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Comparative studies on α-amylases from malted maize (*Zea mays*), millet (*Eleusine coracana*) and Sorghum (*Sorghum bicolor*)

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Abstract

Starch-digesting enzymes were extracted from unmalted, malted, malted and kilned samples of maize (*Zea mays*), millet (*Eleusine coracana*) and sorghum (*Sorghum bicolor*) and the specific activities were determined with soluble starch as substrate. The extract of malted and kilned samples was partially purified by using ammonium sulphate fractionation and the kinetic parameters of the α-amylases were determined using starch as substrate. The heat stability of the enzymes at different temperatures was also established. The results showed that all the malted grains expressed α-amylases in appreciable quantity. Sorghum had the lowest drop in α-amylases activity after kilning. Sorghum α-amylases also had the lowest K_m for starch, and maize, the highest. Amylases from maize were least stable to heat denaturation at all temperatures investigated, while sorghum and millet α-amylases had similar sensitivity to heat inactivation, although sorghum amylases were slightly more resistant. The overall summary showed that α-amylases from maize were least stable to heat denaturation, and also had the lowest affinity for soluble starch. A combination of these factors might have influenced the choice of sorghum grains over maize and millet for commercial malt production.

Keywords: Malt; Starch-digesting enzymes; α-Amylases; Maize; Sorghum; Millet; Stability

1. Introduction

The use of maize and sorghum as brewing adjuncts, in developing countries, is well known (Eneje et al., 2004). Maize is also used in the manufacture of fuel alcohol. The conversion of maize or sorghum starch to alcohol is usually accomplished by the application of exogeneous enzymes, e.g., bacteria α-amylase and fungal amyloglucosidase, to convert the stored starch into monosaccharides, after which it is fermented with yeast to produce ethanol. Malting of grains or cereals could also be a major source of hydrolytic enzymes required for the conversion of starch to simple sugars.

Because of the increasing cost of these exogenous enzymes, breweries in developing countries like Nigeria are increasingly turning to the use of malted sorghum as a substitute for the more expensive malted barley, whose importation and usage has been restricted to encourage the use of local grains for production of beer and other beverages (Bajomo & Young, 1993; Diana & Serna Saldivar, 2005). Often, local maltsters malt sorghum grains in preference to other locally available grains. Specifically, the use of malted maize for brewing is generally not widespread (Eneje et al., 2004), so also is malting of millet. The reason why maize which, has a similar chemical composition to sorghum and is more abundant in many parts of the world, is not popular among the commercial maltsters has not been established, nor is the comparative studies of the α-amylases from these three cereals.

Cereals contain both α and β-amylases although α-amylases account for about 30% of the total protein synthesized during germination (Muralikrishna & Nirmala, 2005). The two amylases can be distinguished from each other. α-Amylases are inactivated at pH around 4.8–5.0, however, at

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this pH range, β-amylases are stable. β-Amylases are inactivated at pH around 6.0–7.0, whereas at this range, α-amylases are stable (Muralikrishna & Nirmala, 2005). α-Amylase is a key enzyme that catalyzes the endo cleavage of α-1,4-glycosidic linkages in reserve starch and releases short oligosaccharides and α-limit dextrins (Moraes, Filho, & Ulhoa, 1999).

The aim of this work is therefore to undertake a comparative study of some properties of α-amylases in these grains, with a view to gain some insight into the suitability of each of these grains for commercial malt production.

2. Materials and methods

2.1. Materials

All the chemicals used were of analytical grade and were purchased from Sigma Chemical Company, St. Louis, MO, USA or BDH Chemical Limited, Poole, England. Soluble starch (from potato) was purchased from Sigma.

The grains were purchased from a local market in Ile-Ife. They were properly identified by Dr. (Mrs) A. Adedeji, of the department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

3. Methods

3.1. Extraction of starch-digesting enzymes from unmalted grains

Total amylases were extracted with 50 mM sodium phosphate buffer, pH 6.0, according to the method of Nirmala and Muralikrishna (2003). Briefly, about 200 g of each grain sample which had been screened to remove broken grains and foreign materials was ground with pestle and mortal, to a fine powder, and then homogenized in a warring blendor in 400 ml of 50 mM phosphate buffer, pH 6.0. The 30% homogenate was centrifuged at 6000 rpm for 30 min to remove the cellular debris, and the supernatant was collected.

3.2. Extraction of starch-digesting enzymes from malted, malted and kilned grain samples

About 200 g of each sample which had been screened was steeped in distilled water for 48 h. The water was changed at 6 h interval to prevent microbial growth that of millet was steeped for 24 h. After steeping, the samples were removed and blotted to remove excess water. They were then spread out in a malting chamber for germination at room temperature for 72 h. The grain samples were divided into two equal parts. One part was homogenized in 200 ml of 50 mM phosphate buffer, pH 6.0 and the homogenate was treated as earlier described for the unmalted extract. The remaining part was immediately placed in a hot air oven which had been preset at 55 °C and allowed to stay for 24 h. The kilned malt was de-rooted by hand and thereafter homogenized in 200 ml of 50 mM phosphate buffer, pH 6.0 and the supernatant was collected after centrifugation. The clear supernatant obtained from the kilned malted samples was subjected to fractional ammonium sulphate precipitation, up to 70%, which precipitated all the starch-digesting enzymes. The precipitate was collected and dialyzed against 50 mM Tris–HCl buffer, pH 7, containing 5 mM $CaCl_2$ and 1 mM EDTA, with several changes of the buffer. The resultant supernatant was centrifuged to remove precipitated proteins and was used for further characterization.

