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Dual role of a natural deep eutectic solvent as lipase extractant and transesterification enhancer

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ABSTRACT

The present work demonstrates the suitability of a natural deep eutectic solvent (DES) composed of glucose and cholinium chloride to act both as lipase extractant and biodiesel enhancer. After having demonstrated the viability of using this DES as segregation agent in aqueous solutions of non-ionic surfactants widely employed as lipase inducers in culture media (Tween 20 and Tween 80), the immiscibility gaps where characterized by ascertaining the binodal curves and tie-lines at several temperatures. Then, the effect of phase forming components concentration on *Candida antarctica* lipase B (CALB) extraction was investigated, concluding that a careful selection of feed composition allows reaching almost 100% of CALB extraction to the DES-rich phase. Additionally, the role of DES as coadjuvant in CALB-catalyzed glycerol-free transesterification was evaluated after an initial optimization of the reaction conditions (enzyme load and DMC:oil molar ratio). It was concluded that the use of the proposed natural DES led to biodiesel conversions 3 times higher than the levels attained with a conventional imidazolium-based ionic liquid ($C_2C_1imC_2SO_4$), or 20% higher than control transesterification reactions (without coadjuvant).

1. Introduction

Transport sector accounts for about 25% of Europe greenhouse gas emissions, being road transport the greatest emitter (70%) (European regulation, 2021). In this scenario, the decarbonisation of this sector cannot exclusively rely on the use of electric alternatives in the short term and the development of low-emission alternative energy sources like biodiesel is a topic in the limelight, as most of mass and good transport are based on diesel cycle.

The industrial process for biodiesel production is carried out at high temperatures (about 333 K) and is based on the use of alcohols as acyl acceptors (specially methanol) to yield alkyl esters (usually fatty acid methyl esters, FAMEs) and glycerine, that are mutually immiscible (Mahmudul et al., 2017). It is well known that 10 kg of glycerol coproduct are obtained for every 100 kg biodiesel (Manaf et al., 2019), which has led to a large excess of this compound (estimated in about 42 billion litres in 2020) and a subsequent fall in its price (about 225%) (Esan et al., 2020). One way to circumvent this shortcoming is the replacement of alcohols by dimethyl carbonate (DMC), which leads to glycerol carbonate formation, instead of glycerol and avoids costly separation stages associated to the conventional process (Lee et al., 2017).

Additionally, this reaction demands the presence of a catalyst, not only for increasing reaction rate, but also for lowering the operation temperatures. However, the current industrial use of chemical catalyst bears additional disadvantages like the high energy consumption (due to the operation at high temperature), the existence of saponification reactions, the absence of catalyst reuse and the generation of acid or alkaline wastewater effluents that have to be neutralized (Moazeni et al., 2019).

The way to dodge these handicaps is to bet in biocatalysis, as the use of lipolytic enzymes (triacylglycerol acylhydrolases EC 3.1.1.3) has already been demonstrated to be effective to further transesterification (Mittelbach, 1990). Additionally, lipase-catalyzed reactions involve other advantages like the high selectivity and the capacity to act both on glyceride-linked and free fatty acids in one step (Wu et al., 2017). The

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elevated lipase cost, mostly associated to purification stages from culture media where they are produced, has been pointed as the main barrier impeding the actual implementation at industrial scale. In this sense, biotechnological lipase production is usually induced by lipidic compounds or surfactants, that are present in the culture media (Balaji et al., 2020). Among the existing alternatives (Álvarez et al., 2019), surfactants from the Tween family have been highlighted as one of the most biocompatible ones. Tween 20 and Tween 80 have been chosen for the present work due to the fact that they are considered GRAS by the US FDA and they are classified as safe food additives (E432 and E433) (Álvarez et al., 2016; European regulation 2021; Álvarez et al., 2015a). These compounds bear the advantage of being segregated by the addition of extractant agents like inorganic/organic salts or ionic liquids, when they are present in aqueous solutions (Álvarez et al., 2012; Álvarez et al., 2015b).

Aqueous Two-Phase Systems (ATPS) are one of the most promising liquid-liquid fractionation technique for the simultaneous purification, fractionation and clarification of biomolecules. In this context, different biomolecules like pectinases (Wolf-Márquez et al., 2017), lipases (Gutiérrez-Arnillas et al., 2015), amylases (Tonova and Bogdanov, 2017), monoclonal antibodies (Muendges et al., 2015) have been successfully recovered from aqueous solutions, which demonstrates the suitability of this technique for biotechnological downstream operations. The main attraction of ATPS is primarily based on an array of well-known advantages like their simplicity, economy and possible biocompatibility when the phase forming components are carefully designed.

