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Studies on Amylase Activity of Pancreatin Obtained from Bovine Pancreas

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Abstract. The main objective of this study was to prepare pancreatin in liquid and powder form and to determine its amylase activity in crude homogenate of animal tissue. Different conditions were optimized for estimation of maximal activity including pH, temperature and substrate concentration. The optimum pH was found to be 6.8. The enzyme was optimally active at 50 °C. The effect of substrate concentration on enzyme activity was also studied and Km was found to be 0.5%.

Keywords: pancreatin, bovine pancreas, amylase activity

Introduction

Pancreatic α-amylase is an important enzyme needed for starch hydrolysis in the small intestine of both nonruminants and ruminants. Alpha amylase, an endoenzyme, hydrolyzes α-1,4-glucosidic bonds in polyglucosans. The location of the bonds in the molecule to be hydrolyzed is selected at random; each cleavage by α-amylase produces a reducing end (Walker and Harmon, 1996).

The enzymes of amylase family have great significance due to the wide area of their potential application. The spectrum of amylase application has widened in many other fields, such as clinical, medicinal and analytical chemistry. Interestingly, the first enzyme produced industrially in 1894 was an amylase from fungal source, which was used as pharmaceutical aid for treatment of digestive disorders (Pandey *et al*., 2000; Crueger and Crueger, 1989). Amylases constitute a class of industrial enzymes covering approximately 25% of the enzyme market (Sidhu *et al*., 1997). Amylase has also significant role in baking industry, brewing industry, papermaking industry etc. (Wojciechowsski *et al.*, 2001; Dauter *et al*., 1991; Fogarty and Kelly, 1980).

Pancreatin contains pancreatic enzymes that function in the digestion of starch, proteins and fats. It principally contains amylase, lipase and protease obtained from bovine pancreas. The main objective of this study was to prepare pancreatin in liquid and powder form. The extract was prepared from certified disease-free bovine pancreas without the addition of other animal enzymes or fermentation enzyme products. This preparation could be of value in the event of faulty digestion due to deficiency of pancreatic secretion and can be used in different formulations of medicinal products in pharmaceutical industry.

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The current study also deals with determination of amylase activity in crude homogenate of animal tissue and optimization of different conditions for estimation of maximal activity including pH, temperature and substrate concentration.

Materials and Methods

Extraction. Fresh bovine pancreas was purchased from local market. It was washed, cut into small pieces, kneaded thoroughly with water and the liquid was strained. Eighty grams of pancreas was placed in a flask of about 500 ml capacity and 200 ml of phosphate buffer (0.2 N, pH 6.8) was added. The mixture was kept for two h with mixing by rotating at frequent intervals and then was homogenized in 200 ml of the same buffer. After filtration of tissue homogenate, it was centrifuged at 6000 rpm in order to remove remaining residue. Extraction was repeated with another 50 ml phosphate buffer. This filtrate was prepared as enzyme source and amylase activity was determined by dinitrosalicylic acid reagent.

Drying techniques. Various drying techniques have been employed to concentrate extract from bovine pancreas like vacuum oven drying, freeze-drying. It was found that vacuum oven drying is time consuming than the freeze-drying method which is suitable as regards stability of the extract.

Enzyme assay. Amylase activity was assayed by the Bernfield method with slight modification. Assay was performed using starch as substrate. The reaction mixture, containing 1.5 ml substrate, 1 ml acetate buffer (pH 6.8), was preincubated at 37 °C for three min. Enzyme (1:1000 diluted) was added and then the tubes were incubated at 40 °C for 5 min. The reaction was stopped by addition of 0.5 ml NaOH. After addition of 1 ml dinitrosalicylic acid, the tubes were placed in boiling water bath for 5 min. After cooling to room temperature, 9 ml of distilled water was added and the absorbance was recorded

at 540 nm. The level of amylase activity was determined by measuring the reducing sugar released from soluble starch (Bernfield, 1951).

Amylase activity unit was expressed as the micromoles of maltose released per min under specified conditions of assay. Optimal conditions for the enzyme activity were studied at temperature, 20-70 °C, substrate concentration, 0.1% - 1.5% and pH, 4.0 - 8.5.

Results and Discussion

The amylase enzyme activity was studied in the crude extract of bovine pancreas and was found to be 9575 U/mg using modified method of Bernfield. Enzyme activity was found to be directly related to the substrate concentration (Fig. 1). There was gradual rise of activity with the increase in substrate concentration, up to 1% with no further increase in the activity. According to Fig. 2, Km was found to be 0.5%. A straight line is obtained when reciprocals of initial velocity are plotted against reciprocals of substrate concentration.

