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Evaluation of *β*-amylase Activity of Sweet Potato (*Ipomoea batatas*) Cultivated in Iran

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ABSTRACT: Besides the high starch content, sweet potato contains endogenous amylases where the two Predominants are ∝- and β-amylases. Sweet potato is thought to be a promising source of β -amylase since β -amylase is one of the major protein in the tubers. This investigation has focused on the properties of β amylase from white-flesh sweet potato that has been grown in Iran as a potential source for industrial applications. Protein was determined using Bradford method and the enzyme activity was evaluated by monitoring of starch hydrolysis by determination of reducing sugar. The effect of pH and temperature on the enzyme activity and stability was determined. β amylase had optimum pH of 5.5 and was stable from pH of 3.5 to 7.5 with the maximum activity at 55°C.

Keywords: *Amylases, β-amylase Activity, Sweet Potato (Ipomoea batatas).*

Introduction

Amylases are enzymes that catalyse the hydrolysis of the 1→4-glycosidic linkages found in polysaccharides. Amylases are widely distributed in plant tissues, for example, in storage tissues such as seeds, nodes and tubers and in vegetative organs, such as leave (Dunn, 1974). The major starch hydrolysing enzyme is believed to be ∝-amylase but in leaves and stems, the amylolytic activity of β-amylase has been shown to be substantial (Dreier *et al*., 1995).

β-amylase was first discovered by Caldwell (1931) in the pancreas. It is an exoenzyme that releases successive maltose units from the non-reducing end of a polysaccharide.

∝ −amylase is probably the most widely distributed among the group and produced by many different types of bacteria, fungi, animals and some plants, while β-amylase is of plant origin and is abundant in sweet potato and in such as wheat and barley (Okon and Uwaifo, 1984).

Sweet potato (*Ipomoea batatas*) is an important food crop worldwide. The main constituents of sweet potato are carbohydrates in particular starch. Besides the high starch content, sweet potato contains ∝ and β-amylases. β-amylase constitutes about 5% of the total soluble protein of the tuberous root (Nakamura *et al*., 1991). The importance of sweet potato as a source of β-amylase has been documented by several workers (Chang *et al*., 1996; Cheong *et al*., 1995; Takahata *et al*., 1994; Jiang *et al*., 1994; Hagenimana *et al*., 1994a; Hagenimana *et al*., 1994b; Toda *et al*., 1993). β-amylase from sweet potato was the first amylase to be obtained in the

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crystalline form (Bernfeld, 1955). Unlike α-amylase, that is localised in the outer layers of the root, β-amylase is ubiquitously distributed throughout the root (Hagenimana *et al*., 1992). Cheong *et al*. (1995) reported that sweet potato β-amylase is a tetramer of identical subunits that are arranged to exhibit molecular symmetries. The tetrameric nature of sweet potato β-amylase uniquely distinguishes it from others that are monomeric (Nakamura *et al*., 1991). The subunit of the enzyme is a single polypeptide consisting of 498 amino acid residues similar to amino acid sequence from soyabean and barley. Different varieties of sweet potato have been shown to exhibit varying levels of β-amylase activities.

Amylases are among the most important enzymes used in several biotechnological applications particularly in starch processing industries to hydrolyse the polysaccharides such as starch into simpler sugars. This is the base for various industrial processes like preparation of glucose syrups. These enzymes are quite important and have applications in various industries (Prakash *et al*., 2011).

β -amylases from soybean, barley, and wheat have been used industrially. However, soybean β -amylase is relatively expensive and barley and wheat β -amylases are lacking in thermo stability. The sweet potato is thought to be a promising source of β -amylase since β -amylase is one of the major proteins in the tubers. Moreover, it has advantages as a crop in being resistant to unfavourable environments such as typhoons, drought, pests, and diseases.

In order to exploit the industrial potential of β –amylase, sweet potato was grown in Isfahan as a source of β-amylase and its activity was evaluated accordingly.

Materials and Methods

The roots of sweet potato were graciously provided by the Potato Research Biotechnology Department, University of Isfahan.

The fresh tuberous roots were washed, peeled and diced and were used just after the collection to avoid high ∝- amylase contamination, considering that ∝ -amylase tends to increase during storage. For β -amylase extraction, 50 g of the potato pieces were added to 100 mL of cold distilled water and pulverized. After centrifugation (7000 × g/20 min) the supernatant (amylase extract) was used for all the assays (Hagenimana *et al*., 1992; Morrison *et al*., 1993).

