ARTICLE

OBC
www.rsc.org/obc

New opportunity for enzymatic modification of fats and oils with industrial potentials

Zheng Guo and Xuebing Xu*

BioCentrum-DTU, Technical University of Denmark, Building 221, DK-2800 Kgs. Lyngby, Denmark. E-mail: xx@biocentrum.dtu.dk; Fax: +45-45884922; Tel: +45-45252773

Received 16th May 2005, Accepted 25th May 2005
First published as an Advance Article on the web 13th June 2005

Novozym 435 (*Candida antarctica* lipase)-catalyzed glycerolysis of commercial oils and fats to produce monoglycerides (MGs) was investigated using a tetraammonium-based ionic liquid (IL) as a reaction medium. A 90% yield of MGs and nearly 100% conversion of triglycerides in this ionic liquid were achieved, markedly higher than in normal solvents. The amphiphilic structure of cocosalkyl pentaethoxi methyl ammonium methosulfate (CPMA·MS) was suggested to be capable of creating a compatible system for glycerol, oils and fats, as well as inducing the shift of reaction equilibrium to the formation of MG. Interestingly, over a wide range of solvent dosage, the higher yields of MG were observed, indicating the good bulky substrate-tolerating capacity of the IL. The universal validity of the protocol was verified by being successfully applied to different commercial oils and fats. Excellent operational stability of the lipase and the reusability of IL were also observed in consecutive batchwise reactions. The results indicate that the protocol developed in this work provides a new environmentally benign "solution" to the enzymatic modification of fats and oils with industrial potentials.

Introduction

Emerging as eco-friendly solvents, ionic liquids (ILs) have many unique properties, such as negligible vapor pressure, good and tunable solvent power, excellent thermal and chemical stabilities and increased stabilities of enzymes in ILs.[1–6] These properties undoubtedly provide quite a few new opportunities and possible solutions for many chemical or biochemical processes.[7–14] As an ubiquitous enzyme, lipase has also been employed as a model enzyme using ILs as reaction media.[14–17] However, previous reports focused on using simple molecules as model substrates to demonstrate the protocols. Being the natural substrates of lipase, few successful examples for enzymatic modification of fats and oils in ILs have been reported so far.[11–15] The gap between theoretical investigations and practical applications is what motivates our current efforts in this area.

Monoglyceride (MG) constitutes the most important food-grade emulsifier, accounting for 75% of the worldwide production of emulsifiers for food industry.[18] Among those approaches for MG production, the glycerolysis of oils and fats is the most widely used due to its high space–time production (1 mol triglyceride yields 3 mol monoglyceride).[19] Currently the used industrial method is the glycerolysis of fats and oils under alkaline catalysis at high temperatures (220–250 °C), which yields 40–60% monoglycerides depending on the glycerol excess and other reaction parameters.[20] The high temperatures and low yield of MG lead to undesirable byproducts, a burned taste and high energy consumption, as well as further molecular distillation processes to obtain pure MG. Furthermore, the chemical method is unsuitable for the modification of some functional oils with polyunsaturated fatty acids, conjugated linoleic acids *etc*., due to their heat sensitivity.[21–22] Enzymatic glycerolysis is receiving more attention as a promising alternative because of its improving reaction conditions and the high selectivity of biocatalysts.[18–20] However, there remains a problem in this area regarding how to create an efficient system with good compatibility for both oil and glycerol, as well as to shift the reaction equilibrium to generate higher yields of MG. Not surprisingly, many protocols, including employment of excessive glycerol, *in situ* removal of MG by temperature-programmed cooling crystallization,[23–24] as well as applications of traditional solvent engineering *etc*.,[25–27] have been reported as methods to improve this concern. These efforts achieved only a limited success because the slow reaction rate at lower temperatures or the introduction of inflammable and toxic solvents into the reaction system made them impracticable in industrial processes, especially for food industry applications. Here for the first time, we have demonstrated an IL reaction system to produce monoglycerides from commercial fats and oils with higher yields.

