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Analysis of Glucose Tolerance of β-Glucosidases from Commercial Preparations

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ABSTRACT

β-glucosidases catalyze the selective cleavage of glucosidic linkages and are an important class of enzymes having significant prospects in industrial biotechnology. This study aimed to quantify the presence of glycosidase activity on three different commercial preparation, and measure its glucose tolerance in different glucose concentrations (5.55–222 mmol/L) and pH values. The Pectinex Ultra SP-L exhibited the highest glucosidase activity and better glucose tolerance at low pH. However at higher pH (7.0) better tolerance was shown by Pectinex Smash XXL. In conclusion, the commercial preparations with pectinolytic activities as the primary activity may also be used as sources of β-glucosidases with biotechnological potential.

Keywords: β-glucosidase activity, glucose tolerance, aromatic compounds.

INTRODUCTION

β-Glucosidases (3.2.1.21) have the ability to remove nonreducing β-d-glucosyl residues from glucoconjugates, including glucosides, 1-O-glucosyl esters, and oligosaccharides (Cairns *et al.* 2015). They are responsible for the release of aromatic compounds in fruits and fermentation products and are among the key enzymes for juice and beverage industries (Agrawal, Verma & Satlewal, 2015). However, the high concentration of glucose contained in juices and wines causes inhibition of β-glucosidase, reducing enzyme activity. Therefore, the search for β-glucosidase with high glucose tolerance is extremely relevant for biotechnological applications (Singhania *et al.* 2013; Chamoli *et al*. 2016).

Thus, this study aimed to evaluate the β-glucosidase activity of different commercial enzyme preparations, measuring their glucose tolerance and the effect of pH on this tolerance.

MATERIAL AND METHODS

Material: Pectinex Ultra SP-L and Pectinex Smash were kindly donated by Novozymes (Spain), and Lallzyme Beta was from Lallemand Wine (France).

Enzymatic activity and protein quantification: The β-glucosidase activity was measured using 4-Nitrophenyl β-D-glucopyranoside (pNPG) as substrate. The mixture consisting of 100 µl of pNPG (9 mM) in 100 mM sodium citrate buffer pH 4.8, 100 µl of the same buffer and 100 µl of enzyme extract incubated at 40 °C for 5 min. The reaction was

stopped by the addition of 1.2 ml of 0.1 M Na_2CO_3 and quantified at 410 nm. One unit of enzyme activity (U) was defined as the amount of enzyme that releases 1 µmol of p-nitrophenol per minute. Protein concentration was measured by the Lowry method (Lowry Rosebrough, Farr, & Randall, 1951), using bovine serum albumin (BSA) as the standard.

Effect of glucose on β-glucosidase activity: Glucose tolerance of the β-glucosidase was carried out measuring the enzyme reaction in the presence of different glucose concentrations (5.55–222 mmol/L) and measuring release of pNP as described above.

Effect of pH on glucose tolerance from β-glucosidase: The effect of pH on the inhibition of β-glucosidase by sugars was also evaluated by incubating the reaction mixture in 0.1 M citrate buffer pH 3.0, 4.8 and 0.1M phosphate buffer pH 7.0. The residual enzyme activity of reaction mixture was measured under standard conditions. The relative activity was defined as the relative value to the activity of the control without glucose.

RESULTS AND DISCUSSION

All commercial enzymes are pectolytic preparations indicated for use in juice and wine production for extraction, clarification and aroma improvement. From the three preparations, Lallzyme beta has a high secondary β-glycosidic activity, while Pectinex Ultra SP-L and Pectinex Smash XXL are polygalacturonases (Dal Magro *et al.* 2016). Preliminarily, the β-glucosidase activity of the three commercial enzyme preparations was evaluated on pNPG as substrate and the results are shown in Table 1. Interestingly, the maximal activity and specific activity of β-glucosidase were found for Pectinex Ultra SP-L with 7.67 $U.mL^{-1}$ and 0.19 $U.mg^{-1}$ respectively. The activity obteined for this preparation was higher than the reported for a mixed culture of Juhász and collegues (2003) (3.07 $U.mL^{-1}$) and by *Fusarium proliferatum* of Gao et al. (2012) (3.31 ± 0.14 $U.mL^{-1}$). Furthermore, the low specific activity values reported were easily increased for simple steps partial purification (date not shown).

Table 1. Analysis of β-glucosidase activity and protein in commercial enzyme preparations

Enzyme Preparation	Activity ($U.mL^{-1}$)	Protein ($mg.mL^{-1}$)	Specific activity ($U.mg^{-1}$)
Pectinex Ultra SP-L	7.67±0.56	40.43±0.14	0.19
Pectinex Smash XXL	0.20±0.00	9.91±0.48	0.02
Lallzyme Beta	0.31±0.01	3.10±0.00	0.10

Values are mean ± SD of three replicates.

