SYNTHESIS OF GERANYL BUTYRATE USING A NOVEL ENZYME-BASED BIOCATALYST DERIVED FROM CARNAUBA STRAW (*Copernicia prunifera*)

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ABSTRACT

The use of biocatalysts in catalysis reactions is an alternative to conventional chemical catalysis. However, the use of soluble enzymes in the industry is limited due to problems related to their stability and difficulty in reuse. Therefore, enzymatic immobilization is presented as a promising alternative that will guarantee a better performance of the enzyme. Thus, the present work aims at the use of carnauba straw (Copernicia prunifera) in the stabilization of enzymes to catalyze the synthesis of scent ester geranyl butyrate. The immobilization process highlighted a point with 5% of biocatalyst, a time of 24 h, ionic strength of 5 mM, and 13% of glutaraldehyde in the activation of the support, which resulted in a yield of 82.54%. The application of the biocatalyst was carried out to confirm the effectiveness of the immobilization process used and resulted in 91.68±0.07% conversion of Geranyl Butyrate. Which highlights the effectiveness of the new biocatalyst developed.

Keywords: Enzyme. Immobilization. Biomass. Geranyl butyrate.

1 INTRODUCTION

With the advancement of research focused on reuse, it was concluded that agro-industrial waste had the potential for reuse[1]. Within this class of residues, there are residues of lignocellulosic materials, which have great potential for use in the most diverse lines of research. These materials are represented by different residues, such as sugarcane bagasse[2], used in the production of ethanol, cashew bagasse, carnauba straw, among others [3]. In studies of potential applications for these materials, the possibility of using them as a support for enzymes was seen.

Enzymes are macromolecules that have the potential to catalyze the most diverse reactions[4]. These macromolecules are obtained from different microorganisms in different ways, thus resulting in the classification as a biocatalyst. These biocatalysts have a high degree of selectivity and purity, thus reducing the generation of by-products in the reactions where they are applied[5]. However, these biocatalysts may suffer interference from the reaction medium, as they are susceptible to denaturation by organic solvents and damage to their catalytic site. In addition to these points, another disadvantage is the impossibility of reusing these enzymes[6].

This immobilization results in the potentization of the catalytic capacities of these enzymes to improve stability and resistance to denaturation in an organic medium and enable the separation of the reaction medium, thus allowing the reuse of this biocatalyst [7,8]. In addition, in the studies, new materials are analyzed to be used as a support and this resulted in the possibility of applying lignocellulosic materials [9,10]. Thus, given the need to apply these biocatalysts formed by lignocellulosic materials, the synthesis of industrial aromas and fragrances (Figure 1) was selected.

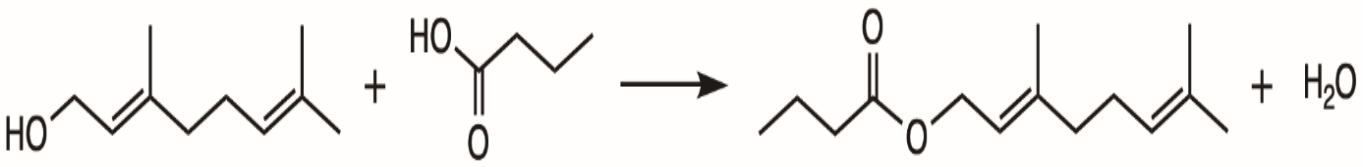

Figure 1 Esterification reaction of geraniol (alcohol) and butyric acid (carboxylic acid) producing geranyl butyrate (flavor ester) and water.

Thus, the present work aims at the use of carnauba straw in the stabilization of enzymes to catalyze the synthesis of industrial aromas and fragrances. This justifies the need to reduce environmental impacts by polluting industrial waste and producing by-products that donate to the environment from the chemical synthesis of aromas.

2 MATERIAL & METHODS

The *Aspergillus oryzae* lipase, commercially known as Eversa® transform 2.0, was obtained from Sigma-Aldrich Brazil Ltda (Cotia, São Paulo, Brazil). The chemical reagents are analytical grade and were purchased from Synth (São Paulo, Brazil) and Vetec (São Paulo, Brazil). The experimental planning based on the Taguchi method was elaborated in the Statistic®10 software (Statsoft, USA).

For the development of this study, an advanced experimental design using the Taguchi method that has a standard L9 orthogonal matrix, in which “L” represents the Latin square and “9” the number of experiments, in its base, was used to investigate four factors (glutaraldehyde concentration, ionic strength, time and enzymatic load) in three levels to determine the best conditions for the immobilization of lipase Eversa® Transform 2.0 (ET2) in CAR@GLU, abbreviated CAR@GLU-ET2, by the covalent method. For this, 0.1 g of CAR@GLU was suspended in 1 mL of buffer with varying ionic strength (5, 50, and 95 mM) and sodium phosphate pH 7.0, containing a load of ET2 protein (1, 3, and 5 mg/g), in the presence of 10uL Triton X-100 0.01%. The system was maintained under constant moderate agitation at 25 °C for the time determined in the planning (6, 15, and 24 h).

