OCL 2015, 22(4) D408
© E. Durand *et al.*, Published by EDP Sciences 2015
DOI: 10.1051/ocl/2015026

OCL
Oilseeds & fats Crops and Lipids
Available online at:
www.ocl-journal.org

REVIEW – DOSSIER
OPEN ACCESS

12TH EURO FED LIPID CONGRESS: OILS, FATS AND LIPIDS:
FROM LIPIDOMICS TO INDUSTRIAL INNOVATION

Are emerging deep eutectic solvents (DES) relevant for lipase-catalyzed lipophilizations?

Erwann Durand*, Jérôme Lecomte and Pierre Villeneuve

CIRAD, UMR IATE, 34398 Montpellier, France

Received 26 May 2015 – Accepted 2 June 2015

Abstract – With the recent interest on green chemistry, the scientists have focused on developing new and more efficient solvents to carry out enzymatic-catalyzed reactions with emphasis on reduced costs, risks and toxicity while improving biodegradability. Among the new available solvents, the multimolecular-based liquids (such as ionic liquids and eutectic solvents) have been the subject of most recent studies. Currently, and mainly due to its environmental and economic features, DES are arousing much interest and curiosity. Regarding the biotransformations with lipases, the so-called "lipophilization" reactions are of major interest. However, they are complex to implement mainly because it is difficult to find a suitable reaction medium. Thus, this review aimed at providing a presentation of these multimolecular-based solvents with general overview of the recent studies dealing with lipase-catalyzed reactions in DES. In addition, emphasis was placed on their strengths and weaknesses, especially with the perspective to be use as efficient and green medium to implement complex and valuable biotransformation such as lipase-catalyzed lipophilizations.

Keywords: Low transition temperature mixtures / deep eutectic solvents / Lipase / Lipophilization

Résumé – Solvants de type eutectiques profonds : milieux réactionnels adaptés aux réactions de lipophilisation biocatalysées par les lipases ? Depuis le début des années 1990 et l'émergence de la « chimie verte », il est indispensable de concevoir des nouveaux procédés, en particulier enzymatiques, qui mettent l'accent sur la réduction des coûts, des risques et de la toxicité tout en améliorant la biodégradabilité. Les nouveaux solvants de type « liquides multimoléculaires » (tels que les liquides ioniques et les solvants eutectiques) ont fait l'objet de la plupart des études récentes. Actuellement, et principalement en raison de ses atouts environnementaux et économiques, les solvants eutectiques suscitent beaucoup plus d'intérêt et de curiosité. Concernant les biotransformations lipasiques, les réactions dites « de lipophilisation » présentent de nombreux avantages. Cependant, ces réactions sont complexes à mettre en œuvre, principalement parce qu'il est difficile de trouver un milieu réactionnel adapté. Ainsi, cette revue a pour objectif de fournir une présentation de ces milieux réactionnels avec un aperçu des travaux récents portant sur les réactions lipasiques dans des solvants eutectiques. En outre, l'accent a été mis sur leurs forces et leurs faiblesses, avec la perspective d'être utilisés comme milieu réactionnel « vert » pour mettre en œuvre des biotransformations complexes telles que les réactions de lipophilisation biocatalysées par les lipases.

Mots clés : Mélanges à faible transition de température / solvants eutectiques profonds / lipase / lipophilisation

1 Introduction

Since the beginning of the 1990s and the emergence of "green chemistry", it is essential to design new processes to reduce or eliminate the use or generation of harmful substances. The objective is to minimize hazards while maintaining or even increasing the efficiency of current processes. That is why, the use of new eco-compatible and efficient reaction media (other than conventional molecular solvents derived from fossil fuels) is now of growing interest.

In this context, the scientific community is craving for a new family of solvent called "Low Transition Temperature Mixtures (LTTM)". These solvents are composed of two or more constituents that are able to self-associate by non-covalent interactions to form a mixture with a significantly lower *liquidus* domain. Among them and probably the most popular subfamily are ionic liquids (IL), which were the first ones to be considered as possible substitutes for conventional organic solvents for chemical or biocatalysis reactions. However, although they are very promising owing to their non-volatility, thermal stability and their ability to solubilize a wide range of molecules, their implementation beyond the laboratory scale is rather poor. Indeed, besides their complex preparation, ionic liquids are very expensive. On the other hand, their non-toxicity previously put forward to justify their use, has recently been questioned. More recently, a new emerging subfamily of LTTM named "deep eutectic solvent (DES)" has been described as a serious alternative to IL, with more realistic and economical features. Just like IL, DES may be

---

* Correspondence: `erwann.durand@cirad.fr`

liquid at room temperature, non-volatile and with an excellent thermal stability. However, in contrast to most of the IL, DES are easy to prepare with a decreased harmfulness and improved biodegradability.

