PRODUCING EMOLIENT ESTERS BY PHYSICAL ADSORPTION USING IMMOBILIZED COMMERCIAL LIPASES

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ABSTRACT

This study aimed to produce emollient esters for the cosmetic industry using commercial lipases from *Pseudomonas fluorences* (LAK), *Pseudomonas cepacia* (LPS) and *Candida rugosa* (LCR) immobilized on chitosan (CHIT) and polyhydroxybutyrate (PHB) by physical adsorption. Ethylene glycol oleate, isoamyl palmitate and ethylene glycol monostearate were produced in batches, which allowed optimizing process conditions and assessing product quality. Lipases from LPS and LAK showed optimal hydrolytic activities, and LPS reached 1276.03 U/g and LAK 1039.11 U/g. LPS was selected to evaluate immobilization on chitosan, resulting in enzyme activity of 437.09 U/g and immobilization yield of 70.44%. Therefore, lipase form LAK was selected for esterification reactions, and immobilized protein yields of 5.38% (5 mg/g) and 8.68% (10 mg/g) were reached, corresponding to immobilization yields of 48.42% and 59. 92%, respectively. Immobilized derivatives were applied in the synthesis of different esters, with emphasis on isoamyl palmitate due to reaching approximately 84% conversion within 8 hours.

Keywords: Immobilization, Support, Lipases, Esters.

1 INTRODUCTION

The cosmetics industry has achieved enormous growth over the last few years and strategies of famous brands tend to demonstrate results both locally and worldwide. One of the main commercial strategies of large companies is to make their products known globally. In the last decade, technological advances and easy access to information have boosted sales and made it simple to purchase cosmetics available in other parts of the globe[1].

The search for sustainable industrial alternatives aligned with the principles of green chemistry makes the implementation of enzyme technology an attractive option for industries. Unlike chemical catalysts, biocatalysts have high specificity, allowing efficient distinction of reactions and substrates, similar parts of molecules (regiospecificity) and optical isomers (stereospecificity). Furthermore, they exhibit remarkable catalytic activities under mild conditions of pressure, pH and temperature. These characteristics make enzyme technology a promising choice, aligned not only with sustainability, but also with the search for specific industrial products[2].

Therefore, the synthesis of emollient esters using immobilized lipases represents a promising approach in the cosmetics industry, given that it stands out for its efficiency and sustainable characteristics. This strategy allows producing desired esters, such as ethylene glycol oleate and isoamyl palmitate in a more controlled and efficient way. Thus, the synthesis of emollient esters via immobilized lipase stands out as an innovative and sustainable alternative in the production of emollient esters on account of meeting the demands of the cosmetic industry[3]. Thus, the main objective of this work was to evaluate the potential of immobilized lipases via adsorption on two organic supports, as well as investigating their application in the synthesis of emollient esters relevant to the cosmetics industry.

2 MATERIAL & METHODS

Lipases from *Pseudomonas fluorences* (LAK), *Pseudomonas cepacia* (LPS), *Candida rugosa* (LCR) from Sigma Aldrich Co, USA, were used. The following were used in the production of esters: oleic acid, stearic acid, monoethylene glycol alcohol, benzyl alcohol and isopropyl alcohol, all analytical grade. The supports used were natural chitosan (CHIT) and polyhydroxybutyrate (PHB). All other reagents used were of analytical grade and purchased from Synth.

Experimental Procedure: Initially, CHIT and PHB were soaked in 95% w/w ethanol and kept at room temperature (± 25 ºC) for 2 h4. Afterwards, supports were vacuum-filtered and washed with distilled water.

Immobilization of lipases by physical adsorption: 10 g of pre-treated support was weighed and added to an enzymatic solution of 190 mL of enzyme loading of 5 and 10 mg/g of support incubated for 8 h at 125 rpm and room temperature (± 25 ºC). At the end of the procedure, immobilized derivatives were filtered and washed with distilled water and then dried.

Analysis: The protein content of samples was evaluated using Bradford assay[5]. The determination of enzyme activity followed the methodology modified by Soares et al 1999[6].

