From Water-in-Oil to Oil-in-Water Emulsions to Optimize the Production of Fatty Acids Using Ionic Liquids in Micellar Systems

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Biocatalysis is nowadays considered as one of the most important tools in green chemistry. The elimination of multiple steps involved in some of the most complex chemical synthesis, reducing the amounts of wastes and hazards, thus increasing the reaction yields and decreasing the intrinsic costs, are the major advantages of biocatalysis. This work aims at improving the enzymatic hydrolysis of olive oil to produce valuable fatty acids through emulsion systems formed by long alkyl chain ionic liquids (ILs). The optimization of the emulsion and the best conditions to maximize the production of fatty acids were investigated. The stability of the emulsion was characterized considering the effect of several parameters, namely, the IL and its concentration and different water/olive oil volumetric ratios. ILs from the imidazolium and phosphonium families were evaluated. The results suggest that the ILs effect on the hydrolysis performance varies with the water concentration and the emulsion system formed, that is, water-in-oil or oil-in-water emulsion. Although at low water concentrations, the presence of ILs does not present any advantages for the hydrolysis reaction, at high water contents (in oil-in-water emulsions), the imidazolium-based IL acts as an enhancer of the lipase catalytic capacity, super-activating 1.8 times the enzyme, and consequently promoting the complete hydrolysis of the olive oil for the highest water contents [85% (v/v)]. © 2015 American Institute of Chemical Engineers *Biotechnol. Prog.*, 31:1473–1480, 2015

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Introduction

Biocatalysis is a sustainable technology for the production of a plethora of chemicals,[1] because of its attractive features such as high specificity, high yields, and low environmental impact, when compared with the chemical processes.[2] In general, the biocatalytic processes are carried at moderate pH values and temperature conditions, and preferably in the absence or using only minor amounts of toxic chemicals.[3] The use of enzymes catalyzing the chemical reactions allows the elimination of various reaction steps, reducing the amount of residues and decreasing or even eliminating the risks, thus increasing the reaction yields and decreasing the intrinsic costs, making biocatalysis a green and sustainable process.

Lipases (E.C.3.1.1.3) are widely found in nature namely, in microorganisms, animals, and plants. Their main function is to catalyze the hydrolysis of triglycerides to free fatty acids (FFAs) and glycerol.[4] However, this class of enzymes is also capable to efficiently catalyze esterifications[5] and transesterifications.[6] Lipases are widely used in industrial processes,[7] because of their high versatility and consequent large range of applications, such as detergent formulations and wastewater treatment.[8] Moreover, this class of enzymes can also be used in the production of fatty acids, flavors,[9] biopolymers,[10] and biodiesel by the transesterification of vegetable oils.[11] They also have a high affinity for, and performance at, oil–water (substrate–media) interfaces due to structural rearrangements of the lipase active-site region. Crystallographic studies show that in the presence of oil–water interfaces, the "lid" (structure around the enzyme active site) is opened, increasing the accessibility of the catalytic residues to the substrate diffusion and exposing the hydrophobic enzyme surface (stabilized by hydrophobic and electrostatic interactions).[12,13] As described in the literature, when surfactants are applied, a significant increase of the lipase activity may be observed, which is related with the increase of the organic–aqueous interface, making these enzymes appropriate biocatalysts for the hydrolysis of fats and oils.[14,15] Surfactants are amphiphilic molecules, which contain a polar and a nonpolar part, and are capable of stabilizing the organic/water interfaces. They can be classified as anionic,[16] cationic,[17] zwitterionic, and nonionic[18] according to their ionic nature. Depending on the amount of water in the medium, the tensioactive agents can form a variety of structures from micelles, when highly diluted, to reverse micelles at very high concentrations.[19,20] Oil-in-water emulsions (o/w emulsions) are structures with the polar part of the surfactant in contact with the water molecules of the

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continuous phase and the nonpolar tails oriented toward the inner part of the structure. When the water content is low and the continuous phase has a nonpolar nature, the hydrophilic parts associate themselves around the water droplets and the hydrophobic tails are oriented toward the organic medium, generating water-in-oil emulsions (w/o emulsions). There are many studies reporting the application of conventional surfactants, such as sodium bis(2-ethyl-1-hexyl)sulfosuccinate, sodium dodecylsulfate, or cetyltrimethylammonium bromide, to improve the enzyme activity (a phenomenon designated as superactivity) resulting from the formation of aggregated/micellar systems.[16,21,22] Many theories explaining the superactivity phenomenon have been proposed,[19] and in the last years, some researchers[23] have reported the application of mixed systems, formed by the traditional surfactants and tensioactive ionic liquids (ILs), aiming at enhancing the enzyme catalytic performance.

