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Full Length Research Paper

Effect of the presence of surfactant and ionic liquids on the esterification of oleic acid catalyzed by immobilized lipase

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Ionic liquids (ILs) and surfactants are widely used in order to increase the catalytic efficiency of lipases. In this sense, the present study aimed to evaluate the potential of different ionic liquids ($C_4MIM.Ac$, $C_4MIM.HSO_4$, $C_4MIM.TF_2N$, $C_4MIM.BF_4$, $C_{12}MIM.I$, and $C_{12}MIM.Cl$), Aliquat 336 and di-2-ethylhexyl sodium sulfosuccinate (AOT) as adjuvants in the esterification of oleic acid with different alcohols catalyzed by Novozyme 435. First of all, it evaluates the effect of alkyl chain length of the alcohol and the positioning of the hydroxyl group. The selection of additives, made with isopropyl alcohol, pointed AOT and $C_4MIM.Ac$ as adjuvants effective on the potential of esterification of the enzyme (an increase of 35% in the potential of esterification). Using methanol, ethanol and 3-methyl-1-butanol, the $C_4MIM.Ac$ was proven not to be efficient, while the AOT shows adjuvant effect in the esterification of oleic acid with methanol (increasing in catalytic efficiency of enzyme in 21%).

Key words: Esterification, di-2-ethylhexyl sodium sulfosuccinate (AOT), Ionic Liquids, Novozyme 435.

INTRODUCTION

The synthesis of esters catalyzed by lipases has been intensively studied as an alternative to the conventional method of synthesis, although the enzymatic synthesis of monoglycerides is not being performed in industrial scale. The choice of lipases as catalysts in processing oils and fats is mainly due to the mild reaction conditions required, which imply low energy consumption and its high selectivity, which in an integrated manner, resulting in better quality products. It is worth noting from environmental point of view that enzymatic process is technically clean and safe (Freitas et al., 2008). Lipases

in organic synthesis are widely used because of its selectivity and specificity (Martín et al., 2008). However, the advantages of the use of lipases bump mainly in difficulty of solubilization of a large part of substrates in water. Thus, biotransformations in aqueous-restricted environments have attracted great interest from the scientific community (Hara et al., 2009).

The use of biocatalysts in non-aqueous media offers advantages as increased solubility of hydrophobic substrates and possibility of driving processes that are thermodynamically unfavorable in water (as esterification and transesterification), beyond the ease product recovery (Klibanov, 2011). Without doubt the organic solvents stood out during long as a non-aqueous medium most used in biocatalysis (Rantwijk and Sheldon, 2007), but the use of organic solvents conventional in enzymatic

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systems can also produce undesirable physico-chemical effects in the structure of biocatalyst thereby reducing their activity or even inactivating it; moreover, it promotes the increase of costs of the process since subsequent removal of this solvent needed (Kaewthong et al., 2005; Arcos and Otero, 1996). In recent years, with the growing evolution of call green chemistry, alternative solvents as supercritical fluids (Fricks et al., 2009), ionic liquids (Pan et al., 2010) and the use of surfactants (Valério et al., 2009) comes; gaining at each point more space in modern biocatalysis with the purpose of increasing the efficiency of lipases and reduce the use of conventional organic solvents.

The use of surfactants may be an alternative for dissolution of substrates miscibility distinct (hydrophilic and hydrophobic) gifts in reaction medium. Non-ionic surfactants, for example, Triton X100 and Tween 80 were commonly used as biochemical assays for many years (Lichtenberg et al., 1983). The systems of reverse micelles arouse interest in particular when using ionic surfactants that promote the formation of microemulsions calls "water in oil" that enable the solubility, even in high concentrations, of hydrophilic compounds and lipophilic system. Most studies with reverse micelles in biological systems employ di-2-ethylhexyl sodium sulfosuccinate (aerosol-OT or AOT) as surfactant (Stamatis et al., 1999; Bartscherer et al., 1995). Unlike other tensoactives, by virtue of their molecular geometry, AOT does not require co-surfactants for the formation of reverse micelles (Stamatis et al., 1999; Singh et al., 1994).