Results and discussion

Reaction behaviors of glycerolysis in ionic liquids

Thermodynamic equilibrium of the overall glycerolysis reaction can be described by eqn. (1) or a more detailed mechanism illustration given by eqn. (2) and (3) (Fig. 1A). Previous work has demonstrated that the above equilibrium could be shifted in the direction favoring the production of monoglycerides by

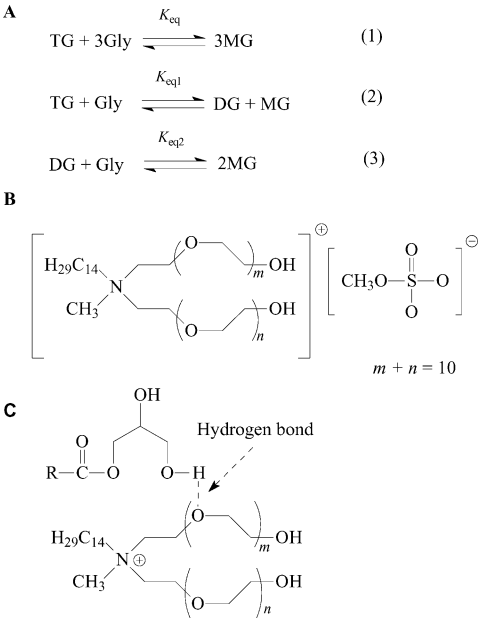

Fig. 1 (A) Thermodynamic equilibrium of the glycerolysis of triglycerides. (B) Structure of cocosalkyl pentaethoxi methyl ammonium methosulfate (CPMA·MS). (C) Proposed mechanism for the decrease of the thermodynamic activity of monoglycerides.

DOI: 10.1039/b506763d

adjusting the solvent system.[25–27] Ionic liquids are claimed to be tailored solvents by the judicious selection of the cation, anion and substituent moiety, making it theoretically possible to tailor an IL molecule to meet the specific requirement of a reaction system, which certainly also includes the enzymatic modification of oils and fats.[1,2,5,18] Therefore we intend to make a new possibility for the enzymatic modification of oils and fats by screening for a suitable molecular structure of ILs. As part of this study, we investigated the enzymatic glycerolysis of sunflower oil in 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) and 1-ethyl-3-methylimidazolium ethylsulfate ([EMIM][$EtSO_4$]). However, the results were disappointing. Little reaction was observed, even though a number of lipases from different sources, such as *Candida Antarctica* (Novozym 435), *Thermomyces lanuginose* (Lipozyme TL IM) and *Rhizomucor miehei* (Lipozyme RM IM), were tested (data not shown). A general phenomenon occurring in these three ILs is that little oil is dissolved in ILs, where clear phase separation could be observed, which is incapable of making an efficient interaction of glycerol and oil and thus yields little reaction.

Encouragingly, the reaction in CPMA·MS gave us a surprise (Fig. 2). Fig. 2A clearly depicts that the enzymatic glycerolysis in CPMA·MS exhibits a significant difference in reaction behaviour when compared to those in the solvent-free system or in *tert*-butanol (proven to be one of the most effective solvents for MG production).[25–28] The first hallmark of the glycerolysis in CPMA·MS is that a markedly higher yield of MG was achieved in comparison to in solvent-free system or using *tert*-butanol. More than 90% yield of MG in CPMA·MS was obtained, compared to only 36% in the solvent-free system and 72% in *tert*-butanol. Another distinction for the reaction in CPMA·MS is that there is always an induction period that occurs during the evolution of the reaction, differing from those in the solvent-free system or in *tert*-butanol (Fig. 2B). The length of the induction period depends on the reaction temperature, which lasts about 7, 2, 1.5 and 1.5 h for the reaction carried out at 40, 50, 60 and 70 °C, respectively. The relative lower initial reaction rate and the strong dependency of reaction time to reach equilibrium on temperature constitute another characteristic of the reaction in CPMA·MS. Usually in solvent-free systems glycerol slows down the reaction by adhering to the enzyme and its support, thus blocking access of hydrophobic substrates as previously reported,[29] which is also observed in this work. The reaction in *tert*-butanol is fast, the equilibrium being reached at 2 h; however, for the reaction in the IL at 70, 60, 50 and 40 °C, it needs 8, 10, 12 and more than 24 h, respectively.

