Biocatalysis and Agricultural Biotechnology 15 (2018) 229–234

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

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

Enzyme-catalyzed production of emollient cetostearyl stearate using different immobilized commercial lipases under vacuum system

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ARTICLE INFO

Keywords:
Emollient
Esterification
Fatty esters
Lipases
Fatty materials

ABSTRACT

This work reports the enzymatic esterification of stearic acid and cetostearyl alcohol for the production of cetostearyl stearate, a widely employed substance in the cosmetic and hygiene personal industry as emollient, as it assists in the consistency of beauty products and provides a soft feeling on the skin. The development of this work presents important results for the scientific literature since just a few references on this subject are available. For the maximization of the enzyme-catalyzed process, two immobilized commercial lipases were tested, NS 88011 and Novozym 435, and a study of reaction conditions was carried out, taking into account the catalyst concentration, enzyme catalyst type, reaction time, vacuum influence, process temperature, substrates molar ratio and agitation. Through the experimental design, the optimum reaction condition was determined as 75 °C, 1:1.5 acid to alcohol molar ratio, 600 mmHg vacuum and 760 rpm agitation. For such condition, high conversion values (99%) were obtained. The quality of the final product was characterized by the acidity index (0.6 mg KOH g^{-1}), iodine index (0% of iodine absorbed/g of sample), hydroxyl index (17.06 mg KOH.g^{-1}), saponification index (133.68 mg KOH g^{-1}) and color analysis.

1. Introduction

The cosmetic industry in Brazil has presented in recent years a large growth, from a net sales tax on sales from R $ 4.9 billion in 1996 to R $ 42.6 billion in 2015. This market growth of products for personal hygiene, perfumery and cosmetics elevated Brazil to fourth position in the world rankings, representing 7.1% of world consumption. The ranking is led by the United States (19%), followed by China (12%) and Japan (7.5%), respectively (ABIHPEC, 2016). Together with such considerable growth, concerns have raised about environmental and social impacts generated by the cosmetic industries (Moral and Angelis, 2012). The personal hygiene and beauty sectors will keep growing but always changing in order to meet the demands from consumers, who increasingly prioritize sustainable products (Gonçalves et al., 2016).

Currently, consumers present a growing interest in products that have low impact on the environment. These consumers are responsible for the emergence and consolidation of a new market niche, the “green” cosmetics, i.e., cosmetics produced with minimal impact on the environment (Fraccascia et al., 2018) and, consequently, new technologies are being developed to attain this goal. These technologies go beyond the idea of recycling, energy savings, combating waste and pollution control, they are, in fact, based on modifications of own manufacturing principles (Veiga et al., 2006).

Due to this fact, cosmetics, toiletries and perfumes industries are making a strict selection of their raw materials, and developing new methodologies for the production of cosmetics, aiming at sustainability (Galembeck and Csordas, 2010). Within these sustainable alternatives it can be mentioned the selection of raw material from alternative sources to petrochemical origin and the use of enzymes as biocatalysts. It is well-known that enzymes offer several advantages over chemical catalysts, such as: ecologically more correct, require milder reaction conditions, are compatible with synthetic substrates, raise the speed of reactions, can introduce the concept of selectivity, and do not present risks to the environment (Warner et al., 2004; Monteiro and Silva, 2009).

In addition, a series of substrates are of great importance for the production of cosmetics and personal care products, for example the fatty materials group, composed primarily of fatty acid derivatives and fatty alcohols. The produced esters, fatty esters produced by fatty acid and fatty alcohols, have a fundamental role to provide features of emollient for cosmetics (scattering, absorption, skin lubricant). These fatty esters can be produced sustainably, with the use of biocatalysts in esterification reactions and through selection of fatty materials derived from sustainable sources (Haag et al., 2005).

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https://doi.org/10.1016/j.bcab.2018.06.012
Received 11 April 2018; Received in revised form 7 May 2018; Accepted 19 June 2018
Available online 20 June 2018
1878-8181/

The esterification reaction between a carboxylic acid and an alcohol is the most commonly used industrial processes to obtain esters. This process releases water and occurs from the replacement of a hydroxyl (-OH) of an acid by an alkoxy group (-OR). The water released in excess by the reaction can facilitate enzyme aggregates and this may cause a decrease of biocatalyst activity - it is believed that one of the reasons for aggregations is the formation of intermolecular bonds disulfides (Yang and Russel, 1996; Khan and Rathod, 2015). Based on this, the reactions were carried out in this work upon the presence of vacuum, so that the excess water was removed from the process, thus preventing a decrease in enzymatic activity and hydrolysis undesirable reaction.

For a high efficiency of the esterification, it is recommended the use of immobilized lipases because they can provide several benefits, such as: increased thermal stability and chemistry of lipase, which allows a better control of the process and the product quality; requires low levels of water in the reactions; and the ease of separation of biocatalyst reaction system allowing the reuse of the enzyme, resulting in economically viable processes (Sabbani et al., 2006; Villeneuve, 2007; Wang and Hsieh, 2008). In this context, the objective of this work was to develop an innovative methodology towards low environmental impact for the synthesis of cetostearyl stearate in a solvent-free system under vacuum using commercial immobilized lipases as biocatalysts.