Ionic liquids (LIs) are liquid salts at room temperature and have been of great interest because unlike organic solvents are not volatile and/or flammable and have a high thermal stability (Rantwijk et al., 2003; Eckstein, et al., 2002). Moreover, as in the case of LIs with imidazoles cations, presented lower toxicity if compared to conventional solvents (Jarstoff, et al., 2003). But, without any doubt, the main advantage to the use of ionic liquids in biocatalysis is the possibility of dissolution of immiscible substrates like alcohols and fatty acids. The literature is wide discussed on examples of the use of LIs in esterification reactions with lipases (Vidya and Chadha 2010; Zhang et al., 2008; Pavlidis et al., 2009; Adamczak and Barnscheuer 2009; Kahveci et al., 2009; Lee et al., 2008; Diego et al., 2009). The possibility of better solubilization of hydrophobic and hydrophilic substrates and the elucidation of the behavior of lipases in aquo-restricted media motivated this work, whose main objective is to evaluate the effect of the presence of ionic liquids and tensoactive (AOT) in the esterification of oleic acid with different chain lengths alcohols catalyzed by lipase Novozyme 435.

MATERIALS AND METHODS

The reactions were conducted on thermostat bath (Nova Técnica) in hermetically sealed reactors with 50 ml of capacity containing oleic acid (Aldrich) and alcohol molar ratio (1:1.62). The enzyme

used in the study was the commercial lipase from *Candida antarctica* (Novozym 435), immobilized on a macroporous anionic resin, acrylic resin (1.4% water, diameter in the range of 0.3 to 0.9 mm was purchased from Novozymes (Araucária, PR, Brazil)) and used as catalyst in all tested systems. In order to verify the effect of chain size of alcohol, reactions were performed in the absence of additive and with different alcohols (Vetec, 99.5% purity): methanol, ethanol, 1-propanol, isopropanol, 1-butanol, isobutanol, tert-butanol, 1-pentanol and 3-methyl-1-butanol. The substrates, in the molar ratio, and the additives studied (10.7 mol% based on number of moles of oleic acid) were added to reactor. The total volume reaction was about 5 ml. All reactions were conducted in the presence of molecular sieves (3.2 mm pellets) in order to remove the water from the esterification reaction and to prevent the reverse reaction. The enzyme was added (3.36 % (w/w) based on oil weight) after stabilization of reaction temperature medium (at 333 K). The following reaction times (tx) were studied: 1, 2, 3, 5, 10 min.

First, the better additives for enzymatic esterification of oleic acid with isopropanol were investigated. The additives tested were ionic liquids with imidazole cation (C₄MIM.Ac, C₄MIM.HSO₄, C₄MIM.TF₂N, C₄MIM.BF₄, C₁₂MIM.I, C₁₂MIM.Cl), Aliquat 336 (a quaternary ammonium salt, that mimics an ionic liquid) and AOT - all reagents Adrich, 99.5 % purity (Figure 1). Esterification reactions of oleic acid with methanol, ethanol and isoamyl alcohol were also performed in the presence of the additives AOT and C₄MIM.Ac. For all experimental conditions were made controls in the absence of enzyme. The conversion of oleic acid was expressed as oleic acid consumed: after the reaction time (tx), aliquots of 0.3 ml were collected in triplicate, weighed, solubilized in 5 ml of acetone: ethanol (1:1) and titrated with NaOH 0.1 M properly standardized. Conversion was calculated according to Eq.1.

$$C(\%) = \frac{(V_{\text{NaOH}} * M)t_0 - (V_{\text{NaOH}} * M)t_x}{(V_{\text{NaOH}} * M)t_0} * 100$$

Where, C is conversion (%), V is volume NaOH (ml) and M is molarity (mol.L⁻¹).