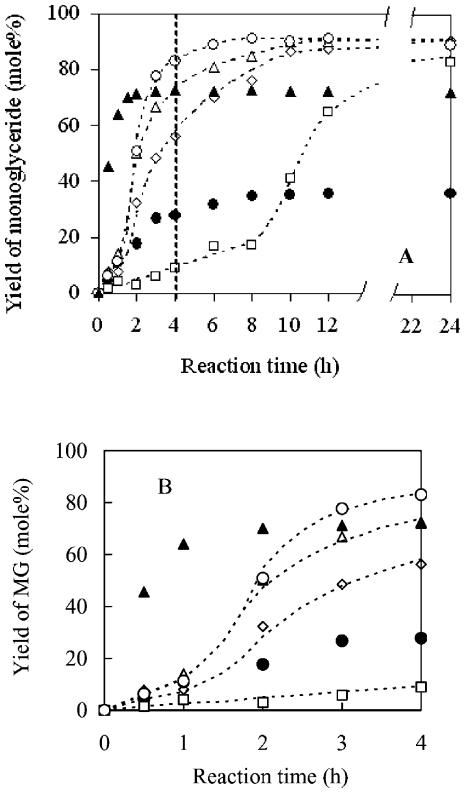

Fig. 2 (A) Different reaction behaviours of the glycerolysis of sunflower oil in CPMA·MS, *tert*-butanol and the solvent-free system (0.5 mmol sunflower oil and 5 × 0.5 mmol glycerol for reactions at □, 40 °C; ◇, 50 °C; Δ, 60 °C; and ○, 70 °C in 2.2 g CPMA·MS and ▲, 50 °C in 2.5 g *tert*-butanol; ●, 2 mmol sunflower oil and 5 × 2 mmol glycerol for reaction at 70 °C in solvent-free system). (B) The sub-figure for the enlarged part of Fig. 2A within 4 h to illustrate the starting reaction stage.

The reaction equilibria shown in Fig. 1A could also be correspondingly described by the following equations:

$$K_{eq} = \frac{a^3_{MG}}{a_{TG} \cdot a^2_{Gly}} = \frac{\chi^3_{MG}}{\chi_{TG} \cdot \chi^2_{Gly}} \cdot \frac{\gamma^3_{MG}}{\gamma_{TG} \cdot \gamma^2_{Gly}} = K_{eq,x} \cdot K_{eq,\gamma} \quad (4)$$

$$K_{eq1} = \frac{a_{DG} \cdot a_{MG}}{a_{TG} \cdot a_{Gly}} = \frac{\chi_{DG} \cdot \chi_{MG}}{\chi_{TG} \cdot \chi_{Gly}} \cdot \frac{\gamma_{DG} \cdot \gamma_{MG}}{\gamma_{TG} \cdot \gamma_{Gly}} = K_{eq1,x} \cdot K_{eq1,\gamma} \quad (5)$$

$$K_{eq2} = \frac{a^2_{MG}}{a_{DG} \cdot a_{Gly}} = \frac{\chi^2_{MG}}{\chi_{DG} \cdot \chi_{Gly}} \cdot \frac{\gamma^2_{MG}}{\gamma_{DG} \cdot \gamma_{Gly}} = K_{eq2,x} \cdot K_{eq2,\gamma} \quad (6)$$

