A HYDROESTERIFICATION REACTION SYSTEM TO PRODUCE XYLOSE ESTERS USING DEGUMMED SOYBEAN OIL AS SUBSTRATE

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ABSTRACT

This study shows the enzymatic synthesis of xylose fatty acid esters carried out in two steps: hydrolysis of degummed soybean oil (DSO) and esterification of the produced and purified free fatty acids (FFA) with xylose. Different lipases immobilized on a hydrophobic support were evaluated in the hydrolysis of DSO and an experimental design was used to determine the variables that influence the reaction. Eversa® Transform 2.0 (EV-Purolite) yielded 93% conversion after 6 h using biocatalyst load of 6.53 wt.%, water/oil mass ratio of 5.5 and temperature of 48.4 °C. Sequential batch strategies showed high operational stability for EV-Purolite and the possibility of concentrating glycerol in the heavy phase of the reaction medium. In the esterification stage, lipase from Thermomyces lanuginosus (TLL-Purolite) and lipase B from Candida antarctica (CALB-Purolite) showed better performance than lipase from porcine pancreas (PPL-Purolite) and EV-Purolite. About 17% of FFAs was consumed in the synthesis of xylose fatty acid esters using an enzyme load of 5 wt.% of TLL-Purolite and FFA/xylose molar ratio of 5. Sequential esterification batches showed low operational stability of TTL-Purolite due to the desorption of TLL from the support. The final mixture of the reaction medium containing xylose fatty acid esters showed emulsifying properties similar to those of commercial surfactant.

Keywords: degummed soybean oil, lipase catalyzed hydrolysis and esterification, experimental design, xylose fatty acid esters, emulsifying properties.

1 INTRODUCTION

Sugar esters derived from fatty acids are surfactants with a set of very interesting properties: they are non-ionic, biodegradable, odorless, non-irritating, non-toxic with high emulsifying/detergent effect, etc. Therefore, sugar fatty acid esters (SFAEs) are widely applied in pharmaceutical, cosmetic, detergents, food and biomedical industries. SFAEs can be produced from either monosaccharides or oligosaccharides with different fatty acids or their derivatives. The most used sugars are sucrose, glucose, fructose, xylose, lactose or sorbitol (Gonçalves et al., 2021b). The use of xylose (acyl acceptor) in the synthesis of SFAEs has been sparsely evidenced in the literature, this gap opens the horizon for the development of further research in this area.

The synthesis of SFAEs can be performed using chemical or enzymatic catalysts (Gonçalves et al., 2021b). The enzymatic synthesis is an interesting alternative that provides advantages such as mild reaction conditions, high selectivity that avoid the formation of by-products, easy product separation and simple operation. Indeed, the synthesis of various sugar ester surfactants catalyzed by lipases in organic solvents have been described in the literature. SFAEs can be obtained by direct esterification of free fatty acids or transesterification between monoalkyl esters and the desired sugar (Gonçalves et al., 2021b; Yoo et al., 2007). Hydroesterification, a sequential process of oil/fat hydrolysis followed by purification of fatty acids and their further esterification, has not been described in the literature in these kinds of processes. The main advantage of this strategy is the removal of glycerol after the hydrolysis and purification step of FFAs. This allows this polyol not to act as a competitive alcohol to the acyl acceptors used in the esterification reaction (Monteiro et al., 2023).

In this study, the purpose is to obtain xylose esters through a hydroesterification strategy in a two-step enzymatic process, starting by the enzymatic hydrolysis of degummed soybean oil (DSO) to obtain a FFAs-rich fraction. Different lipases immobilized on Purolite Lifetech EC8806F were evaluated in this step. The most relevant variables (DSO/water mass ratio, temperature, and enzyme load) that influence the hydrolysis process using immobilized lipases were studied. Next, the esterification of free fatty acids (FFA) with xylose in the presence of solvent was catalyzed by ET, TLL, CALB, or PPL immobilized on Purolite Lifetech EC8806F. Finally, the emulsifying capability of the produced xylose esters was evaluated by measuring the stability of a water-in-kerosene emulsion.

2 MATERIAL & METHODS

All other reagents were of analytical grade.

2.1 Enzyme immobilization, standard activity assay and protein quantification

Enzyme immobilization followed the protocol described by Tacias-Pascacio et al. (Tacias-Pascacio et al., 2016). Standard activity assays and protein quantification followed the procedure described by Beisson et al. (Beisson et al., 2000) and Bradford (Bradford, 1976), respectively.