Protein concentration was determined by Bradford method (Bradford, 1976), using bovine serum albumin (BSA) as standard.

The enzymatic activity was determined using starch as the substrate (1% w/v in 100 mM citrate-phosphate buffer at pH 6.0). The hydrolysis of starch at 50℃ was monitored by determination of the reducing sugar using dinitrosalicylic acid method at 540 nm .A standard curve was prepared with maltose. One β-amylase activity unit (U) was defined as the amount of enzyme capable of producing 1 μMol of maltose per minute under assay conditions.

The enzymatic activity was determined as described above, using different pH values (citrate-phosphate buffer for pH 3.0–8.0 range and glycine –NaOH buffer for pH 9.0 and 10.0) and the effect of pH on the activity of enzyme was measured at 50°C.

The thermal and pH stabilities were determined as described below where the aliquots of the enzyme samples were incubated at different pH values for 30 min, at 25°C, and the remaining activity was assayed at pH of 6.0 for pH stability determination. When thermal stability was assayed, aliquots of the enzyme were incubated at pH of 6.0 and temperature of 60℃. Samples of these suspensions or solutions were withdrawn, placed in the ice baths for 30 sec and the remaining activities were assayed. For both studies the initial activity is regarded as 100% and the residual

activity was expressed as the percentage of the initial activity.

Experiments were performed in triplicate order and the values are the means of at least three independent experiments. Standard deviations were always under 10%.

Results and Discussion

The β-amylase from commonly grown sweet potato in Isfahan was assessed as a source of industrial enzyme. The result of the study indicated the presence of beta amylase activity in the sweet potato. The extracted enzyme approximately presented 3.2±0.5 mg protein mL^{-1}. Sweet potato protein concentration is highly dependent on the environmental conditions and cultural management practices. In over 300 lines of sweet potatoes grown in Taiwan under similar conditions within a single season indicated that the total protein varied from 1.27% to 10.07% on fresh weight basis with the majority falling between 4% to 5% (Li, 1974). One hundred seedlings from seven parental clones grown in America in a single location for a season ranged from 4.38% to 8.98% with the mean value of 6.29% (Dickey *et al*., 1984). Within cultivars, there are significant variations among the roots from the same plant as well as variations between the plants (Bradbury *et al*., 1985). Location within a field and field-to field variability also causes significant differences in protein concentration within cultivars. Environmental conditions, including climate, soil, incidence of pests and diseases which vary with location, season, and year, have a greater effect on some cultivars more than others. Total protein variation between and within cultivars showed significant effects of environment, genotype, and interaction between genotype and environment (Collins and Walter, 1982).

Cultural management techniques, including plant spacing, irrigation, fertilization, variations in planting and harvest time, also affect the total protein concentration.

The activity and the specific activity of the β-amylase in sweet potato were 55.69 μmole/min and 16.37 μmole/min/mg, respectively. Different varieties of sweet potato have been shown to exhibit varying degrees of β-amylase activity. According to Morrison, Pressey, and Kays (1993) the staple-type lines of sweet potato have higher levels of β-amylase synthesis than the traditional-type lines and the staple-type lines have shown β-amylase protein contents of 361 to 374μg/ g in fresh root while the traditional-type lines had levels in the range of 12–60 μg/g.

Takahata *et al*. (1994) reported that β-amylase activity ranged between 600 and 1300 μmol/min/g fresh weight for six sweet potato lines. Working with two varieties of sweet potato, Chang *et al*. (1996) showed that β-amylase isolated from different varieties of sweet potato had different specific activities and kinetic constants. Enhancing the β-amylase levels in sweet potatoes has potential cost efficiency advantages in glucose syrup production.

Sweet potato is reported to contain fairly high level of β-amylase activity (146 U ml-1) among the plants (Bernfeld *et al*., 1955). Enzymes and here β-amylase is a protein compound that its building blocks comprise principally of carbon and nitrogen (Lehninger, 1981). The higher the nitrogen and carbon contents of the soil implies higher capacity for the synthesis of proteins and consequently β-amylase (Dziedoave *et al*., 2010).

