TITLE: IMMOBILIZATION OF THE *Aspergillus ochraceus* TANNASE AND APPLICATION OF THE DERIVATE IN ENZYMATIC HYDROLYSIS OF TANNINS OF GRAPE JUICE AND BLACK TEA

AUTHORS: CAVALCANTI[1], R. M. F. C.; MAESTRELLO[1], C. C.; GUIMARÃES[2], L. H. S.

INSTITUTION: [1]CHEMISTRY INSTITUTE OF ARARAQUARA – UNESP (AV. PROF. FRANCISCO DEGNI 55, JARDIM QUITANDINHA, CEP 14800-900 ARARAQUARA – SP, BRAZIL); [2]FACULTY OF PHILOSOPHY, SCIENCES AND LETTERS OF RIBEIRÃO PRETO – USP (AV. BANDEIRANTES 3900, VILA MONTE ALEGRE, CEP 14040-901 RIBEIRÃO PRETO – SP, BRAZIL)

ABSTRACT:
The enzyme tannin acyl hydrolase (EC 3.1.1.20), also known as tannase, hydrolyzes esters and lateral bonds of hydrolysable tannins, releasing glucose and gallic or ellagic acid. Tannase is potentially applied in the food industries for clarification of juices and teas and removal undesirable phenolic compounds, improving significantly the quality of feeding. Therefore, the present study aimed to immobilize *Aspergillus ochraceus* tannase in different supports, the characterization of tannase immobilized and the application of the best derivative to hydrolyze tannins in grape juice and black tea. The filamentous fungus *A. ochraceus* was cultivated in Khanna medium with 2% (w/v) tannic acid for 72 hours, 30 ºC and 120 rpm. The crude filtrated was dialyzed for 24 hours and the tannase was precipitated in acetone (1:2 v/v). The enzyme was immobilized by adsorption, covalent bonding and encapsulation methods, using Amberlite IR 120, sodium alginate crosslinked in $CaCl_2$ and $MnCl_2$, DEAE-Sephadex A25 and glass beads. The highest yield (100%), activity recovery (73.84%) and immobilization efficiency (100%) was obtained using sodium alginate as matrix (3% w/v) reticulated with $MnCl_2$ (0.1 mol L^{-1}). The sodium alginate derivative presented maximal tannase activity at 50 ºC and pH 5. In addition, the enzyme activity was maintained about 70% after 6 cycles of reuse. The treatment of the grape juice and black tea with sodium alginate derivative for 120 min at 30 ºC reduced the tannins content by 48.34% and 23.28%, respectively, increase the gallic acid content and flavonoids. Grape juice and black tea were also clarified after treatment, and the pH values of these beverages did not show significant differences when compared to untreated samples. Thus, immobilized *A. ochraceus* tannase presents potential for industrial application in the food sectors offering advantages as recovery and derivative reuse.

Keywords: Clarification, immobilization, sodium alginate, Tannin acyl hydrolase

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