Solvent-free ethyl oleate synthesis mediated by lipase from *Candida antarctica* Bfree

Síntese de oleato de etílio sem solventes mediada pela lipase de *Candida antarctica* Bfree

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ABSTRACT

Lipases are the more usable enzymes for this purpose, due to the ability to recognize a wide variety of substrates and catalyze a high number of reactions. In this context, the objective of the present study using Lipase B from *Candida antarctica* B to ethyl oleate ethyl synthesis solvent-free in function of the studied variables, mass catalyst free and temperature.A maximum conversion of ethyl oleate was observed with 56% of conversion after 40 minutes of reaction time. The oleate esters of ethyl were reported for having applications interesting in various industrial fields, such as, food, aromatic products, cosmetics, detergents, flavorings and pharmaceuticals.
Kewyords: Esters, enzyme,ethyl oleate, catalysis, esterification.

RESUMO

As lipases são as enzimas mais utilizáveis para este fim, devido à capacidade de reconhecer uma grande variedade de substratos e catalisar um elevado número de reações. Neste contexto, o objetivo do presente estudo foi utilizar a Lipase B de *Candida antarctica* B para a etil-oleato etil-síntese livre de solventes em função das variáveis estudadas, massa catalítica livre e temperatura. Uma conversão máxima de oleato de etila foi observada com 56% de conversão após 40 minutos de tempo de reação. Os ésteres oleato de etilo foram relatados por terem aplicações interessantes em vários campos industriais, tais como, alimentos, produtos aromáticos, cosméticos, detergentes, aromatizantes e farmacêuticos.
Palavras-chave: Ésteres, enzima, oleato de etila, catálise, esterificação.

INTRODUCTION

Lipases are biocatalysts of great importance in different areas, being able to catalyze reactions in aqueous or organic media. Furthermore, these enzymes are capable of using several substrates being stable in a wide range of pH and temperatures (NETA et al., 2012).

Lipase B *Candida antarctica* One of the cheap and commercially available nonmicrobial enzymes is which has high selectivity and specificity, mild reaction conditions, wide pH range, activity in anhydrous reaction mixtures as demonstrated for esterification and transesterification reactions, allowing to obtain products with high purity, reduction of co-products and/or toxic waste, consequently reducing the environmental impact (TOMIN et al., 2010; DHAKE et al. 2013; HIRATA et al. 2016).

Promoting the esterification between fatty acids and ethanol producing oleate esters. Ethyl oleate ester (ethyl cis-9-octadecanoate) (ITSEKSON et al., 2011; DESHMUKH et al., 2013; KHAN&RATHOD, 2015) is obtained by the esterification reaction between oleic acid and alcohol, is useful as biological additive, PVC plasticizer, water resisting agent and for hydraulic fluid. Due to several advantages of enzyme catalysis in organic solvents, the synthesis of ethyl oleate and other esters of oleic acid have been studied by exploiting the catalytic activities of enzymes (ITSEKSON et al., 2011; DESHMUKH et al., 2013; KHAN & RATHOD, 2015; KHAN et al., 2015; NOVAK et al., 2016).

Considering the peculiarities of each process, where depending on the operating conditions used for its production can interfere or contribute positively or negatively to the enzymatic synthesis, so the objective of this study is to evaluate the performance of *Candida antarctica* free lipase B in the synthesis of ethyl oleate using ethyl alcohol and oleic acid in a solvent-free system.

MATERIAL AND METHODS

Materials

Lipase B de *Candida antarctica* - CALB (Novozyme NZL-102), oleic acid (Vetec, 99%), ethanol (Merck, P.A), dichloromethane (Vetec, 95%)and Toluene diisocyanate (TDI).

Synthesis of ethyl oleate ester

Lipase B of*Candida antarctica* - CALB (Novozyme NZL-102) was prepared (Enzyme solution of 10%) (catalyst free) according described by Nyari et al. (2016).

The synthesis of ethyl oleate ester was carried out in triplicate (n=3) in 50 mL glass flask keeping constant molar ratio oleic acid to ethanol in 1:1 (5 g). The product of the reaction was kept under refrigeration (8°C) to posterior analysis. The ethyl oleate ester was evaluated by gas chromatography (CG-FID Hewlett–Packard (HP), CG-6890C, column Sigma-Aldrich ß-DEX 325 (30m x 0.25mm x 0.25µm) and RT-WAX column - 30 m x 0.32 mm). A reaction, under same conditions but without enzyme, was conducted as a standard reaction.

