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Extraction and study of kinetic parameters of variety of sprouted pulses β-amylases

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Abstract

Various pulses are a good source of β-Amylase and this enzyme could find promising application of hydrolysis of starch. Hydrolysis of starch by β-amylases has couple of food and pharmaceutical applications. In the present work, β-Amylases from a variety of germinating pulses (*Vigna radiata*, *Cicer arietinum* (black), *Vigna mungo*, *Glycine max*, *Cicer arietinum*(white) were extracted and characterized for their optimum pH, temperature, time of incubation, substrate concentration, effect of $CaCl_2$ as well as their thermal stability. Various pulses were germinated and then extracted with Phosphate buffer (pH 7.0). The amylase assay was done by Dinitrosalicylic acid method (DNS). Optimum pH was found in the range of 5.5 to 8.5 and Thermal stability of pulses of β-Amylase was found up to 72ºC with optimum temperature in the range of 40ºC to 50ºC. As well as 6% of $CaCl_2$ was good activator to enhance the enzyme activity with optimum conditions.

Keywords: *Vigna radiata*; *Cicer arietinum* (black); *Vigna mungo*; *Glycine max*; *Cicer arietinum*(white); pulses β-amylases

Introduction

Legumes/pulsess are the most important plant food material and they are the concentrated cheap sources of protein for the vegetarian population. Initially the term amylase was used originally to designate enzymes capable of hydrolyzing α-1,4- glucosidic bonds of amylose, amylopectin, glycogen andtheir degradation products (Bernfeld, 1955; Fisher and Stein, 1960; Myrback and Neumuller, 1950). β-amylase (EC3.2.1.2) (alternative names: 1,4-α-D-glucanmaltohydrolase; glycogenase; saccharogen amylase) is also synthesized by bacteria, fungi, and plants. Working from the non-reducing end, β-amylase catalyzes the hydrolysis of the second α-1,4glycosidic bond, cleaving off two glucose units (maltose) at a time.

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During the ripening of fruit, β-amylase breaks starch into maltose, resulting in the sweet flavor of ripe fruit. γ-Amylase (EC3.2.1.3) (alternative names: Glucan 1,4-α-glucosidase; amyloglucosidase; Exo-1,4-α-glucosidase; glucoamylase; lysosomal α-glucosidase; 1,4-α-D-glucanglucohydrolase)will cleave α (1-6) glycosidic linkages, as well as the last α (1-4) glycosidic linkages at the non-reducing end of amylose and amylopectin, yielding glucose. β-Amylase (1,4-α-D-Glucan maltohydrolase; EC 3.2.1.2) plays a central role in the complete degradation of starch into fermentable sugars during the germination or malting of cereal grains (Okamoto, K. and H. Kitano, 1980). β-amylase, ubiquitous in nature, have been isolated, purified and characterized from a number of animal, plant, fungal, as well as bacterial sources. The β-Amylase is calcium metallo-enzymes, many times completely unable to function in the absence of calcium. Amylases are ubiquitous in nature and have been isolated, purified and characterized from a number of animal, plant, fungal as well as bacterial sources (Kumar *et al*, 2009). Starch depolymerization by amylases is the basis for several industrial processes such as preparation of glucose syrups, brewing and bread making. Cereal amylases play a very important role in the starch metabolism in developing as well as germinating cereals and have gained importance in supplementary foods, breweries and starch saccharification (Adewale *et al*, 2006). Pulses are

important sources of protein in the diets of millions of people in the world (Millward & Nutr., 2004). However their contribution to the nutrition of the consumer is limited, principally due to poor digestibility and anti nutritional factors (Davilla *et al*, 2003). Amylase is expressed in cotyledons of germinated Vigna mungo seeds and is responsible for the degradation of starch that is stored in the starch granule. Vigna mungo is an exalbuminous legume, in which the cotyledonary cells are filled with starch grains as the main energy storage component. During seedling growth, the level of amylase increases continuously more than 3 times throughout 6^{th} day (Koshiba T & Minamikawa T, 1983). Chickpea (*Cicer arietinum*) is the world's third most important pulse crop after common bean (*Phaseolus vulgaris*) and pea (*Pisum sativum*). In this present work, β- amylases were extracted from variety of sprouted pulses such as *Vigna radiata*, *Cicer arietinum* (black), *Vigna mungo*, *Glycine max*, *Cicer arietinum*(white) and their comparative characterization was done.

Material and Methods

Pulses

Variety of pulses such as *Vigna radiata*, *Cicer arietinum* (black), *Vigna mungo*, *Glycine max*, *Cicer arietinum*(white) were purchased from a local market in New Delhi, India. Grains were surface sterilized with 0.5% sodium hypochlorite solution washed several times and soaked in distilled water for 24-48 hours. Sprouting was carried out for 3 days at 25ºC in dark on petri-dishes layered with moistened filter paper.

Extraction of pulses β-Amylases

Cotyledons from 3-day old seedlings from germinating seeds were homogenized using mortar and pestle in 0.05 M sodium phosphate buffer (pH 7). 4-6 ml of buffer was added gm/weight of pulses sprouts in pestle mortar and homogenised well at 4°C. Extract was filtered through two layers of muslin cloth and centrifuged for 15 min at 4°C at 8000rpm. The supernatant was collected which contained enzyme and stored at 4°C.