where TG, DG, MG and Gly are triglyceride, diglyceride, monoglyceride and glycerol, respectively. K_{eq}, K_{eq1} and K_{eq2} are the equilibrium constants; a_i, x_i and γ_i represent the thermodynamic activity, mole fraction and activity coefficient of component *i*, respectively. $K_{eq,x}$, $K_{eq1,x}$ and $K_{eq2,x}$, as well as $K_{eq,\gamma}$, $K_{eq1,\gamma}$ and $K_{eq2,\gamma}$ are the corresponding constants denoted by mole fractions and activity coefficients. For a given temperature K_{eq} is constant, however the thermodynamic activity of a component depends on its nature and specific reaction system. The change of the thermodynamic activity of a substrate governs the equilibrium shift. In hydrophilic solvents there is stronger interaction between hydrophilic monoglyceride and solvents than that which occurred between hydrophobic triglyceride or diglyceride and solvents, which result in the lower thermodynamic activity of monoglyceride and higher a_{TG} and a_{DG} values. It has been known that ILs are among the most complex solvents dependent on their broad diversity of anion, cation and attached substituents, which lead to a number of different and often simultaneous solute–solvent interactions, including dispersive, hydrogen bonding and charge–charge action.[30,31] In this work, the unique molecular structure of CPMA·MS, as indicated in Fig. 1B, might give a reasonable explanation for the results in Fig. 2, in which the hydrophobic moiety of the alkyl group and ionic property provide a compatible medium for both oil and glycerol, making them soluble in this IL synchronously. Perhaps this could explain the poor reactivity of oils and fats in the other three ILs mentioned above, due to the poor solubility of oils and fats in these ILs. Furthermore, the moiety of ployethoxyl alcohol in the CPMA·MS molecule could form hydrogen bonds with the hydroxyl group in the glycerol moiety of monoglyceride, as suggested in Fig. 1C, which made the hydroxyl group of monoglyceride unreactive. Of course, the possibilities of the formation of hydrogen bonds between MG and the anion moiety of IL could not be ruled out, though almost no reaction was observed in [EMIM][$EtSO_4$] where similar anion structure existed. The formation of hydrogen bonds will greatly reduce the thermodynamic activity and activity coefficient of MG, which shifts the equilibrium to the formation of MG (eqn. (4)–(6)). If the above hypothesis is correct, glycerol and water molecules in this system with lower activities should be a logical conclusion due to their capability of forming hydrogen bonds with the IL molecule. The lower water activity in the IL has been verified by a_w measurements, in which 1.4% water content only results in

0.108 of a_w at 24 °C. As a result, a lower free fatty acid (FFA) content (<5%) in the final products in the IL system, generated as by-products from hydrolysis, provides an indirect proof for the lower activity of H_2O in the IL and also supports our assumption from another side. Lower activity of glycerol might be one of the factors accounting for the slow initial reaction rate in the IL system, though the reason perhaps could mainly be attributed to the mass-transfer resistance. Therefore, it is most likely that the action of CPMA·MS in this system is not only just as a solvent but also as a "trap" for MG molecules, which made the reaction favour the synthesis of monoglyceride.

Substrate-tolerating capacity of CPMA·MS

To further identify the role of IL molecules in the reaction system and to test its tolerating capacity for substrates, glycerolysis of sunflower oil against varying molar ratios of oil to IL was performed (Table 1). Herein we introduce two terms, *i.e.* "tolerating capacity" concerning substrate and "buffering power" concerning CPMA·MS. The former refers to smaller amounts of IL being able to produce more product and the latter refers to a similar high yield of MG achieved over a wide range of molar ratio of oil–IL. The results in Table 1 show that similar high conversion of oil and high yield of monoglyceride could be obtained when the molar ratio of oil–IL is less than 1.2. Table 1 also reveals that although the $K_{ep2,x}$ value proportionally declines with the increasing molar ratio, the value of $K_{ep1,x}$ does not significantly decease, which results in a bigger overall equilibrium constant, $K_{ep,x}$, and a higher conversion of oil. Another interesting suggestion from the results is that the tolerating capacity of CPMA·MS molecule to adapt MG molecule is not unlimited, but the "buffering power" is higher. Taking into account the yield of MG and conversion of oil at an oil–IL molar ratio of 1.2 (Table 1), as well as one oil molecule generating three MG molecules, it is easily estimated that one mole of IL molecules could effectively "trap" three moles of monoglyceride molecules. This is of course an arbitrarily estimated value and no obvious changing border was observed. It is definitely of importance for the industrial interest of this approach, which makes it more competitive in cost since a lower amount of IL could give a higher productivity.

Universal validity of the reaction protocol

Effects of fatty acid chain length and solvent polarity on monoglyceride formation and equilibrium position in traditional solvents have been intensively studied.[32–34] However, little is known about the regiospecificity and acyl chain dependency of lipases and their influence on equilibrium shifting in IL systems. Commercial oils and fats from different sources have varied fatty acid profiles. As mentioned above, our concern is whether this system could be effectively applied to different types of oils and fats. To verify the universal validity of the protocol, we selected another four representative commercial oils and fats as testing materials, namely palm stearin, rapeseed oil, fish oil and CLATG, rich in palmitic acid (56%), oleic acid (58%), polyunsaturated fatty acid (20 : 5ω3 + 22 : 5ω3 + 22 : 6ω3, 33%), and conjugated linoleic acid (80%), respectively (Table 2). As expected, higher conversion and higher yield of MG were obtained for all tested oils and fats. Free fatty acid contents in the final products were all less than 2%. These results confirmed the general applicability of this technology and the validity of our suggested mechanisms.