The kinetic study was conducted to evaluate the effect of the reaction time (0 to 90 minutes) in terms of conversion to ethyl oleate. The variables studied were mass catalyst (0.018 - 0.582 g) and reaction temperature (36-64°C), keeping mechanical (Shaker - MA 420 - 600 Watts, Marconi)fixed stirring at 160 rpm and reaction time in 40 minutes (NETA et al. 2012).

The conversion was evaluated by gas chromatography (GC-Mass Shimadzu 2010) using a 5-column (Agilent, J & W. Scientific, USA) capillary column (30 m x 0.25 mm x 0.25 um). Samples were prepared by dissolving 10 µl of the final product in 98 ml of n-heptane and 10 µl of MSTFA (N-Methyl-N- (trimethylsilyl) trifluoroacetamide), with the injection of 1 µl follow conditions described by Souza et al. (2015). The conversion was calculated based on the reduction of the limiting reagent peak area.

The optimization of ethyl oleate ester synthesis was carried out based on experimental factorial design – delineation central composite rotational (DCCR 2^2). Software Statistica 5.0 (Statsoft Inc) was used to assist the design, statistical analysis, and optimization of the reaction conversion ($p < 0.05$).

RESULTS AND DISCUSSION

Synthesis of ethyl oleate ester

Figure 1 shows the evolution of ethyl oleate ester conversion (90 minutes) for lipase from *Candida antarctica* B free, according to the condition designed by the full DCCR2^2 (Table 1).

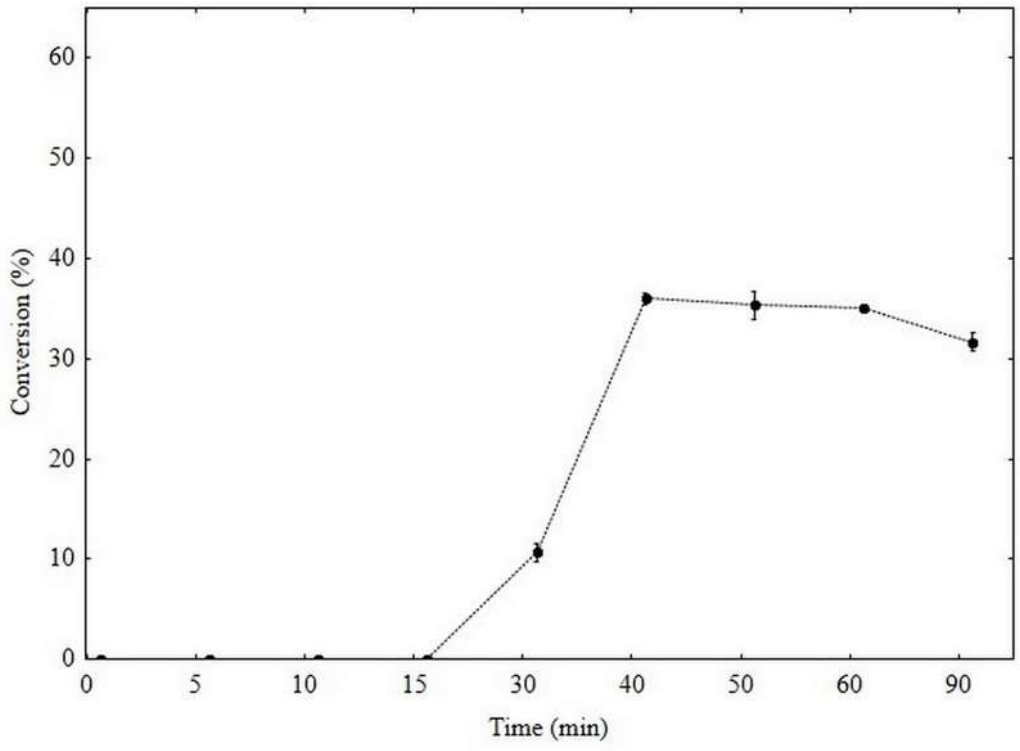

Figure 1. Kinetics of ethyl oleate conversion for lipase from *Candida antarctica* B free.