In the last years, a new type of neoteric solvents, has been demonstrated to be suitable aqueous phase separation inducers in aqueous solutions of non-ionic surfactants (Fernández et al., 2020; Álvarez et al., 2021). These compounds, named Deep Eutectic Solvents (DES), have been demonstrated to improve the efficiency of lipase-catalyzed transesterifications, so the hypothesis that they can be simultaneously used for both lipase extraction and biodiesel production catalysis is plausible. These compounds can be commonly synthesized by just mixing a hydrogen bond acceptor, HBA, (e.g. quaternary ammonium salts) and a hydrogen bond donor, HBD, (e.g. alcohols, amino acids, carboxylic acids, etc.) at specific ratios. Their production is comparably more straightforward and economic than that of ionic liquids, and their biocompatibility and toxicity may be tuned by carefully selecting the parent materials. Among the available options, cholinium-based HBA are an appealing choice due to their demonstrated low cost and environmental impact. The great biocompatibility of cholinium-based DES with lipases and their role as agents for the extraction of commercial lipases from aqueous solutions has already been remarked in recent research works of our group (Fernández et al., 2020; Álvarez et al., 2021; Morandeira et al., 2017). Besides, DES were proved to be suitable adjuvants in lipase-catalyzed transesterification reactions with alcohol-based acyl acceptors (Bacic et al., 2021; Zhang et al., 2016), although there are no studies tackling their effect in DMC-based biocatalytic biodiesel production.

In this work, the suitability of a natural DES (cholinium chloride: glucose) to generate immiscibility windows in aqueous solutions of Tween 20 and Tween 80 at several temperatures was demonstrated. After describing the experimental solubility and tie-line data, different empirical models were employed to characterize the biphasic region. Once the immiscibility curve was defined, lipase B from *Candida antarctica* (CALB) was extracted since it is considered one of the most robust biocatalysts for transesterification reactions (Ortiz et al., 2019). Then, a biocatalytic complex combining DES and CALB was applied to transesterification reaction at 313.15 K in order to check the viability of this integrated strategy to simultaneously extract lipases and apply them in biodiesel production processes.

2. Material and methods

All the compounds and methods employed in the manuscript are detailed below in order to ease the reproducibility of the experimental data.

2.1. Materials

Cholinium chloride (ChCl, 98% purity) and $C_2C_1imC_2SO_4$ (95% purity) were purchased from Sigma-Aldrich and were kept at 323.15 K and $2{\cdot}10^{-1}$ Pa to remove solvents and moisture. Trizma base (99% purity), ethanol (99% purity), propanol (98% purity), and *p*-nitrophenyl laurate (98% purity) were also acquired from the same company. Sodium carbonate (99% purity), glucose (99% purity), calcium chloride (99% purity), Tween 80 and Tween 20 were provided by Scharlau chemicals. Methanol (99% purity) was sold by Fischer Chemical while hydrochloric acid (37% purity) was bought in Panreac. Free and immobilized lipase B from *Candida antarctica* were kindly donated by Novozymes being their activity using *p*-nitrophenyl laurate as a subtrate 11530 U/L and 10000 U/g.

2.2. DES preparation

An amount of ChCl (HBA) and glucose (HBD) was introduced in a glass tube to yield a final molar ratio of 2:1 and a homogeneous liquid was got after heating at 373.15 K for 5 h to get a water content below 2%.

2.3. Characterization of the immiscibility region

The immiscibility region was characterized by the cloud point method (Álvarez et al., 2014) at the selected temperatures (F200 ASL digital thermometer with uncertainty of ±0.01 K). Different binary mixtures (Tween + DES) covering all the composition range were prepared in thermostatted glass tubes, and water was added until turbidity disappeared, which allows recording the liquid-liquid equilibrium data. The compositions were gravimetrically ascertained in an analytical balance (Sartorius Cubis MSA 125P-100-DA balance, ± 10^{-5} g).

Tie-lines (*TLs*) were carried out as described elsewhere (Álvarez et al., 2014). In brief, a mixture with composition corresponding to the immiscibility region was prepared in a glass ampoule and, after a stirring step, it was left to settle for one day to allow a complete phase splitting. Once the layers were separated, they were characterized by analysing the density (Anton Paar DSA 5000 M digital vibrating tube densimeter, uncertainty of $\pm 2{\cdot}10^{-5}$ g cm^{-3}) and refractive index (Dr. Kernchen ABBEMAT WR, uncertainty of $\pm 4{\cdot}10^{-5}$). Mili-Q water and tetrachloroethylene were employed for calibration following manufacturer indications.

2.4. Enzyme extraction

Six different compositions in two different *TLs* were selected to carry out the enzyme separation in aqueous mixtures of free CALB and surfactant. Enzyme activity was evaluated after splitting the layers once a centrifugation step at 1000 g for 30 min at 313.15 K was completed.