Comparison of the yields of different oils and fats in Table 2 reveals that CLATG with high purity gave the highest yield of MG. Other commercial oils consisting of diverse fatty acid profiles yield different results, indicating the selection of IL for the different acyl moieties of TG and the subsequent effect on the equilibrium of the reaction. Therefore, the work in this area employing pure homogeneous TG will help to figure out the interactions between IL and glyceride molecules with varied chain lengths and their contribution to equilibrium shifting. This work is being carried out in our group.

Effect of IL on lipase activity and operational stability

Recently Welton and his coworker[35] investigated the molecular states of water in ILs and identified that water molecules in ILs could form H-bonded complexes with the anion moiety of ILs. The strength of H-bonding depends on the basicity of anions.[36] The work of Lau *et al.*[37] demonstrated that ILs affected the structure and activity of lipases and also found that this effect was related to the anion of ILs. According to their results, the anion of CPMA·MS was among those with strong basicity, which had strong dehydrating ability and generated a negative effect on the activity of enzymes. The influences have been observed in this work, as indicated in Fig. 3, in which the activities of lipases are denoted by the changes of relative content of TG and MG with time. After the 24 h reaction, the immobilized lipase from *Rhizomucor miehei* only achieved 15% conversion of TG and even less conversion for the immobilized lipase from *Thermomyces lanuginose*. It is postulated that the strong dehydration of IL might account for the low activity of lipases. Novozym 435 exhibited a different behavior, where 99% conversion of TG was obtained with 89% yield of MG

Table 1 Effects of the molar ratio of sunflower oil to ionic liquid

Molar ratio of oil–IL	0.2 : 1	0.4 : 1	0.8 : 1	1.2 : 1	1.6 : 1	6.3 : 1	8.1 : 1
Conversion of oil (mole%)	98.60	99.07	98.60	97.29	96.63	79.37	78.65
Yield of monoglyceride (mole%)	91.88	90.41	88.11	86.68	80.77	66.74	54.88
Percentage of FFA in mixture (mole%)	5.85	4.03	2.77	1.49	0.77	0.25	0.33
$K_{ep,x}$	32.29 ± 2.51	57.4 ± 12.51	19.72 ± 1.44	8.24 ± 0.74	3.50 ± 0.32	0.08 ± 0.01	0.03 ± 0.00
$K_{ep1,x}$	3.29 ± 0.02	10.39 ± 1.91	6.71 ± 0.14	4.64 ± 0.43	3.44 ± 0.21	0.24 ± 0.05	0.20 ± 0.03
$K_{ep2,x}$	9.81 ± 0.80	5.46 ± 0.40	2.94 ± 0.28	1.80 ± 0.04	1.05 ± 0.04	0.32 ± 0.04	0.13 ± 0.02

Reaction conditions: 70 °C, Novozym 435: 5% of oil weight, 600 rpm, molar ratio of oil–glycerol, 1 : 5 and 24 h

Table 2 Glycerolysis in IL employing different commercial oils or fats

[a]Oils or fats	Sunflower oil	Rapeseed oil	Palm stearin	Fish oil	CLATG
Conversion of oil (mole%)	98.90	95.58	97.13	99.87	97.90
Yield of monoglyceride (mole%)	89.09	88.61	90.70	88.20	93.30
Percentage of FFA in mixture (mole%)	1.81	1.40	1.10	1.75	0.40

[a] Calculation of the average molecular weight of each oil species is based on its FA composition according to GC analysis. Reaction temperature, 70 °C and reaction time, 24 h.

