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RESEARCH ARTICLE

Starch degrading α-amylases from different sources-A comparative study

B. Usha and K.P.J. Hemalatha*

Dept. of Biochemistry, Andhra University, Visakhapatnam 530 003, Andhra Pradesh, India.

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Corresponding Author

K.P.J. Hemalatha

Abstract

α-amylases have potential application in a wide number of industrial processes. They can be obtained from several sources, such as plants, animals and microorganisms. The results of the present study indicate that the amylases from different origin showed different pH and temperature optima. The microbial amylases were more susceptible to increased salt and sucrose concentrations. Ascorbic acid had minimum effect on microbial amylases where as the little millet amylase showed increase in activity. Sodium propionate inhibited the activity of the microbial amylases to the maximum extent.

INTRODUCTION

Bread is one of the most common, relatively low cost, traditional foods around the world. For decades enzymes such as malt and fungal alpha-amylase have been used in bread making. Due to the changes in the baking industry and the ever increasing demand for more natural products, enzymes have gained real importance in bread formulations. New and rapid advances in biotechnology have made a number of exciting new enzymes available to the baking industry. Alpha amylase (1, 4-α-D-glucan glucanohydrolase, EC 3.2.1.1) is an endo-enzyme that randomly hydrolyzes complex starches into simple sugars which are further fermented by yeast. A proper balance of naturally occurring amylase in wheat flour is desirable in order to produce bread that is properly fermented with richly colored crust and well developed flavor. Alpha amylases occur naturally in wheat flour but sometimes the endogenous activity is not sufficient to yield fermentable sugars, consequently, flours are supplemented with exogenous α-amylases. In flours with weak α-amylase activity, carbon dioxide development can be increased/accelerated by adding damaged starch or exogenous α-amylase (Wong, 1995). Commercial α-amylases can be obtained from fungal, cereal or bacterial sources.

Enzyme activity is greatly dependent on environmental conditions, i.e., medium conditions (pH, temperature, water activity and ionic strength) and the presence of different molecules that could modify their catalytic center (Rubenthaler *et al*., 1965; Pomeranz and Finney, 1975; Dragsdorf and Varriano-Marston, 1980). Furthermore, some differences are associated with their origin (Bird and Hopkins, 1954; Valjakka *et al*., 1994). Therefore, it is necessary to have a thorough knowledge of the enzyme properties for their efficient application. In the present study, the hydrolytic activity of α-amylases from different sources (fungal, little millet, bacterial) were tested under different conditions (pH and temperature). The effect of some common ingredients and additives used in bread making on different α-amylases was studied to envisage the behaviour of these enzymes during the bread making process.

Material and Methods

Commercial α-amylases which are produced from *Bacillus licheniformis* and *Aspergillus oryzae* (Product Code: A3404 and A6211 respectively) were purchased from Sigma Chemical Company, St. Louis, Missouri, U.S.A and α-amylase isolated and purified from germinating *Panicum sumatrense* (little millet) (Usha and Hemalatha, 2011) were used to compare the kinetic parameters such pH, temperature and the of effect of some ingredients and additives used in bread making on the α-amylase activity.

Enzyme assay

Amylase was assayed according to the procedure of Bernfeld (1955). Gelatinized soluble starch (1%, 1 ml) in sodium acetate buffer (50 mM, pH 5.0) was incubated with appropriately diluted enzyme at 45°C for 30 min. The reaction was stopped by adding DNS reagent (1 ml). One unit of enzyme activity was defined as μ mol maltose equivalent released/min under the assay conditions.

Effect of pH on α-amylase activities from different sources

Alpha amylase activities (50 U each) were determined at various pH values using buffers such as acetate and phosphate buffers (pH 3.0-8.0) 0.05 M concentration with 1% soluble starch as substrate. The relative activity at different pH values was calculated, taking the maximum activity obtained as 100%.

Effect of temperature on α-amylase activities from different sources

Alpha amylase activities (50 U each) were determined at temperature range of 30-80°C (with an interval of 10°C) with 1% soluble starch as substrate. The relative activity at different temperatures was calculated, taking the maximum activity obtained as 100%.

Effect of some ingredients and additives used in bread making on the α-amylase activities from different sources

The effect of sodium chloride (1-5 mM), sucrose (0.5-3.0 mM), ascorbic acid (0.2-1.0 mM) and sodium propionate (0.5-2.5 mM) on α-amylase activity was studied. Alpha amylase from various sources (50 U each) were preincubated for 30 min in 50 mM acetate buffer (pH 5.0) at 45°C, supplemented with salt, sugar, ascorbic acid and sodium propionate, testing several final concentrations in the range customarily utilized in bread formulation and assayed for α-amylase activity according to the procedure of Bernfeld (1955).