The effect of pH on the enzyme activity (Figure 1) and stability (Figure 2) indicates that the β-amylase of sweet potato is active in the pH range of 3.5 to 7.5. This suggests that the enzyme would be useful in processes that require wide range of pH changes. pH optimum and stability results indicate that the optimum pH was at pH of 5.5 for β-amylase activity. However, its activity dramatically is decreased by just

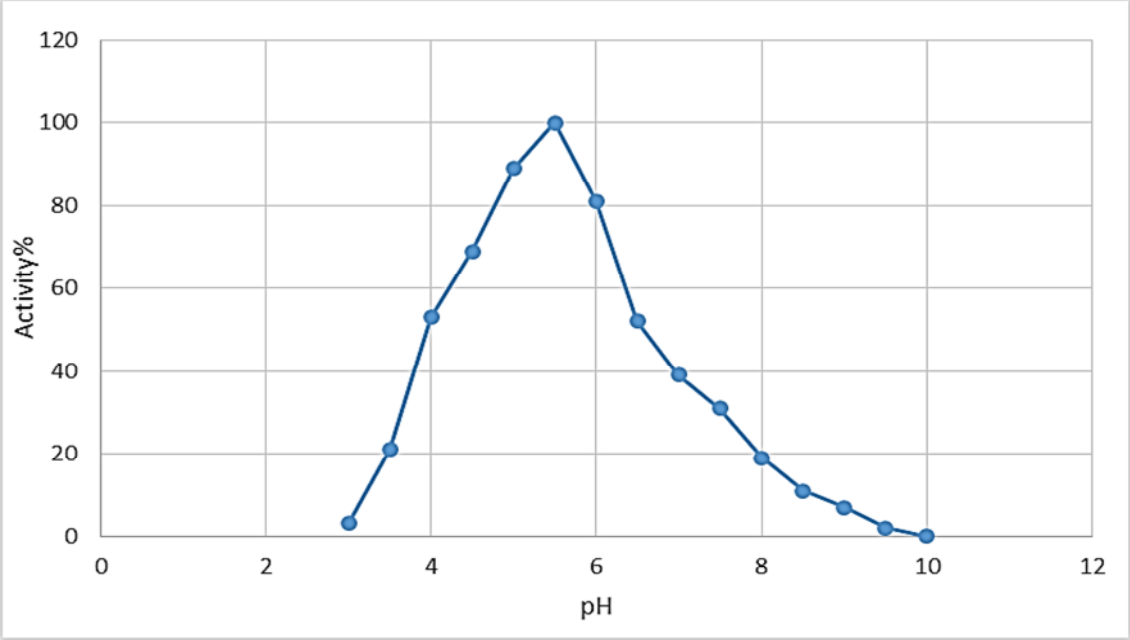

Fig. 1. The effect of pH on β-amylase activity

moving one pH unit away from the optimal pH value. The obtained results concerned that the pH optimum is in good agreement with the reports for β- amylase from other sources such as nodes of sugar cane (Oyefuga *et al*., 2011) and barley (Gessler and Birch, 1985). Yamamoto *et al*. (1988) also reported an optimum pH levels between 5 to 6 for β-amylase from *Bacillus subtilis.* However, it is worthy to note that the enzyme was active over a wide pH range. The enzyme cannot withstand the incubation at pH < 4.0 or pH > 7.5 for 30 minutes. This might be due to the dissociation of the enzyme (Tavano *et al*., 2013).

The effect of temperature on the activity and stability of the sweet potato β-amylase is shown in Figures 3 and 4. The results indicated a gradual increase in the enzyme activity at 20 – 55°C. The optimal temperature for the maximum activity of the sweet potato β-amylase (55°C) is similar to the Brazilian sweet potato β-amylase (Tavano *et al*., 2013). The optimal temperature reported for β-amylase from pea epicotyl (Lizotte *et al*., 1990) was 40°C but this result showed a better thermostable enzyme that has its optimum temperature at 55°C. The time needed to reach 50% of residual activity was about three hours. β-amylase retained only 14% of its initial activity after 12h at 60°C and was nearly inactive after 24h. Nakayama and Kono (1959) reported similar findings for sweet potato β-amylase. This temperature of inactivation of the enzyme might be attributed to the formation of incorrect conformation due to the processes such as hydrolysis of the peptide chain, destruction of amino acid and aggregation (Schokker and Van Boekel, 1999). Since this enzyme is thermostable, it could be a target for the production of β-amylase from a cheap plant source.