Assay of β-Amylase

Assay of β-Amylase was done by dinitrosalicylic acid method (DNS) (Bernfeld, 1955). Soluble starch (1%) in 0.05 M sodium acetate buffer (pH 7.0) was incubated with appropriately diluted enzyme at 45ºC for 15 min. The reaction was stopped by the addition of 2ml of DNS reagent. One unit of enzyme activity was defined as µ-mole maltose equivalent released /min at optimum conditions. Amylase activity was measured spectrophotometrically at 570 nm. One unit of enzymatic activity is defined as the amount of enzyme that produces 1 µmol of maltose per minute (Robert R.B. & Evan R.K., 2003, Garen A. & Levinthal C., 1960).

Study of kinetic parameters of pulses β-amylases

pH optima

Pulses β-Amylases activities were estimated at different buffers of 0.05M having pH range from 2.5 to 10.5. The relative enzyme activity at different pH was determined by incubating enzyme extracts in different buffers for 15 minutes at 37º and the relative activities were measured under standard assay conditions by DNS method.

Optimum incubation time

The effect of Pulses β-Amylases activities were studied at different time ranging from 5min to 25min with an interval of 5 min. The relative enzyme activity at different time intervals was determined by incubating enzyme extracts different time period of incubation (5min to 25 min) for 15 minutes and the relative activities were measured under standard assay conditions by DNS method.

Optimum Temperature

Pulses β-Amylases activities were estimated at different temperature range of 20-70°C (with an interval of 10°C). The relative enzyme activity at different temperature was determined by incubating enzyme extracts in different 20- 70°C for 15 minutes and the relative activities were measured under standard assay conditions by DNS method.

Effect of substrate concentration

Pulses β-Amylases activities were estimated at different starch concentration (0.25% - 1.25%). The relative enzyme activity at different temperature was determined by incubating enzyme extracts at different concentrations in the range of 0.25% - 1.25% for 15 minutes and the relative activities were measured under standard assay conditions by DNS method.

Effect of $CaCl_2$ concentration

Pulses β-Amylases activities were estimated at different $CaCl_2$ concentrations (2%-8%). The relative enzyme activity at different temperature was determined by incubating enzyme extracts at different $CaCl_2$ concentrations (2%-8%) for 15 minutes and the relative activities were measured under standard assay conditions by DNS method.

Results and Discussion

The pH optimum for pulses β-Amylases activity was found to be stable in the pH range of 4.0-7.5 with maximum activity at pH 5.5 and in contrast, *Glycine max* β-Amylase had optimum pH 8.5 with optimum time of incubation in the range 15-25 minutes. These results were comparable to earlier reports (Saleh A. & Abdulrahman M., *et al*., 2009 and Mar *et al*., 2003)

who reported that the optima pH of different α-amylase of wheat have broad pH optima range from 5.0 to 7.0. Optimum temperature for pulses β-Amylases was found in the range of 40°C to 50°C. With the increase in temperature, enzyme activity was increased sharply with gradual increase in temperature up to 50ºC. However, 21% of decrease of enzyme activity was found with increase in temperature above 50ºC indicating loss in the active conformation of the enzyme. These results were agreed with previous reports (Chakraborty K. et al., 2000). Thus, the present study was showed maximal thermal stability at 72°C which was more than free enzyme (40°C) and pretty comparable to earlier reports (Junior AB., et al., 2009). For the study of effect of substrate concentration, 0.5 % of enzyme activity was progressively increased with the increase in starch concentration up to 1.2.5%. Our present study was also showed that at 6% $CaCl_2$ concentration leads to maximum increase in pulses β-Amylases activity. Amylases activity of Ca^{2+} ions enhancement of is based on its ability to interact with negatively charged amino acid residues such as aspartic and glutamic acid which resulted in stabilization as well as maintenance of enzyme conformation. In addition, calcium is known to have a role in substrate binding (Sprinz C., 1990).

Hence, pulses β-Amylase was extracted from variety of sprouted pulses [*Vigna radiata, Cicer arietinum* (black), *Vigna mungo, Glycine max, Cicer arietinum*(white)] and their kinetic parameters were studied such as pH optima, optimum temperature, effect of time of incubation, effect of substrate concentration and effect of $CaCl_2$ concentration. From the results of present study, we concluded that the pH optima for β-Amylases from variety of sprouted pulses was found to be 5.5 with the exception in case of *Glycine max* which had 8.5, thermal stability at 65ºC to 72ºC, time of incubation in the range of 15-25, optimum substrate concentration in the range of 1%-1.25% and optimum 6% $CaCl_2$ acted as activator for maximal pulses β-amylases activities.

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Table 1: Comparative kinetic parameters of varity of pulses β-amylases

Kinetic Parameters	***Vigna radiata***	***Cicer arietinum* (black)**	***Vigna mungo***	***Glycine max***	***Cicer arietinum* (white)**
pH	5.5	5.5	5.5	8.5	5.5
Temperature	40ºC	50 ºC	40 ºC	50 ºC	50 ºC
Time of Incubation	20 mins	20 mins	20 mins	15 mins	25 mins
Substrate concentration	1%	1%	1%	1%	1.25%