

TITLE: SYNTHESIS OF FLAVOR ESTERS USING LIPASE OF *YARROWIA LIPOLYTICA* PRODUCED BY SOLID STATE FERMENTATION IN SOYBEAN MEAL AND THE EFFECT OF PH AND TEMPERATURE IN ITS ACTIVITY

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Lipases (acylglycerol acylhydrolases, EC 3.1.1.3) constitute one of the most important groups of biocatalysts due to their wide range of applications. Their ability to catalyze esterification reactions made them a useful biocatalyst to produce several esters employed in food, beverage, cosmetic and pharmaceutical industry, such as flavor esters. This study aimed the production of two different ethyl flavor esters (Ethyl octanoate and ethyl decanoate) using lipase produced by *Yarrowia lipolytica* in solid state-fermentation (SSF) employing soybean meal supplemented with 1.5% of soybean oil. The influence of pH and temperature on lipase hydrolysis activity was also evaluated at 25°C, 37°C and 50°C in pH 3 to 9. The fermented solids of soybean meal were completely dried in a lyophilizer and used as biocatalyst. Lipase hydrolytic activity was measured by the titrimetric (Metrohm 916 - Ti-Touch) method using olive oil as substrate (5% v/v) in the different conditions of pH and temperature listed above. Esterification reactions were performed in closed 15 mL batch reactors thermostated and magnetically stirred. Substrates were used in a molar ratio 1:1 in 15 mL of hexane, temperature was set to 40°C. The conversion progress was monitored by taking quadruplicate samples (100 µl) after 2, 4, 6, 8 and 24h. Control assays (blanks) were considered samples took before the biocatalyst addition. Free medium chain acids in each sample were analyzed by titration using a Metrohm 916 -Ti-Touch autotitrator. The esterification reaction using ethyl alcohol and octanoic acid as substrate after 8 h presented 88% of conversion against 77.4% using ethyl alcohol and decanoic acid after the same time. Nevertheless, ethyl decanoate reached a conversion of 89.8% after 24 h, ethyl octanoate showed 87.4%. Regarding the lipase activity in different conditions of pH and temperature at 25°C and 37°C higher activities were obtained in pH 8, 69.9 U/g and 102 U/g, respectively. However, at 50°C lipase activity showed maximum activity in pH 7 (67.8 U/g). Lower activities were obtained in pH 3 and 4 for all temperatures tested. The biocatalyst obtained from a low cost feedstock showed potential to perform flavor ester (high added value) synthesis in a short period of time. Moreover, lipase of *Y. lipolytica* showed great ability to act in the different ranges of pH and temperature tested in this work, showing in a first moment great application versatility.

Keywords: Ethyl octanoate, ethyl decanoate, *Yarrowia lipolytica*, Lipase, Solid-state fermentation

Development Agency: CNPq