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Lipase-catalyzed glycerolysis in ionic liquids directed towards diglyceride synthesis

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ABSTRACT

This work examined the lipase-catalyzed glycerolysis of triglycerides (TG) in a list of commercially available ionic liquids (ILs) with varied cations and anions for the purpose of developing an efficient reaction protocol for diglyceride (DG) production and to understand whether ILs could assist the reaction systems. The reaction performances (reaction rate, TG conversion and DG yield) were found to be greatly dependent on the structure and property of ILs. The reactions in [TOMA·Tf_2N] and Ammoeng 120 produced comparable yield of DG to those most efficient conventional systems but with less by-products. Temperature enhancement generally yields positive effect on the conversion of TG, which was much more significant for the ILs with high viscosity. Unusually, increasing substrate concentration in many types of ILs led to enhanced conversion and yield; whereas the increase of glycerol/TG ratio resulted in a dramatic improvement of the reactions in the ILs with strong acidic anions. This work also sorted out some promising IL candidates, namely the ILs with good DG formation selectivity and the ones being able to achieve high TG conversion, which offered possibility to design binary IL systems. Overall, this study presented the first attempt concerning evaluation and characterization of lipase-catalyzed glycerolysis of TG for DG production in IL-based systems.

1. Introduction

Diglycerides (DGs) are natural components of edible oils, which also represent surface-active molecules widely used as emulsifiers in food industry. DG exists in two regioisomeric forms, *sn*-1,2(2,3)-DG and *sn*-1,3-DG, with a natural isomeric ratio of approximately 3:7. The main reason for DG oil to gain a particular interest recently is that consumption of the oil rich in DG, especially the 1,3-isomer, is proved to have positive effects on human health, namely, suppression of both postprandial serum triglycerides (TG) elevation and body fat accumulation [1]. It was shown that energy value of DG oil was nearly 98% of TG oil with similar fatty acid composition. The bioavailability [2] and absorption rates [3] of the two types of oils were not different from each other. Thus, the health benefits from DG oil are supposed to be the results of their different metabolic fates after absorption by intestinal epithelial cells. There have been a great number of studies focusing on enzymatic production of DG oils in recent years [4–10].

Enzymatic reactions were interestingly shown to be unique and sometimes even more effective in anhydrous solvents than in aqueous condition, the natural environment for enzymes. Hence, nonaqueous phase biocatalysis was boosted as a major area of interest over the last two decades. However, the use of organic solvents was undesirable in most cases due to environmental impacts and, particularly, safety concerns for food applications. To overcome these drawbacks, ionic liquids (ILs) are suggested to be alternative media for enzymatic reactions. The unique properties of ILs, such as near-zero vapor pressure, high chemical and thermal stability, and tunable physicochemical properties, made these compounds popular as a medium for chemical and biochemical reactions [11]. From the biocatalytic point of view, ILs are reported to have protective effects on enzymes for stability enhancement and to be recoverable and recyclable [12]. The most interesting nature of ILs for a synthetic scientist might be their tunable properties, which made it possible to control reaction progress or to optimize reaction towards the desired direction in IL-mediated reaction systems.

The majority of enzymes reported to be active in ILs so far are lipases. Since Lau et al. [13] published their pioneering work involving lipase-catalyzed alcoholysis, ammoniolysis and perhydrolysis, extensive studies concerning lipase-catalyzed reactions in ILs were conducted, such as direct esterification [14] and transesterification [15,16], enantioselective transesterification [17–19], enantioselective hydrolysis [20], enantioselective acylation of amines [21], and enzymatic production of biodiesel [22]. There have been studies on enzymatic glycerolysis for

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monoglycerides (MGs) production as well [23,24]. However, little work is available in the literature concerning enzymatic glycerolysis for production of DGs in ILs [11].

This study, thus, aimed to explore the possibility of using ILs in enzymatic glycerolysis for DG synthesis and find out how ILs can play a role to improve the conversion and product selectivity in a reaction equilibrium containing glycerol, MG, DG, and TG. A list of ILs with different cations and anions were tested for lipase-catalyzed glycerolysis of triolein. The effects of the properties of ILs on reaction efficiency, in terms of TG conversion and DG yield (defined as the percentage of the desired product in the final reaction mixture), were evaluated and compared with representative conventional solvents. A preliminary evaluation on the effects of variations of reaction parameters on glycerolysis was made as well.