RESULTS AND DISCUSSION

The size chain effect of alcohol is shown in Figure 2. It is assumed that the formation of enzyme acylated as an intermediary of reaction is a crucial step during esterification and the conversion to ester depends on the accessibility of the alcohol for the formation of this intermediate (Liu et al., 2009). In this sense, alcohols of short chain creates a specific geometry around the active site, which favors the recognition of alcohol as they offer less steric impediment, which would facilitate the stabilization of the intermediate acylated. This fact explains greater activity of esterification with methanol and ethanol; however, in the case of reactions catalyzed by lipases, another factor must be mentioned: the solvent polarity. It is known that lipases have greater stability in media containing less polar solvents, like hexane (Log P > 3) and lower stability in more polar solvents as ethanol (Log P = -0.24) (Kragl et al., 2002). Whereas with primary alcohols and with more than three carbons, the Novozyme 435 presented greater power of esterification with alcohols that possess higher Log P values (3-methyl-1-butanol > 1-pentanol > 1-butanol > 1-propanol). For

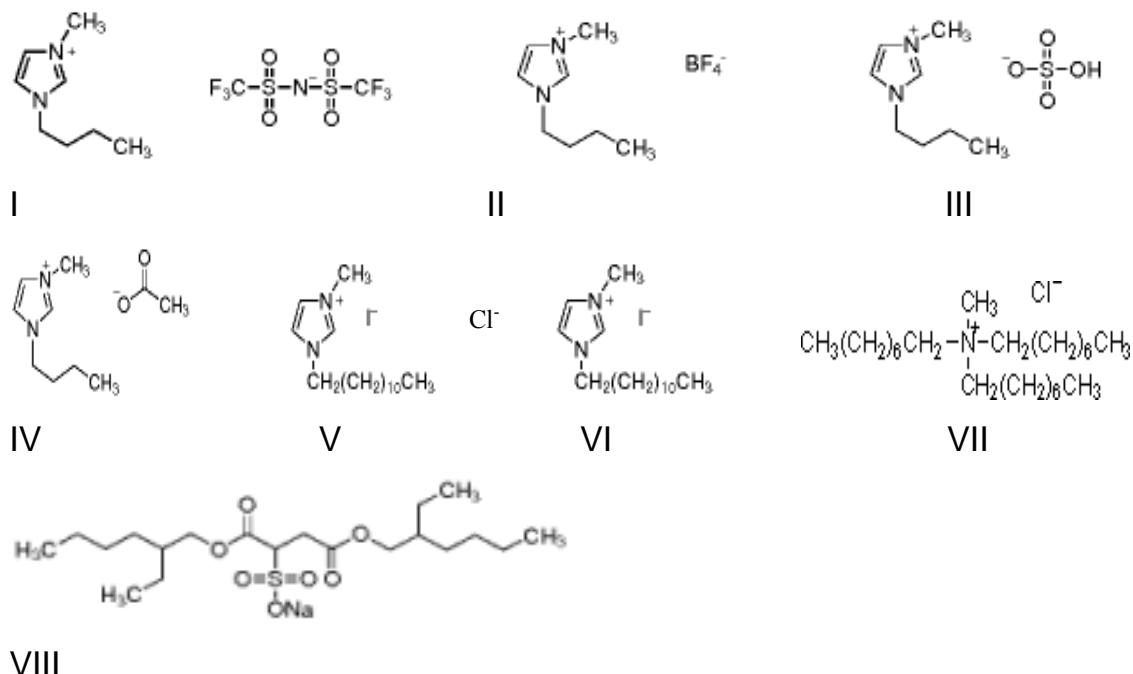


Figure 1. Additives tested. IIs: I-VIII: **I.** 1-butyl-3-methylimidazolium bis (trifluoro methyl sulfonyl) imide ($C_4MIM.TF_2N$); **II.** 1-butyl-3-methylimidazolium tetrafluoroborate ($C_4MIM.BF_4$); **III.** 1-Butyl-3-methylimidazolium hydrogen sulfate ($C_4MIM.HSO_4$); **IV.** 1-Butyl-3-methylimidazolium acetate ($C_4MIM.Ac$); **V.** 1-Dodecyl-3-methylimidazolium chloride ($C_{12}MIM.Cl$); **VI.** 1-Dodecyl-3-methylimidazolium iodide ($C_{12}MIM.I$); **VII.** Trioctylmethylammonium chloride (Aliquat 336[®]); **VIII.** Di-2-ethylhexyl sodium sulfosuccinate (AOT).