In the field of biocatalysis, whereas the studies on IL are deeply documented, the published papers dealing with biotransformation reactions in DES are scarce. Concerning the lipase-catalyzed reactions, in addition to their exhaustive and intense applications in the lipids' transformation, these enzymes may also be suited in so-called "lipophilization reactions" for the synthesis of new molecules with high-value-added (surfactants or lipophilized antioxidants). However, such biotransformations are somewhat complex to implement with often limited yields and slow kinetics. These complications are mainly due to the difference in polarity of the two substrates involved and the difficulty in finding a suitable medium for solubilization, while allowing a good biocatalytic activity (Villeneuve, 2007). Besides these experimental issues, there is a great interest and attractive challenge in finding, creating and developing new and green media to implement such biotransformation processes. That is why, this review aimed at examining the potential of new and "green" media (with focus on DES) for the lipase-catalyzed lipophilization of molecule with great potential (*e.g.* phenolic compounds).

2 LTTM, a new family of promising green media

Regardless of the application within the different scientific fields, the role of the solvent holds a major place. Since the beginning of the green chemistry concept, one can point out that the new emerging solvents with potential "green" assets are almost exclusively composed of at least two molecules. Moreover, the unique common property of these new multimolecular-based solvents is their low transition temperature (solid-liquid) compare to ones of their constituents. In summary, these new green media are formed by the association of two or more components that are able to self-associate through intermolecular interactions (mainly Van der Waals interactions, hydrogen bonding or ionic forces) to form a mixture with a solid-liquid transition temperature significantly lower than that of each individual component. That is why we got into the habit of naming these solvents "Low Transition Temperature Mixtures (LTTM)". The two most widespread subfamilies of LTTM are Deep Eutectic Solvents (DES) and ionic liquids (IL). IL are multi-component liquid salts that differ from the molten salts because of the melting point below 100 °C (arbitrarily set in reference to the boiling point of water), but a large number of them are liquid at temperature around 25 °C. IL are constituted of a cation, usually organic, combined with an organic or inorganic anion. The cations are quaternary phosphoniums or ammonium salts (tetraalkylammoniums, tetraalkylphosphoniums, alkylpyridiniums, triazoliums, pyrrolidiniums or imidazoliums) generally large and asymmetric that may be functionalized with amines, alcohols, ethers, carboxylic acids, esters, thiols, vinyls, allyls, alkynes or nitriles. The anions may be organic or inorganic with different sizes, electronegativities or polarizabilities. Thus, the huge choice of combinations anions/cations ($> 10^6$) offers the possibility of adjusting the physicochemical characteristics of the medium. Consequently, IL are capable of dissolving a wide range of organic and inorganic compounds with very different polarities. Their application as reaction solvent has many advantages because of a wide range of thermal and chemical stability, high conductivity (range from 0.01 to 1.5 S/m), non-volatility (decomposition temperature typically over 300 °C) and non-flammability. Nevertheless, the use of IL as reaction media for chemical or enzymatic synthesis faces with many problems. Indeed, their controversial ecotoxicological data and environmental safety (Ranke *et al.*, 2004; Stolte *et al.*, 2006), associated with their high costs, their difficulties to synthesis and their tedious purification, are considerably limiting their implementation on an industrial scale. In conclusion, although the IL have original physico-chemical properties compared to other solvents typically encountered in chemistry, the widespread use of IL is deeply affected by economical and environmental imperatives. That is why, we believe that currently the use of such solvents will be only realistic for low productions of specific and very high-value molecules. To overcome IL's drawbacks while maintaining their advantages, the use of DES is now rapidly emerging in the literature. These solvents were initially described by Abbott an co-workers as any mixture characterized by an significant ("deep") decrease in the melting point compared to its individual counterpart (Abbott *et al.*, 2004, 2003). Most of these mixtures are actually obtained by mixing an ammonium or phosphonium salt with a hydrogen-bond donor such as acids, amides, amines, alcohols, etc... as a result, numerous opportunities to prepare DES because of the high flexibility to choose the components (Kareem *et al.*, 2010). Compare to IL, DES display many advantages such as a low price, a very easy preparation without any purification steps (they are simply prepared by mixing the components), and most of them are biodegradable (Durand, Lecomte, Villeneuve, 2013). Thus, the number of publications dedicated to their use has considerably increased in many fields of research such as metal recovery, gas purification, catalysis, chemical fixation of CO_2 (Li *et al.*, 2008; Su *et al.*, 2009), extraction of phytochemicals from plant material (Dai *et al.*, 2013; Nam *et al.*, 2015) or green solvents for biocatalysis (Alemán *et al.*, 2015; Durand *et al.*,2012; Durand, Lecomte, Baréa, 2013; Gorke *et al.*, 2008; Lindberg *et al.*, 2010; Maugeri *et al.*, 2013; Zhao *et al.*, 2011, 2013). Recently, the term Natural Deep Eutectic Solvents (NADES) was introduced in literature as a new subfamily of LTTM, to label any mixture combining molecules abundantly present in the cellular constituents (*e.g.* primary metabolites) (Choi *et al.*, 2011). In their article entitled "Are natural deep eutectic solvents the missing link in understanding cellular metabolism and physiology", Choi *et al.* (2011), postulated that although their accurate role and presence in living cells are still in investigation, NADES may be an alternative medium to water and lipids, and would explain numerous biological processes such as biosynthesis or storage of various non-water soluble metabolites and macromolecules.