Ionic liquids[24] are salts composed by bulky asymmetric organic cations with disperse charge and small inorganic or organic anions with a low melting point. These compounds are an attractive alternative to organic solvents, as they have a large number of interesting and unique properties, among which a "designer solvent" character, imposed by a large number of potential anion/cation/alkyl chain combinations,[25] allowing the manipulation of their physicochemical, thermodynamic, and biological properties.[26] Their tunable nature allows their design for a specific reaction to improve its yield and selectivity.[27] Their application has been studied in organic catalysis,[28,29] polymerization,[30,31] and biocatalysis either as organic media[27,32] or as surfactants in aqueous environments.[23,33–36] Many studies on the effect of ILs on the enzyme properties have been reported.[37–43] Despite the significant number of publications describing the possibility of forming micelles using ILs with long alkyl chains, it was only in 2010 that their use as surfactants to induce superactivity in the *Candida antarctica* lipase B (CaLB) was reported.[44] The increment on activity was explained by the formation of aggregates because of the tensioactive nature of the IL. In this context, this work proposes the application of tensioactive ILs (with long alkyl chains) on the formation of emulsion systems, aiming at increasing the catalytic performance of CaLB to catalyze the hydrolysis reaction, producing valuable fatty acids from a commercial olive oil. A Portuguese olive oil with low acidity was chosen as substrate, as it is composed by valuable fatty acids, namely, oleic, linoleic, and palmitic acids. CaLB was adopted as it is widely used industrially because of its high stability. Furthermore, this enzyme is a valuable scientific and industrial tool because it does not require a hydration shell to be active and has a relaxed substrate specificity and good operational stability, even when contacting with ILs.[45–49]

Material and Methods

Chemicals

The commercial CaLB was kindly supplied by Novozymes A/S, Bagsværd, Denmark. A commercial olive oil, Rosmaninho Virgem Extra, was used as substrate. The olive oil with low acidity (0.3%) was produced by Cooperativa de Olivicultores de Valpaços, being purchased in a local market in Aveiro.

The ILs used were trihexyl(tetradecyl)phosphonium chloride ($[P_{6,6,6,14}]Cl$), kindly offered by Cytec, and 1-decyl-3-methylimidazolium chloride ($[C_{10}mim]Cl$), acquired at Iolitec (Ionic Liquid Technologies). Disodium phosphate dodecahydrate (≥99.0%) and potassium phosphate monobasic (≥99.5%) were purchased from Sigma-Aldrich®, sodium hydroxide (≥98.0%) from Eka Chemicals, pure potassium hydroxide from Pronolab, pure citric acid, ethanol, and acetone (99.99%) were purchased from Fisher Scientific, and hydrochloric acid (37%) was acquired from Fisher Chemical. The chemicals used in this study are of analytical grade.

Emulsion characterization

Emulsion Preparation. To prepare the emulsion, 10 mL of olive oil and 10 mL of an aqueous solution (buffer solution) of each IL (0.250 M) were mixed using an ultrasonic homogenizer equipment (130 W; Sonics-Vibra Cell™) for 10 min at 20 W in an ice bath. To optimize the emulsions preparation, several water/olive oil volumetric ratios [from 1 to 50% (v/v)], IL concentrations (from 0.020 M to 0.500 M), and buffer solutions (sodium phosphate buffer or McIlvaine buffer) or water were investigated and adjusted.

Emulsification Activity. For the determination of the emulsification activity (EA), 10 mL of emulsion was transferred into a test tube and centrifuged at 4,200*g* and 298.1 (± 1.0) K in a Thermo Scientific Heraeus Megafuge 16 R. After 15 min, the emulsified layer volume (V_w in mL) and the total volume (V_t in mL) were measured, and the EA[50] was calculated according to the following equation:

$$EA = \frac{V_w}{V_t} \times 100. \quad (1)$$

Viscosity. The viscosity of the emulsions prepared was determined at 310.15 K and atmospheric pressure, using an Anton Paar (model SVM 3000) automated rotational Stabinger viscometer–densitometer. The temperature uncertainty is ±0.02 K in the temperature range analyzed, and the relative uncertainty of the dynamic viscosity is ±0.35%. Further details about the equipment and method can be found elsewhere.[51]

Phase Inversion. A stainless steel electrode was connected to a conductometer to measure the conductivity of the emulsions. The olive oil and buffer solutions conductivity were determined as 68 μS cm^{-1} and 20 mS cm^{-1}, respectively. Therefore, a significant variation in the conductivity could be observed with the phase inversion. A fresh emulsion was made for each oil composition tested.