2.5. Transesterification reaction

15 mL jacketed glass flasks were employed to carry out the transesterification reaction using sunflower oil and dimethyl carbonate (DMC) as substrate and acyl acceptor, varying the molar ratio oil:DMC from 1:3 to 1:9 and the immobilized lipase B from *C. antarctica* from 10% to 30% (w/w regarding oil weight). The reaction mixture was allowed to proceed under constant stirring at 250 rpm for 24 h at 313.15 K, and a sample was reserved for FAME analysis.

2.6. Lipolytic activity determination

The hydrolysis of *p*-nitrophenyl laurate was selected to quantify lipolytic activity (Deive et al., 2013). The reaction mixture containing 100 μL of *p*-nitrophenyl laurate (2.5 mM in ethanol) and 800 μL of Tris HCl buffer 50 mM (pH 8) (containing 20 mM $CaCl_2$) was kept at 313.15 K for 5 min, prior to adding 100 μL of the sample. After 20 min, the reaction was stopped with 250 μL of 1 M Na_2CO_3 and it was kept in an ice bath for 10 min. After centrifugation at 17709*g* for 10 min at 277.15 K, the supernatant absorbance was quantified at 400 nm (Unicam Helios β, Thermo Electron Corp spectrophotometer). The amount of enzyme catalysing the release of 1 μmol of *p*-nitrophenol per minute was defined as one activity unit (U/L). In all cases, the measurements were carried out by triplicate, and a blank sample was included as control.

2.7. Water content determination

Water content in DES was quantified by Karl-Fischer titration (Metrohm 899 coulometer) after introducing a single drop of the eutectic mixture and imidazolium-based ionic liquid, that had been previously weighted.

2.8. FAME analysis by gas chromatography

EN 14103 method was employed for FAME quantification in an Agilent GC-7820A equipment with a flame ionisation detector (GC-FID) and an Agilent HP-88 capillary column (30 m × 0.25 mm I.D. x 0.25 μm film thickness). The injector and detector temperature was maintained at 523.15 and 553.15 K, and 1 mL/min of He was employed as carrier gas. The split flow was set at 250 mL/min and the temperature cycle was as follows: after keeping the temperature at 393.15 K for 1 min, it was increased to 448.15 K (10 K/min) and maintained for 10 min prior to a heating step up to 493.15 K (3 K/min) and a holding period of 3 min.

The sample was prepared as described: 100 mg of sample was mixed with 100 mg of internal standard (methyl nonadecanoate C19:0) in a 15 mL vial. 10 mL of toluene were added and the mixture was vigorously stirred prior to be transferred to a 2 mL vial. The FAME content (%) was calculated using the following equation:

$$\text{FAME}\ (\%) = \left(\frac{\sum A - A_{is}}{A_{IS}}\right).\left(\frac{w_{is}}{w}\right)100 \quad (1)$$

where ΣA is the sum of the methyl esters (C16:0-C24:1) peak areas, A_{IS} is the peak area associated to the internal standard, w_{is} is the weight of internal standard and *w* is the sample weight (mg).

3. Results and discussion

Prior to demonstrate the capacity of the selected natural DES composed of cholinium chloride and glucose to extract CALB, it is crucial to find and exhaustively characterize the biphasic region.

3.1. Characterization of immiscibility region

The influence of the temperature in the solubility data is an issue of practical importance for proposing these systems in lipase extraction. More specifically, given that CALB displays optimum temperature about 313.15K (Siódmiak et al., 2015), the thermal stability of the ATPS at several temperatures was investigated. The biphasic boundaries of the systems composed of DES, Tween 20 or Tween 80 and water have been ascertained at 303.15 K, 313.15 K and 323.15 K, and the data can be visualized in Fig. 1 and Tables S1 and S2.

The analysis of literature data evidences two clear trends depending on the phase forming components. Polymer-salts-based systems lead to greater immiscibility regions when temperature is increased, as confirmed in previous research works of our group (Álvarez et al., 2019). On the other hand, an opposite trend was reported for ATPS composed of inorganic and organic salts (Li et al., 2013). This information makes one hypothesizing that the use of the selected polymeric surfactants would entail greater biphasic windows at higher temperature values. The reason for this striking behaviour may be found in a complex balance of interactions between DES, Tween and water. More specifically, ethoxylated groups present in Tween non-ionic surfactants are less prone to interact with water molecules at elevated temperatures. Actually, conformers displaying low or negligible dipole moments are predominant at higher temperatures, so hydrogen bonds between surfactant and water are broken while surfactant-surfactant interactions are prompted (Lindman et al., 2016). This rationale is corroborated when the experimental data shown in Fig. 1 are analysed, as the binodal curves are closer to the water vertex for greater temperatures, no matter the surfactant employed. On the other hand, it is clear that the differences between temperatures are maximized in the low surfactant concentration region (right part of the ternary diagrams in Fig. 1), which is obviously due to the DES capacity to interact with water molecules. It is clear that the use of the proposed DES involves an equilibrium shift toward phase disengagement at increased temperatures due to the existence of water-water, water-DES and DES-DES hydrogen bonds, that will undoubtedly impact the final interplay balances. So it can be concluded a lower critical solution temperature, leading to a LCST-type diagram.