3 Interest and challenge of Lipophilization reactions

Lipophilization consists in a strategy where the hydrophilicity/lipophilicity balance of a selected hydrophilic

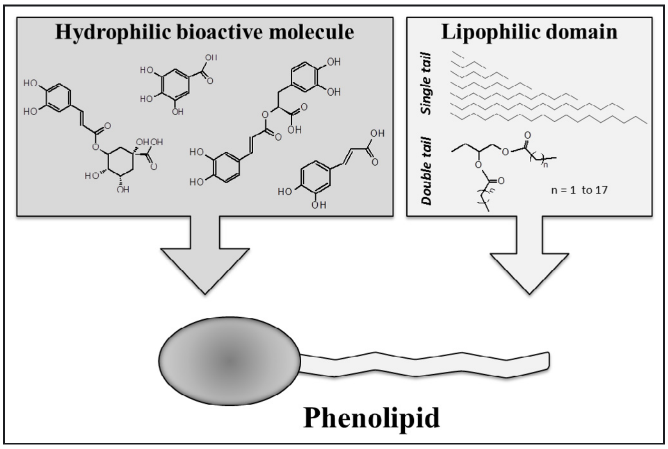

Fig. 1. General strategy for phenolipid conception.

bioactive molecule is adjusted by a covalent linking with a lipophilic domain. These reactions are mainly aimed at lowering the polarity of the hydrophilic molecule, and many examples of lipophilization are now found in the literature. This can be onto a sugar, an amino acid, a protein, a water soluble vitamin or alternatively a (poly)phenol. Among them, phenolic acids represent a large and diverse group of secondary metabolites that are naturally widespread in the plant kingdom and of particular interest. Indeed, these products are known for their potential protective role against oxidative damage and their action as natural antioxidants in various foods, cosmetics or pharmaceutical systems was already proved (Huang and Cai, 2009; Soobrattee *et al.*, 2005; Vanden Berghe, 2012). However, most of the phenolic acids express their functional properties in a hydrophilic environment, resulting in a few efficient and advanced applications in formulated-lipid dispersions. Thus, a practical approach to implement these compounds in such formulation is to increase their hydrophobicity. In that sense, the lipophilization reactions are particularly advantageous and effective, and may be seen as a vectorization key unlocking the lipid barrier encountered by the bioactive molecule while maintaining its original functional properties. In the case of phenolic acids, the methods commonly used consists in attaching on the reactive carboxyl group, either a single or double tail lipophilic domain (usually an aliphatic with a different carbon backbone) resulting in new molecule with emulsifying properties, and often greatly improved antioxidant activity (Durand *et al.*, 2015; Laguerre *et al.*, 2010). This reaction can be performed on simple phenolic acids derived from benzoic acid (hydroxybenzoic acids), cinnamic (hydroxycinnamic acids) or more complex polyphenolic acids such as chlorogenic acid and rosmarinic acid with single or double tail (Fig. 1). Lipophilization of the phenolic antioxidants can be carried out chemically or enzymatically. The chemical strategies are faintly used because of the instability at high temperatures and alkaline environments of these molecules. Concomitantly, the enzymatic alternative (using lipase as biocatalyst) has many advantages: milder reaction conditions, selectivity, reduction of by-products, less purification steps, and also allows to implement more environmentally friendly processes in agreement with this new era of green chemistry. However, one of the main difficulties encountered to realize such lipase-catalyzed lipophilizations is optimizing the synthesis (in terms of yield and kinetics) with two substrates having different polarities. Therefore, it is essential to select a medium in which the two substrates will be soluble, at least partially, and where the activity/stability of the enzyme can be maintained at a satisfactory level. Indeed, in nonpolar solvents such as hexane which lipases generally maintain good activity, the hydrophilic phenolics have very low solubility. Inversely, in polar organic media, the phenolic compounds have good solubility but lipases often present limited activity. Thus, there is a technical dilemma in choosing an effective system in which a polar substrate (*e.g.* phenolics) and nonpolar (a long chain alcohol, for example) will have both high solubility and where the enzymes maintain a good activity (Figueroa-Espinoza and Villeneuve, 2005). Nevertheless, there are some reports dealing with esterification of phenolic acids with fatty alcohols in organic solvents, solvent-free conditions (the role of which was ensured by the excess of alcohol) or in IL (Pang *et al.*, 2013; Weitkamp *et al.*, 2006; Yang *et al.*, 2012). According to these studies, the lipase-catalyzed reactions in organic solvents are preferable and a binary mixture of solvents seems to be the best solution to achieve this kind of reaction since it allows to finely tune both the solubility of the substrates and the lipase's activity/stability. However, these enzymatic reactions are often limited in terms of performance with often extremely long reaction times. In addition, the use of a significant amount of biocatalyst and very high alcohol to phenolics molar ratios was often observed, which may cause poor mass transfer and difficulties when it comes to separation and purification of the final products. In the end, although these reactions have showed tremendous potential and interesting perspective, they are still complex to implement because of the difficulty in finding a suitable reaction medium. Besides this, the recent interest on green chemistry has forced the scientists to pay attention to other criteria (cost, risk and toxicity, biodegradability, etc.) when it comes to develop new and more efficient solvents to carry out enzymatic-catalyzed reactions.