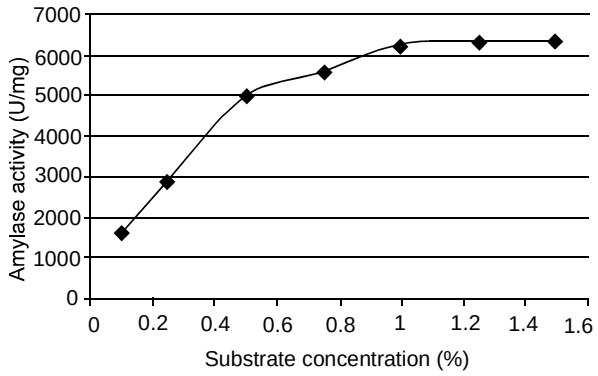

Fig. 1. Effect of substrate concentration on amylase activity of bovine pancreatin.

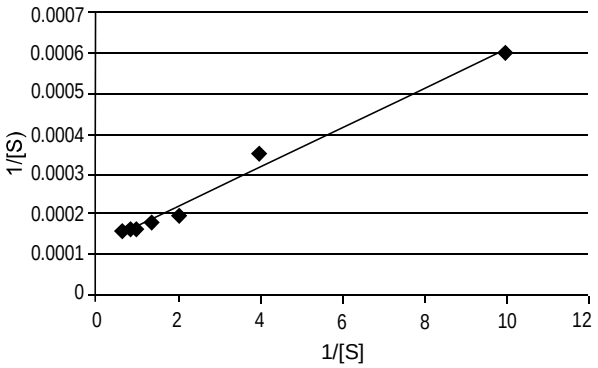

Fig. 2. Lineweaver -Burk plot showing Km value.

The effect of temperature on amylase activity was determined at different temperatures ranging from 20 to 70 °C. Enzyme activity gradually increased with increase in temperature. The maximum activity was achieved at 50 °C which may correspond to the native confirmation, after which there is sharp decline in activity with increase in temperature (Fig. 3). Comparable study showed that as the temperature is increased beyond the optimum temperature, the vibration energy of entire amylase molecule also increases. This puts a strain on the weak interactions that hold the enzyme together and change the native confirmation of enzyme so the activity lessens (Borgstrom *et al*., 1993). Another study explained the phenomenon of denaturation concluding that at higher temperature all hydrophobic and hydrophilic bonds are broken and the three dimensional structure of protein destabilizes (Yamasaki, 2003).

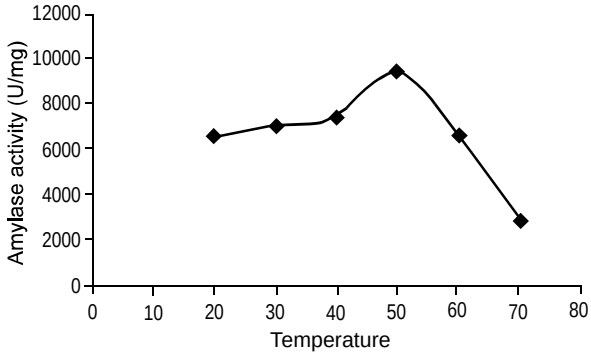

Fig. 3. Effect of temperature on amylase activity of bovine pancreatin.

Kinetic studies of amylase showed that enzyme is highly active in pH range of 6-7. The optimum pH was found to be 6.8 (Fig. 4). Suitable hydrogen concentration is attributed to the formation of enzyme substrate complex. The activity of enzyme falls on either side of the hydrogen ion concentration. The evidence shows that the range of pH of amylase varies from source to source (Teles *et al*., 2004).

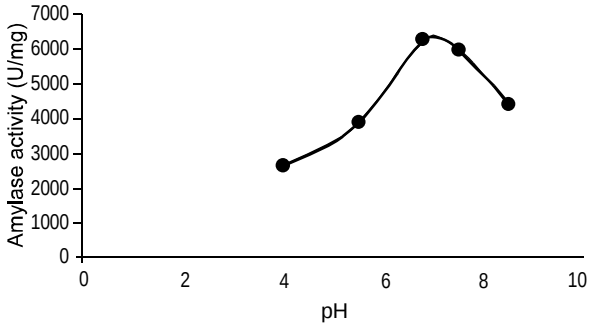

Fig. 4. Effect of pH on amylase activity of bovine pancreatin.

Extracted pancreatins have wide applications in foods, biotechnology and pharmaceutical industries. Use of the enzyme reported in this study may have several advantages over the existing state of the art, the most important being that it does not require such cost intensive filtrations to obtain microbe-free preparations, thereby making it economically feasible. Although bacterial and fungal amylases have already been used as detergent additives, their main drawback is that they require cost-intensive techniques to obtain microbe-free preparations (Phadtare *et al*., 1993). Moreover bovine pancreatin can be easily available for commercial use.

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