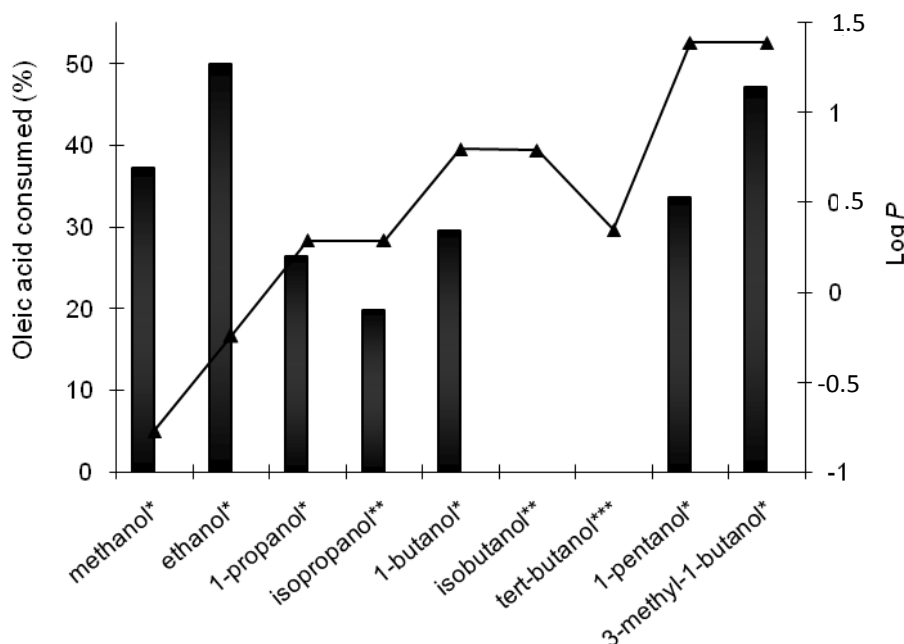


Figure 2. Effect of chain size of alcohol and positioning the hydroxyl. *OH on primary carbon, **OH on secondary carbon, ***OH on tertiary carbon.

secondary alcohols, Novozyme 435 shows lower catalytic power compared with primary alcohols (1-propanol > isopropanol), and showed no activity with isobutanol and

tert-butanol, secondary and tertiary alcohols, respectively, which shows that the construction of intermediary reaction depends strongly on accessibility of