4 Lipase-catalyzed lipophilisation in DES

Compared with other applications, the use of DES for biocatalysis reactions has been little studied, likely because the use of strong hydrogen-bond donors forming such mixtures (*e.g.* urea) is well known to denature proteins. However, some studies have shown that DES could be used as promising solvent or co-solvent in biocatalysis reactions. As observed in the ring opening reactions of 1R, 2R-trans-2-methylstyrene oxide into its corresponding vicinal diol, using potato epoxide hydrolase (StEH1) (Lindberg *et al.*, 2010), where the DES as co-solvent significantly increased the Km (up to 20 times) of the enzyme. In addition, the DES allowed to solubilize 1.5 times more substrates than the reference solution (sodium phosphate buffer 0.1 M). Among the DES tested, cholinium chloride:glycerol (ChCl:Gly) appeared to be the most effective to increase regio-selectivity while preserving enzyme activity. Similar results were observed with the conversion of the epoxy-styrene using EHAD1 epoxide hydrolase (Gorke *et al.*, 2008) where the conversion, only 4.6% in the buffer solution, was increased to 92% with 25% (v/v) addition of ChCl:Gly. Interestingly, a similar addition of organic solvents (DMSO or acetonitrile) did not provoke significant improvement, suggesting that the effect was not simply due to the increased solubility of substrates, but related to the inherent properties of the DES. Moreover, it has been observed that the efficiency of proteases-catalyzed reactions (subtilisin and α-chymotrypsin) in ChCl:Gly have an activity and selectivity that depends on the water content of the mixture (Zhao *et al.*, 2011). Thus, a small amount of water (about 5% v/v) may increase the enzymatic activity while decreasing the selectivity. The positive effect of low water content in DES was also detected for peptide synthesis using protease as biocatalyst (Maugeri *et al.*, 2013). Finally, 10% to 25% (v/v) addition of ChCl:Gly in acetate buffer allowed to significantly improve (up to 3 times) the esterase activity for the hydrolysis of p-nitrophenyl acetate (Gorke and Kazlauskas, 2009). Regarding the lipase-catalyzed reactions in such solvents, the first works were carried out in 2008 by the team of the Prof. R.J. Kazlauskas (Gorke *et al.*, 2008; Gorke and Kazlauskas, 2009). These preliminary studies aimed at investigating the potential of several lipases to catalyze the transesterification of ethyl valerate with butanol in some DES. Among them, lipase B from *Candida antarctica* showed the highest activity, and the best bioconversions were obtained in ChCl:Gly and ChCl:Urea. In addition, the best results observed in DES based on glycerol or urea, were five times higher than those observed in IL (BMIM[BF_4] or BMIM[Tf_2N]), but similar to that observed in toluene. Interestingly, unexpected good lipase stability in DES was observed, despite the presence of denaturing substance, with a superior stability to those observed in the aqueous solutions of the individual component. Indeed, a 75% loss of activity was detected after only 90 min in an aqueous solution of urea, while it was almost negligible (1%) in the DES based on the same urea as component. This surprising stability is actually explained by the strong hydrogen-bond network within the DES, which lowers the diffusion of the constituents to the protein core. The DES forms hydrogen bonds with the surface residues of the enzyme which, instead of denaturation, may stabilize the tertiary structure of the enzyme (Monhemi *et al.*, 2014; Wu *et al.*, 2014). This is also the reason why a very low reactivity of DES' counterparts is often observed. Later, Zhao *et al.* (Zhao *et al.*, 2013) have confirmed the potential of DES as an alternative medium to organic solvents or IL for biotransformation catalyzed by lipases. They showed that DES made with cholinium chloride and glycerol (ChCl:Gly) could be used as "green" solvent for biodiesel preparation by enzymatic transesterification. A screening of different lipases has shown that the best activity (assessed by soybean oil methanolysis) was, once again, obtained with the immobilized lipase B from *Candida antarctica* (iCALB). They also confirmed the remarkable stability of iCALB in a DES based on cholinium acetate and glycerol (ChOAc:Gly), with a residual activity maintained at 92% and 50% after 48 h and 168 h. Finally, Borse *et al.* were able to accomplish with success a multi-component synthesis of a series of novel pyrimidinedione- and pyrimidinetriones-based compounds using the *Rhizopus Oryzae* lipase, with reaction yields between 73% and 95% in only 6 h (Borse *et al.*, 2013). Also, the influence of the DES has proven to be beneficial and critical to the reaction since lower yields were obtained using other reaction media such as water, methanol, dioxane or DMF. Concomitantly, our group has been interested in conceiving the first lipase-catalyzed lipophilization in DES. We firstly conducted preliminary studies to evaluate the best experimental conditions before initiating any investigation for such complex and sensitive biocatalysis reaction. In a first study (Durand *et al.