



Enhanced synthesis of feruloylated acylglycerols by the lipase-catalyzed transesterification of glyceryl monoferulate with different acyl donors using ionic liquids as reaction solvents

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ABSTRACT

Feruloylated acylglycerols (FAG) can be used as antioxidants and UV absorbing ingredients in food and cosmetics. In this work, FAG was prepared by the lipase-catalyzed transesterification of glyceryl monoferulate (GMF) with different acyl donors using ionic liquids (ILs) as reaction solvents. The effect of different imidazolium ILs (BF_4^- , PF_6^- and TF_2N^-) and acyl donors (monoacylglycerols and diacylglycerols) on the transesterification and lipase selectivity for FAG formation were compared. The effect of reaction parameters (temperature, enzyme concentration, substrates ratio and time) on the reaction were also studied. The results showed that FAG preparation can be enhanced using monoolein (MO) and distearin (DS) as acyl donors. High transesterification activity and excellent lipase selectivity for lipophilic FAG formation were achieved using $[\text{C}_{18}\text{MIM}]\text{PF}_6$ as reaction solvent. The activation energy to form the lipophilic FAG by transesterification using MO as an acyl donor was 37.2 kJ/mol, which was lower than that of DS (92.9 kJ/mol). The activation energy to form the hydrophilic glyceryl diferulate by the esterification of GMF with feruloyl formed by the hydrolysis of another GMF (21.5 kJ/mol) using MO as an acyl donor was lower than that of DS (61.9 kJ/mol).

1. Introduction

Ferulic acid (FA), (E) 4-hydroxy-3-methoxycinnamic acid, is a phenolic acid in some plants (vegetables and fruits) and Chinese medicinal herbs (Kikuzaki et al., 2002; Ou and Kwok, 2004). Recently, due to their broad biological activities (antioxidant, UV-protecting and radical-scavenging activities, antiviral and antitumor activities) (Graf, 1992; Khan and Rathod, 2015; Shi et al., 2017; Sørensen et al., 2014), FA and its derivatives have attracted increasing interest in some industries. Feruloylated structured lipids, including lipophilic feruloylated monoacylglycerols (FMAG) and feruloylated diacylglycerols (FDAG), are potent antioxidants and UV absorbing ingredients in foods and cosmetics (Antonopoulou et al., 2016; Katsoura et al., 2009; Zheng et al., 2010; Wang and Shahidi, 2014).

Due to heat-sensitivity and susceptibility to the oxidation of FA, enzymatic methods have been widely used for the preparation of lipophilic feruloylated acylglycerols (FAG) (Antonopoulou et al., 2017; Graf, 1992; Liu et al., 2013; Sorour et al., 2012). In previous reports, some feruloyl donors, such as, FA, ethyl ferulate (EF), or vinyl ferulate,

were selected and used for FAG preparation (Compton et al., 2000; Sun et al., 2007). Glyceryl monoferulate (GMF) is one type of hydrophilic derivative of FA (Matsuo et al., 2001), which can be found ubiquitously in plants (Cooper et al., 1978; Graça and Pereira, 2000). There are two available hydroxyl groups in GMF, which makes GMF a potential reaction intermediate for the preparation of FA derivatives. However, no information about FAG preparation using GMF as a feruloyl donor was found.

Ionic liquids (ILs), due to their non-flammability, non-volatility, thermal stability, and controlled miscibility, have been used as reaction solvents (Hu et al., 2009; Kaar et al., 2003; Mai and Koo, 2014; Moniruzzaman et al., 2010; Vekariya, 2017). For example, imidazolium ILs (tetrafluoroborate $[\text{BF}_4]^-$, hexafluorophosphate $[\text{PF}_6]^-$, and bis [(trifluoromethyl)sulfonyl]imide $[\text{TF}_2\text{N}]^-$) used as reaction solvents, can enhance enzyme stability and reaction selectivity (Aspras et al., 2017; Gao et al., 2015; Gholivand et al., 2017; Katsoura et al., 2006; Kim et al., 2003; Vafiadi et al., 2009; Zhao et al., 2017). However, no available information about the effect of ILs on the lipase-catalyzed transesterification of GMF using monoacylglycerols (MG) or