Regarding the effect of Tween 20 and Tween 80 on the immiscibility area, the data presented in Fig. 1 reveal that the longer alkyl chain of the latter furthers the surfactant-surfactant interactions to the detriment of

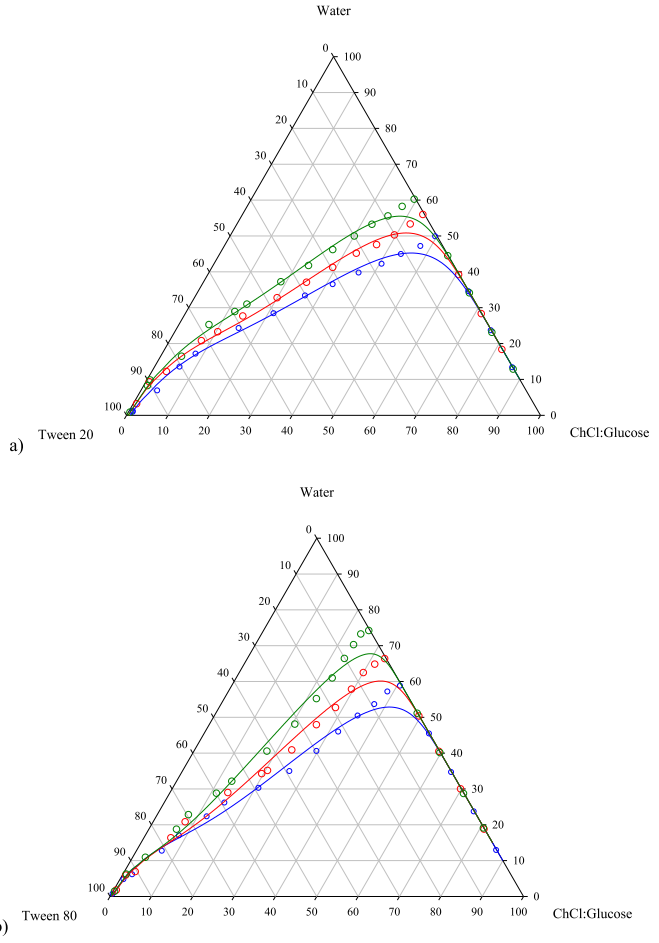

Fig. 1. Experimental solubility data for: a) {Tween 20 (1) + ChCl:glucose (2) + H_2O (3)} and b) {Tween 80 (1) + ChCl:glucose (2) + H_2O (3)} at 303.15 K (○), 313.15 K (○), 323.15 K (○).

the hydrogen bonding with water molecules. This phenomenon is reflected in an easier phase disengagement of Tween 80 than Tween 20. In this sense, a possible quantitative explanation can be given on the basis of the hydrophilic lipophilic values (HLB) of both surfactants. This parameter indicates the balance of the size and weight of the selected surface active compounds, and usually varies between 0 (low hydrophilicity) and 20 (high hydrophilicity). Then the higher affinity of Tween 20 for water molecules is demonstrated by the greater HLB value (16.7) than that recorded for Tween 80 (15.0) (Álvarez et al., 2016).

A comprehensive characterization of the segregation capacity of the selected DES must include the tie lines (*TLs*) determination, as it yields useful information about the phase forming components distribution between the DES and Tween-rich phases. The data obtained can be visualized in Figs. 2 and 3, and they are compiled in Table S3. It can be noticed that longer *TLs* lead to surfactant-rich phases bearing high purity values (almost 100%) for both Tween 20 and Tween 80. These data can be quantitatively supported by calculating the tie-line length (*TLL*) and slope (*S*):

$$TLL = \left[\left(w_1^{I} - w_1^{II}\right)^2 + \left(w_2^{I} - w_2^{II}\right)^2\right]^{0.5} \quad (2)$$

$$S = \frac{w_1^{I} - w_1^{II}}{w_2^{I} - w_2^{II}} \quad (3)$$

where w_1 and w_2 are the surfactant and DES compositions, and the superscripts I and II refer to the surfactant- and DES-rich phase. These data are also listed in Table S3, and reveal the direct relationship between *TLL* and DES/surfactant composition in upper and bottom phases. Greater *TLL* values are associated with higher surfactant and DES concentrations. Contrarily, increased slopes are correlated with lower *TLL* values, a behaviour already reported in previous research works using cholinium-based neoteric solvents (Álvarez et al., 2016).