After carrying out the esterification process [11], the samples were analyzed in triplicate in Erlenmeyer flasks containing 0.1 g of the sample, 2.5 mL of standardized ethyl alcohol, and 3 drops of phenolphthalein. The 0.1 molar NaOH solution was added gradually until the color change to a subtle pink occurred. After titration, the volume values used were inserted into Equation 1 to obtain the acid number (AI).

$$AI\ \left(\frac{mgNaOH}{g}\right) = \frac{MM_{NaOH}.M_{NaOH}.f.V_{NaOH}}{m} \quad (1)$$

Where, MM NaOH (g/mol) is the molar mass of NaOH; M NaOH (mol/L) is the molarity of the NaOH solution; f is the correction factor determined by NaOH standardization; V NaOH is the volume of NaOH used during the titration, and m (g) is the mass of the sample to be studied.

The conversion of butyric acid into aroma esters from the application of the new biocatalyst is given by Equation 2, where IAi represents the initial acidity value given by the amount of acid added in the reaction, and IAf to the final acidity value, equivalent to the remaining acids in solution, not used by the enzyme.

$$Conversion\ (\%) = \frac{IAi-IAf}{IAi} x100 \quad (2)$$

3 RESULTS & DISCUSSION

Thus, with the analyzes carried out, the optimized reaction conditions were defined to guarantee greater immobilization yields. Thus, it was defined that the best time was presented at Level 3, with 24 h (L3), the best percentage of glutaraldehyde in the activation of the support was 12% (L2), the percentage of biocatalyst was defined as 5% (L3) and, finally, the ionic strength stood out in Level 1, at a concentration of 5 mM (L1). In theory, the yield presented at this optimized point would be 84.34%. However, after the experimental application of this point, the yield was 82.54%.

The application of CAR@GLU-ET2 as a biocatalyst was carried out under optimized conditions of molar ratio 1:5 (v/v), 15% biocatalyst load, at 50 °C in 6 hours and resulted in 91.68±0.07% conversion into Geranyl Butyrate, from the reaction between butyric acid and geraniol. The expected response for this reaction would be 95.98 %. Conversion using free lipase was 85.17 ± 0.06%. Thus, the use of CAR@GLU-ET2 increased the conversion of this reaction, although it did not reach the value expected by the planning.

Following this, the characterization of the produced geranyl butyrate was carried out. In the ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of the reaction medium, the signals of the hydrogen atoms for the formed ester can be observed. Expansions for the highlighted hydrogen atoms in the structure have been inserted to identify and assign the signals to their respective chemical shift values and multiplicities.The signals near δ 5.40 and δ 5.09 have been attributed to the hydrogen atoms of the sp^2 carbons present in the carbon chain of geranyl butyrate. This chemical profile leads to a signal with multiplet characteristics, due to the characteristic splitting for alkenes. Due to a higher s orbital character compared to methyl groups, these hydrogen atoms undergo greater deshielding, generated by the increased electronegativity of the double bond.

The signal at δ 4.15 is a doublet, corresponding to the multiplicity and chemical shift attributed to the hydrogen directly bonded to the oxygen of the ester group. This hydrogen is directly bonded to an electronegative atom and an sp^2 carbon bonded to a single hydrogen, justifying the deshielding observed by the shift and the doublet multiplicity. The triplet observed at δ 2.30 is characteristic of methylene hydrogens in the α positions to the carbonyl of esters. The multiplicity of this signal corresponds to the proximity of these hydrogens to a methylene group, -CH2. The signals observed in the region near δ 2.30 are related to the methylene and methyl groups common to the rest of the carbon chain of the ester. Finally, the signal at δ 0.96 has been attributed to the methyl group at the end near the ester carbonyl, as it exhibits greater shielding and lower chemical shift, along with the corresponding multiplicity. In the spectrum, the peak for the deoxygenated carbon present in esters is close to δ 177.7, confirming the formation of the ester and the consumption of the substrate, geraniol.

4 CONCLUSION

The increase in immobilization time contributed to greater interaction between activated support (CAR@GLU) and the biocatalyst (ET2), thus ensuring a more efficient immobilization. Variations in glutaraldehyde and ionic strength were a few determinants of the process. The application of the new biocatalyst (CAR@GLU-ET2) presented the expected results, based on theoretical studies carried out and its effectiveness was superior to the use of lipase Eversa® in its free form since the yield increased by approximately 85% to 91%.

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