Biocatalysis and Agricultural Biotechnology 16 (2018) 373–377

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Enzymatic esterification for the synthesis of butyl stearate and ethyl stearate

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ARTICLE INFO

Keywords:
Butyl stearate
Ethyl stearate
Lipase
Novozym 435
Enzymatic esterification

ABSTRACT

This work reports the enzymatic synthesis of butyl stearate and ethyl stearate, two esters with important industrial properties, ranging from cosmetic applications to emollient characteristics (butyl stearate) to food industries, imparting flavor and aroma to beverages and foods (ethyl stearate). Here, reactions were carried out using Novozym 435 as biocatalyst, considering different stirring levels and substrates molar ratios, to determine optimal conditions to produce both esters. The kinetics under optimized conditions, varying the substrates molar ratio was performed and the influence of the fractional addition of the alcohol in the reaction was also verified. It was observed for butyl and ethyl stearate that satisfactory results (92%) occurred when batch kinetics was carried out for 24 h at 60 °C, 1% (by weight of substrates) of the enzyme, agitation of 250 rpm and molar ratio of 1:2 and 1:5, respectively. Enzyme reuse up to five and three cycles led to satisfactory results for butyl stearate and ethyl stearate, respectively. This work represents, therefore, a novelty in the production of these esters by enzymatic route, leading to a high conversion of products, using a low amount of biocatalyst, proving the relevance of the production of these esters by enzymatic means, which makes the process ecologically correct and still add value to the final product.

1. Introduction

Biocatalysis is becoming an important tool for the production of fine chemicals, mainly from the point of view of environmental sustainability and in the long term, adding value to existing products, but produced through classical methods such as the chemical route. Lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3) are quite stable in non-aqueous solvents and, because of their excellent properties, are often used in organic synthesis for industrial application (Patel et al., 2015). Biocatalysts, as they are called enzymes, have attractive characteristics such as versatility, substrate selectivity and enantioselectivity, limited use of hazardous reagents, reduction of undesirable by-products and moderate temperatures, and catalysis pressures (Voulgaris et al., 2015). Other advantages in the use of enzymatic catalysts are: non-toxicity, wide range of pH in which enzymes act, low probability of occurrence of secondary reactions and reduction of energy costs. The main disadvantages associated with enzymatic catalysis are the high cost of the enzymes and the increase of the reaction time (Mata et al., 2018).

Esterification is a reaction consisting of the condensation of the carboxylic acid and the alcohol, forming ester and water. This reaction is slow, when no catalyst is used, which may be chemical or biological. In the case of using a biocatalyst, the enzymes are used to increase the rate of reaction (Rajendran et al., 2009).

One possible product of esterification is flavor esters, which are significant and versatile compounds that can be used as important ingredients in food, beverages, cosmetics, pharmaceuticals, chemicals and personal care products such as perfumes and lotions, creams, shampoos, soaps among others (Sá et al., 2017).

This study presents an optimization of the main parameters for the synthesis of two important esters, butyl stearate and ethyl stearate. The first one is an ester obtained by the esterification reaction of stearic acid and butanol, which is used as solvent and dispersing agent for creams and cream lotions and has been important for the cosmetic industry since it often acts as a substitute for mineral oils and vegetables. It also has emollient properties, with the advantage of not leaving the greasy sensation in the skin and providing the sensation of softness. Ethyl stearate is obtained by the esterification of stearic acid and ethanol and is widely used in the perfume industry in fragrances in small concentrations, can be used as artificial flavor, are generally used as additives to enhance the aroma of a beverage or a food. However, butyl stearate and ethyl stearate are usually produced by esterification in the presence of a homogeneous acidic catalyst such as concentrated sulfuric

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https://doi.org/10.1016/j.bcab.2018.09.008
Received 12 July 2018; Received in revised form 5 September 2018; Accepted 10 September 2018
Available online 11 September 2018
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acid, and the main drawbacks of this production process include that the catalyst is corrosive to the equipment, the existence of side reactions, the fact that the catalyst cannot be easily separated from the reaction mixture, a large amount of water is required, and discharged wastewater is harmful to the environment (Peters et al., 2006; Chen et al., 2011).

This work represents, therefore, a novelty in the production of these two esters by enzymatic route, having very promising aspects such as the high conversion and the low amount of catalyst used, besides the possibility of reuse, making them interesting products for future industrial application, besides the substitution of conventional methods by alternative routes, giving greater added value to the final product.

2. Materials and methods

2.1. Materials

As substrates for the esterification reactions the following chemicals were used: vegetable stearic acid (ALMAD, Fats and Derivatives - Brazil) and alcohols, n-butanol (Rhodia Solvay) and ethanol (95% purity, Vetec). Novozym 435 lipase was used as catalyst; this lipase is obtained from *Candida antarctica* and immobilized on macroporous acrylic resin, and kindly provided by Novozymes (Araucária/Brazil).