3.3. Enzyme assay

For the determination of α-amylase activity, an aliquot of the enzyme was incubated at room temperature with 1% soluble starch in 10 mM phosphate buffer, pH 7.0, for 10 min. The final concentration of the soluble starch in the incubation mixture was 0.5%. Boiled enzyme solution was used for the blank control. The amount of reducing sugar liberated from the soluble starch was then determined by Somogyi–Nelson method (Sugita, Kawasaki, Kumazawa, & Deguchi, 1996). One unit of the amylase activity was defined as the amount of enzyme which liberated reducing sugar equivalent to 1 μg of D-glucose per minute from soluble starch at 25 °C.

3.4. Protein concentration determination

The method of Lowry, Rosebrough, Farr, and Rondall (1951) was used to determine the protein content in the samples. Bovine serum albumin was used as the standard protein.

3.5. Determination of kinetic parameters

For the determination of kinetic parameters, an aliquot of the starch-digesting enzymes was incubated for 10 min with soluble starch, dissolved in 10 mM phosphate buffer, pH 7.0, whose concentration was varied from 0.1% to 1%, while other components were kept constant. The amount of reducing sugar formed was then estimated from where the units of activity were calculated. The data obtained was analyzed by double-reciprocal plot.

3.6. Thermal stability studies

Partially purified amylases in 50 mM phosphate buffer, pH 6.0, were incubated at a temperature range of between 30 and 100 °C. Aliquots were taken at 10 min interval and assayed for residual activity, taken that of the unincubated control as 100%. The percentage residual activity remaining was then plotted against time for different temperatures.

4. Results and discussion

4.1. Amylase induction

After steeping, starch-digesting enzymes were induced considerably in all the three grain samples. It should be noted that in the conditions chosen for the extraction and assay, almost all other amylases, except α-amylases would have been inactivated. The activity of α-amylases in unmalted samples was negligible. Malted sorghum α-amylases had the highest specific activity of 270 U/mg protein. The specific activity of malted millet and maize amylases were 133 and 52 U/mg protein respectively. After kilning at 55 °C for 24 h, specific activity of amylases in malted sorghum decreased by about 27% while the decrease in specific activity for maize and millet α-amylases was 44% and 30%, respectively.

4.2. Kinetic parameters

The values of the kinetic parameters obtained for each of the α-amylases from malted grain samples are shown in Table 1. A typical double reciprocal plot for the determination of the kinetic parameter is shown in Fig. 1. Sorghum α-amylases had the lowest K_m for starch (1.82 mg/ml) while maize α-amylases had a value about 7-fold higher. K_m of millet α-amylases was 3-fold higher than that of sorghum α-amylases. This result suggests that among the three amylases compared, sorghum α-amylases had the highest affinity for soluble starch whereas maize α-amylases had the least.

Table 1
Kinetic parameters of α-amylases from kilned malted extracts

Grain sample	K_m (mg/ml)	V_{max} (U/min)
Maize	12.5 ± 2.2	714 ± 50
Sorghum	1.8 ± 0.16	570 ± 35
Millet	5.8 ± 0.68	620 ± 96

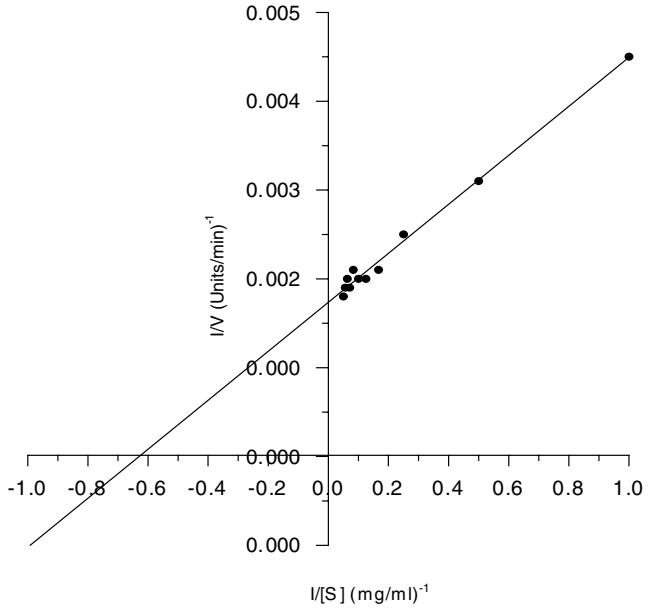

Fig. 1. A typical Lineweaver–Burk plot of the activity of α-amylases from kilned malted sorghum for the determination of kinetic parameters.