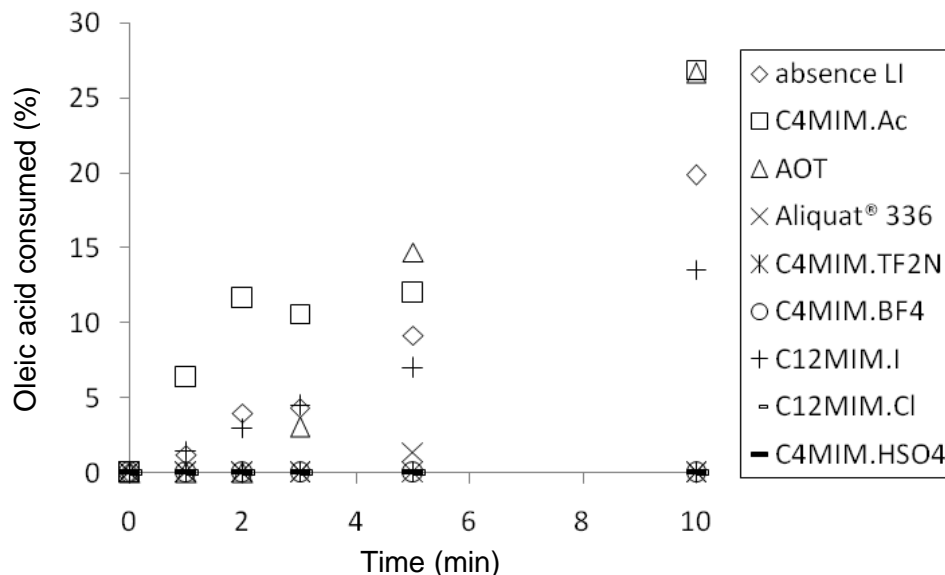


Figure 3. Selection of additives: LIs, AOT and Aliquat 336 (10,7 mol%). Substrates: oleic acid and isopropyl alcohol, molar ratio (1:1.62).

the alcohol to binding site, since the isobutanol presents Log *P* similar to the corresponding primary alcohol (1-butanol).

For the selection of additives on the esterification of oleic acid, we use isopropyl alcohol as alkyl chain donor alcohol. The results are shown in Figure 3. Novozyme 435 was inactivated in the presence of C₄MIM.HSO₄, C₄MIM.TF₂N, C₄MIM.BF₄, C₁₂MIM.Cl and Aliquat 336 and show activity in the presence of esterification C₄MIM.Ac, AOT and lower intensity C₁₂MIM.I. The stability of lipases in LIs dependent strongly on factors such as hydrophobicity, polarity, basicity, viscosity and their ability to form hydrogen bonds (Gu and Li, 2009). Normally LIs series C₄MIM presented similar polarities, but differ in terms of water solubility. C₄MIM.TF₂N is sparingly soluble in water (1.4 % (w/v) whereas C₄MIM.BF₄ is totally soluble in water (Park and Kazlauskas, 2003), which favors interaction with the structural water of the enzyme, and is important for keeping the catalytic active conformation. Also, multiple hydrogen bonds and other strong interactions can occur that lead to unfolding of the protein (Martin et al., 2008). C₄MIM.TF₂N despite its higher hydrophobicity, more easily disperse the negative charge in 3 fluorine atoms in its anionic moiety, which causes less destabilization of hydrogen bonds of the protein and not favorable to esterification, this fact can be explained by the greater solubilization difficulty of the substrates. The inactivation of the enzyme in the presence of C₄MIM.HSO₄, C₁₂MIM.Cl, Aliquat 336, can be justified by the strong destabilization of the intramolecular hydrogen bonds of the enzyme, since these anions are derived from extremely strong acids (HCl, H₂SO₄).

The enzyme show larger activity esterification (mainly

with shorter reaction times (1 to 3 min, conversion from 6.37 to 10.61 %) with C₄MIM.Ac, which can be justified by the lower interference of anionic portion of the LI in the structure of enzyme, since it comes to LI with anion derived from weak acid (H₃CCO₂H), with lower avidity by hydrogen bonds in the structure of the enzyme. In the presence of C₁₂MIM.I the enzyme shows potential for esterification intermediary (conversion 13.55 % in 10 min). The presence of C₁₂MIM.I was more beneficial than in the presence of C₄MIM.HSO₄, C₁₂MIM.Cl, Aliquat 336, C₄MIM.BF₄ and C₄MIM.TF₂N and less efficient in the presence of AOT and C₄MIM.Ac, because the anion is derived from less strong acid than HCl and H₂SO₄, however, it has higher cationic portion (12 carbons) causing reaction medium more viscous which hinders the mass transport properties and the access of substrates to the active site of the enzyme (MacFarlane et al., 2001; Park and Kazlauskas, 2003).