Results

Effect of pH on α-amylase activities from different sources

The effect of pH on the various α-amylase activities is shown in Figure-1. The pH affected the three α-amylases tested in different ways. The greatest activities were observed at pH higher than 4.0, below that a marked drop of activity was displayed. The fungal α-amylase showed optimum activity at pH 4.5 and remained constant at pH higher than 4.5. A different behavior was observed with α-amylases from little millet and bacterial source. The first showed a hyperbolic activity curve reaching a maximum at pH 5.0, whereas the bacterial α-amylase displayed a continuous increase of activity as the pH increased and exhibited maximum activity at pH 6.5.

Effect of temperature on α-amylase activities from different sources

The effect of temperature on the hydrolytic activities of various α-amylases are shown in Figure-2. As expected, the enzyme activity increased with the temperature, but the trend was different depending on the origin of the enzyme. The bacterial α-amylase showed the highest increase with the temperature. The temperature profile was studied between 30-80°C. Maximum activity was obtained at 40°C for fungal, 50°C for little millet amylase and 70°C for bacterial amylase. From the Figure-2, it is clear that at 80°C, over 90% activity was retained by the

bacterial α-amylase, while the little millet α-amylase retained 82% of activity at 60°C, but an increase in temperature to 70°C showed a sharp decrease in relative activity upto 50%.

Effect of NaCl on α-amylase activities from different sources

The addition of NaCl decreased the α-amylase activity in the range of salt tested (Figure-3). However, when the activities of α-amylases from different origins were compared, a diverse grade of inhibition was observed. The little millet α-amylase was less inhibited by salt than those from fungal or bacterial retaining 50% of its activity at 5 mM NaCl while at the same concentration, the bacterial α-amylase and fungal α-amylase exhibited enzyme activity of 40% and 25% respectively.

Effect of Sucrose on the α-amylase activities from different sources

The effect of the presence of sucrose in the reaction media on the α-amylase activity has also been analyzed (Figure-4). The α-amylase activity showed a continuous decrease with increasing concentrations of sucrose. However, little millet α-amylase was inhibited in lesser proportion than α-amylases from fungal or bacterial sources. The little millet α-amylase exhibited 70% activity at 3.0 mM sucrose; whereas fungal α-amylase retained 38% of its activity at the same concentration. Bacterial α-amylase was drastically inhibited by sucrose, as less than 20% of its activity remained at 1.0 mM sucrose.

Effect of Ascorbic acid on α-amylase activities from different sources

The presence of growing concentrations of ascorbic acid hardly affected the α-amylase activity, with the exception of the little millet α-amylase, which showed a marked increase of its activity as the ascorbic acid concentration was increased (Figure-5).

Effect of Sodium propionate on α-amylase activities from different sources

The effect of various concentrations of sodium propionate on α-amylase activity is shown in Figure-6. An inhibitory effect of the enzyme activity should be expected because the salts of propionic acid are used as anti-microbial agents, it is desirable to check how these salts affect α-amylases from various sources. The addition of sodium propionate inhibited the α-amylase activity but to differing extents depending on the enzyme origin. The little millet α-amylase was the most stable, retaining more than 48% of its activity at 2.0 mM sodium propionate. A drastic inhibition by this salt was observed in case of the other α-amylases, with the bacterial α-amylase exhibiting 23% activity and fungal α-amylase being the most affected retaining only 12% of its activity at 2.0 mM sodium propionate.

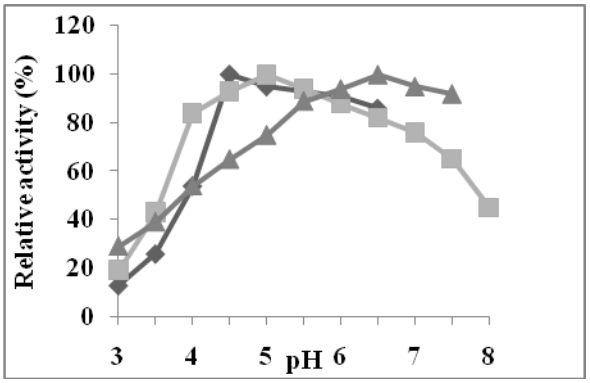

Fig: 1 Effect of pH on the (♦) fungal, (■) little millet and (▲) bacterial α-amylase activity.