Olive oil enzymatic hydrolysis

Reaction Kinetics. The hydrolysis reaction was carried out in glass bottles of 5 mL at 310 (± 1) K and under constant agitation (100 rpm), using an incubator shaker IKA® KS 4000 ic control. Each hydrolysis reaction was carried out using emulsions (total volume of 25 mL) previously prepared (20 min) in the ultrasonic homogenizer at 20 W. The reaction was initiated by adding 25 μL (or 0.69 mg) of an aqueous solution of CaLB and 1 mL of emulsion. After the reaction time was established (different for each sample), the samples were removed from the incubator, and 7 mL of an acetone–water–ethanol (1:1:1) solution was added to stop the hydrolysis reaction. The content of FFAs produced was measured by titration, using a KOH solution (0.500 M) and phenolphthalein as indicator. Each experiment was performed in triplicate.

The kinetics of the reaction was expressed as the amount of FFAs produced (represented by the acidity ratio, I'_a) and the hydrolysis extension.

The percentage of hydrolysis was calculated from the acidity ratio and the total amount of fatty acids as described by the following equation:

$$\%\text{Hydrolysis} = \frac{I'_a \times 100}{I'_s}, \quad (2)$$

where I'_a represents the amount of KOH (mmol) necessary to neutralize the fatty acids released from 1 g of olive oil, as represented by the following equation:

$$I'_a = I_a(t) - I_a(t=0), \quad (3)$$

where

$$I_a = \frac{V_{KOH} \times M}{m_{oil}}. \quad (4)$$

The saponification index, I'_s, is defined as the amount of KOH (mmol) needed to carry out the esters saponification and to neutralize the released fatty acids per gram of olive oil.

$$I'_s = \text{TFA} - \text{FFA}. \quad (5)$$

Lipase Activity. The hydrolysis reaction was carried out in 20-mL glass bottles at 310 (± 1) K and under constant stirring at 100 rpm, using an incubator shaker IKA® KS 4000 ic control. The reaction was initiated by adding 125 μL (3.45 mg) of lipase (or water in the control system) into the reaction mixture composed of 5 mL of the emulsion previously prepared. After a given time (which is dependent on the linear region of the reaction for each set of specific conditions), 350 μL of the reaction media was collected and placed in an Erlenmeyer containing 2 mL of an acetone–water–ethanol solution (1:1:1) to stop the reaction. The amount of FFAs released in the reaction mixture was estimated by titration, using a KOH aqueous solution (0.500 M) and phenolphthalein as indicator. Each experiment was performed in triplicate. One unit of lipase activity is defined as the amount of enzyme which produces 1 μmol of fatty acids per minute at the assay conditions. The CaLB activity was calculated by the following equation:

$$\text{Activity}\ \left(\mu\text{mol min}^{-1}\,\text{mL}^{-1}\right) = \frac{(V_s/V_{reac} - V_c/V_{reac}) \times M \times 1{,}000}{t}, \quad (6)$$

where V_s and V_c represent the volume of KOH used to titrate each sample and the control (in mL), respectively, M represents the molality of the KOH solution (in mol L^{-1}), t represents the time of reaction (in min), and V_{reac} represents the volume of the reaction solution (in mL).

The relative activity, used to compare the enzymatic activity between systems with and without ILs, is given by the following equation:

$$\text{Relative activity} = \frac{\text{CaLB activity in systems with IL}}{\text{CaLB activity in systems without IL}}. \quad (7)$$

Results and Discussion

In this work, the effect of oil-in-water and water-in-oil emulsions formed by the tensioactive ILs on the enzymatic hydrolysis of vegetable oils aiming at producing added-value fatty acids was studied.