Conclusion

This study, has shown that sweet potato β-amylase that has been grown in Iran could serve as an alternative source of plant β-amylase with desirable technological properties. However in term of industrial purification, further studies concerned with selecting the high β-amylase content from different cultivars is recommended.

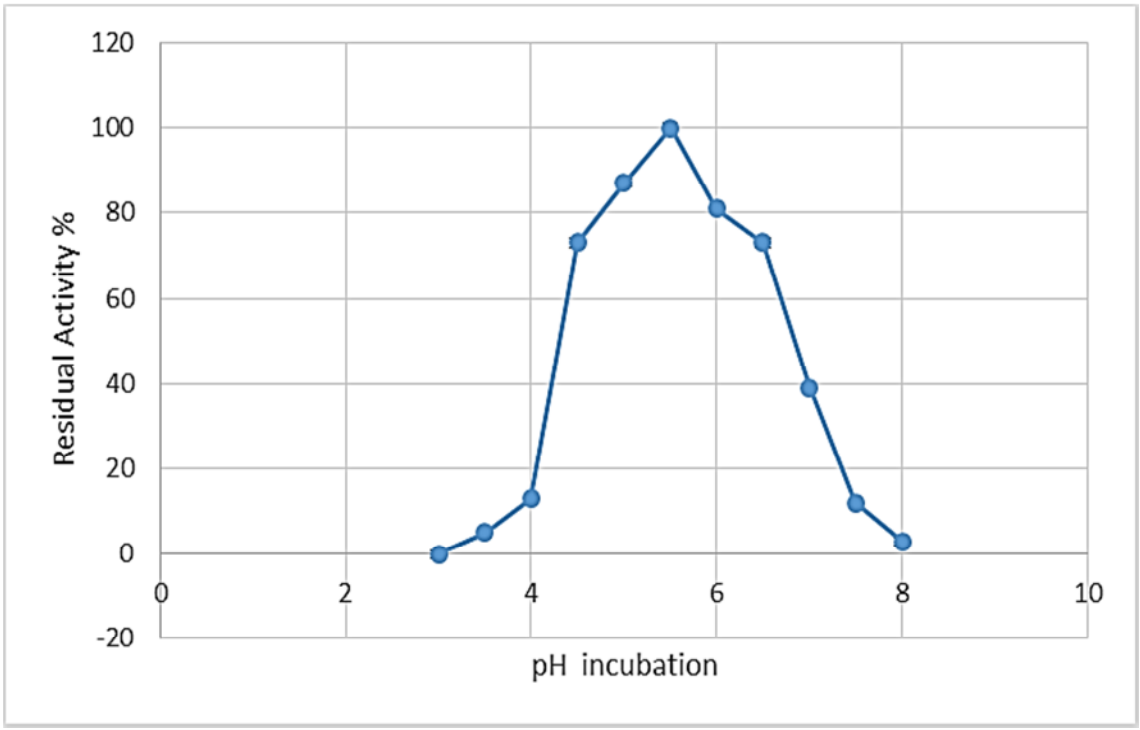

