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Hydrolysis of Cereal Starches Using Purified Little Millet α-Amylase

Biochemistry

KEYWORDS : little millet, α-amylase, cereal starches, gelatinized, hydroysis.

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ABSTRACT

The conversion of starch to value-added products by enzyme-catalyzed reactions represents the largest industrial use of enzyme molecules. The results in the present investigation show that little millet α-amylase has the ability to digest raw starch as well as pregelatinized starch. A very promising characteristic of this enzyme is that even at a very low concentration of starch, production of maltose was maximum reducing the demand of higher substrate volume. Starch degradation was greatly affected by temperature.

INTRODUCTION

Starch is a storage polysaccharide present in seeds, tubers and leaves of various plants. In nature, starch occurs in the form of inert granules arranged in a polycrystalline state that differ in size and structure depending on plant species (Singh *et al.*, 2003). The shape and size of starch granules varies (Tester and Karkalas, 2002; Tester *et al.*, 2004 a) with plant source. The morphology and the surface of the granule, the amylose content, the crystalline structure or the presence of amylose-lipid complexes were shown to be limiting factors for hydrolysis of the starch granule. The rate and extent of α-amylase hydrolysis of starches from different plant sources has been quantified by Ring *et al.* (1988) and Fukai *et al.* (1994).

The relative efficiency of many cereal α-amylases with respect to the various starch degradation is essential as this information will be very useful for the appropriate use of different cereal malts in the brewing industry as a source of α-amylases as well as starches which can be used as adjuncts. In this study, experiments were carried out to check the ability of little millet α-amylase to hydrolyze several cereal starches.

MATERIALS AND METHODS

Hydrolysis of non-gelatinized starches

Non-gelatinized cereal starches (5 mg, 0.5% w/v) in sodium acetate buffer (50 mM, pH 5.0) were used for hydrolysis. Purified enzyme 10 U was added to it, mixed thoroughly and was incubated at 45°C for 60 min. Maltose produced after enzymatic breakdown was measured by Bernfeld method (1955).

Hydrolysis of gelatinized starches

Cereal starches (5 mg/1 ml) suspended in boiling water bath (96°C) sodium acetate buffer (50 mM, pH 5.0) were gelatinized at boiling temperature, cooled to 45°C and incubated with amylase (10 U each) (Nirmala and Muralikrishna, 2003). Maltose produced after enzymatic breakdown was measured by Bernfeld method (1955).

Effect of substrate concentration on hydrolysis of gelatinized starches

Different substrates at concentrations (1-8 mg/ml) were mixed separately with enzyme 10 U each and incubated maintaining optimum incubation time for different starchy materials from the previous experiment and maltose produced was measured by Bernfeld method (1955).

Effect of temperature on hydrolysis of gelatinized starches

Fixed concentration of different substrates (5 mg/1 ml) were mixed separately with enzyme 10 U and incubated maintaining optimum incubation time for different starchy materials at varying temperatures and maltose produced was measured by Bernfeld method (1955).

RESULTS

Panicum sumatrense α-amylase purified according to Usha and Hemalatha [18] was used to hydrolyse various cereal starches.

Hydrolysis of non-gelatinized and gelatinized starches

The little millet α-amylase can act on both gelatinized and non-gelatinized form (raw starch). Table-1 shows that the effect of gelatinization on the extent of hydrolysis achieved depended on the type of starch. The susceptibility of wheat, sama and barley starches to amylolytic enzyme increased on gelatinization.

Table-1
Hydrolysis of non-gelatinized and gelatinized starches

Starch type	Maltose produced (mg/ml)	
	Non-gelatinized	Gelatinized
Barley	4.89 ± 0.32	8.39 ± 0.78*
Corn	3.12 ± 0.25	3.65 ± 0.32
Sama	6.06 ± 0.44	10.02 ± 1.10*
Rice	5.44 ± 0.40	6.87 ± 0.62
Wheat	6.67 ± 0.53	12.45 ± 1.32*

Mean ± SD (N=6)

* $p < 0.05$ considered as significant difference.

The amount of maltose liberated from gelatinized rice and corn starch did not differ significantly from that released from non-gelatinized starches. These results indicate either that these starches are retrograded after gelatinization or that other factors contribute to incomplete digestion of these starches. Among the various cereal starches tested, the amount of maltose liberated was maximum for wheat in both non-gelatinized (6.67 mg of maltose) and gelatinized form (12.45 mg of maltose). The results indicate that there is a significant increase ($p < 0.05$) in maltose production about 86% on gelatinization of wheat starch. Similarly, in gelatinized condition hydrolysis of barley and sama starches showed about 71 and 65% increased production of maltose (significant, $p < 0.05$) among the substrates tested. While hydrolysis of corn and rice starches showed an increase in maltose production about 16% and 26% respectively which is statistically not significant.

Effect of substrate concentration on hydrolysis of gelatinized starches

Concentration of different substrates were varied and subjected to degradation with little millet α-amylase (Figure-2). Wheat starch (5 mg/ml) increased the production of maltose steadily and reached maximum production (12.45 mg). At the same concentration of sama starch (5 mg/ml), 10.02 mg of maltose was produced. Barley starch (5 mg/ml) produced 9.35 mg of maltose. Higher concentration of substrate decreased saccharification rate. Corn starch showed very little production of maltose (3.65 mg) at 5 mg/ml concentration. Rice starch showed highest production of maltose at 6 mg/ml concentration.