Emulsion optimization

In this work, the use of ILs as emulsion-stabilizing agents is studied. The effect of different IL concentrations, water content used in the emulsion formulation, buffer type, pH media, and temperature on the EA were evaluated; the results are presented in Figure 1. The effect of two ILs with proved tensioactive nature, namely, $[P_{6,6,6,14}]Cl$ (CMC = 0.023 M; unpublished data) and $[C_{10}mim]Cl$ (CMC = 0.048 M),[44] was investigated at concentrations from 0.020 to 0.425 M (Figure 1a). These results suggest a positive effect promoted by the presence of both ILs, demonstrating that the EA is maintained constant and at high levels above 0.05 M. From these results, 0.100 M of concentration for both ILs was selected as the most adequate for the following studies. The effect of the water/olive oil volumetric ratio (varying from 10 to 90% of water) in the EA was also tested; the experimental data are shown in Figure 1b. It was concluded that the emulsion stability was strongly dependent on the water amount and has an inverse bell shape behavior, that is, the emulsion stability decreases while increasing the water content until 50% (v/v), which seems to be justified by the increase in the viscosity (Supporting Information Figure S1), and then increases with the water concentration. These results suggest that emulsions with high stability are obtained when the difference in the concentration of both components is high, that is, in the extreme scenarios of water and oil concentration. This tendency is justified by the fact that intermediate concentrations promote the occurrence of the emulsion phase inversion, as supported by the conductivity measurements depicted in Supporting Information Figure S2. Considering that one of the main objectives of this work was the study of the hydrolysis reaction at high oil concentrations, using water-in-oil emulsions to simplify the simultaneous recovery and purification of the fatty acids, the optimum condition found for a complete hydrolysis in a stable emulsion was 10% (v/v), that is, 0.10 mL of water per milliliter of olive oil.

The effects of the ionic strength and the pH media using different salt additives were also investigated (Figures 1c,d) toward the EA. The study of the ionic strength was carried out by using a sodium phosphate buffer (0.100 M) and the McIlvaine buffer (0.150 M); the results were compared with the effect promoted by the addition of distilled water. Regarding the pH effect, four distinct pH solutions (pH 5, 6, 7, and 8) based on the McIlvaine buffer were applied. From the results depicted in Figures 1c,d, it seems that the ionic strength and the pH of the aqueous phase have no significant effect (<0.025) on the emulsion stability. Moreover, a nonsignificant effect on the emulsion stability was achieved when different temperatures [from 298 to 313 (± 1) K] were tested (Figure 1e), which is in agreement with the literature.[52]

Optimization of the hydrolysis reaction conditions

The optimization of the hydrolysis conditions of a low-acidity (0.3%) olive oil to produce added-value fatty acids were tested by applying water-in-oil emulsions to superactivate the enzyme. Before the optimization of the reaction conditions, olive oil was characterized regarding the initial