From the results, it was observed the maximal conversion of 38%, after 40 minutes of reaction time. From this results, it was possible to relate the initial reaction velocity with the agitation type. From the obtained results, for the optimization assay of ethyl oleate synthesis, the reaction times was fixed in 40 minutes.

According to Liu et al. (2008), the reaction time can be linked to the increase in the reaction rate, which can be obtained using ultrasonic agitation system, mainly due the formation of microscopic droplets in the system, increasing the interfacial area by increasing surface contact reducing mass transference limitations between substrate and catalyst.

Table 1 shows the full DCCR 2^2 for the synthesis of ethyl oleate ester for lipase from *Candida antarctica* B free, as a function of the studied variables, mass catalyst free (g) and temperature (°C).

Table1. Matrix offull2^{2}DCCR experimental design (real and coded values)for lipase from *Candida antarctica* B free with responses in terms of ethyl oleate conversion.

Run	Mass catalyst (g)	Temperature (°C)	Oleic acid conversion (%)
1	-1 (0.1)	-1 (40)	28.3 ± 2.1
2	-1 (0.1)	1 (60)	26.8 ± 1.7
3	1 (0.5)	-1 (40)	35.3 ± 1.8
4	1 (0.5)	1 (60)	28.8 ± 2.5
5	-1.41 (0.018)	0 (50)	46.6 ± 2.2
6	1.41 (0.582)	0 (50)	53.8 ± 0.1
7	0 (0.3)	-1.41 (35.9)	37.8 ± 2.7
8	0 (0.3)	1.41 (64.1)	46.9 ± 1.7
9	0 (0.3)	0 (50)	54.8 ± 2.4
10	0 (0.3)	0 (50)	56.5 ± 2.1
11	0 (0.3)	0 (50)	54.9 ± 1.9

* Fixed parameters: substrate mass 5 g, oleic acid to ethanol molar ratio 1:1, reaction time 40 min and 160 rpm of mechanical agitation.

According to the results, the maximum conversion in terms of oleic acid was obtained in the assay 9, 10 and 11of 56% conversion after 40 minutes of reaction time.

From the assays 1, 2, 3 and 4, it was observed that conversion was directly proportional to the reaction temperature and independent of the mass catalyst, indicating a positive effect of the temperature in the oleic acid conversion. It should be noted that the highest conversions (above 50%) (assays 6, 9, 10 and 11) were observed for the temperatures = 50°C after 40 minutes of reaction time.

The temperature is consistent with the endothermic nature of the esterification reactions, which is characterized by the reversibility, that is, it presents a chemical equilibrium, indicates that it occurs with heat absorption, providing an equilibrium in the reaction system, shifting the reaction for the products side, increasing reaction yield.The temperature effect can be related to the reduction in the system viscosity, reducing the mass transfer limitation.

This trend can be linked to start the process of heat inactivation of the enzyme provided by the temperature increase (≥ 50°C). According to Inouque et al. (2011) the enzymes in soluble form (free) are very delicate and fragile. If the molecule absorbs a lot of energy, can occur conformational changes in their active sites, often irreversible, and may even completely denature the enzyme.

Another variable of extreme importance, combined with temperature was the mass catalyst, according to the Table 1, it was possible to observe an increase in the oleic acid conversion with the increase of the mass catalyst (assays 3 and 4). It should be noted that the highest conversions (above 50%) (assays 6, 9, 10 and 11) were observed for the mass catalyst ≥ 0.5 g in 40 minutes of reaction time.

The increase in the biocatalyst content increases the number of active sites present in the reaction favoring the conversion. According to Orellane-Coca et al. (2005) and Colombo et al. (2015), an excess in the catalyst content is necessary to keep the enzyme activity/conversion during the reaction time.

Figure 2 presents effects between variables, the results were treated statistically with a confidence level of 95% ($p < 0.05$), which describes the ethyl oleate conversion as a function of the independent variables (factors) analyzed (mass catalyst and temperature).