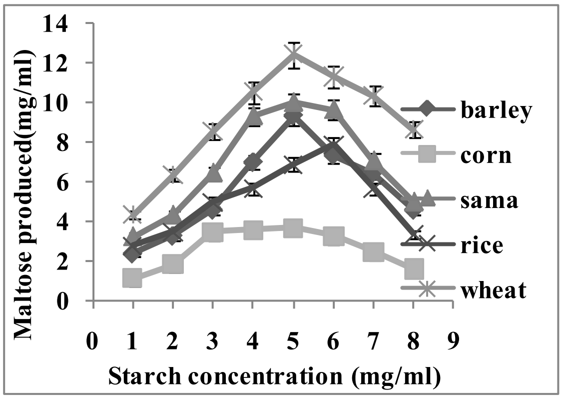

Fig: 1 Effect of substrate concentration on hydrolysis of gelatinized starches

Effect of temperature on hydrolysis of gelatinized starches

Starches at a concentration of 5 mg/ml were subjected to hydrolysis with little millet α-amylase at different temperatures. As it can be seen in Figure-2, while all substrates exhibited better reaction rates upon increase of temperature to 50°C, the effect of temperature on the hydrolysis was more significant in the case of wheat and sama starches with an increase in maltose liberated by 40 and 35% respectively. The amount of maltose produced from wheat, sama, barley, rice and corn starches at 50°C was about 1.4, 1.35, 1.3, 1.25 and 1.14 times higher respectively, compared to the maltose produced at 45°C.

The starch degrading ability of enzyme decreased on increase of temperature to 60°C exhibiting a loss of 18% for wheat starch. Significant loss of activity at 60°C was shown for corn starch with a decline of 59% activity and 48% for rice starch. Further increase in temperature to 70°C showed a sharp decrease in activity showing a maximum loss of 75% activity for corn starch, followed by rice starch which showed a loss of 70% activity. The enzyme retained 53% activity with wheat starch at 70°C.

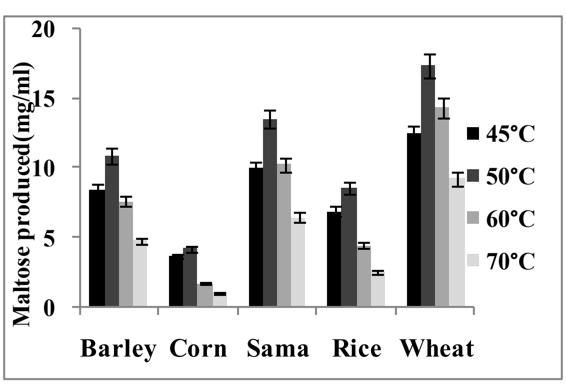

Fig:2 Effect of temperature on hydrolysis of gelatinized starches

DISCUSSION

Enzymatic saccharification of raw starches without thermal gelatinization saves energy and reduces cost of starch processing (Shiau *et al.*, 2003). However, due to densely packed polycrystalline state with inter and intramolecular bonds, raw starch is resistant to enzymatic attack as evidenced from lower productivity than on gelatinized starches (Hamilton *et al.*, 1999).

When starch is gelatinized the semi-crystalline nature of granules becomes totally amorphous and the starch becomes digestible by amylases (Dettori-Campus *et al.*, 1992; Tester and Sommerville, 2000; Tester and Karkalas, 2002; Konsoula and Kyriakides, 2004; Tester *et al.*, 2004 a, b). The α-amylase can progressively digest starch granules more and more as they gelatinize (Tester and Sommerville, 2000) although this is dependent on the moisture content available to support the gelatinization process and the temperature profile the starch is exposed to (Tester and Sommerville, 2000; Slaughter *et al.*, 2001 a, b).

In the present study, hydrolysis of various starches using purified little millet α-amylase was investigated. Gelatinization of wheat, sama and barley starches increased their susceptibility to little millet α-amylase degradation in comparison to their native form. Literature reports indicate very little hydrolysis of native starch granules when subjected to hydrolysis by α-amylase isolated from pearl millet (Beleia and Marston, 1981), wheat and barley (MacGregor, 1983) and cereal/ragi (Nirmala and Muralikrishna, 2003 c).

The extent of substrate degradation was greatly affected by temperature. The increase in reducing power could be ascribed either to enhancement of the activity of the thermostable α-amylase at higher temperatures or to swelling of the amorphous regions of starch granules. The little millet α-amylase exhibited maximum hydrolytic activity on increase of temperature to 50°C. A decline in enzyme activity was observed on further increase in temperature to 70°C. This may cause limitation for the usage of the enzyme in the industry which require highly thermostable enzymes but may be favorable for use in the baking industry that requires complete inactivation of the enzyme at high temperatures (Vihinen and Mantsala, 1989).

CONCLUSIONS

The purified little millet amylase may not be applied to hydrolyze, starches which gelatinize at high temperatures. However, there may be applications for mashes or starches obtained from starch sources that have low gelatinization temperatures. Moreover, the little millet α-amylase could be used for hydrolysis of starches/mashes if the gelatinized starch is cooled down to the optimum temperature of activity of this amylase.

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