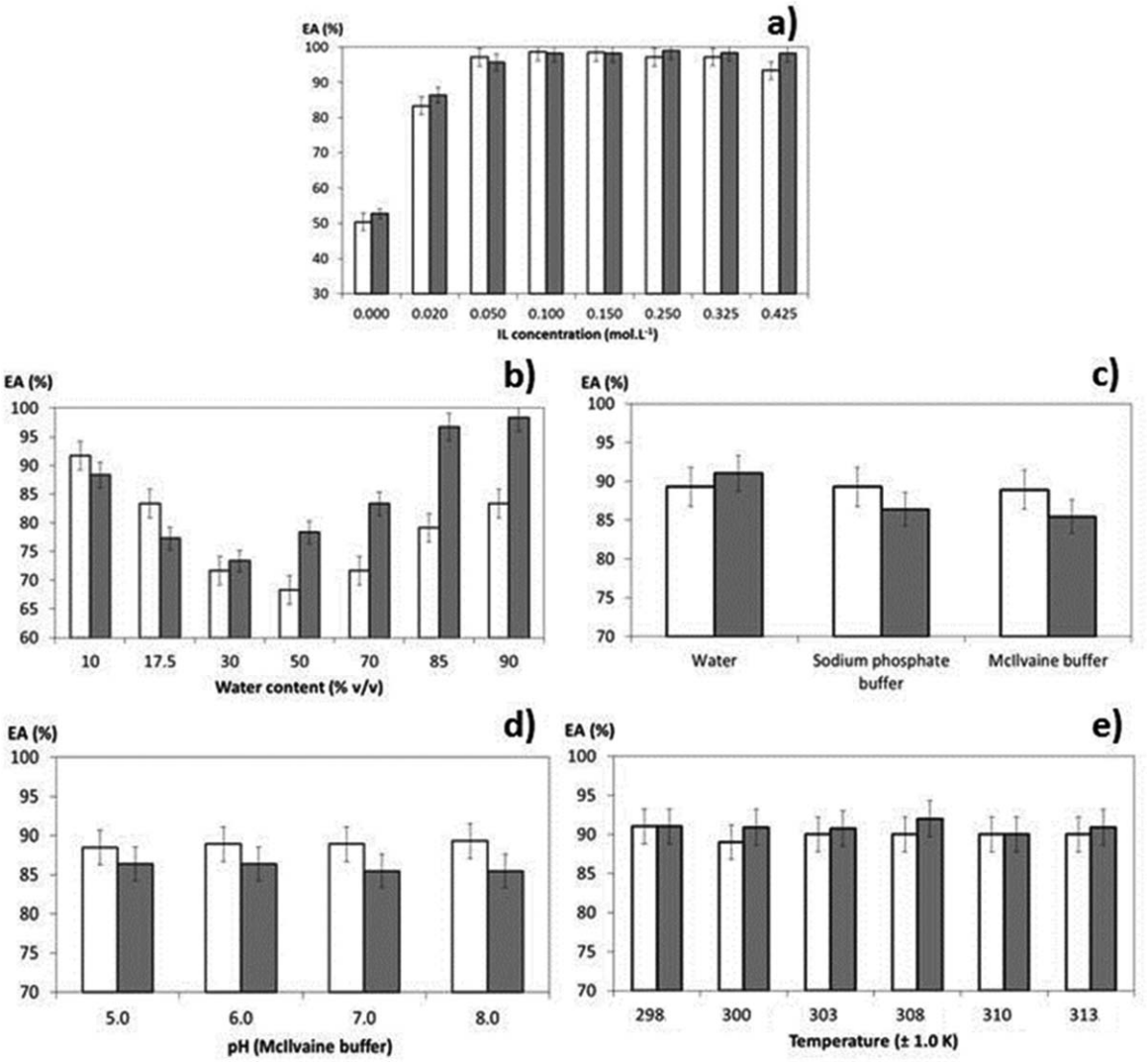

Figure 1. Effect of different parameters on the emulsification activity.

(a) IL concentration (in mol L^{-1}): (□) $[P_{6,6,6,14}]Cl$ and (■) $[C_{10}mim]Cl$; (b) water content (%, v/v) presented in the emulsion formulation: (□) $[P_{6,6,6,14}]Cl$ (0.100 M) and (■) $[C_{10}mim]Cl$ (0.100 M); (c) ionic strength; (d) pH using the McIlvaine buffer: (□) $[P_{6,6,6,14}]Cl$ and (■) $[C_{10}mim]Cl$; and (e) temperature: (□) $[P_{6,6,6,14}]Cl$ and (■) $[C_{10}mim]Cl$.

and total concentration of FFAs. The initial concentration determined was 0.0058 ± 0.0007 mmol of FFAs per gram of olive oil or 0.0051 ± 0.0006 mmol FFA per milliliter of olive oil. The total amount of FFAs determined was 3.45 ± 0.02 mmol per gram of olive oil (or 3.04 ± 0.02 mmol per milliliter of olive oil).

To optimize the hydrolysis reaction conditions, the impact of ILs on the enzyme superactivity was studied. The control system, that is, the system without ILs presented an initial velocity of 2.55 μmol mL^{-1} min^{-1}. Because the hydrolysis extension under these initial conditions was shown to be extremely low (less than 3.5%), a careful optimization of the reaction conditions was performed, taking into account the agitation speed, the enzyme concentration, and the temperature of reaction, aiming at maximizing the hydrolysis yield.