3.2. Modelling of experimental data

Mathematical modeling can be considered a tool of particular importance and a practical significance for exhaustively characterizing the liquid-liquid equilibria, since it facilitates the process implementation at greater scale and ease the separation simulation with relevant software tools. So different empirical equations were applied to describe the liquid-liquid equilibrium (Álvarez et al., 2012):

$$w_1 = A \cdot exp\left(Bw_2^{0.5} - Cw_2^3\right) \quad (4)$$

$$w_1 = A + Bw_2^{0.5} + Cw_2 + Dw_2^2 \quad (5)$$

$$w_1 = exp\left(A + Bw_2^{0.5} + Cw_2 + Dw_2^2\right) \quad (6)$$

where w_1 and w_2 are mass fraction percentages bearing the same meaning as that mentioned above, and *A*, *B*, *C*, and *D* are the fitting parameters, that were obtained after minimization of the standard deviation (σ) through solver function in Microsoft Excel:

$$\sigma = \left(\frac{\sum_i^{n_{DAT}} \left(z_{exp} - z_{adjust}\right)^2}{n_{DAT}}\right)^{1/2} \quad (7)$$

being z_{exp} and z_{adjust} the experimental and the theoretical values, and n_{DAT} meaning the number of data. The values of the fitting parameters and standard deviations are listed in Tables S4, S5 and S6 and evidence the suitability of Merchuk equation (4) to describe the experimental data, as the deviations are lower for the studied temperatures no matter the surfactant under study.

On the other hand, *TL* data were correlated by means of two well-known models like Othmer-Tobias and Bancroft equations (Álvarez et al., 2012; Othmer and Tobias, 1942):

$$\left(\frac{1 - w_1^{I}}{w_1^{I}}\right) = n\left(\frac{1 - w_2^{II}}{w_2^{II}}\right)^m \quad (8)$$

$$\left(\frac{w_3^{II}}{w_2^{II}}\right) = k\left(\frac{w_3^{I}}{w_1^{I}}\right)^r \quad (9)$$

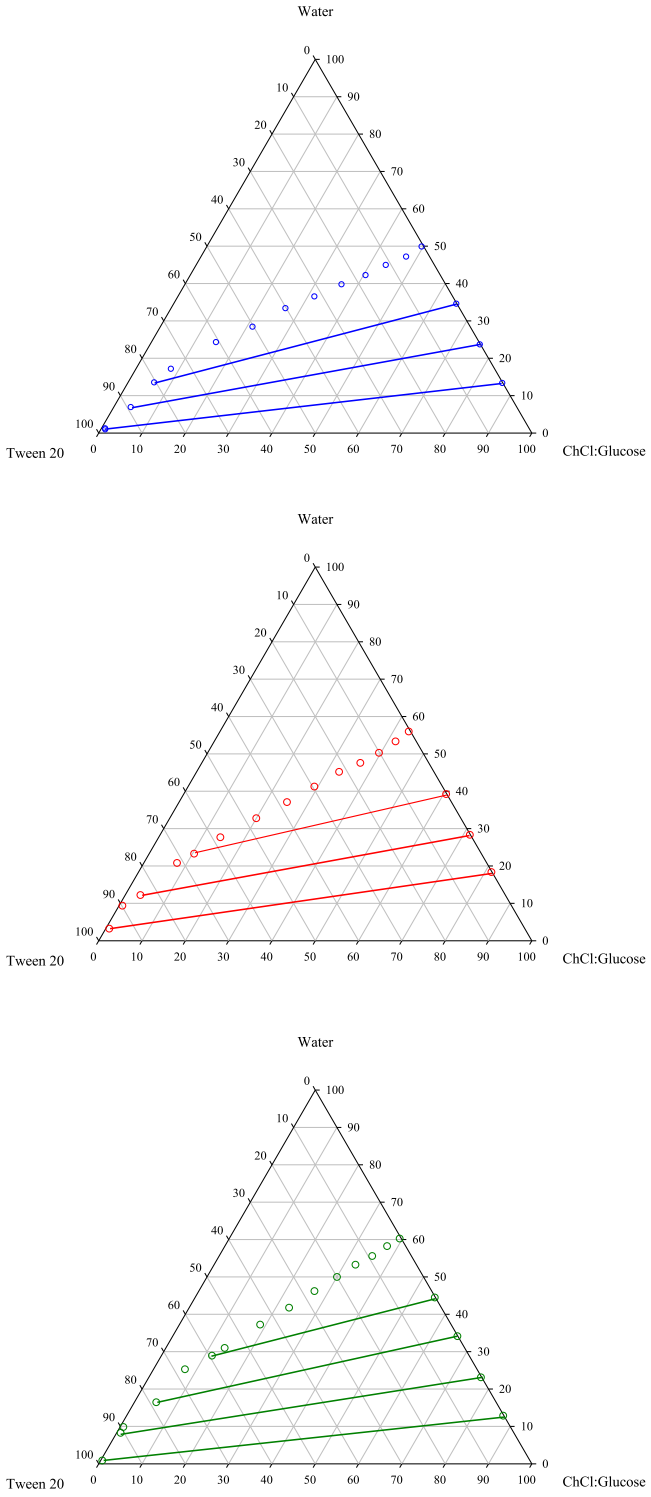

Fig. 2. *TL* data for: {Tween 20 (1) + ChCl:glucose (2) +H_2O (3)} at 303.15 K (○), 313.15 K (○), 323.15 K (○).