Novozyme 435 presented power of esterification appreciable in the presence of AOT, with conversion of 14.71 % in 5 min, while in the absence of additive this value was of 9.18 % (Figure 3). This anionic surfactant promotes the formation of microemulsions thermodynamically: non numeric scale droplets dispersed in an organic phase stabilized by a surfactant, the so called reverse micelles. These structures are relatively orderly and are characterized by their diameter and defined by the enormous interfacial area, which obviously favors the reactions mediated by lipases that have their mechanism of action, in most cases, activation in regions of interface (Stamatis et al., 1999; Bartscherer et al., 1995). Among the additives studied, the presence of AOT and C₄MIM.Ac were favorable to potential esterification of oleic acid with isopropanol catalyzed by Novozyme 435.

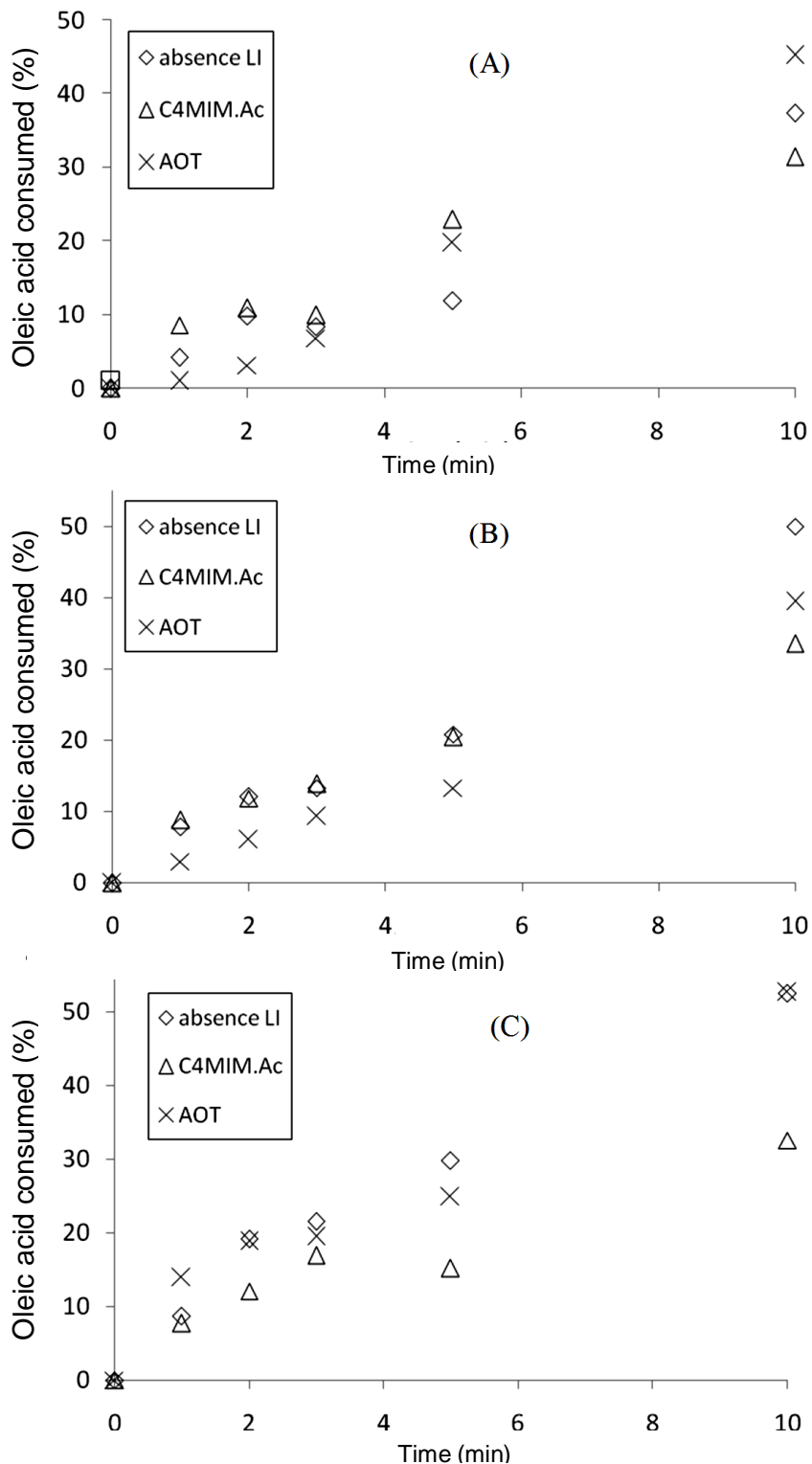


Figure 4. Effect of AOT and C₄MIM.Ac (10.7 mol %) for consumption of oleic acid: A, methanol; B, ethanol; C, 3-methyl-1-butanol. Molar ratio acid: alcohol (1:1.62).