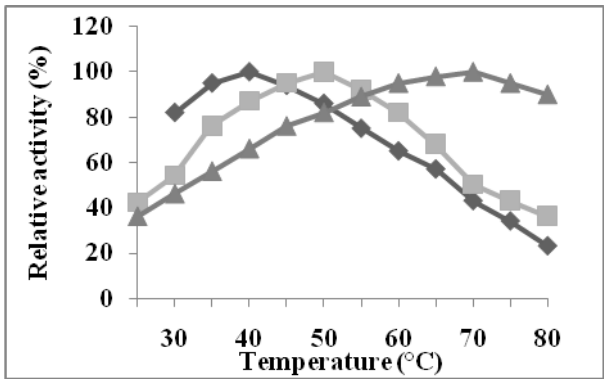

Fig:2 Effect of temperature on the (♦) fungal, (■) little millet and (▲) bacterial α-amylase activity.

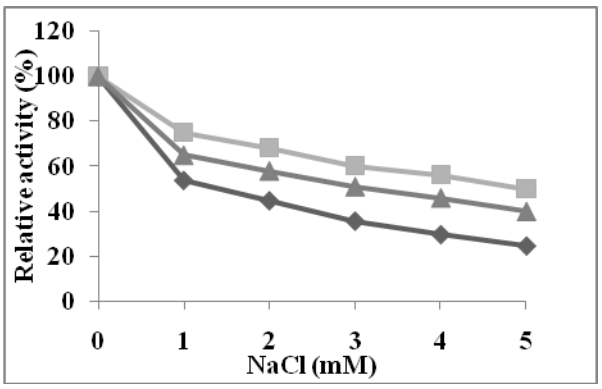

Fig: 3 Effect of varying NaCl conc. on the (♦) fungal, (■) little millet and (▲) bacterial α-amylase activity.

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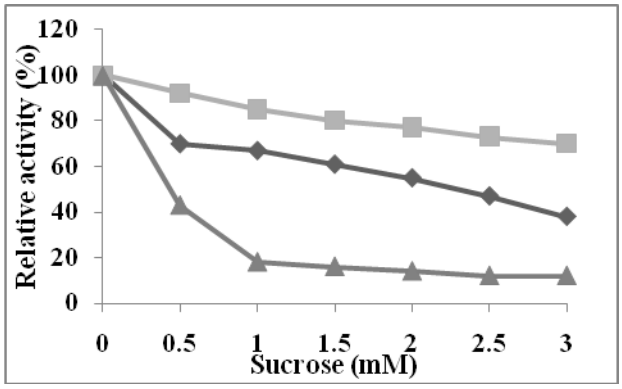

Fig: 4 Effect of varying conc. of sucrose on the (♦)fungal, (■) little millet and (▲) bacterial α-amylase activity

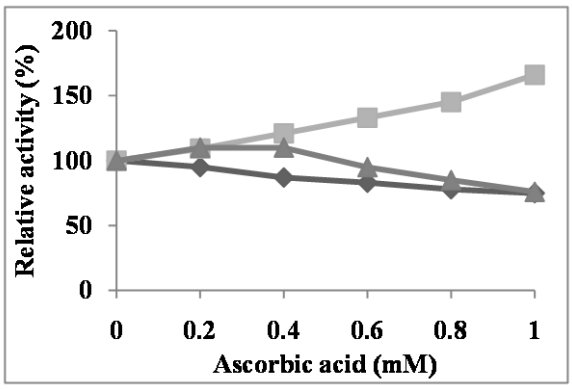

Fig: 5 Effect of varying ascorbic acid conc. on the (♦) fungal, (■) little millet and (▲) bacterial α-amylase activity.

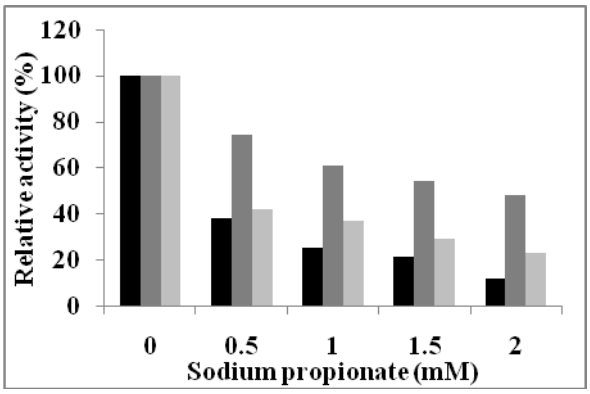

Fig: 6 Effect of varying sodium propionate conc. on the (♦) fungal, (■) little millet and (▲) bacterial α-amylase activity.

