investigated. The effect of reaction variables on the transesterification was optimized using response surface method (RSM), and kinetic analysis in the IL was also evaluated. Among the tested ILs, excellent FAGs selectivity (-0.63) was achieved using [Emim]TF₂N as the reaction solvent. Moreover, [Emim]TF₂N also showed protective effect on the enzyme stability at high temperature ($110\text{ }^{\circ}\text{C}$). High EF conversion ($99.7 \pm 0.3\%$) and FAGs yield ($68.7 \pm 1.0\%$) were also obtained at the optimized conditions (reaction temperature of $95\text{ }^{\circ}\text{C}$, reaction time of 28 h, and enzyme load of 15% (w/w, relative to the weight of substrates)). The activation energies (E_a) of EF conversion, FGs and FAGs formation were 44.4, 41.4, 59.9 kJ/mol, respectively. K_mA , K_mB and V_{max} were 0.02, 0.12 mol/L, and 4.54 mmol/(L·h), respectively. The kinetics of transesterification of EF with distearin in [Emim]TF₂N agreed with the Ping-Pong Bi-Bi mechanism without substrates inhibition.

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1. Introduction

Ferulic acid ((*E*)-4-hydroxy-3-methoxycinnamic acid, FA) is a phenolic compound widely existing in agricultural products, beverages and Chinese herbs. FA has free radical scavenging, UV absorption, and antioxidant abilities [1–5]. However, the poor solubility of FA in various oil-based or multiphase systems limits the application of FA in cosmetics, food and pharmaceutical fields. To improve the situation, FA has been modified by using lipophilic moieties [6,7]. Due to the heat sensitivity and susceptibility of FA at high temperature, enzymatic preparation of lipophilic feruloylated derivatives has attracted more attention than chemical processes [6,8,9].

Feruloylated acylglycerols (FAGs), referring to the combination of feruloylated monoacylglycerols (FMAGs) and feruloylated diacylglycerols (FDAGs), are lipophilic structured lipids of FA that have antioxidant activities and UV-absorbing properties [10–13]. According to previous reports, lipophilic FAGs can be produced by transesterification of EF or

FA with different feruloylated acceptors, such as, triacylglycerols and monoacylglycerols [9–13]. However, triacylglycerols as feruloylated acceptors have great stearic hindrance, which leads to low reaction efficiency and long reaction time. Monoacylglycerols as feruloylated acceptors are prone to the formation of hydrophilic glyceryl ferulates in solvent-free or ionic liquids (ILs) system. Therefore, distearin, with a lower stearic hindrance than triacylglycerols and more lipophilic FAGs formation than monoacylglycerols, is the best choice as feruloylated acceptors for FAGs preparation [14].

ILs, owing to the excellent solubility, designability, non-volatility, non-flammability, etc., have been used as environmentally friendly reaction media and reaction promoters [15–26]. However, there is no available information on using ILs as reaction solvents in the transesterification of EF with distearin for the selective preparation of FAGs.

In this work, the effect of different ILs as a reaction medium on the transesterification of ethyl ferulate (EF) with distearin and the selective formation of FAGs were investigated. Moreover, the protective effect of ILs on lipase at high temperatures was also evaluated. The effects of reaction variables including reaction temperature, enzyme load and time

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on EF conversion and FAGs yield in ILs were also studied and evaluated using response surface method (RSM). The thermodynamics and kinetics were also analyzed.

2. Materials and methods

2.1. Materials and reagents

Novozyme 435 (*Candida antarctica* lipase immobilized on poly acrylic resin by adsorption, 10,000 PLU/g solid enzyme) was obtained from Novozymes A/S (Bagsvaerd, Denmark). Ethyl ferulate (EF, purity > 99%) was procured from Suzhou Chang Tong Chemical Co., Ltd. (Suzhou, China). Distearin was purchased from Jinan Shengtong Chemical Technology Co., Ltd. (Jinan, China). ILs (all $\geq 99\%$), including 1-ethyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide ([Emim]TF₂N), 1-butyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide ([Bmim]TF₂N), 1,3-dimethylimidazolium bis[(trifluoromethyl)sulfonyl]imide ([Mmim]TF₂N), 1-ethyl-3-methylimidazolium hexafluorophosphate ([Emim]PF₆), 1-butyl-3-methylimidazolium hexafluorophosphate ([Bmim]PF₆), 1-octyl-3-methylimidazolium hexafluorophosphate ([Omim]PF₆), 1-decyl-3-methylimidazolium hexafluorophosphate ([C₁₂mim]PF₆), 1-tetradecyl-3-methylimidazolium hexafluorophosphate ([C₁₄mim]PF₆), 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim]BF₄), and 1-hexyl-3-methylimidazolium tetrafluoroborate ([Hmim]BF₄), were purchased from Shanghai Cheng Jie Chemical Co., Ltd. (Shanghai, China). Methanol and glacial acetic acid were of High Performance Liquid Chromatograph (HPLC) grade. All other reagents were of analytical grade.