Fig. 2. The effect of pH on β-amylase stability

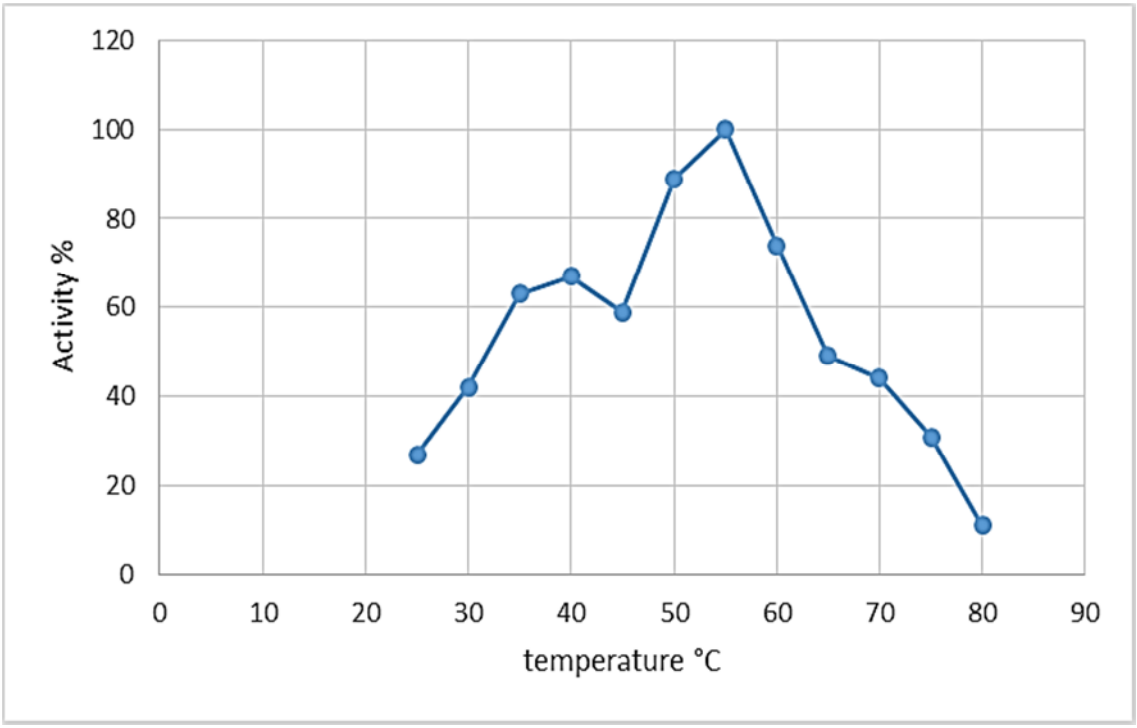

Fig. 3. The effect of temperature on the activity of β-amylase from white-flash sweet potato

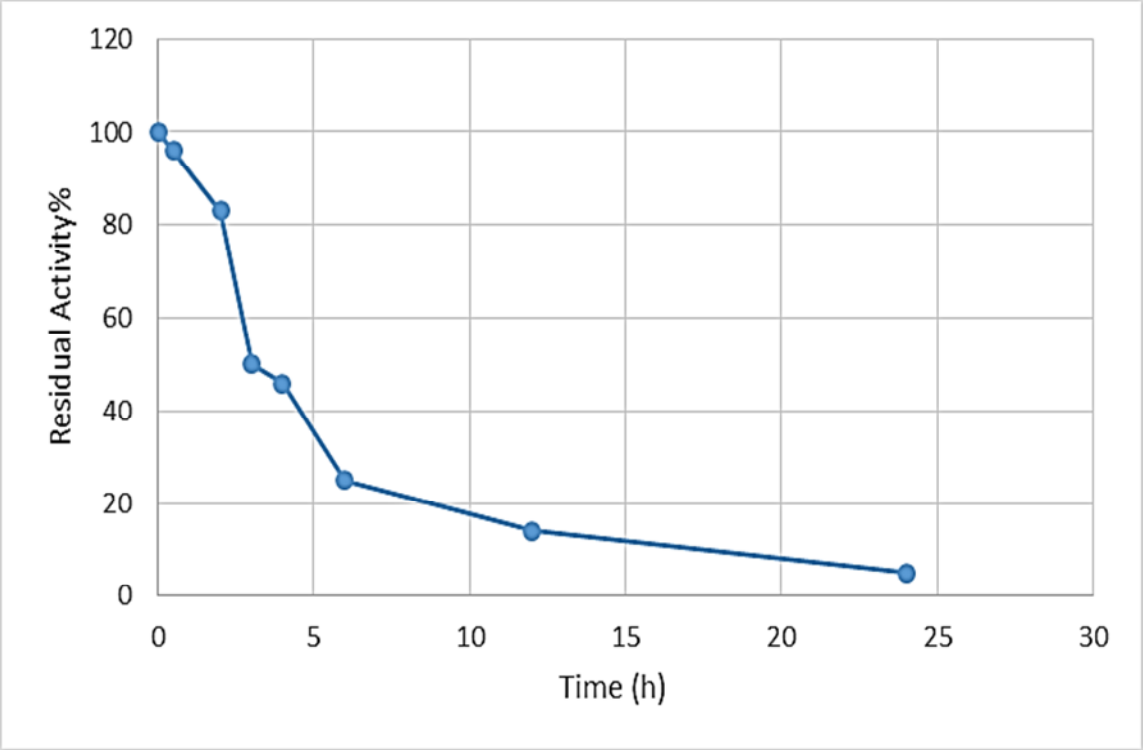

Fig. 4. Thermal inactivation of β-amylase at 60◦C and pH of 6.0.
Remaining activity was assayed at 50◦C (The initial activity is regarded as 100%).

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