4.3. Heat stability studies

The result of thermal stability studies on α-amylases from malted sorghum, millet and maize are depicted in Figs. 2–4. All the amylases were stable to heat denaturation at 30 °C. Sorghum and millet α-amylases were relatively stable to heat inactivation at 50 °C whereas maize α-amylases were unstable loosing about 80% activity within 1 h of incubation. Interestingly, 50 °C is within the range of temperature usually used for kilning (50–55 °C). This

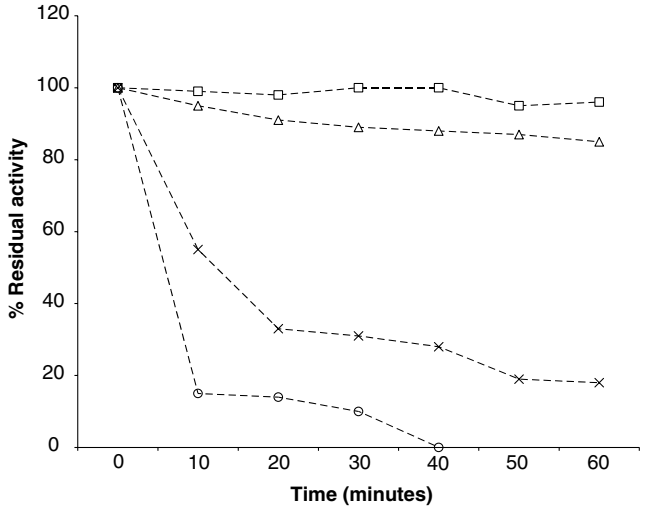

Fig. 2. Effect of temperature on the stability of sorghum α-amylases at 30 °C, (□-□); 50 °C, (△-△); 80 °C, (×-×) and 100 °C, (○-○). The experimental details are as stated in the text.

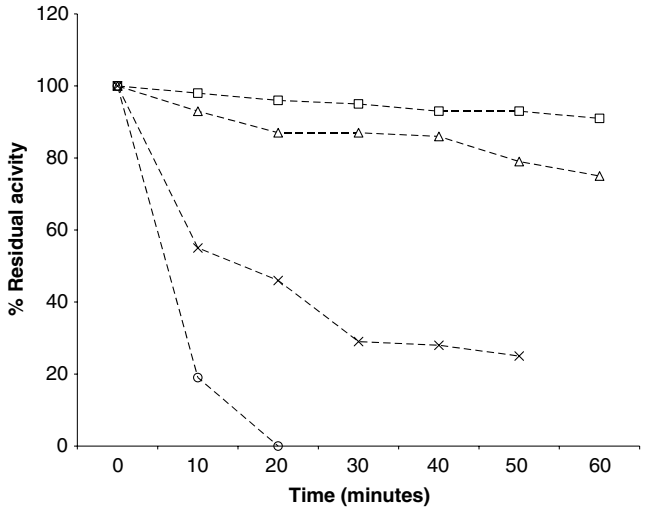

Fig. 3. Effect of temperature on the stability of millet α-amylases at 30 °C, (□-□); 50 °C, (△-△); 80 °C, (×-×) and 100 °C, (○-○).

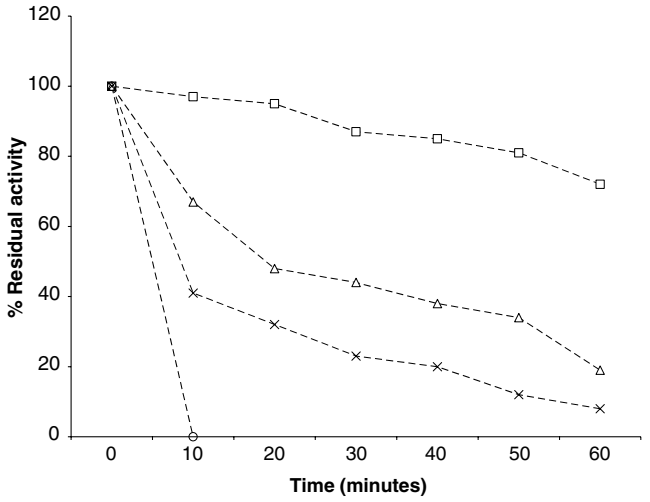

Fig. 4. Effect of temperature on the stability of maize α-amylases at 30 °C, (□-□); 50 °C, (△-△); 80 °C, (×-×) and 100 °C, (○-○).

suggests that maize α-amylases activity could easily be lost during the kilning process. At 80 °C, there was no activity remaining at 10 min of incubation for maize α-amylases, whereas sorghum and millet α-amylases still retained about 18% and 20% residual activity, respectively. Overall, maize α-amylases were least stable to heat denaturation at all the temperatures studied, while sorghum α-amylases were most stable. This is also consistent with the specific activities obtained after kilning for the three grain amylases where it was observed that sorghum α-amylases had the lowest drop in specific activity.

In conclusion, the data generated in this report showed that α-amylases from maize were least efficient in the bioconversion of starch to simple sugars. This observation probably explains why traditional maltsters do not use maize grains for commercial malt production.

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