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BIOCATALYTIC PRODUCTION OF CITRONELLYL ESTERS BY DIFFERENT ACYLATING AGENTS: A KINETIC COMPARISON STUDY

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1. INTRODUCTION

Terpene esters are active compounds of essential oils obtained from several plants, showing application as flavor and fragrance components in cosmetics (AKACHA E GARGOURI, 2015; SÁ et al., 2017) as well as in the food and pharmaceutical industries (DHAKE et al., 2011; SERRI et al., 2006; YADAV & LATHI, 2004). Among the promising compounds, attention has been given to esters obtained from citronellol (SANTOS et al., 2019), used as a flavor in perfumes, food, and beverages for fruity-floral nuances (MOHAMAD et al., 2015). Nonetheless, it is possible to naturally find a variety of terpene esters, where the most common are those with short carbon chains. Nowadays, the most terpene esters are obtained by chemical synthesis (ABDULLAH et al., 2009; MOHAMAD et al., 2015). Nevertheless, applied protocols show limitations such as the use of dangerous chemical reagents and catalysts, with high temperatures and pressure, generating by-products. Besides, extensive purification steps are also required (SÁ et al., 2017; YOU et al., 2011). For these purposes, biocatalysis can lead to a wide variety of esters with regio-, chemo- and enantioselectivity, through the application of biocatalysts in systems that require lower temperatures and more environmentally conditions (FRYSZKOWSKA e DEVINE, 2020; PELLIS et al., 2018). Among enzymes applied in biocatalysis, lipases are one of the most suitable for the synthesis of monoterpene esters, presenting high activity in organic solvents, good performance with different substrates in a broad range of temperatures as well as do not need cofactors (DJAMNJANOVIC et al., 2012; YOU et al., 2011). Considering the necessity for pursuing new systems for obtaining citronellol esters, this study brings the production, purification, and identification of those compounds, resultant fromacylating agents with different sizes, using (Novozym®435) as catalyst.

2. MATERIAL AND METHODS

Esterification reactions were conducted using propionic, butyric, valeric, hexanoic, octanoic, lauric, myristic, stearic, and oleic acids as acyl donors. Each reaction was conducted in a conic flask from the mixture of citronellol and the acyl donor, in 1:1 molar ratio, heptane, 3Å molecular sieves, and Novozym®435, under 150 rpm at 70°C. Samples were taken until 6 h reaction. Ester conversion was determined concerning citronellol decay by GC-MS equipped with a DB-5MS column. The best reaction time was selected and repeated scaling up three times. Products purification was performed by preparative thin-layer chromatography. The products were analyzed by GC- MS. and elucidated by Nuclear Magnetic Resonance. Statistical analysis was performed using GraphPad Prism version 7.0. Kinetic parameters were determined from the non-linear regression with Michaelis-Menten model, using ANOVA and Tukey's multiple comparison test, with 95% confidence (α=0.05).

3. RESULTS

Up to 6 h reaction all esters seemed to achieve not less than 91% conversion (Figure 1). More precisely, in 2h reaction-time the production varied from 71% to about 97% (citronellyl butyrate), followed by citronellyl hexanoate (96%). Both showed a plateau after 2h reaction-time, with higher than 98% conversion (Figure 1). The reaction performed with butyric acid seemed to present the higher initial rate (V_0), followed by myristic and oleic acids. Lauric acid presented the lower V_0 and V*max*. The reaction conducted with propionic acid exhibited the higher V*max*, followed by butyric acid. The

esterification method provided a minimum of 15 $g.L^{-1}$ in esterified products, in 2 h, with all acyl donors tested, reaching over 35 $g.L^{-1}$ in case of stearate and oleate esters, followed by myristate and laurate, that achieved near to 35 and almost 30 $g.L^{-1}$ production, respectively. Citronellyl propionate exhibited the lower productivity, as well as citronellyl butyrate, which, even though presented the second higher V*max,* higher initial rate (V_0), and conversion (%). It was observed that the carbon chain size shows influence on the conversion in citronellyl esters. The statistical analysis showed a significant difference (α=0.05) as long as the carbon chain increased, explained by the difference in density, viscosity, evaporation rate, and unsaturation on the acyl donor. The purification step resulted in nine products obtained with over 95% purity, with the exception of citronellyl oleate, that presented 90% purity. The preparative TLC showed to be an efficient method of purification, being a fast, stable, and cost-efficient chromatographic separation technique.

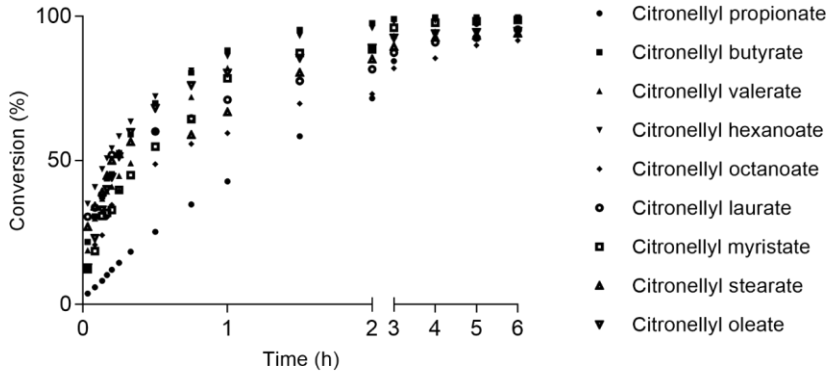

Figure 1. Kinetic of citronellyl ester production by the esterification reaction. (Esterification reactions conducted in an orbital shaker at the conditions: molar ratio 1:1; 100 mM of citronellol, 5 $U.mL^{-1}$ (unities of enzymatic activity by mL of reaction) of Novozym®435, heptane as a solvent, 150 rpm, and 70°C).

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