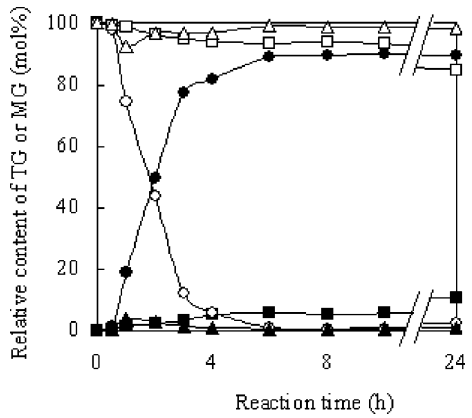

Fig. 3 Glycerolysis activities of the immobilized lipases from different sources in CPMA·MS (relative content of TG: □, Lipozyme RM IM; Δ, Lipozyme TL IM and ○, Novozym 435; and MG: ■, Lipozyme RM IM; ▲, Lipozyme TL IM and ●, Novozym 435). Reaction conditions: 100 mg lipase catalyzed glycerolysis of 0.5 mmol sunflower oil and 2.5 mmol glycerol at 60 °C.

after 6 h. This result agrees with the previous observation that *Candida antarctica* lipase B could retain its catalytic activity even in water-depriving solvents.[38] It is assumed that this enzyme has a special structure to bind the essential water for catalysis and consequently can work well at low water activities.

To further validate the stability of enzyme in reuses and the reusability of the Novozym 435–CPMA·MS system, we conducted a consecutive batchwise reaction. Fig. 4 depicts the residual activity profiles denoted by conversion of sunflower oil, yield of monoglyceride and free fatty acid content. The results showed that no significant activity loss was observed even after ten consecutive reactions with the duration of 10 h per batch and a few ethyl ether extraction processes. Similar operational stability of *Candida antarctica* lipase in other IL systems was also observed in previous work.[12,39,40] The reusability of the IL–enzyme system not only demonstrates the practical usability of the method in actual applications, but also indicates the commercial competence in comparison with other approaches.

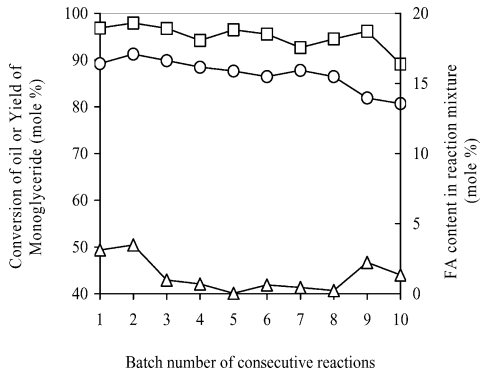

Fig. 4 Reusability of CPMA·MS and Novozym 435 in the consecutive batchwise glycerolysis of sunflower oil (□, conversion of sunflower oil; ○, yield of monoglyceride; and Δ, free fatty acid content in the final reaction mixture of each batch).

Conclusions

In conclusion, we have developed a new and highly efficient reaction system to carry out the enzymatic modification of oils and fats in ionic liquids. Higher yield of the desired product, better tolerating capacity of IL for substrate and considerable stability of enzyme in consecutive reuses demonstrate that this eco-friendly technology has industrial application potentials. Furthermore, the work clearly shows the structure–function relationship of ionic liquids and how the reaction system affects the selectivity of enzymes and reaction equilibrium. We believe that this work will initiate more attention on applying ILs to the biomodification of oils and fats and a thorough evaluation of its application potentials.

Experimental

Materials

Novozym 435 (from *Candida antarctica*), Lipozyme TL IM (from *Thermomyces lanuginosa*) and Lipozyme RM IM (from *Rhizomucor miehei*) were provided by Novozymes A/S (Bagsvaerd, Denmark). Glycerol (minimum 99%) was purchased from Sigma-Aldrich Co. (St. Louis, USA). Sunflower oil and rapeseed oil were obtained from Aarhus United (Aarhus, Denmark), triglycerides of conjugated linoleic acids from Cognis Deutschland GmbH (Monheim, Germany), palm stearin from Golden Hope (Kuala Lumpur, Malaysia) and fish oil from Maritex (Aarhus, Denmark). 1-Butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]), 1-ethyl-3-methylimidazolium ethylsulfate ([EMIM][$EtSO_4$]) and cocosalkyl pentaethoxi methyl ammonium methosulfate (CPMA·MS) were procured from Solvent Innovation Gmbh (Köln, Germany) and of 98% minimum purity. Other chemicals and reagents were all of analytical grade and used as received.