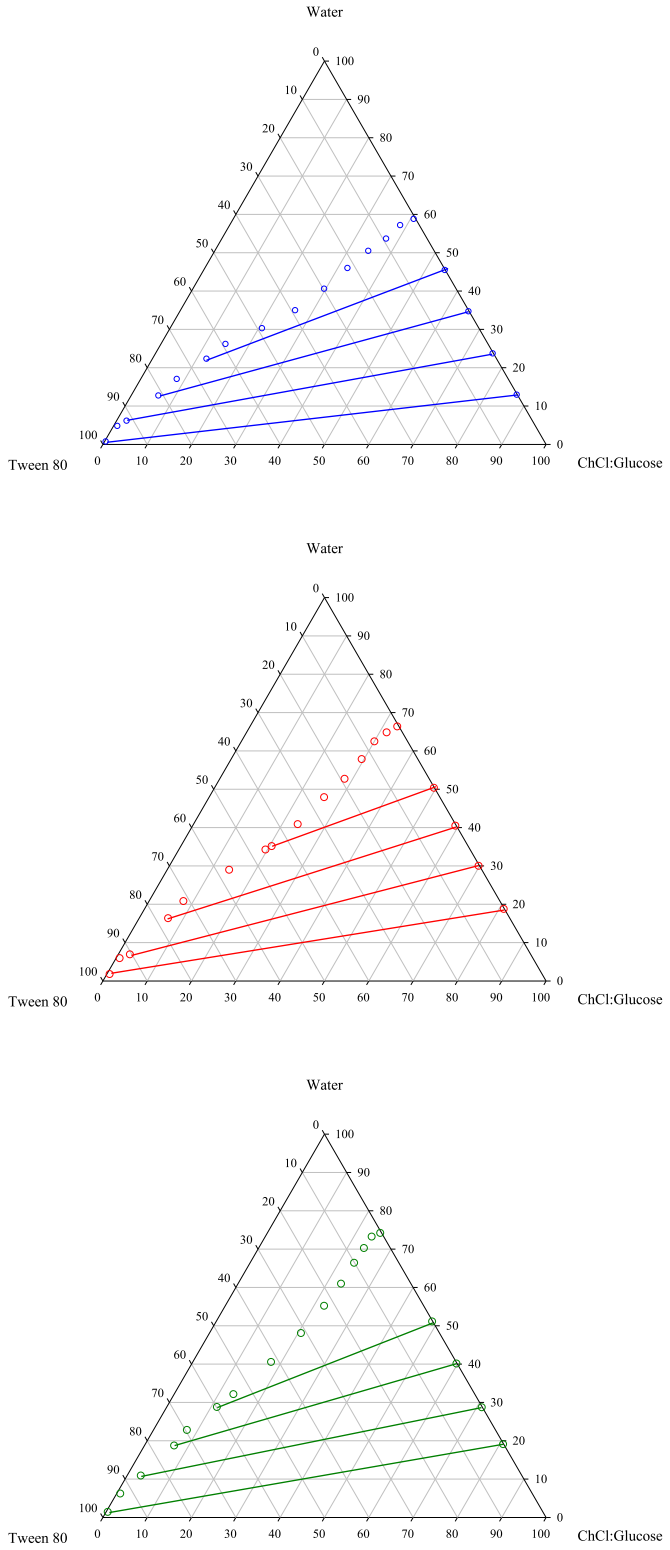

Fig. 3. *TL* data for: {Tween 80 (1) + ChCl:glucose (2) +H_2O (3)} at 303.15 K (○), 313.15 K (○), 323.15 K (○).

where w_1, w_2 and w_3 are Tween, DES and water compositions. The superscripts I and II have the same meaning as that mentioned above, and the values of the parameters n, m, k, and r are obtained after minimizing the standard deviations, as previously mentioned (equation (7)).

The data presented in Table S7 allow concluding that Bancroft model is the one leading to a better description of the *TL* data, as deviations are halved in most of cases under study.