2. Materials and methods

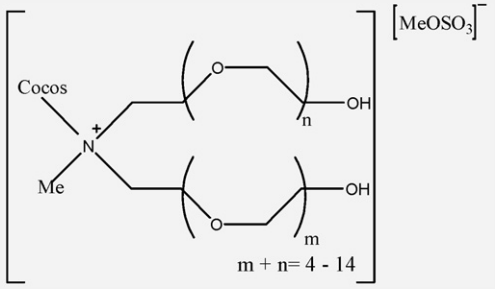
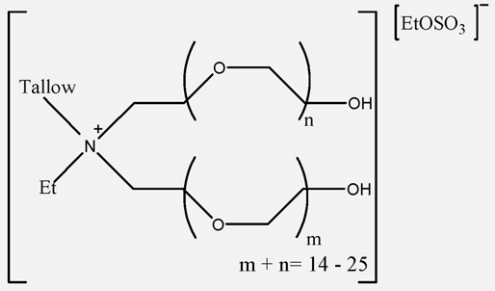
2.1. Materials

Triolein of 90% purity was purchased from Dr. Frischer GmbH (Bremen, Germany) and glycerol of minimum 99% purity was from Sigma–Aldrich Co. (St. Louis, MO). Novozym 435® (*Candida antarctica* lipase B), Lipozyme RM IM® (*Rhizomucor miehei* lipase), and Lipozyme TL IM® (*Thermomyces lanuginosus* lipase) were provided by Novozymes A/S (Bagsvaerd, Denmark). 1-Butyl-3-methylimidazolium tetrafluoroborate ([BMIM]·[BF_4]), 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM]·[PF_6]), 1-butyl-3-methylimidazolium trifluoromethanesulfonate ([BMIM]·[CF_3SO_3]), 1,3-dimethylimidazolium dimethylphosphate ([DMIM]·[DMP]), 1-ethyl-3-methylimidazolium 2(2-methoxyethoxy)ethylsulfate ([EMIM]·[$MDEGSO_4$]), 1-ethyl-3-methylimidazolium *n*-octylsulfate ([EMIM]·[$OctSO_4$]), 1-methyl-3-octylimidazolium tetrafluoroborate ([OMIM]·[BF_4]), 1-methyl-3-octylimidazolium hexafluorophosphate ([OMIM]·[PF_6]), trioctylmethylammonium bis(trifluoromethylsulfonyl)imide ([TOMA]·[Tf_2N]), 1-butyl-3-methylpyridinium dicyanamide ([BMPy][$N(CN)_2$]), 1-butyl-1-methylpyrrolidinium dicyanamide ([BMPyo]·[$N(CN)_2$]), 1-ethyl-3-methylpyridinium perfluorobutanesulfonate ([MeEtPy]·[$C_4F_9SO_3$]), 3-methyl-1-octylpyridinium tetrafluoroborate ([MeOcPy]·[BF_4]), cocosalkyl pentaethoxy methyl ammonium methylsufate (Ammoeng 100), tetraalkyl ammonium sulfate (Ammoeng 102), and quaternary ammonium sulfate (Ammoeng 120) were purchased from Solvent Innovation GmbH (Cologne, Germany), while trioctylmethylammonium trifluoroacetate ([TOMA]·[TFA]) was from Merck (Darmstadt, Germany). The molecular structures of Ammoeng series of ionic liquids are given in Table 1. All solvents used for analysis were of chromatographic grade.

2.2. Experimental procedure for glycerolysis in ILs

In a typical reaction, 1 mmol of triolein (0.885 g) and 0.5 mmol of glycerol (0.045 g) were mixed with 1 g ionic liquid in a 25 mL jacketed reactor. The substrates and the IL are pre-mixed by magnetic agitation at 700 rpm, thereafter the reaction was initiated by the addition of lipase (10 wt% based on the oil mass). The reaction was thermostated at 60 °C by circulated water bath for 48 h with magnetic agitation at 700 rpm. Aliquots of 20 μL were withdrawn at desired time, and analyzed by thin layer chromatography coupled with a flame ionization detector (TLC-FID). Parallel reactions in *tert*-butanol and *tert*-pentanol were conducted in 25 mL jacketed reactors with 5 mL solvent containing 4 mmol oil, 2 mmol glycerol and lipase in the amount of 10 wt% based on the oil mass, at 60 °C for 24 h with 200 rpm stirring. For all the ILs tested, a control reaction without the presence of

Table 1
The molecular structures of Ammoeng series of ionic liquids.

Name	Structure
Ammoeng 100 (cocosaklyl pentaethoxy methyl ammonium methylsulfate)	
Ammoeng 102 (tetraalkyl ammonium sulfate)	
Ammoeng 120 (quaternary ammonium sulfate)