Typical experimental procedure for glycerolysis

Typical experimental procedure for glycerolysis of oils or fats in IL: 2 mmol oil or fat and 5 × 2 mmol glycerol were mixed with 2 mL (2.2 g) cocosalkyl pentaethoxi methyl ammonium methosulfate (CPMA·MS) in a 25 mL jacketed vial by magnetic stirring. The reaction was initiated by the addition of 100 mg Novozym 435 (*Candida antarctica* lipase B) and conducted at the desired temperature, controlled by the circulated water. The evolution of the reaction was monitored by sample withdrawal and the TLC–FID analysis after sample dissolving in chloroform–methanol (2 : 1 v/v). All reactions were performed in duplicate. The developing solvents for TLC–FID consist of *n*-hexane, diethyl ether and acetic acid (35 : 35 : 1 v/v/v). Area percentage on a glycerol-free basis was used as weight for the calculation of the conversion of oil and yield of monoglyceride.[41] Fatty acid composition of oils and fats from commercial sources was analysis by GC.[42] Water content in solvents and substrates was determined by Karl Fischer titration. The water activity (a_w) of ILs with specific water content was measured with an Aqualab Water Activity Meter (Decagon Devices, Inc., Washington, USA) at the set temperature.

Reusability of IL–lipase system

Procedure for consecutive batchwise glycerolysis using recycled IL and enzyme: the first batch glycerolysis of 2 mmol sunflower oil and 10 mmol glycerol with 150 mg Novozym 435 was conducted at 60 °C and 400 rpm for 10 h. At the end of reaction the reaction mixture was extracted by 25 mL diethyl ether and centrifuged for separation. The IL layer containing unreacted glycerol and enzyme was subjected to twice extraction with 25 mL diethyl ether. The resulting IL with the suspension of enzyme and remaining glycerol was employed for the next batch, initiated by the addition of 20 mmol fresh oil and 35 mmol glycerol to compensate for its consumption in the previous batch. The above procedure was repeated nine times. The sample withdrawn from the reaction mixture at the end of each batch was subjected to TLC–FID analysis.

References

1 *Ionic Liquids in Synthesis*, ed. P. Wasserscheid and T. Welton, Wiley-VCH, Weinheim, 2003.