Synthesis of emollient esters: a previous treatment was carried out using hexane to reduce the percentage of water so as not to affect the formation of esters. Hexane was treated using 4 g of magnesium sulfate and left to rest at room temperature for 2 hours[6]. The esterification of immobilized lipase was determined by the formation of aromatic esters: isoamyl palmitate ((isoamyl alcohol reaction (0.06 M) with palmitic acid (0.06 M)); ethylene glycol oleate ((reaction of ethylene glycol alcohol (0.06 M) with oleic acid (0.06 M)); ethylene glycol monostearate ((reaction of ethylene glycol alcohol (0 .06 M) with stearic acid (0.06 M)).

All reactions took place at 40°C using dry mass of lipase (free and immobilized) of 0.1 g. In each of the three assays, control with the absence of the enzyme was used. The reaction was started by adding the immobilized lipase to the reaction medium (20 mL) in a closed 100 mL flask using a rotary shaker at 200 rpm. Aliquots of 100 µL were removed from the reaction medium at time zero and after predetermined intervals (2, 4, 6, 8, 10 and 24 h).

The concentration of fatty acids was determined by titrating aliquots dissolved in 15 mL of a solution of acetone and ethanol (1:1) using a 0.02 M NaOH solution and 1% phenolphthalein as indicator. The percentage of esterification was related to the percentage of acid consumed.

3 RESULTS & DISCUSSION

Lipases from LPS and LAK achieved the highest hydrolytic activity among enzymes under evaluation, reaching values of 1276.03 U/mL and 1039.11 U/mL, respectively. In contrast, lipase from LCR showed the lowest activity, i.e. only 295.44 U/mL. To investigate the influence of the immobilization process on PHB and CHIT supports, lipase from LPS was selected due to its superior performance. Detailed results are presented in Table 1.

Table 1. Enzyme immobilization parameters for support selection: Hydrolytic activity of the immobilized derivative $AH_{Imobilized}$ (U/g); Immobilized protein (PI) (mg/g) and Immobilization Yield (RI) (%).

Immobilized Derivative	$AH_{Immobilized}$ (U/g)	PI (mg/g)	RI (%)
LPS-PHB	238.13	4.51	61.54
LPS-CHIT	437.09	5.16	70.44

CHIT demonstrated superior results compared to PHB in the immobilization parameters under evaluation. The produced immobilized derivative presented an AH_{Imob} of 437.09 U/g with 5.16 mg/g of PI and an RI of 70.44%. However, LPS immobilized in PHB also achieved RI greater than 60%, reaching 4.51 mg/g of immobilized protein support. As CHIT demonstrated better results in the immobilization process, it was used as support for the remaining commercial lipases with different enzyme loadings (5 and 10 mg/g) in which hydrolytic activities before and after immobilization, recovery of hydrolytic activity (%), immobilized protein (mg/g) and immobilization yield (%) were evaluated. These results are described in Table 2.

Table 2. Immobilization protocols via physical adsorption – CHIT support.

Lipase (mg/g support)	AH Free (U/mL)	AH Immobilized (U/g)	AH (%) Recovery	PI (mg/g)	RI (%)
LAK (5mg/gsupport)	778.12 ± 99.93	455.56 ± 31.02	58.54	5.38	48.42
LAK (10mg/gsupport)	890.68 ± 64.02	476.82 ± 14.66	53.53	8.68	59.92
LPS (5mg/gsupport)	1312.17 ± 132.36	407.70 ± 17.48	31.07	3.55	63.68
LPS (10mg/gsupport)	1370.79 ± 133.58	365.14 ± 30.58	44.30	9.43	54.29
LCR (5mg/gsupport)	771.96 ± 29.74	341.99 ± 19.98	26.64	5.75	60.32
LCR (10mg/gsupport)	1105.35 ± 26.35	320.52 ± 50.36	29.00	8.98	49.80

LAK lipase was the most efficient, reaching similar values of hydrolytic activity for both immobilization protocols: 455.56 ± 31.02 U/g for 5 mg/g of support and 476.82 ± 14.66 for 10 mg/g g of support, as well as reaching hydrolytic activity recovery above 50%. However, lipase from LPS, despite obtaining immobilization yield greater than 55%, faced a loss of activity between 55% and 68% after the immobilization process. LCR lipase presented the lowest results, with hydrolytic activity was in the range of 320 to 342 U/g and an immobilization yield of approximately 29%.