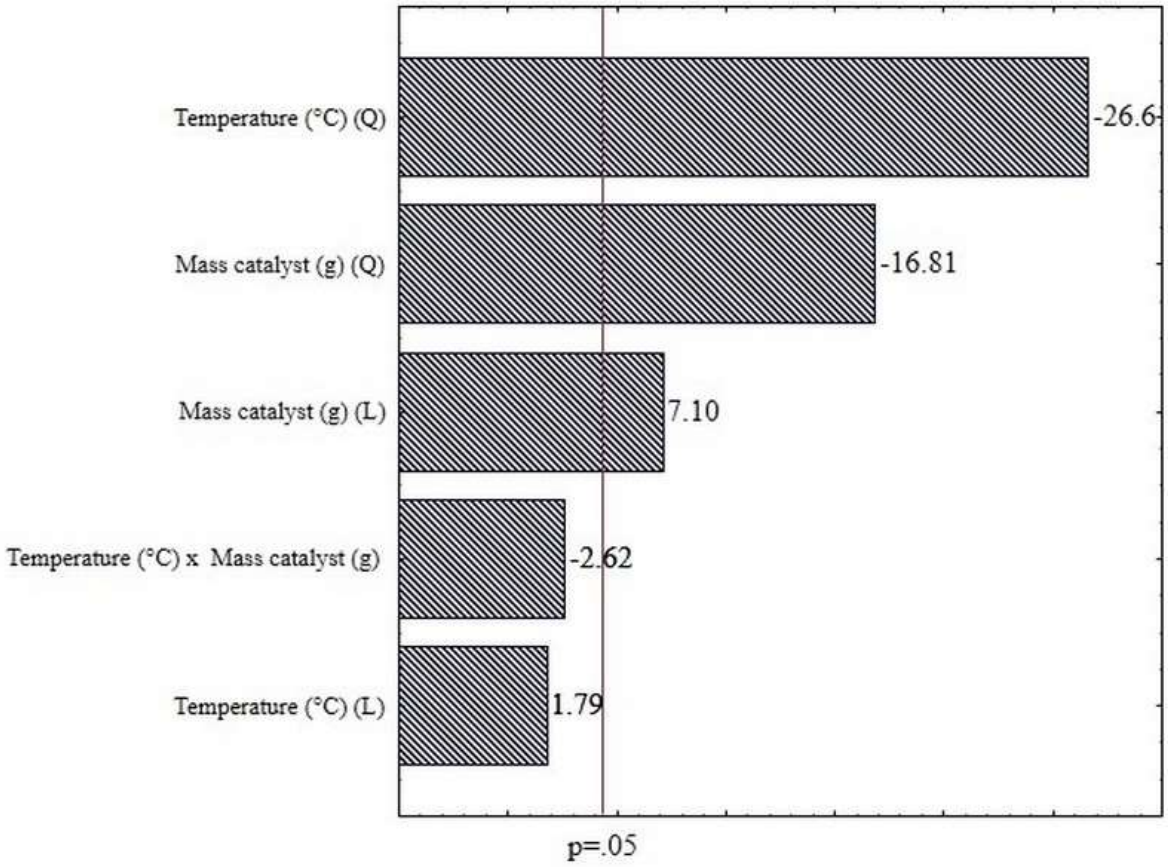

Figure 2. Pareto of ethyl oleate conversion for lipase graph from *Candida antarctica* B free.

We observed that both variables catalyst mass (g) and temperature (°C) showed significant negative quadratic effect, is the greater the mass of the catalyst and temperature in the reaction medium, the lower the conversion obtained. For the mass of the catalyst (g) (linear) presented a significant positive effect, presenting sufficient quantity for the reaction to occur. Temperature (linear) and the interaction between the variable mass of the catalyst and temperature had no significant effect.

CONCLUSIONS

In our study an optimum conversion of 56% in the ethyl oleate synthesis was found using *Candida antarctica* B Lipase B free after a time of 40 minutes of reaction in solvent-free system. This fact is considered promising since in the current literature in most cases systems are employed with the use of solvents such as hexane, in addition to reaction times superior to that used in our study.

We can identify efficiency of a system using enzymes, especially lipases when we obtain conversions greater than 50% in time relational of less than 1 hour. According to the characteristics of the final product obtained, the oleate ester of ethyl, has among its characteristics liquid form at room temperature, colorless to slightly yellowish, insoluble in water and soluble in organic solvents.

Among the possible applications, it can be used in the treatment of fragrances in the food and pharmaceutical industry, as plasticizers and lubricants in the chemical and biological industry, as well as

emulsifiers and emollients in the cosmetic industry.Therefore, we can conclude that the obtained data show a promising perspective to overcome the known disadvantages of the route catalyzed by chemical products.

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