Production and purification of recombinant xylanases for application in juice clarification

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Abstract

Endo-β-1,4-xylanases (EC 3.2.1.8) are able to hydrolyse xylan, the second most abundant polysaccharide in plant cell walls. In this work, two recombinant xylanases derived from *Orpinomyces* xynA gene were studied: one with mutations A226T and V135A (XM) and the other without mutations (XNM). They have been cloned in *E. coli*/pET24b, using IPTG as inducer and purified using ion exchange chromatography (Q-Sepharose). After purification, only one band of 25 kDa, corresponding to xylanases, was visualized on SDS-PAGE. Specific activities and Km against arabinoxylan were 12.844 $U.mg^{-1}$ and 0.0021 $mg.mL^{-1}$ for XNM and 10.794 $U.mg^{-1}$ and 0.0014 $mg.mL^{-1}$ for XM. The mutations improve thermo stability. The half-life of XNM was 220 min and 31 min at 50 °C and 60 °C, while for XM the values were 440 and 71 min at 50 °C and 60 °C, respectively. The clarification assays were conducted at 60 °C, 100 rpm for 60 min, using 50U xylanase/mL apple juice. The parameters available was pH, A660 (clarity), L* (luminosity), C (chroma) and h* (hue). All the juices tended to orange while only enzymatically treated with XM had an increase in color intensity (chroma / C) and higher content of soluble solids (sst) of 4,7 °brix. The results indicate that the processing of apple juice with xylanases can promote clarification and improvement of its properties.

Palavras-chave: xylanase, juice clarification, apple

Apoio financeiro: CNPq, FAPEMIG, CAPES, Bioagro/UFV

Passarinho, Amanda Tafuri Paniago; Mamede, Mariana Siqueira Lacerda; Oliveira, Eduardo Basílio de; Guimaraes, Valeria Monteze; "PRODUCTION AND PURIFICATION OF RECOMBINANT XYLANASES FOR APPLICATION IN JUICE CLARIFICATION", p. 35 . In: **Anais da V Jornada Acadêmica Internacional de Bioquímica [= Blucher Biochemistry Proceedings, v.1, n.1]**. São Paulo: Blucher, 2015. DOI 10.5151/biochem-jaibqi-0073