2.2. Enzymatic transesterification

EF and distearin (molar ratio of EF to distearin of 1:1) were mixed with ILs (1:1, relative to the total weight of all substrates) in 25-mL round-bottom flasks, which were incubated in a water bath to the required temperature. Then, Novozyme 435 was added to the reaction mixtures. Reactions were carried out at 10 mm Hg vacuum pressure for the ILs system. Samples (20 μ L) were withdrawn at specified time intervals.

2.3. HPLC analysis

The reaction progress was analyzed by HPLC and HPLC-ESI-MS according to the previous reports [11,27]. Reaction selectivity for FAGs production was defined as follows:

$$\text{FAGs selectivity} = \frac{(\text{FMAGs} + \text{FDAGs}) \text{ yield}}{\text{EF conversion}} \quad (1)$$

2.4. Experimental design and statistical analysis

A three-level-three-factor Box-Behnken experimental design was employed. The factors and their levels selected for the investigation of FAGs production were reaction temperature (60, 85, 110 °C), reaction time (4, 20, 36 h), and enzyme load (5, 10, 15%). The effect of reaction factors on EF conversion and FAGs yield were evaluated using the model. The use of 3^k factorial tests allowed an efficient estimation of a second order polynomial regression. The mathematical relationship relating the factors to EF conversion and FAGs yield could be calculated by the second-order polynomial equation:

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

where Y is the predicted response (EF conversion or FAGs yield), and X_i and X_j represent the independent variables. The regression coefficients

β_0 , β_i , β_{ii} , and β_{ij} are the intercept, linear, quadratic, and interaction terms, respectively.

To ensure the validity of the results, all experiments were performed at least in triplicate. The results were expressed as averages \pm S.E.M. The significance of the difference was estimated using a two-way analysis of variance (ANOVA). Statistical significance was considered at $p < 0.05$.

3. Results and discussion

3.1. Effect of ILs as reaction media

The effect of different ILs on the transesterification of EF with distearin was evaluated. For all tested ILs, EF conversion increased with reaction progress (Fig. 1A). However, for those ILs with BF₄⁻, EF conversions were lower than 60%, which were all lower than those of PF₆⁻ and TF₂N⁻ at 48 h (~99%). The time that reaction reached equilibrium in [Bmim]BF₄ or [Hmim]BF₄ (>48 h) was longer than that of ILs

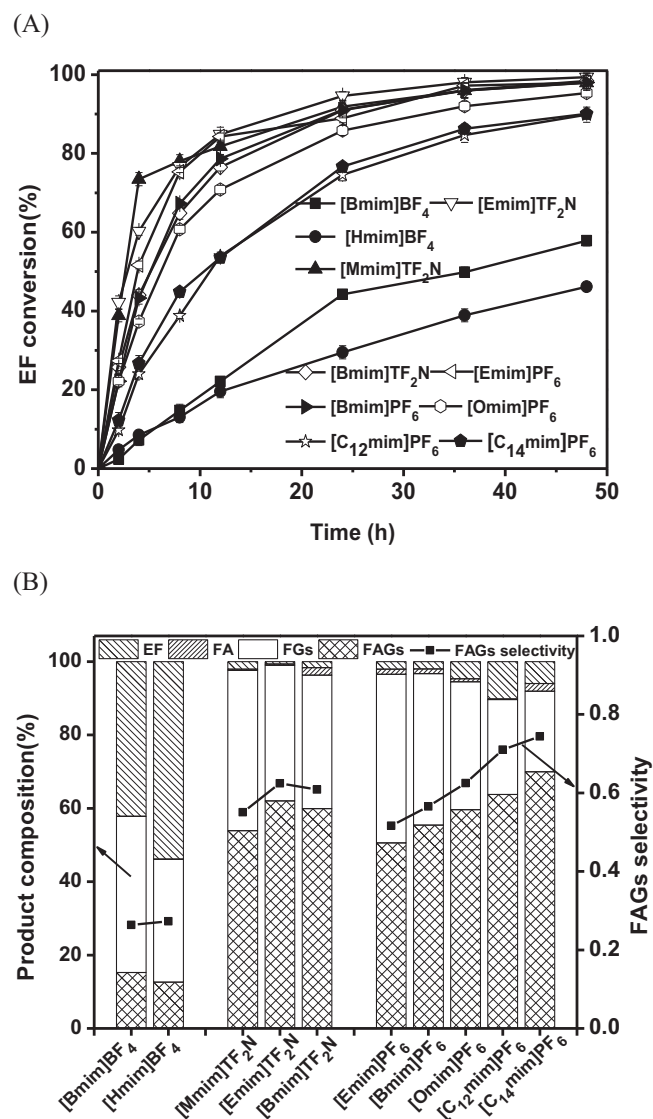


Fig. 1. (A) Effects of ILs on transesterification of EF with distearin. (B) Product composition and FAG selectivity in different ILs (48 h). Reaction condition: EF/distearin = 1:1 (mol/mol), ILs: substrates = 1:1 (w/w), enzyme load 10% (relative to the weight of all substrates), 70 °C, 90 kPa.

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