EFFECT OF TEMPERATURE IN α-AMYLASE AND AMYLOGLUCOSIDASE PRODUCED FROM ENGLISH POTATO RESIDUE VIA FERMENTATION IN SOLID STATE

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ABSTRACT

The objective of the present study was to use the English potato residue as a substrate of the solid state fermentation (SSF) of the fungus Aspergillus niger for the production of the enzymes alpha amylase and amyloglucosidase. A spores solution of concentration 109 was used. The fermentations were conducted at 30 °C in a bacteriological oven, with 35%, 60% and 75% moisture content, at times of 48, 72 and 96 hours. Fermentation was carried out with and without the presence of the inducer (1 g corn starch). The study variable was the optimum temperature, necessary for the inclusion of enzymes in industrial routes. The temperatures analyzed were 30, 40, 50, 60 and 70 °C. The highest α-amylase activity was at 30 ° C yielding 192.8 U / g, in the presence of the inducer the temperature with the best activity was 70 ° C yielding 258.8 U / g. Glucoamylase presented a lower temperature preference, 30 ° C without the inductor and 40 ° C with the presence of inductor, with an activity of 230.41 U / g and 313.81 U / g respectively.

1. INTRODUCTION

The solid state fermentation (SSF) plays a prominent role in processes of biological detoxification using filamentous fungi, besides representing an interesting process to obtain products of biotechnological interest at low cost (Amorim, 2011), such as amylases. The use of solid state fermentation is related to: enzymes production and protein enrichment of agroindustrial residues.

The potato species *Solanum tuberosum* L. is commonly known as English potato, carbohydrate-rich and an important source of phosphorus and B-complex vitamins (Garmus et al., 2009). The disposal of certain residues from the potatoes processing in the environment can cause pollution problems, these residues can be used as a substrate for the production of enzymes and other biotechnological products.

There are several types of amylolytic enzymes and they have uses in various types of industries such as paper, textiles, baking, syrup production, alcohol, beverages, among others. (Cruz et al., 2011). For the production of this enzyme are used fungi, such as *Aspergillus niger*. Due to these factors, the aim of this work is to analyze the effect of alpha-amylase and glucoamylase produced by solid-state fermentation, using as substrate the potato peel flour and as fermentation agent the filamentous fungus *Aspergillus niger*.

2. MATERIALS AND METHODS

2.1. Solid state fermentation

Erlenmeyers (250 ml) were used, with 10 g of the dried and milled residue. The fermentations were conducted in a bacteriological oven with a temperature of 30 °C 60% moisture content, and this process was carried out without and with the presence of inducer (1 g of corn starch). The erlenmeyers were autoclaved at 1.0 atm for 15 minutes and after cooling, inoculated with the spore solution. *Aspergillus niger* was inoculated into a potato dextrose agar medium (PDA) and incubated for 7 days at 35°C in a temperature control incubator (SL 222; Solab) then spores were collected using a 0.01% Tween 80 solution. Spores in suspension were counted using a Neubauer chamber and a binocular microscope. The destructive sampling was performed at 72 hours to determine the enzymatic activity. After the fermentation process, 50 mL of distilled water was added to perform the mechanical extraction (pressure filtration) of the enzymatic extract and then centrifuged at 3000 rpm for 5 minutes to remove fine solids (Santos et al., 2016).

2.2. Enzymatic activities

To determine the enzymatic activity of amyloglucosidase, the method chosen was based on the dosage of reducing sugars produced by the degradation of the starch. 1.5 ml of phosphate buffer solution with pH7 at 50 mM, 0.5 ml of enzyme extract and 1.0 ml of dinitrosalicylic acid (DNS) were added into the test tubes. The blank of the analysis contained 1.0 mL of DNS and 10 mL of distilled water. The samples were incubated in a bacteriological oven at 30 °C for 30 minutes, the reaction was stopped with the addition of 0.2 mL of NaOH. The tubes were immersed in boiling water for 5 minutes, after which 10 mL of distilled water were added for further measurement to absorbance at 540 nm by spectrophotometry (Digital Spectrometer 325-1000NM Mod. GT7220) (Ghose, 1987).

The α-amylase activity was determined from the addition, in test tubes, of 0.5 ml of the enzyme extract, 1.5 ml of a 10 g / L starch solution with a phosphate buffer at pH 7 and 50 mM. Samples were inoculated in a bacteriological oven at 30 °C for 30 minutes and the reaction was quenched with 0.2 mL of NaOH. After that, 1 mL of potassium iodide and 10 mL of distilled water were added. The blank of the analysis contained 1 mL of potassium iodide and 10 mL of distilled water. The measurement was carried out at 660 nm in the digital spectrophotometer 325-1000NM Mod. GT7220 (Archibald et al., 1988.)

2.3. Optimum temperature

For the temperature analysis the best results of amyloglucosidase and alpha amylase were used. The samples were inoculated in a bacteriological oven at temperatures of 30, 40, 50, 60 and 70 °C for 30 minutes at each temperature. For the determination of the enzymatic activities, the processes already described previously were followed.

3. RESULTS AND DISCUSSION

In figure 1 are presented the values obtained during the tests with the temperatures of 30, 40, 50, 60 and 70 ° C based on three experimental repetitions.

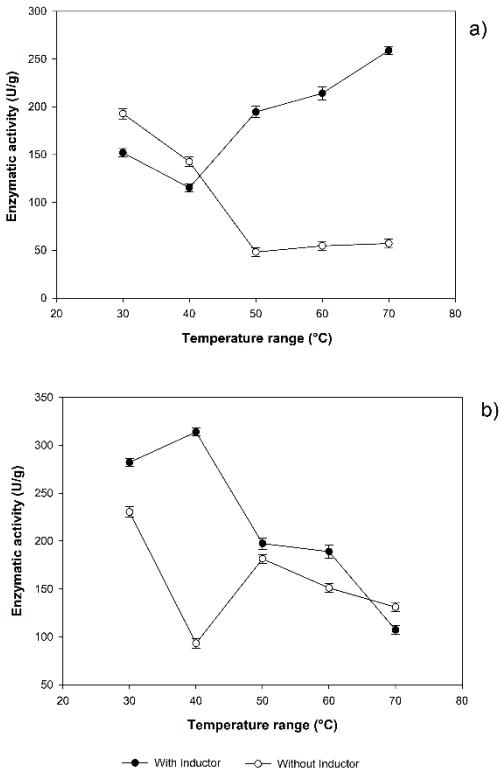

Figure 1 - Optimum temperature evaluation of α-amylase (a) and amyloglucosidase (b) with and without inducer.

In the study of the optimal temperature of the alpha amylase (Figure 1a), the reactions were higher at lower temperatures, however, the activities with the presence of the inducer had a higher quantification from the temperature of 50 °C and at the temperature of 70 °C occurred the highest performance, of 258.80 U / g.

The activity of the amyloglucosidase (Figure 1b) with and without the presence of the inducer had a good response in relation to the enzymatic activities in the studied temperature range. In the tests with the presence of the inductor, the optimum temperature was 40 °C with an activity of 313.81 U / g. At temperatures above 40 °C the activities were smaller, but significant. In the tests without the presence of the inducer, the enzymatic activity with temperature of 30 °C was the most relevant with an activity of 230.41 U / g.

5. REFERENCES

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