3.3. DES-based biocatalyst extraction and biodiesel production

After having characterized the immiscibility region, the systems were proposed for analysing their suitability in lipase extraction operations. Then, CALB was separated from aqueous solutions containing both Tween 80 and Tween 20 in order to emulate the aqueous milieu where lipases are commonly synthesized. Since the optimum temperature of CALB is near 313.15 K, the extraction experiments were carried out at this value. Additionally, the influence of the feed concentration in two different TLs for both Tween 20 and Tween 80 was researched and the results were analysed in terms of active lipase extraction capacity, defined as follows:

$$\textit{Active enzyme extraction}\ (\%) = \frac{A^{II}\cdot V^{II}}{A^{i}\cdot V^{i}}100 \tag{10}$$

where A and V are the initial (i) and DES-rich phase (II) lipolytic activity (U/L) and volume (L).

The results presented in Table 1 point out the great influence of the feed composition and *TLs* in the CALB extraction percentage. It is noticeable that the operation in *TLs* closer to the water vertex (Figs. 2 and 3) involves much higher enzyme extraction values, even up to 100%, which is relevant as no deleterious effects on the lipase activity are imposed by the phase forming components. A plausible explanation to this behaviour may be the higher lipase robustness in aqueous environments, as it has been demonstrated that the functionality of proteins is greatly dependent on the hydration level (more specifically, a minimum of 0.3 g of water per gram of protein has been remarked) (Laage et al., 2017). Another reason for the enhanced lipase extraction may be attributed to the different chemical environment faced at the studied feed compositions, as hydrogen bonding balance is completely altered when water content is reduced. At the same time, viscosity is clearly increased at lower water content, which would explain the lower lipase migration to the DES-rich phase at these compositions.

On the other hand, it can also be confirmed the greater suitability of Tween 80 to yield higher extraction levels, probably due to the well-known CALB interfacial activation observed when more hydrophobic compounds are present in the extraction media (Rodrigues et al., 2019; Guo et al., 2020). In summary, the modulation of the feed concentration is a valuable tool to find the right point allowing a complete lipase extraction to achieve a biocatalytic milieu able to act on transesterification reactions.

Then, as no information can be found in literature about the effect of DES on glycerol-free biodiesel production, the optimization of the immobilized CALB load and the DMC:oil molar ratio has been tackled after 24 h of sunflower oil transesterification reaction time at 313.15K. The analysis of the contour plots of FAME conversion (%) shown in Fig. 4 reflects that the increase in lipase load is beneficial for biodiesel production, and the operation at molar ratios (DMC:oil) over 6 and CALB loads over 20% allows reaching FAME conversions surpassing 75%. This fact demonstrates the viability of obtaining biodiesel without facing the costly separation stages (due to glycerol formation) and the presence of chemical catalysts (leading to the necessity of implementing acid or alkaline water treatment), together with the operation at mild temperatures (313.15 K instead of 333.15K).

In order to determine whether the glucose-based DES could be a suitable coadjuvant in CALB-biocatalyzed glycerol-free biodiesel production, these operating conditions have been selected. This hypothesis is based on the positive effect of different ionic liquids on the lipase-catalyzed transesterification yield (Gutierrez-Arnillas et al., 2016). Then, two different controls were included: one with a conventional neoteric solvent (the ionic liquid $C_2C_1imC_2SO_4$), and another one without adding any solvent. The results obtained after 24 h at 313.15 K

Table 1
CALB extraction at different feed concentrations in systems {Non-ionic surfactant (1) + ChCl:Glucose (2) + H_2O (3)} at 313.15 K.

Tween 20												
Feed	w_2	w_1	w_2	w_1	w_2	w_1	w_2	w_1	w_2	w_1	w_2	w_1
	14.98	79.23	43.77	44.72	68.84	15.17	19.60	54.04	34.10	34.89	49.35	15.01
E (%)	14.4 ± 1.21		23.3 ± 3.13		36.0 ± 8.1		75.6 ± 3.1		66.9 ± 6.0		84.6 ± 6.7	
Tween 80												
Feed	w_2	w_1	w_2	w_1	w_2	w_1	w_2	w_1	w_2	w_1	w_2	w_1
	15.28	79.99	44.73	44.04	68.68	14.86	15.05	64.85	34.65	35.75	49.43	15.10
E (%)	25.3 ± 0.2		17.6 ± 0.2		31.3 ± 0.1		99.9 ± 1.34		76.1 ± 0.61		15.7 ± 0.20	