2.2 DSO enzymatic hydrolysis to produce FFA

The different immobilized lipases were evaluated in the hydrolysis of DSO, using an enzyme load of 1 wt.% (considering the oil mass), water/oil mass ratio of 1, a temperature of 35 °C and an agitation of 250 rpm for 1 h. The effects of temperature (31.6 – 48.4 °C), water/oil mass ratio (0.48 – 5.52) and enzyme load (1.48 – 6.52 wt.%) in the hydrolysis reaction were studied using a central rotational composite design (2^3 with three central points and six axial points). The reactions were carried out in sealed Erlenmeyer flasks (250 mL capacity) using 2 g of DSO under agitation at 250 rpm for 0.5 h. After this period, aliquots were collected to titrate with 125 mM KOH and determine the conversion. After validating the experimental data, a kinetic profile of DSO hydrolysis over time was evaluated. Aliquots were collected throughout the reaction to analyze base consumption to determine conversion.

2.3 Enzymatic esterification to produce fatty octyl esters

The FFA-rich fraction produced in the hydrolysis step was washed with distilled water (2 times) and separated from the heavy phase (mainly composed of water and glycerol) by centrifugation at 3,600 rpm for 10 min at 25 °C. Purified FFA was dried in an oven at 70 °C for 24 hours. An esterification reaction was carried out using xylose as the acyl acceptor (FFA/xylose molar ratio of 5) in heptane as solvent (FFAs and xylose concentration of 35 and 7 mmol L^{-1}, respectively). Molecular sieves were used to remove the water produced during the reaction (adsorption capacity of 0.23 mg water/mg molecular sieve). TLL, EV, PPL, or CALB immobilized on Purolite were used as biocatalysts (5%, w/v). The immobilized enzyme that presented the best potential for the esterification of xylose with FFAs was selected to determine the conversion profile over time. The esterification reactions were carried out in a shaker at a temperature of 40 °C and agitation of 175 rpm. The consumption of KOH necessary to neutralize FFA was used to determine the conversion of FFA to xylose fatty acid esters. That is, yields refer to FFAs consumption.

3 RESULTS & DISCUSSION

TLL, EV, PPL and CALB were immobilized on Purolite for their application in the hydroesterification of DSO for the synthesis of xylose fatty esters. The immobilization was very fast, reaching high enzyme adsorption values in a short period of time. TLL, CALB and EV showed low recovery of tributyrin activity despite showing high immobilization yield, while PPL showed the opposite behavior. Immobilized lipases showed better performance in the conversion of DSO to FFA compared to free enzymes. EV-Purolite provided conversions 1.4 times higher than those achieved with free Eversa, while TLL-Purolite showed 2 times higher conversion than its liquid formulation. Comparing the performance of immobilized lipases, EV-Purolite showed statistically better performance than the other lipases, reaching a conversion 36 times greater than that observed for PPL-Purolite. We selected immobilized EV for further experiments due to the lower price of this enzyme.

The optimized variables for DSO hydrolysis were water/oil mass ratio of 5.5, enzyme load of 6.5 wt.% and temperature of 48.5 ºC. To improve the yields, we prolonged the reaction for longer times. EV-Purolite showed good performance in the conversion of DSO to FFA, reaching 93% conversion after 6 h of reaction. After 16 h, the FFA yield reached 98%. The composition of the FFAs of the used oil, calculated in this study, showed that it was composed of 5.99 ± 0.13 wt.% stearic acid, 9.50 ± 0.24 wt.% palmitic acid, 23.01 ± 0.36 wt.% oleic acid, 46.13 ± 0.27 wt.% linoleic acid and 8.10 ± 0.04 wt.% linolenic acid. The final pH of the reaction medium was between 4.0-4.8, this pH decrease can justify the slowdown of the reaction.

The operational stability of EV-Purolite in DSO hydrolysis was evaluated. It was observed that it was possible to reuse the immobilized biocatalyst for at least 5 sequential batches, without altering its performance in the hydrolysis reaction. Furthermore, the procedure used during the EV-Purolite reuse trials showed the possibility of concentrating glycerol in the heavy phase, since both the biocatalyst and the heavy phase were reused throughout the recycling. The accumulation of glycerol in the reaction medium did not affect the performance of the immobilized enzyme, probably because the high degree of hydrophobicity of the support prevented the concentration of glycerol on the biocatalyst. This concentration of the glycerol via successive reaction cycles can make purification and reuse of the produced glycerol more feasible.

EV, TLL, CALB and PPL immobilized on Purolite were evaluated in the esterification of the purified FFAs-rich phase with xylose. CALB-Purolite and TLL-Purolite did not show any statistical difference in the synthesis of xylose fatty acid esters. EV seemed to be the least efficient biocatalyst for this reaction. The reaction catalyzed by CALB-Purolite and TLL-Purolite reached conversions of around 9.9% (acid conversion) after 1 h of reaction, which represents about 50% of the xylose modified as monoester. CALB immobilized in Lewatit VP OC 1600 (Lipozyme 435) has been previously used for the synthesis of xylose fatty acid esters obtaining high yields of xylose oleate, palmitate and laurate (Gonçalves et al., 2021a). The use of TLL immobilized on Purolite for the synthesis of xylose fatty acid esters was not evidenced in literature.