2. Material and methods

2.1. Materials

Two commercial lipases, NS 88011 (Lipase B from *Candida antarctica* immobilized on hydrophobic polymer resin) and Novozym 435 (Lipase B from *Candida antarctica* immobilized on macroporous of acrylic resin), both kindly donated by the company Novozymes S.A. (Araucária, Brazil), were used in this work. Two substrates were used in the esterification reactions, vegetable stearic acid and cetostearyl alcohol (ALMAD - Brazil). For the product characterization other chemicals were used: diethyl ether (Vetec), ethyl alcohol 99.8% (Vetec), potassium hydroxide (Vetec) and phenolphthalein indicator solution, both of analytical grade.

2.2. Enzymatic synthesis of cetostearyl stearate

To perform the esterification reactions, it was used a stainless-steel reactor of 300 mL (Autoclave Engineers, A303060B86000 model), with available mechanical agitation, temperature control by an internal coil coupled to a thermostatic bath (Microchemistry, MQBTZ99 model-20) and vacuum gauge. The reaction vessel was connected to a vacuum pump (Quimis, Q35502 model). The amount of substrates was calculated to occupy a volume of approximately 200 mL in the reactor. The value of the individual mass of these reactants varied as the molar ratio of the reaction changed.

2.3. Preliminary tests

Preliminary tests were performed using stearic acid and cetostearyl alcohol as substrates, under the conditions 1:1 M ratio (acid:alcohol), agitation 600 rpm, temperature of 75 °C, 600 mmHg vacuum and reaction time of 6 h. Initially, two immobilized lipases (Novozym 435 and NS 88011) were tested, in different concentrations (0.5; 1 and 2 wt% in relation to the total mass of the substrates). Along with these tests, it was also performed reaction without the presence of the enzyme (blank). Ester production was accompanied by the decrease of the acidity of the reaction medium and the conversion was calculated by the difference between the initial and the final acidity values, determined by the AOCS Cd 3d-63 method.

From the results of the preliminary tests on selection and concentration of the enzyme, it was carried out the evaluation of the esterification kinetics to produce cetostearyl stearate under the following conditions: lipase NS 88011 (1 wt%, based on the total mass of substrates), 600 rpm, 75 °C, 600 mmHg vacuum and 1:1 substrates molar ratio. Samples were taken from the reaction medium at each 1 h interval during 6 h of reaction.

2.4. Optimization of cetostearyl stearate synthesis

For the optimization step, a 2^3 central composite rotational design was adopted, with triplicate experiments at central point, totalizing 17 experiments, in which the independent variables studied were: temperature (61.6–78.4 °C), molar ratio of stearic acid to cetostearyl alcohol (1:0.83–1:1.67) and agitation (250.8–889.2 rpm), keeping constant the enzyme concentration (1 wt% NS 88011) and reaction time at 2 h.

2.5. Kinetic experiment

After reaction conditions optimization for the synthesis of cetostearyl stearate, a kinetic experiment was carried out under the following conditions: NS 88011 lipase (1 wt%), 75 °C, 760 rpm, vacuum of 600 mmHg and molar ratio of 1:1.5 (acid/alcohol). Aliquots of approximately 1 mL were taken at predetermined times (1–48 h) to measure the acid value.

2.6. Biocatalyst reuse

Reuse study was performed with lipase in several cycles of synthesis of cetostearyl stearate. The reuse of the biocatalyst was based on work by Lerin et al. (2011). In this study we used the conditions: lipase NS 88011 (1 wt%), 75 °C, 600 rpm, vacuum of 760 mmHg, molar ratio of 1:1.5 (acid/alcohol) and reaction time of 48 h. After each experiment, the enzyme was separated from the reaction medium, subjected to successive washings with n-hexane (10 mL each) and oven drying at 40 °C for 4 h. After this step, the lipase was kept in a desiccator, using silica gel blue with the drying agent, for at least 12 h and then used in a new reaction. The evaluation of each enzyme cycle was performed by monitoring the ester production.

2.7. Analytical methodology for reaction conversion

2.7.1. Determination of acid content

The determination of the acid content was conducted following the AOCS Cd 3d-63 method. Briefly, approximately 3 g of solution and 3–4 drops of phenolphthalein were diluted in 50 mL of 1:1 (v/v) ethanol to ether solution. Such a solution was then titrated with KOH 0.1 M, under vigorous agitation until subtle color change. The solution acidity was then determined according to:

$$AI = \frac{V_{KOH}.C_{KOH}.\ MFA}{10.m} \quad (1)$$

where AI is the acidity, i.e., the content of the fatty acid (wt%), V_{KOH} is the KOH solution volume (mL) employed in the titration, C_{KOH} is the molarity of KOH solution (mol L^{-1}), MFA is the molar mass of stearic acid, and m_s is the sample mass (g).

2.7.2. Determination of reaction conversion

The conversion determination was done through the relation between the indexes of final and initial acidity, by:

$$C(\%) = \frac{(AI_f - AI_i)}{AI_i}.100 \quad (2)$$

where AI_f is the final acidity index (mg KOH g^{-1}) and AI_i is the initial acidity index (mg KOH g^{-1}).