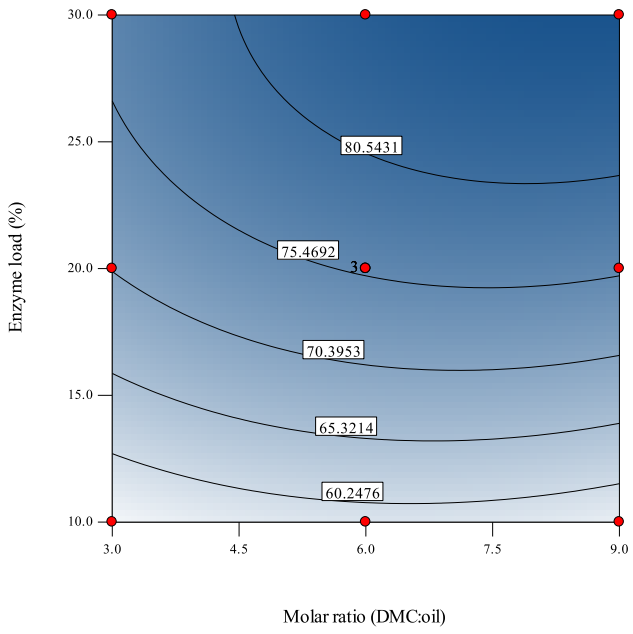

Fig. 4. Contour plot of FAME conversion (%) at different enzyme loads (% w/w) and molar ratios DMC:oil.

are presented in Fig. 5, and demonstrate that the conventional imidazolium-based ionic liquid led to FAMEs conversion more than 2 fold-lower than the control reaction (without coadjuvant) no matter the concentration under study. On the other hand, it becomes obvious the superiority of ChCl:glucose DES over the ionic liquid (almost three times higher, up to about 90%) and even in the control reaction (about 20% higher) at the lowest DES concentrations. These data are in agreement with previous results reporting the greater suitability of DES than imidazolium ionic liquids in traditional biodiesel production reactions (yielding glycerol) (Merza et al., 2018). Additionally, it has been already reported that the presence of choline chloride-based DES is suitable for leading to a biodiesel meeting the international standard specifications (EN14214 (Hayyan et al., 2014), so the present data goes beyond these results by removing glycerin by-product and betting in a biocatalytic strategy. More specifically, the use of the proposed natural DES involves benefits in terms of both process economy and environmental sustainability as conventional strategies usually entail the pollution of great amounts of water. Then, future research works should analyse the possibility of reusing DES after a recovery stage using e.g. a solvent like acetone, as pointed oud in a recent review (Mamtani et al., 2021). In this sense, different research works have already demonstrated the possibility of employing many conventional strategies (salt recrystallization, distillation, antisolvent extraction or cooling) for recovering the DES (Shahbaz et al., 2011).

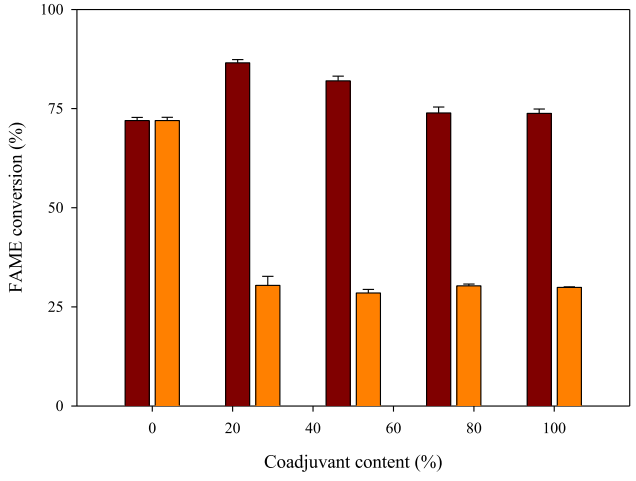

Fig. 5. Effect of different DES (■) and conventional IL (■) concentrations on FAMEs conversion (percentage regarding oil content).

In summary, the viability of using a glucose-based DES both as lipase extractant and further, as lipase-catalyzed transesterification coadjuvant has been demonstrated for glycerol-free reactions, which points out the pertinence of betting in these new biocompatible solvents to improve the sustainability of biodiesel production process.

4. Conclusions

The effectiveness of using ChCl:glucose DES as lipase extractant was demonstrated in this work after exhaustively characterizing and modelling the immiscibility region at 303.15, 313.15 and 323.15 K. In this sense, the operation at different feed concentrations in several tie-lines was proved to be crucial to attain high levels of CALB extraction to the DES-rich phase. Then, the positive effect of DES addition to CALB-biocatalyzed glycerol-free transesterification was demonstrated, as near 90% of FAME conversion was reached, surpassing the values recorded for the control reaction and the imidazolium-assisted reaction (about 72% and 30%). In summary, the present research work confirms the suitability of ChCl:glucose DES to be concomitantly employed both as lipase extractant and transesterification coadjuvant.

CRediT authorship contribution statement

Andrea Fernández: All the authors have contributed to the writing and critical revision of the manuscript. **María A. Longo:** are responsible of the financial support. **Francisco J. Deive:** are responsible of the financial support. **María S. Álvarez:** have designed the work and planned the experiments, that were carried out in the laboratory by, are responsible of the financial support, have approved the final version. **Ana Rodríguez:** have designed the work and planned the experiments, that were carried out in the laboratory by, have approved the final version.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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