*, 2012) we showed that the nature of the constituents, namely the salt and the hydrogen-bond donor (HBD), could strongly influence the physicochemical properties of the DES and the resulting biocatalytic activities. For example, by changing the nature of the salt in combination with glycerol as HBD, the performance in the alcoholysis reactions of vinyl laurate with alcohols of different alkyl chain lengths were deeply affected. The same observation may be drawn by changing the nature of the HBD in association with the cholinium chloride (ChCl) as salt. Moreover, we observed and confirmed that some DES could react and compete with the substrates in the lipase-catalyzed reactions leading to the formation of undesirable by-products, which may be a serious weakness to their development. Among the different DES tested, ChCl:U and ChCl:Gly have clearly shown the best results since they allowed excellent bioconversions with minor influence of the polarity of the nucleophilic substrate, and with no apparent reactivity of their constituents. While providing a hopeful step forward, all the results collected from the literature so far, were performed with substrates that had little affinity with the DES. Thus, one of the evident difficulty would be the realization of lipase-catalyzed reactions with substrate(s) highly disposed to create strong interactions (mostly hydrogen bonding) with the DES' matrix. Indeed, it was previously noticed that the very low reactivity of constituents was due to the strong and well-organized non-covalent intermolecular bonds established in such mixture. Thus, as far as the lipase-catalyzed lipophilizations, the challenge faced by this study is two-fold: the reactivity of hydrophilic substrate (*e.g.* phenolics) locks up in DES, and lipase-catalyzed reactions with substrates of two different polarities. In a recent work (Durand, Lecomte, Baréa, 2013), our group highlighted the necessity to add significant quantities of water to promote lipase-catalyzed lipophilization (with 1-octanol) of phenolic coumpounds (coumaric and ferulic acids) in DES (ChCl:U and ChCl:Gly). For example, very high conversion rates were achieved in ChCl:U with water contents of 8% and 10% (w/w), respectively 93% and 98%. As observed in previous studies, a clear catalytic superiority of iCALB in ChCl:U compare to ChCl:Gly was obtained. In addition, these studies have revealed that the very low lipase activity/stability in the neat or weakly hydrated DES for such complex experiments could be explained by both the strong association of the substrate into the DES' matrix and the very low thermodynamic water activity ($a_w < 0.02$). Indeed, significant enhancement in lipase initial activity (67 times faster in ChCl:U) was observed when a_w was increased up to 0.15. Also, water in DES significantly preserved the enzyme catalytic activity over the time. Regarding the pH values in the DES-water binary mixture, the gradual addition of water did not cause a significant variation but the acidic or basic state of the DES depends on the nature of the HBD. The secondary role played by the pH in lipase's catalytic activity was confirmed by the drastic differences obtained between DES based on HBD of similar chemical nature displaying similar pH values. Finally, in a recent study our team went to investigate the structure and supramolecular organization of these solvents in order to understand how the different components (quaternary ammonium salt, HBD, water and substrates) involved in these mixtures could contribute to their functional properties and affect the lipase activity/stability (Durand *et al.*, 2014). This work, carried out with the greatest DES to promote lipase-catalyzed reactions (ChCl:U), provided interesting information on the availability of water to contribute to the functional properties in the environment (hydrolysis activity, hydration of the enzyme). It has been demonstrated the existence of two thermodynamically different areas, which determine the influence and effect of the water. Thus, for a water to ChCl molar ratio below 1.75, water will interact mainly with the salt (ChCl) through very strong intermolecular bonds. Its availability will be such that it can marginally participate in the competitive hydrolysis reactions or properly intervene in the hydration state of the enzyme. However, for a water to ChCl molar ratio above 1.75, the probability of water to participate in its various activities will be greatly increased. Similarly, we have demonstrated that urea and the nucleophilic substrate could have a positive effect on reducing hydrolysis yields. The first reducing a_w, the second acting as a co-solvent to protect the product against the reactivity of water. These experiments provided substantive clarifications to finely adjust the conditions to favor lipase-catalyzed lipophilizations in DES. Thus, given the difficulties encountered when performing such biotransformations, it was possible to synthesize with high yields, a full range of lipophilic derivatives of ferulic and coumaric acids from C4 to C16 (aliphatic chain).