Figure 2a shows the enzymatic activity as a function of the agitation rate, from 100 to 400 rpm. The results suggest that high agitation rates have a favorable impact on the reaction, which is justified by the reduction of the droplet size and the consequent increase of the oil–water interface, the specific region where the hydrolysis takes place, thus promoting the increase in lipase activity.[53]

The effect of the lipase concentration, from 0.28 to 8.31 mg protein per milliliter, was studied (at 400 rpm) to verify the region of linearity regarding the enzyme activity versus the lipase concentration.[53] The experimental data depicted in Figure 2b show a fast increase in the lipase activity with the enzyme concentration, as the ratio between the free interfacial area and the lipase concentration increases. In this case, all enzyme molecules seems to be available to adsorb the substrate, thus promoting the hydrolysis reaction at the water/oil interface, the maximum of this behavior being achieved at an enzyme concentration of 2.08 mg protein per milliliter, enzyme concentration adopted in the following studies. This maximum is explained by the progressive decrease of free enzyme molecules at the oil/water interface able to catalyze the reaction, as discussed by other authors.[54,55]

The temperature effect in the lipase catalytic activity was also investigated from 308 to 322 (± 1) K, as shown in

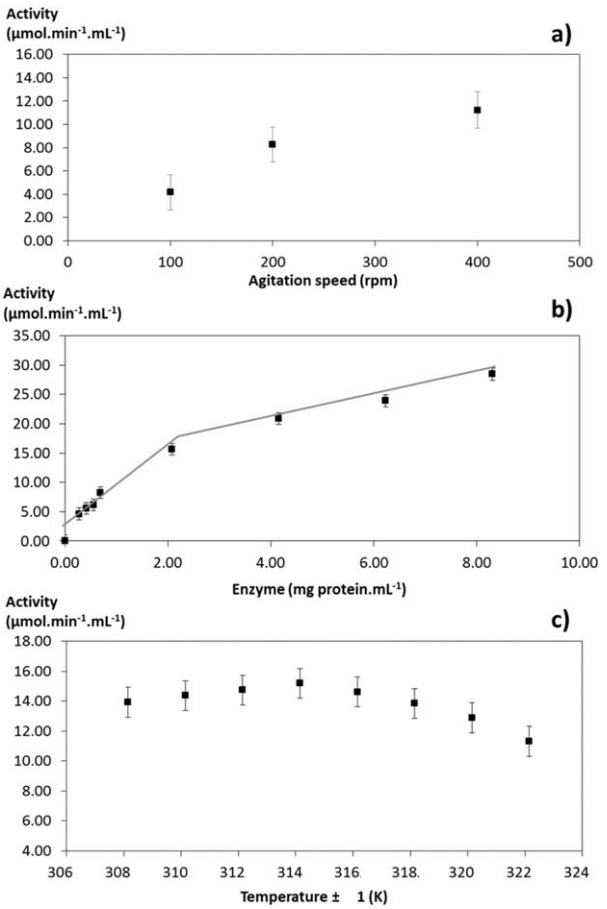

Figure 2. Effect of different conditions on the lipase activity.

(a) Agitation speed at 310 (± 1) K, pH 7, and 0.69 mg of CaLB per milliliter; (b) lipase concentration at 400 rpm, 310 (± 1) K, and pH 7; and (c) temperature at 400 rpm, pH 7, and 2.08 mg of CaLB per milliliter.

Figure 2c. In general, the results suggest that no significant variations were observed, with the enzyme activity independent of the temperature of the hydrolysis, up to 314 (± 1) K, above which a decrease in the enzymatic activity is observed, probably due to enzyme denaturation.[55]

CaLB performance in IL-based emulsions

As discussed, the kinetics of an enzymatic reaction is influenced by the reaction conditions applied. After establishing the best conditions for the hydrolysis reaction, 314 (± 1) K, 400 rpm, and 2.08 mg protein per milliliter, the hydrolysis of the olive oil catalyzed by CaLB was followed during 90 min (Supporting Information Figure S4). Considering these results, the initial velocity of the reaction was identified from the exponential phase slope (between 0 and 10 min) as 19.67 μmol mL^{-1} min^{-1}. By comparison with Supporting Information Figure S3 (before optimization), it is evident of the importance of the optimization for an enzymatic reaction, because not only the initial velocity is eight times higher for the optimized systems but the hydrolysis extension was also increased.