Discussion

Amylases find application in many of the industrial processes such as in food, detergents, textiles, paper industry and for the hydrolysis of starch (Gupta *et al*., 2003; Konsoula and Kyriakides, 2004; Tanyildizi *et al*., 2005). Alpha amylases used in baking are cereal enzymes from barley malt and microbial enzymes from both fungi and bacteria (Hebeda *et al*., 1990, 1991). Amylases from plant and microbial sources have been employed for centuries as food additives (Pandey *et al*., 2000; Adewale *et al*., 2006; Adewale and Oladejo, 2009). Alpha amylases used in starch industry must be active and stable at low pH, but stability at high pH values are required in the detergent industry (Paula and Perola, 2010). Most bacterial α-amylases are optimally active at slightly acidic to near neutral pH (Collins *et al*., 1993; Freer, 1993; Morgan and Priest, 1981). In the present investigation, the little millet α-amylase was active at an optimum pH 5.0 while bacterial α-amylase was active at pH 6.0 and fungal α-amylase at pH 4.5. The results show that these enzymes are active in pH range suitable for starch industry.

Fungal alpha-amylase is inactivated in 2-3 minutes at 65-75ºC. Cereal alpha amylases are slightly more thermo-stable and remain active during early stages of starch gelatinization. Bacterial amylases have even higher thermal stability and survive the baking temperatures (Dragsdorf and Varriano, 1980). This causes extensive starch hydrolysis during baking and results in production of sticky and gummy bread crumb (Olesen, 1991). Hence the

amount of α-amylases particularly those from bacterial sources added to bread formulations must be controlled carefully. A recent trend is to use intermediate temperature stable (ITS) α-amylases (Hebeda *et al*., 1990, 1991; Kulp, 1993; Ahuja, 1998). Olesen (1991) found that this feature renders the enzyme to be useful for baking industry through avoiding stickiness in bread. In the present study, the temperature profile was studied between the temperatures 30-80°C. Maximum activity was obtained at 50°C for little millet α-amylase. The enzyme retained 82% of activity at 60°C, but an increase in temperature to 70°C showed a decreased activity. However, the bacterial α-amylase was most thermostable and showed maximum activity at 70°C and fungal amylase was the most thermolabile.

Salt has some technological functions, such as to increase dough stability, firmness and ability to retain fermentation gases. Salt has a precise effect on fermentation: the higher the concentration of salt, the lower is the rate of fermentation with the same yeast level (Asghar *et al*., 2006). In the present study, the addition of salt promoted a decrease of the α-amylase activity in the range of salt assayed. Salt dropped off the activity of flour α-amylase as reported by Harinder and Bains (1987) and Hanee *et al*. (2009). The little millet α-amylase was less inhibited by salt than those from fungal or bacterial. Cristina *et al*. (2001) reported similar results for endogenous wheat amylase and malt amylase. In consequence, it is suggested that when doughs were supplemented with fungal or bacterial α-amylases this inhibition should be considered in order to adjust the enzyme dosages.

Sucrose used in baking processes provides sweet taste, furnishes fermentable sugars to yeast, confers colour to bread crust, and improves the texture. Results from the present study, show that the little millet α-amylase was inhibited in lesser proportion than α-amylases from fungi or bacteria in the presence of sucrose. This behavior was also envisaged by Adams (1953), who reported the inhibition promoted by the addition of growing concentrations of sucrose on the *Aspergillus oryzae* α-amylase. Cristina *et al*. (2001) reported bacterial α-amylase was drastically inhibited by sucrose.

Ascorbic acid is used to develop the structure and bread volume of the dough and to enhance dough strength. It has been widely used as an oxidant in the baking goods (Asghar *et al*., 2006; Grant and Sood, 1980). Vitamin C is able to modify the pH of the reaction medium to an optimum pH for wheat amylase activity (Alina *et al*., 2005). In the present investigation, it was seen that ascorbic acid hardly affected the activity of bacterial and fungal α-amylase. However, there was a pronounced increase in little millet α-amylase activity. Alina *et al*. (2005) reported vitamin C as a strong activator of wheat flour α-amylase. Similar results were reported by Cristina *et al*. (2001) and Hanee *et al*. (2009) for flour α-amylase.

Commonly used additives in baking include anti-microbial agents such as different salts of propionic acid. Sodium propionate prevents mold growth in bread and baked goods. In the present investigation, sodium propionate showed an inhibitory effect on the enzyme activity depending on the enzyme origin. Fungal α-amylases were the most susceptible followed by bacterial α-amylases. Similar results were reported by Cristina *et al*. (2001).

Conclusion

Enzyme activities, in addition to the differences associated with their origins, are strongly affected by the process conditions and the presence of other compounds in the medium. Therefore α-amylases with characteristics suitable for the industrially relevant process conditions and applications have to be appropriately selected as per the demand.

Acknowledgements

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