2 *Ionic Liquids as Green Solvents*, ed. R. D. Rogers and K. R. Seddon, ACS Symposium Series 856, American Chemical Society, 2002.
3 R. Sheldon, *Chem. Commun.*, 2001, **23**, 2399–2407.
4 J. Dupont, R. F. de Souza and P. A. Z. Suarez, *Chem. Rev.*, 2002, **102**, 3667–3692.
5 U. Kragl, M. Eckstein and N. Kaftzik, *Curr. Opin. Biotechnol.*, 2002, **13**, 565–570.
6 N. Jain, A. Kumar, S. Chauhan and S. M. S. Chauhan, *Tetrahedron*, 2005, **61**, 1015–1060.
7 T. Itoh, E. Akasaki, K. Kudo and S. Shirakami, *Chem. Lett.*, 2001, 262–263.
8 S. H. Schöfer, N. Kaftzik, P. Wasserscheid and U. Kragl, *Chem. Commun.*, 2001, 425–426.
9 J. A. Laszlo and D. L. Compton, *Biotechnol. Bioeng.*, 2001, **75**, 181–186.
10 K.-W. Kim, B. Song, M.-Y. Choi and M.-J. Kim, *Org. Lett.*, 2001, **3**, 1507–1509.
11 P. Lozano, T. de Diego, D. Carrié, M. Vaultier and J. L. Iborra, *Chem. Commun.*, 2002, **7**, 692–693.
12 M. T. Reetz, W. Wiesenhöfer, G. Franciò and W. Leitner, *Chem. Commun.*, 2002, **9**, 992–993.
13 M. Eckstein, M. V. Filho, A. Liese and U. Kragl, *Chem. Commun.*, 2004, **9**, 1084–1085.
14 R. M. Lau, F. van Rantwijik, K. R. Seddon and R. A. Sheldon, *Org. Lett.*, 2000, **26**, 4189–4191.
15 S. Park and R. J. Kazlauskas, *J. Org. Chem.*, 2001, **66**, 8395–8401.
16 L. Gubicza, N. Nemestóthy, T. Fráter and K. Bélafi-Bakó, *Green Chem.*, 2003, **5**, 236–239.
17 S. Park and R. J. Kazlauskas, *Curr. Opin. Biotechnol.*, 2003, **14**, 432–437.
18 J. C. Bellot, L. Choisnard, E. Castillo and A. Marty, *Enzyme Microb. Technol.*, 2001, **28**, 362–369.
19 I. Elfman-Börjesson and M. Härröd, *J. Am. Oil Chem. Soc.*, 1999, **76**(6), 701–707.
20 U. T. Bornscheuer, *Enzyme Microb. Technol.*, 1995, **17**, 578–586.
21 Z. Guo and Y. Sun, *Biotechnol. Prog.*, 2004, **20**, 619–622.
22 Y. Watanabe, Y. Yamauchi-Sato, T. Nagao, T. Yamamoto, K. Ogita and Y. Shimada, *J. Mol. Catal. B: Enzym.*, 2004, **27**, 249–254.
23 G. P. McNeill, S. Shimizu and T. Yamane, *J. Am. Oil Chem. Soc.*, 1991, **68**, 6–10.
24 R. Rosu, Y. Uozaki, Y. Iwasaki and T. Yamane, *J. Am. Oil Chem. Soc.*, 1997, **74**(4), 445–450.
25 P. J. Halling, *Biotechnol. Bioeng.*, 1990, **35**, 691–701.
26 A. E. M. Janssen, A. van der Padt, H. M. van Sonsbeek and K. van't Riet, *Biotechnol. Bioeng.*, 1993, **41**, 95–103.
27 X. Rendón, A. López-Munguía and E Castillo, *J.Am. Oil Chem. Soc.*, 2001, **78**, 106–1066.
28 T. Yang, M. Rebsdorf, U. Engerud and X. Xu, *J. Agric. Food Chem.*, 2005, **53**, 1475–1481.
29 E. Castillo, V. Dossat, M. Alain, J. S. Condoret and D. Combes, *J. Am. Oil Chem. Soc.*, 1997, **74**, 77–85.
30 J. L. Anderson, J. Ding, T. Welton and D. W. Armstrong, *J. Am. Chem. Soc.*, 2002, **124**, 14247–14254.
31 J. L. Kaar, A. M. Jesionowski, J. A. Berberich, R. Moulton and A. J. Russell, *J. Am. Chem. Soc.*, 2003, **125**, 4125–4131.
32 G. P. McNeill, D. Borowitz and R. G. Berger, *J. Am. Oil Chem. Soc.*, 1992, **69**, 1098–1103.
33 S.-J Kuo and K. L. Parkin, *J. Am. Oil Chem. Soc.*, 1996, **73**, 1427–1433.
34 G. Boswinkel, J. T. P. Derksen, K. Van't Riet and F. P. Cuperus, *J. Am. Oil Chem. Soc.*, 1996, **73**, 704–711.
35 L Cammarata, S. G. Kazarian, P. A. Salter and T. Welton, *Phys. Chem. Chem. Phys.*, 2001, **3**, 5192–5200.
36 L. Crowhurst, P. R. Mawdsley, J. M. Perez-Arlandis, P. A. Salter and T. Welton, *Phys. Chem. Chem. Phys.*, 2003, **5**, 2790–2794.
37 R. M. Lau, M. J. Sorgedrager, G. Garrea, F. van Rantwijk, F. Secundo and R. A. Sheldon, *Green Chem.*, 2004, **6**, 483–487.
38 W. Piyatheerawong, Y. Iwasaki, X. Xu and T. Yamane, *J. Mol. Catal. B: Enzym.*, 2004, **28**, 19–24.
39 P. Lozano, T. de Diego, D. Carrié, M. Vaultier and J. L. Iborra, *Biotechnol. Lett.*, 2001, **23**, 1529–1533.
40 T. Itoh, S. Han, Y. Matsushita and S. Hayase, *J. Mol. Catal. B: Enzym.*, 2003, **26**, 41–45.
41 T. Tatara, T. Fujii, T. Kawase and M. Minagawa, *Lipids*, 1983, 732–736.
42 H. Zhang, X. Xu, H. Mu, J. Nilsson, J. Adler-Nissen and C.-E. Høy, *Eur. J. Lipid Sci. Technol.*, 2000, **102**, 411–418.