Many β-glucosidases are inhibited by glucose during the fermentation process, which restricts their activity and the liberation of aroma volatile compounds. Therefore, high resistance to glucose inhibition is one of the most important features of ideal β-glucosidases for industrial applications (González-Pombo et al. 2011; Chen, Li & Zong, 2012). The Pectinex Ultra SP-L retained 50 % of its activity in the presence of almost 200 mmol/L of glucose at pH 3.0, whereas Pectinex Smash XXL exhibited similar result at pH 7.0. However, Lallzyme Beta showed no significant differences between pH 3.0 and 4.8, with a slight decrease of its activity at pH 7.0 (Figure 1). The results indicated that while Pectinex Ultra SP-L and Lallzyme Beta exhibited maximal activities at lower pH values, the opposite was observed for Pectinex Smash XXL. According to de Giuseppe et al. (2014), the comparative analysis of active sites of high, moderate and low glucose tolerance β-glucosidases indicated

that beyond the shape and the +2 subsite, the electrostatic properties of the deep active-site entrance also determine the degree of tolerance to glucose. Thus, the structural characteristics and charges of each enzyme preparation should influence its tolerance glucose. However, more studies should be conducted to understand ration between pH and glucose tolerance.

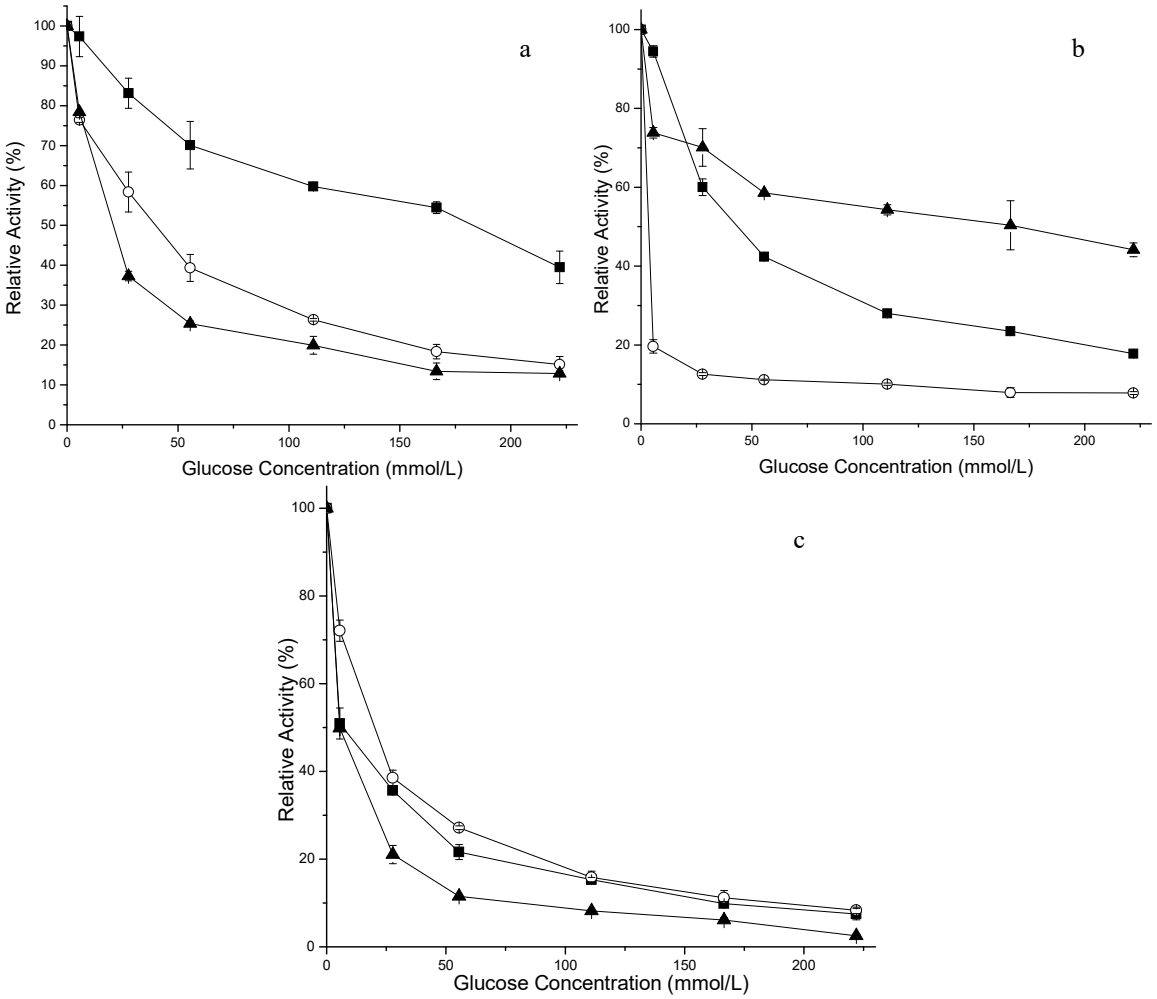

Fig 1. Effect of pH on the glucose tolerance. (a) Pectinex Ultra SP-L; (b) Pectinex Smash XXL; (c) Lallzyme Beta. (■) pH 3.0; (○) pH 4.8; (▲) pH 7.0. Residual activities were assayed at 40 ºC. The values shown represent means ± SD from triplicate assays (n=3) carried out with three separate preparations of β-glucosidase.

CONCLUSIONS

The most effective β-glucosidase was the commercial preparation Pectinex Ultra SP-L presenting volumetric activity of 7.67±0.56 $U.mL^{-1}$ and specific activity of 0.19 $U.mg^{-1}$. This preparation also was presented good glucose tolerance at pH 3.0, showing that your application in beverages with low pH as juices and wines is possible. On the other hand, the enzyme preparation Pectinex Smash XXL was less inhibited by glucose at high pHs.

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