Abbreviations: Mg, monoacylglycerols; DG, diacylglycerols; TG, triacylglycerols; MO, monoolein; MS, monostearin; DO, diolein; DS, distearin; FFA, free fatty acids; GMF, glyceryl monoferulate; GDF, glyceryl diferulate; FMAG, feruloylated monoacylglycerols; FDAG, feruloylated diacylglycerols; FAG, feruloylated acylglycerols; EF, ethyl ferulate; FA, ferulic acid

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diacylglycerols (DG) as acyl donors was found.

In the present study, FAG was successfully prepared by the enzymatic transesterification of GMF with different acyl donors in the IL system. The effect of different imidazolium ILs (BF_4^- , PF_6^- and TF_2N^-) as reaction media and the structure of different acyl donors (MG and DG) on the enzymatic transesterification and reaction selectivity were compared. The effects of various reaction parameters (enzyme concentration, substrate ratio, reaction time and temperature) on the transesterification and enzymatic selectivity for FAG formation were also evaluated. The reaction scheme of the enzymatic transesterification in IL was also proposed.

2. Materials and methods

2.1. Materials

Novozym 435 (EC 3.1.1.3, an immobilized lipase B from *C. antarctica*) was obtained from Novozymes A/S (Bagsvaerd, Denmark). Ethyl ferulate (purity > 99%) was obtained from Suzhou Chang Tong Chemical Co., Ltd. (Suzhou, China). Glycerol (purity > 99%) was purchased from Tianjin Kermel Chemical Co., Ltd. (Tianjin, China). Two types of MG, including monoolein (MO) and monostearin (MS), were purchased from Shanghai Jing Chun Reagent Co., Ltd. (Shanghai, China). Two types of DG, including diolein (DO; a mixture of $21.8 \pm 1.9\%$ MG, $47.3 \pm 2.1\%$ DG, $25.9 \pm 1.5\%$ triacylglycerols (TG), and $5.0 \pm 1.1\%$ free fatty acids (FFA)) and distearin (DS; a mixture of $16.3 \pm 2.0\%$ MG, $44.0 \pm 2.6\%$ DG, $37.2 \pm 1.8\%$ TG, and $2.5 \pm 1.0\%$ FFA) were purchased from Jinan Dowin Chemical Technology Co., Ltd. (Jinan, China).

All ILs, including 1-ethyl-3-methylimidazolium hexafluorophosphate ([Emim]PF₆), 1-octyl-3-methylimidazolium hexafluorophosphate ([Omim]PF₆), 1-decyl-3-methylimidazolium hexafluorophosphate ([C₁₀mim]PF₆), 1-dodecyl-3-methylimidazolium hexafluorophosphate ([C₁₂mim]PF₆), 1-tetradecyl-3-methylimidazolium hexafluorophosphate ([C₁₄mim]PF₆), 1-octadecyl-3-methylimidazolium hexafluorophosphate ([C₁₈mim]PF₆), 1,3-dimethylimidazolium 1-butyl-3-methylimidazolium hexafluorophosphate ([Bmim]PF₆), bis[(trifluoromethyl)sulfonyl]imide ([Mmim]TF₂N), 1-ethyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide ([Emim]TF₂N), 1-butyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide ([Bmim]TF₂N), 1-ethyl-3-methylimidazolium tetrafluoroborate ([Emim]BF₄), 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim]BF₄), and 1-hexyl-3-methylimidazolium tetrafluoroborate ([Hmim]BF₄), were obtained from Shanghai Cheng Jie Chemical Co., Ltd. (Shanghai, China). Methanol (HPLC grade) and glacial acetic acid (HPLC grade) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. The preparation of glyceryl monoferulate (GMF)

GMF was prepared according to our previous method (Sun and Chen, 2015). Briefly, EF was mixed with glycerol (molar ratio, 1:1) in 100-mL round bottom flasks, which was catalyzed by Novozym 435 (60 mg/mL) at 85 °C in [Bmim]PF₆. The reaction mixture was mixed at 300 rpm using a magnetic stirrer for 10 h under 10 mmHg vacuum pressure. Next, the IL solution of the transesterification mixture was obtained by filtration to remove the immobilized Novozym 435. Then, GMF was separated using deionized water and purified by recrystallization according to our previous methods (Sun et al., 2014). Finally, high-purity GMF ($96.7 \pm 1.7\%$) was obtained.