Tests with the ILs ($[P_{6,6,6,14}]Cl$ and $[C_{10}mim]Cl$) were carried out at 10% (v/v) of water, temperature of 314 (± 1) K, agitation of 400 rpm, and enzyme concentration of 2.08 mg protein per milliliter. Supporting Information Figure S5

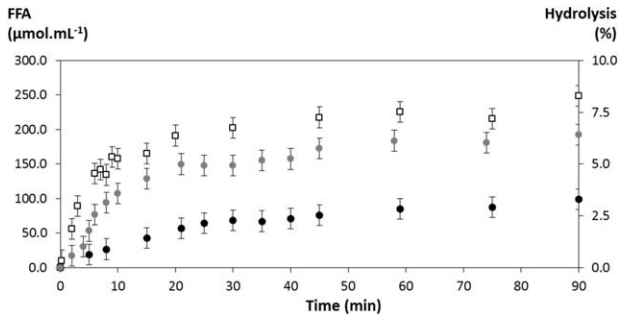

Figure 3. Kinetics of CaLB-catalyzed hydrolysis of olive oil at 314 (± 1) K, 400 rpm, 2.08 mg protein per milliliter, and 10% (v/v) of water in the emulsion preparation with (•) $[P_{6,6,6,14}]Cl$ and (•) $[C_{10}mim]Cl$ and (□) in the absence of ILs (only with sodium phosphate buffer).

shows how the enzymatic activity is affected by the presence of both ILs. Under these conditions, the ILs studied here presented a negative effect in the enzymatic activity, unlike previously observed for the hydrolysis of *para*-nitrophenyl laurate.[44] To achieve a better understanding of this behavior, the kinetics of the hydrolysis was followed for both ILs, as depicted in Figure 3. The results showed a negative effect in the hydrolysis of the olive oil when the ILs (in particular phosphonium) are applied as surfactants in the emulsion preparation. Despite the low hydrolysis extension, the amount of FFAs (with long alkyl chains) produced is high. As mentioned, the fatty acids commonly produced in the hydrolysis of olive oil (mainly palmitic and oleic acids) are characterized by a pronounced surface activity, which means that they may interact with the interface of the emulsions, consequently promoting the inhibition of the enzyme catalytic activity.[56] The behavior reported here contradicts the observations of Das and coworkers,[61] who described a better performance from the lipase when larger head-group size surfactants were applied. Actually, it is possible to conclude that the lipase activity in this particular case seems to be regulated by other factors than the head-group size, namely, interactions between $[P_{6,6,6,14}]Cl$ and the fatty acid molecules responsible for the change in the emulsion molecular packing, thus showing an adverse impact in water-in-oil emulsions and specifically in this enzymatic reaction.

As the water-in-oil emulsions were shown to be inadequate, the reaction was carried out at different amounts of water (previously tested in the emulsion stability studies) aiming at understanding how the change of the emulsion from water-in-oil to oil-in-water could influence this reaction. Figure 4 shows an increase in the hydrolysis performance with the increase of the water content used in the emulsion formation. This increment is observed both in the absence and presence of these ILs; however, this behavior seems to be more pronounced in the presence of ILs, in particular when $[C_{10}mim]Cl$ is used. Actually, a complete hydrolysis (≈100%) is achieved when $[C_{10}mim]Cl$ is introduced into the system. These results show the positive effect of the presence of ILs for the high amounts of water, as the relative activity increases with the water–oil ratio, and it only shows a deleterious effect when the water–oil ratio is 50% (v/v) and 70% (v/v). These exceptions are explained by the phase inversion from water-in-oil to oil-in-water emulsions (Figure 1b and Supporting Information Figure S2), the latest being less stable due to the phase inversion. This

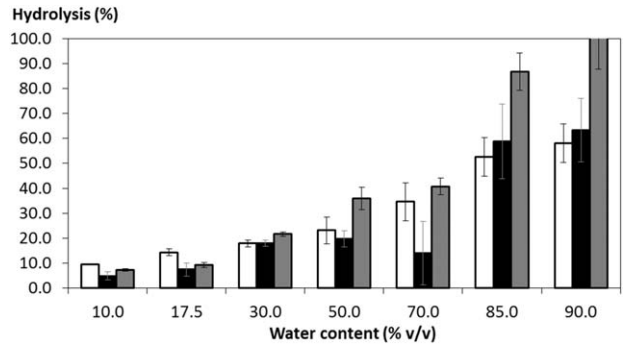

Figure 4. Olive oil hydrolysis as function of the water content (%, v/v) in the emulsion with (■) [$P_{6,6,6,14}$]Cl and (■) [C_{10}mim]Cl and (□) in the absence of ILs (only with sodium phosphate buffer).