Figure 1a shows the course of the esterification reaction of the purified FFA with xylose catalyzed by TLL-Purolite. About 17% of FFAs was consumed in the synthesis of xylose fatty acid esters (considering the FFA/xylose molar ratio of 5). Gonçalves et al. [3] achieved similar xylose modifications when Lipozyme 435 was used for the synthesis of xylose palmitate and laurate. Figure 1b shows the operational stability of TLL-Purolite in esterification for synthesis of xylose esters. It was observed that the performance of the biocatalyst worsened with each reaction batch. This result may be related to the TLL desorption from the support due to the formation of molecules with surfactant/detergent properties. To check this possibility, the enzyme remaining in the support was

followed by SDS-PAGE. The electrophoresis showed the decrease in the intensity of the protein band, confirming that a large percentage of TLL was released to the medium during the reaction.

The final mixture of the reaction medium containing xylose fatty acid esters demonstrated good emulsifying properties, presenting an emulsification index (EI) of 15%. This result is in the same order of magnitude observed for sucrose monolaurate (EI = 12%), in addition, it corroborates what was evidenced by Gonçalves et al. (Gonçalves et al., 2021a). These findings indicated that xylose fatty acid esters have potential for use as emulsifying agent in various industrial applications.

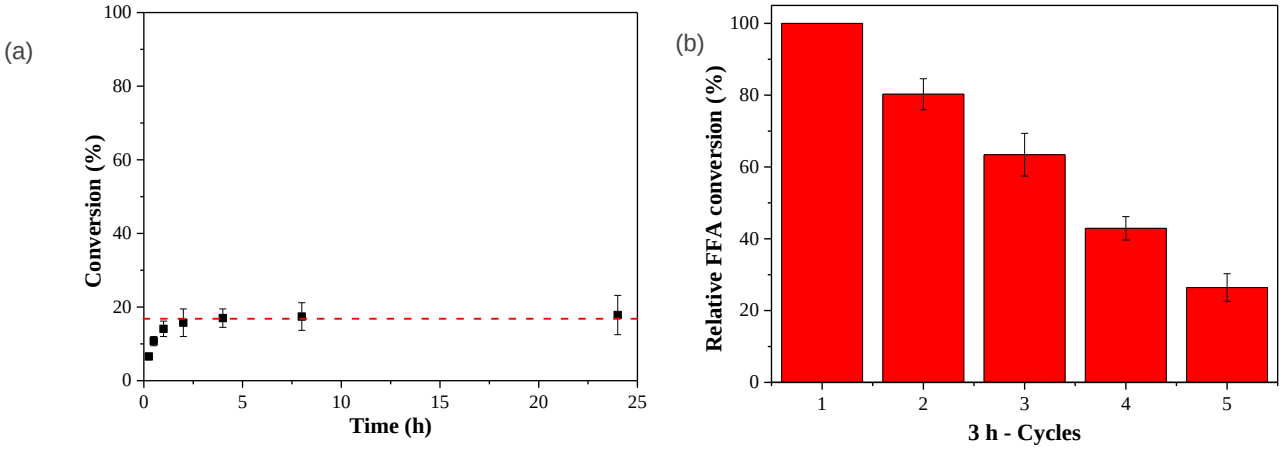

Figure 1. (a) FFA conversion profile in the synthesis of xylose esters catalyzed by TLL-Purolite. The dotted line represents the expected maximum conversion of FFA-rich fraction obtained from the DSO hydrolysis. (b) Operational stability of TLL-Purolite in the esterification reaction for the synthesis of xylose esters. Assay conditions: enzyme load of 10 wt.% (considering the FFA), FFA/xylose molar ratio of 5, temperature of 40 °C and agitation of 175 rpm.

4 CONCLUSION

This article shows the feasibility of the approach used for the enzymatic synthesis of xylose fatty acid esters carried out in two steps. DSO hydrolysis catalyzed by EV immobilized on Purolite allowed to achieve a high yield of FFA. Furthermore, the possibility of reusing the immobilized enzyme and the heavy phase for glycerol concentration without affecting the performance of the biocatalyst was demonstrated. In the esterification step, TLL immobilized on Purolite C18 successfully resulted in the synthesis of xylose fatty acid esters. However, the immobilized lipase showed losses of a large percentage of its activity during five batches of 3 hours of esterification, due to the desorption of the enzyme from the support. Ongoing research in our laboratory suggests the use of crosslinking agents or heterofunctional supports to minimize this problem. The final mixture of the reaction medium containing xylose fatty acid esters has emulsifying properties similar to those of commercial surfactant.

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