5 Conclusion

This review aimed at analyzing whether or not DES may be a viable medium for enzymatic synthesis of molecules with great value, as realized through the lipase-catalyzed lipophlizations. Although the number of studies dealing with enzymatic catalysis in DES is still relatively low, they all share the same conclusion: the enzymes may be active. DES must be seen as new entity where the components are strongly connected to one another through a well-organized tridimensional system with optimum interactions *via* inter- and intramolecular hydrogen-bonding, resulting in properties that may be different than the neat molecules. Moreover, any substance able to associate within this matrix would be highly solubilized and strongly associated. Regarding the lipase-catalyzed lipophilizations in such media, it was observed that a small quantity of water is recommended to promote activity. Moreover, in such hydrated systems, our group described a structural organization which suggests that any substrate able to associate within this matrix would be highly solubilized and strongly associated, which could limit its reactivity. Although the role of HBD is still ambiguous, this review showed a strong importance and influence on the biotransformation. We believe that it is involved in the conformation state of the enzymes. Also, the HBD reactivity is strongly decreased when use to create a DES, but some of them may still be reactive that leads to unwanted by-product. Therefore, the ideal option would be to use a HBD that cannot compete in the reaction. Another solution to address this issue would be to use the substrate as both HBD and substrate. For example, it has been demonstrated that ChCl and p-coumaric acid could form a DES with a melting point at 67 °C (molar ratio of 1: 0.5), which corresponds to a decrease in transition temperature of phenolic acid around 150 °C (Maugeri and Domínguez de María, 2012). In other words, would it be possible to render liquid the substrate by creating intermolecular affinities with another molecule, making this latter enable to react in reactions? This association will probably involve a reduction in the availability and reactivity of the substrate, but there are some experiments showing that the HBD could remain highly reactive (Durand *et al.*, 2012).