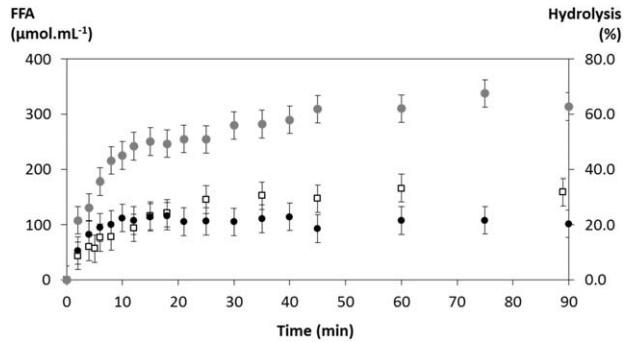

Figure 5. Kinetics of CaLB-catalyzed hydrolysis of olive oil at 314 (± 1) K, 400 rpm, 2.08 mg protein per milliliter, and 85% (v/v) of water in the emulsion preparation with (•) [$P_{6,6,6,14}$]Cl and (•) [C_{10}mim]Cl and (□) in the absence of ILs (only with sodium phosphate buffer).

significant increment in the enzymatic activity was investigated by us[44] and others,[57,58] and it is known as superactivity. In this case, the relative activity increases up to a maximum of 1.8-fold, and a complete hydrolysis is reached. According to these results, 85% (v/v) of water content was chosen to compare the hydrolysis kinetics for the three systems, as shown in Figure 5. The main results evidence that both ILs increase the initial velocity of the hydrolysis reaction (6.81 and 14.12 μmol mL^{-1} min^{-1} for [$P_{6,6,6,14}$]Cl and [C_{10}mim]Cl, respectively) when compared with the control system, that is, without ILs [4.40 μmol mL^{-1} min^{-1}]. However, only [C_{10}mim]Cl shows to be advantageous, as the amount of FFAs (≈325 μmol mL^{-1}) and the hydrolysis extension (≈70%) are higher than the results found for the control system (without ILs). The activation of CaLB by the imidazolium IL may be related with the similarity between the imidazolium cation and the histidine amino acid, an important residue of the catalytic lipase triad responsible for facilitating the hydrolysis reaction. Hence, the imidazolium group, when present in the interface, promotes the increase of the water nucleophilicity by the hydrogen bond interactions established, consequently improving the enzyme activity.[59,60]

Finally, the effects of the reaction media pH and the ionic strength of the bulk system were also evaluated by the application of various buffers, namely, the sodium phosphate buffer and the McIlvaine buffer (Figure 6). The enzyme seems to be more active in an alkaline pH ≈ 8 (results found

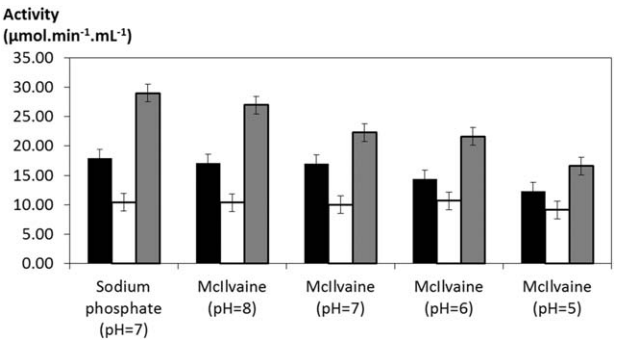

Figure 6. Influence of type and pH buffer in the lipase activity: (■) buffer (absence of ILs), (□) [$P_{6,6,6,14}$]Cl, and (■) [C_{10}mim]Cl.

for the McIlvaine buffer at 0.150 M). However, the ionic strength seems to have the more significant impact on the hydrolysis reaction, because despite the lower pH ≈ 7 when the sodium phosphate buffer (0.100 M) is applied, the results were better.

Conclusions

The application of long alkyl chain ILs to the formation of emulsions on the enzymatic hydrolysis of olive oil to produce valuable fatty acids was investigated. This work reports a complete study of the conditions to be taken into account, not only regarding the emulsion formulation but also the hydrolysis reaction performance. In this context, the whole emulsion spectrum was considered, from water-in-oil to the oil-in-water emulsions, showing significant variations in terms of enzyme stability because of the relevant differences found in the emulsion structures and hydrolysis extension.

By the appropriate optimization of the reaction conditions, it was possible to superactivate CaLB by using tensioactive ILs. The lipase activity was increased to 1.8 times at 314 (± 1) K, 400 rpm, and 2.08 mg protein per milliliter, with the emulsion prepared by using 85% (v/v) of water and 0.100 M of each IL.

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