2.3. Enzymatic transesterification of GMF with MG or DG

GMF (1.5 mmol/mL) was reacted with MG (or DG) (molar ratio of GMF to MG (or DG), 1:0.5) in a 25-mL flask. Different ILs (3 mL) were used as reaction media. The reaction mixture (reactants and ILs) was incubated at various temperatures and mixed at 200 rpm under 98 kPa,

and then Novozym 435 (50 mg/mL) was added to the reaction mixture. Meanwhile, control experiments without lipase or IL were carried out.

2.4. Effects of reaction variables

The factors and levels of reaction variables selected were as follows: reaction temperature (70, 80, 90, 100 and 110 °C), enzyme load (10, 30, 50, 70 and 90 mg/mL), molar ratio of substrates (1:0.1–1:10, mol/mol), and reaction time (h).

2.5. Analysis and characterization

HPLC analysis was performed on Waters 2695 with a C18 reverse phase column (5 μm, 250 mm × 4.6 mm) fitted with a dual absorbance detector (Waters 2487) at 325 nm. Solvent A (methanol) and solvent B (water, 0.5% v/v glacial acetic acid) were the mobile phases, and the flow rate was 1 mL/min. The elution sequence was a linear gradient from 50% (v/v) A to 90% A (v/v) over 10 min, then to 100% A for 30 min, followed by 100% A for 20 min at 35 °C. Products were identified with their major ions detected by HPLC-ESI-MS according to our previous report (Sun et al., 2007). The selectivity for FAG formation could be defined as follows:

$$\text{FAG Selectivity} = \frac{\text{FAG Yield (\%)}}{\text{GMF Conversion (\%)}}$$

2.6. Statistical analysis

All experiments were carried out at least three times. The results were recorded as the mean value ± SD. A two-way analysis of variance (ANOVA) was used to evaluate the differences in mean values. Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Effect of MG and DG as acyl donors

The structure of acyl donors will affect the performance of the acylation reaction and the physic chemical and biological properties of the esters formed. Four types of MG and DG, including monooleate (MO), monostearin (MS), dioleate (DO), and distearin (DS), were used as acyl donors for the enzymatic transesterification of GMF to prepare the lipophilic FAG (Table 1). Compared with a solvent-free system, [C₁₈MIM]PF₆, one type of IL, showed a significant effect on GMF conversion and FAG selectivity. Especially for MO, GMF conversion increased from 19.4% (solvent-free system) to 62.6% (IL system), and FAG selectivity also increased from 56.3% (solvent-free system) to 87.4% (IL system). However, when DS was used as an acyl donor in the IL system, the maximum GMF conversion and FAG selectivity increased to $77.9 \pm 2.7\%$ and $89.0 \pm 0.1\%$, respectively, which were all higher than those of solvent and solvent-free systems (Compton et al., 2000). These results can be ascribed to the presence of the lower steric hindrance of stearic acid in DS than that of oleic acid in DO. In the transesterification products using DS and DO as feruloyl acceptors, the lipophilic FAG yields were $77.9 \pm 2.7\%$ and $69.7 \pm 1.4\%$, respectively, which were higher than those ($55.9 \pm 1.4\%$ and $62.6 \pm 0.4\%$) of MS and MO. These differences were attributed to more available fatty acyl donors of DG than that of MG. A similar effect of fatty acyl donors could also be found in other reactions (Ma et al., 2012).

3.2. Effect of ILs as reaction solvents

No FAG was found in the product of the reaction without enzymes, which suggested that ILs used in the work could not catalyze the reaction. Compared with solvent-free and solvent systems, ILs, as a reservoir for both hydrophilic and hydrophobic substrates and products,

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