References

Abbott AP, Boothby D, Capper G, Davies DL, Rasheed RK. 2004. Deep Eutectic Solvents formed between choline chloride and carboxylic acids: Versatile alternatives to ionic liquids. *J. Am. Chem. Soc.* 126: 9142–9147.

Abbott AP, Capper G, Davies DL, Rasheed RK, Tambyrajah V. 2003. Novel solvent properties of choline chloride/urea mixtures. *Chem. Commun.* 70–71.

Alemán M, Bou R, Guardiola F, *et al.* 2015. Antioxidative effect of lipophilized caffeic acid in fish oil enriched mayonnaise and milk. *Food Chem.* 167: 236–244.

Borse BN, Shukla SR, Sonawane YA, Shankerling GS. 2013. Synthesis of some novel pyrimidinedione and pyrimidinetrione derivatives by a greener method: Study of their antimicrobial activity and photophysical properties. *Synth. Commun.* 43: 865–876.

Choi YH, van Spronsen J, Dai Y, *et al.* 2011. Are natural deep eutectic solvents the missing link in understanding cellular metabolism and physiology? *Plant Physiol.* 156: 1701–1705.

Dai Y, van Spronsen J, Witkamp GJ, Verpoorte R, Choi YH. 2013. Natural deep eutectic solvents as new potential media for green technology. *Anal. Chim. Acta* 766: 61–68.

Durand E, Lecomte J, Baréa B, *et al.* 2012. Evaluation of deep eutectic solvents as new media for Candida antarctica B lipase catalyzed reactions. *Process Biochem.* 47: 2081–2089.

Durand E, Lecomte J, Baréa B, *et al.* 2013. Evaluation of deep eutectic solvent–water binary mixtures for lipase-catalyzed lipophilization of phenolic acids. *Green Chem.* 15: 2275.

Durand E, Lecomte J, Villeneuve P. 2013. Deep eutectic solvents: Synthesis, application, and focus on lipase-catalyzed reactions. *Eur. J. Lipid Sci. Technol.* 115: 379–385.

Durand E, Lecomte J, Baréa B, *et al.* 2014. Towards a better understanding of how to improve lipase-catalyzed reactions using deep eutectic solvents based on choline chloride. *Eur. J. Lipid Sci. Technol.* 116: 16–23.

Durand E, Bayrasy C, Laguerre M, *et al.* 2015. Regioselective synthesis of diacylglycerol rosmarinates and evaluation of their antioxidant activity in fibroblasts. *Eur. J. Lipid Sci. Technol.*, in press.

Figueroa-Espinoza MC, Villeneuve P. 2005. Phenolic acids enzymatic lipophilization. *J. Agric. Food Chem.* 53: 2779–2787.

Gorke JT, Srienc F, Kazlauskas RJ. 2008. Hydrolase-catalyzed biotransformations in deep eutectic solvents. *Chem. Commun.* 1235–1237.

Gorke JT, Kazlauskas RJ. 2009. Enzymatic processing in deep eutectic solvents. US PATENT, N° 20090117.

Huang WY, Cai YZ, Zhang Y. 2009. Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. *Nutr. Cancer* 62: 1–20.

Kareem MA, Mjalli FS, Hashim MA, Alnashef IM. 2010. Phosphonium-based ionic liquids analogues and their physical properties. *J. Chem. Eng. Data* 55: 4632–4637.