Figure 1 presents the results obtained from esterification of emollient esters of interest to the cosmetics industry: isoamyl palmitate (1A), ethylene glycol oleate (1B) and ethylene glycol monostearate (1C), using immobilized lipase from LAK as reaction catalyst at two different enzyme concentrations. Control without enzyme and one with free enzyme were selected to evaluate the efficiency of immobilized lipase regarding conversion.

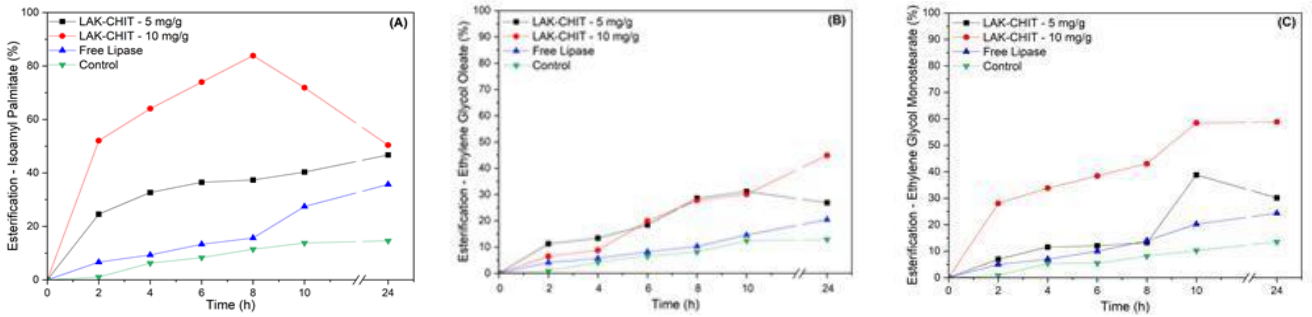

Figure 1 Percentage of esterification of esters: Isoamyl palmitate (A); Ethylene glycol oleate (B); Ethylene glycol monostearate (C)

The synthesis of isoamyl palmitate (Figure 1A) indicated that the LAK-CHIT system with 10 mg/g of support achieved the highest esterification within 8 h (83.88%), with a drop in conversion afterwards. A possible explanation for such a fact is the hydrolysis of ester formed due to the amount of water formed in the reaction. In this context, both the use of free and immobilized biocatalysts demonstrated lower ester conversion.

The synthesis of ethylene glycol oleate (Figure 1B) occurred within 24 h with higher enzyme loading (10 mg/g), and greater esterification conversion (45%) was observed. A lower yield in oleic acid esterification occurred due to its greater affinity with water molecules formed during the reaction. This interference endured, even with increased enzyme loading. However, reactions with immobilized LAK still showed superior results compared to free lipase and control.

Analyzing the esterification percentage of ethylene glycol monostearate ester (Figure 1C), LAK-CHIT (10 mg/g) achieved conversion of 58.46% within 10 h. LAK-CHIT with lower enzyme loading and controls presented lower results than those observed for LAK-CHIT (10 mg/g).

In the synthesis of isoamyl oleate and ethylene glycol monostearate esters, both using ethylene glycol alcohol, it was observed that the immobilized derivative has greater affinity for stearic acid compared to oleic acid. This is evidenced by the slower conversion profile in the synthesis of isoamyl oleate compared to ethylene glycol monostearate. These results, together with data on the synthesis of isoamyl palmitate, suggest that the prepared biocatalyst has greater affinity for saturated acids (stearic acid and palmitic acid) than for acids with unsaturation in their constitution, such as oleic acid.

4 CONCLUSION

Lipase from LAK enzyme with enzyme loading of 10 mg/g of support was the most efficient in the immobilization method, resulting in a biocatalyst with a high concentration of adsorbed protein and high catalytic activity, both in hydrolysis and esterification reactions.

In the synthesis of ethylene glycol oleate, the test with the immobilized derivative obtained the best percentage of ester formation within 24 h, reaching 44.91%. In the case of the synthesis of ethylene glycol monostearate, the immobilized derivative showed the best ester formation in 10 h (58.46%).

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