The power adjuvant these additives were tested utilizing methanol, ethanol and isoamyl alcohol as donors the grouping acyl, the results are shown in Figure 4.

For methanol, with reaction time lower or equal to 5 min no additives was efficient, but with 10 min of reaction AOT showed itself effective, increasing the potential for

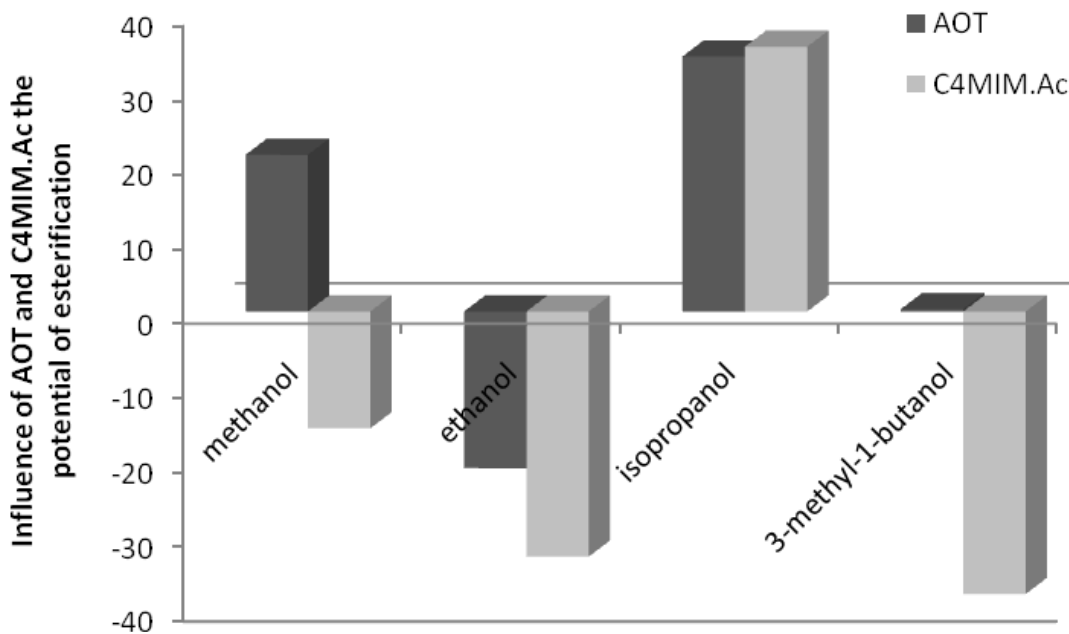


Figure 5. Influence of AOT and C₄MIM.Ac on the esterification of oleic acid with different alcohols catalyzed by lipase.

esterification by 20%. For the ethanol no additives proved efficient in increasing potential for esterification of Novozyme 435. For isopropanol, both the AOT and C₄MIM.Ac increased the potential of esterification of the enzyme by approximately 35%. For 3-methyl-1-butanol, the AOT shows no influence in catalytic power of the enzyme while the C₄MIM.Ac influenced negatively the enzymatic activity (Figures 4 and 5).

Conclusions

Among the tested additives, AOT and C₄MIM.Ac are potential adjuvants in esterification reaction of oleic acid with isopropanol catalyzed by Novozyme 435. The power adjuvant C₄MIM.Ac and AOT in esterification reactions strongly dependent on the nature of the alcohol used.

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