Laguerre M, López Giraldo LJ, Lecomte J, *et al.* 2010. Relationship between hydrophobicity and antioxidant ability of "phenolipids" in emulsion: A parabolic effect of the chain length of rosmarinate esters. *J. Agric. Food Chem.* 58: 2869–2876.

Li W, Zhang Z, Han B, *et al.* 2008. Switching the basicity of ionic liquids by CO2. *Green Chem.* 10: 1142–1145.

Lindberg D, de la Fuente Revenga M, Widersten M. 2010. Deep eutectic solvents (DESs) are viable cosolvents for enzyme-catalyzed epoxide hydrolysis. *J. Biotechnol.* 147: 169–171.

Maugeri Z, Domínguez de María P. 2012. Novel choline-chloride-based deep-eutectic-solvents with renewable hydrogen bond donors: levulinic acid and sugar-based polyols. *RSC Adv.* 2: 421.

Maugeri Z, Leitner W, Domínguez De María P. 2013. Chymotrypsin-catalyzed peptide synthesis in deep eutectic solvents. *Eur. J. Organic Chem.* 4223–4228.

Monhemi H, Housaindokht MR, Moosavi-Movahedi AA, Bozorgmehr MR. 2014. How a protein can remain stable in a solvent with high content of urea: insights from molecular dynamics simulation of Candida antarctica lipase B in urea: choline chloride deep eutectic solvent. *Phys. Chem. Chem. Phys.* 16: 14882–93.

Nam MW, Zhao J, Lee MS, Jeong JH, Lee J. 2015. Enhanced extraction of bioactive natural products using tailor-made deep eutectic solvents: application to flavonoid extraction from Flos sophorae. *Green Chem.* 17: 1718–1727.

Pang N, Gu SS, Wang J, *et al.* 2013. A novel chemoenzymatic synthesis of propyl caffeate using lipase-catalyzed transesterification in ionic liquid. *Bioresour. Technol.* 139: 337–342.

Ranke J, Mölter K, Stock F, *et al.* 2004. Biological effects of imidazolium ionic liquids with varying chain lengths in acute Vibrio fischeri and WST-1 cell viability assays. *Ecotoxicol. Environ. Saf.* 58: 396–404.

Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T. 2005. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. *Mutat. Res.-Fund. Mol. Mech. Mutagen.* 579: 200–213.

Stolte S, Arning J, Bottin-Weber U, *et al.* 2006. Anion effects on the cytotoxicity of ionic liquids. *Green Chem.* 8: 621-629.

Su WC, Wong DSH, Li MH. 2009. Effect of Water on Solubility of Carbon Dioxide in (Aminomethanamide + 2-Hydroxy- N, N, N-trimethylethanaminium Chloride). *J. Chem. Eng. Data* 54: 1951–1955.

Vanden Berghe W. 2012. Epigenetic impact of dietary polyphenols in cancer chemoprevention: Lifelong remodeling of our epigenomes. *Pharmacol. Res.* 65: 565–576.

Villeneuve P. 2007. Lipases in lipophilization reactions. *Biotechnol. Adv*. 25: 515-536.

Weitkamp P, Vosmann K, Weber N. 2006. Highly efficient preparation of lipophilic hydroxycinnamates by solvent-free lipase-catalyzed transesterification. *J. Agric. Food Chem.* 54: 7062–7068.

Wu BP, Wen Q, Xu H, Yang Z. 2014. Insights into the impact of deep eutectic solvents on horseradish peroxidase: Activity, stability and structure. *J. Mol. Catal. B. Enzymatic* 101: 101–107.

Yang Z, Guo Z, Xu X. 2012. Enzymatic lipophilisation of phenolic acids through esterification with fatty alcohols in organic solvents. *Food Chem.* 132: 1311–1315.

Zhao H, Baker GA, Holmes S. 2011. Protease activation in glycerol-based deep eutectic solvents. *J. Mol. Catal. B Enzymatic* 72: 163–167.

Zhao H, Zhang C, Crittle TD. 2013. Choline-based deep eutectic solvents for enzymatic preparation of biodiesel from soybean oil. *J. Mol. Catal. B Enzymatic* 85-86: 243–247.

Cite this article as: Erwann Durand, Jérôme Lecomte, Pierre Villeneuve. Are emerging deep eutectic solvents (DES) relevant for lipase-catalyzed lipophilizations? OCL 2015, 22(4) D408.