2.2. Enzymatic synthesis of ethyl stearate and butyl stearate

Towards the synthesis of butyl stearate and ethyl stearate, reactions were performed in orbital shaker in hermetically sealed 250 mL erlenmeyers. The temperature was regulated through a forced air system with a high homogenization and agitation based on the orbital movement. Initially, the condition of 60 °C, 200 rpm, 5 h of reaction, 1 wt% enzyme (based on the mass of acid), and 1:3 acid to alcohol molar ratio was employed for the two esters synthesis with the lipase Novozym 435. Then, a central composition rotation design (CCRD) 2^2 was executed to evaluate the effect of agitation level and substrates molar ratio on the reaction conversion of both esters synthesis, keeping constant the temperature of 60 °C, 1 wt% enzyme (based on the mass of acid) and 5 h reaction (Table 1).

In the optimized condition, a kinetic study was carried out, keeping constant the temperature of 60 °C, agitation of 250 rpm, 1 wt% enzyme (based on the mass of acid) for both esters and molar ratio (alcohol to alcohol) of 1:1 and 1:2 for butyl stearate and 1:5 for ethyl stearate.

First, stearic acid was added to the reactor vessel and complete solubilization was observed, then the alcohol (ethanol or butanol) was added. Afterwards, about 1 g of sample was taken to determine the acid number (time zero) and then the biocatalyst was added. At pre-determined times aliquots were taken for acidity measurement for further conversion calculation. All results were analyzed using Statistica® 10 (Statsoft Inc., Tulsa, OK, USA), considering a significance level of 95% ($p < 0.05$).

2.3. Evaluation of fed batch kinetics

At this stage, the alcohol fractional addition strategy was used to verify the conversion to butyl stearate and ethyl stearate esters using the same conditions of kinetics in batch mode (item 2.2.). In this step, the temperature was 60 °C, 250 rpm of agitation, 24 h reaction, 1 wt% enzyme in relation to the stearic acid mass, molar ratios (acid to alcohol) of 1:1 and 1:2 for butyl stearate and 1:5 for ethyl stearate and the alcohol was added in three equal fractions during the first three hours.

2.4. Acidity index

The acidity index gives an important information on the evaluation of the amount of acid consumed in the esterification reaction, i.e., the higher the amount of acid reacted with the alcohol, the lower the acidity indexes, the latter being defined as milligrams of potassium hydroxide needed to neutralize one gram of sample. This index was determined as cited by Pereira et al. (2018), according to the methodology by AOCS Cd 3d-63, and was calculated by Eq. (1).

$$AI = \frac{56,\ 1xC_{KOH}xV_{KOH}}{m} \quad (1)$$

where AI is the acidity index (wt%), V_{KOH} is KOH solution volume (mL) employed in the titration, C_{KOH} is the concentration of KOH solution (mol/L), *56.1* is the molecular mass of potassium.

2.5. Conversion of butyl stearate and ethyl stearate

Esters conversions were calculated by the consumption of stearic acid according to Pereira et al. (2018), where this was the relationship between the final and initial acidity indexes given by Eq. (2) and the experimental errors were based on replicates of the tests.

$$C(\%) = \frac{(AI_f - AI_i)}{AI_i}x100 \quad (2)$$

where *C(%)* is ester conversion, AI_f is the final acidity index (mg KOH/g) and AI_i is the initial acidity index (mg KOH/g). The experimental

Table 1
Experimental design matrix (coded and real values) with responses in terms of conversion of butyl stearate (BS) and ethyl stearate (ES). Experimental conditions: temperature of 60 °C, 1 wt% of Novozym 435 (based on the weight of stearic acid) and 5 h of reaction.

Run	Molar Ratio[a]	Agitation (rpm)	Experimental conversion (%) (BS)	Predicted Conversion[c] (%) (BS)	RED[b] (%) (BS)	Experimental conversion (%) (ES)	Predicted Conversion[d] (%) (ES)	RED[b] (%) (ES)
1	−1 (1:3)	−1 (150)	84	80.49	4.17	71	73.37	−3.67
2	1 (1:7)	−1 (150)	61	61.77	−1.26	80	79.11	1.22
3	−1 (1:3)	1 (250)	86	81.71	4.98	76	75.33	0.96
4	1 (1:7)	1 (250)	72	71.99	0.01	90	86.07	4.80
5	−1.41 (1:2.18)	0 (200)	84	88.79	−5.71	66	64.46	2.56
6	1.41 (1:7.82)	0 (200)	70	68.74	1.78	73	76.07	−4.64
7	0(1:5)	−1.41(129.5)	64	65.25	−1.96	85	83.60	1.80
8	0(1:5)	1.41 (270.5)	71	73.32	−3.27	87	89.98	−3.66
9	0(1:5)	0 (200)	80	80.98	−1.22	85	85.32	−0.41
10	0(1:5)	0 (200)	81	80.98	0.02	86	85.32	0.86
11	0(1:5)	0 (200)	81	80.98	0.02	85	85.32	−0.41

[a] Stearic acid to alcohol ratio.
[b] Relative Error Deviation - $RED = \left(\frac{C_{exp} - C_{pred}}{C_{exp}}\right)x100$.
[c] Calculated according to Eq. (3).
[d] Calculated according to Eq. (4).

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