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Characterization of an α-amylase from sorghum (*Sorghum bicolor*) obtained under optimized conditions

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Sorghum malt α-amylase can compete with bacterial α-amylase in industrial applications, if sufficiently stable and produced in a large enough quantity. Conditions for maximal α-amylase production in sorghum malt and the physico-chemical properties of the α-amylase so produced are reported in this study. Sorghum grains were steeped in buffers with varying pH (4.0–8.0) for 24 h, at room temperature, and germinated for another 48 h to obtain the green malt. The buffer that induced the highest quantity of α-amylase was chosen as the optimal pH and served as the medium for further steeping experiments conducted at different temperatures (10, 20, 30, 40, 50 and 60°C). The α-amylase activity in the extract was determined in order to obtain the optimum temperature for α-amylase induction at this particular pH. For the purpose of comparison, the α-amylase produced at the optimum pH and temperature was purified to apparent homogeneity by a combination of ion-exchange and size-exclusion chromatography, and further characterized. Eight-fold higher α-amylase activity was induced in pH 6.5 buffer at 20°C compared with water, the traditional steeping medium. The K_m and V_{max} were estimated to be 1.092 ± 0.05 mg mL^{-1} and 3516 ± 1.981 units min^{-1}, respectively. The activation energy of the purified amylase for starch hydrolysis was 6.2 kcal K^{-1} mol^{-1}. Chlorides of calcium and manganese served as good activators, whereas $CuSO_4$ inhibited the enzyme with a 42% loss in activity at 312 mM salt concentration. Copyright © 2012 The Institute of Brewing & Distilling

Keywords: α-amylase; optimized conditions; pH; sorghum malt; temperature

Introduction

The α-amylases (1,4-α-D-glucan-4-glucanohydrolase; EC 3.2.1.1) are ubiquitous enzymes produced by plants, animals and microbes, and they play a dominant role in carbohydrate metabolism. They are endoglycosidases and catalyse the random hydrolysis of internal α-1,4-D-glycosidic linkages in starch and dextrins, generating smaller dextrins and oligosaccharides with a C_1–OH group in the α-anomeric configuration. Their specificity can vary depending on the source. The α-amylases from fungal and bacterial sources have dominated applications in industrial sectors *(1)*.

Generally, grains are known to contain a varying quantity of amylases (both α- and β-amylases). Unmalted sorghum has no β-amylase and there is very little when the grain is malted. Barley contains a large quantity of α- and β-amylases, which develop during the malting process, when the β-amylase is activated from a bound form, thus increasing the hydrolytic activity *(2)*. Sorghum has been reported to be a good source of α-amylase in comparison to other available grains such as rice, maize or millet owing to the relatively high heat stability of the α-amylase in the intact malt *(3,4)*. Amylases from plant and microbial sources have been employed for centuries as food additives *(1,3,5)*. Barley amylases have been used in breweries and fungal amylases have been widely used for the preparation of oriental foods. In spite of the wide distribution of amylases, microbial sources (fungal and bacterial amylases) are used for industrial production owing to advantages such as cost-effectiveness, consistency, lesser requirement for time and space for production and ease of process modification and optimization *(1)*. Among bacteria, *Bacillus* sp. are widely used for thermostable α-amylase production to meet industrial needs. *Bacillus subtilis, B. stearothermophilus, B. licheniformis* and *B. amyloliquefaciens* are known to be good producers of α-amylase, and these have been widely used for commercial production of the enzyme for various applications. In many developing countries, thermostable amylases from the bacteria or fungal sources are expensive, since they are often imported. Previous work has established that large quantities of α-amylase are induced in sorghum when such grains are malted using traditional techniques *(3)*. Exogenous α-amylase from such malted sorghum could compete with bacterial or fungal α-amylases, if sufficiently stable and available in large quantities. This work attempts to establish the optimum conditions for the production of α-amylase in sorghum grains, and to characterize the amylase so produced. Production of stable grain α-amylase in large quantity could in principle be a good substitute for microbial α-amylase.

Materials and methods

Materials

All reagents were of analytical grade. Soluble starch was obtained from BDH Chemicals Ltd (Poole, England). Sorghum grains were purchased from a local market in Sabo, Ile-Ife, Nigeria. The grains

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were identified as *Sorghum bicolor* by the Department of Botany, Obafemi Awolowo University, Ile-Ife. The grains were a white Fara Fara cultivar harvested in April 2009.

Induction of α-amylase

Malting was carried out following an earlier procedure established by Adewale *et al. (3)* and Adewale and Oladejo *(5)*, by steeping about 100 g of sorghum grains, which had been screened to remove broken grains and other extraneous materials, in 10 mM acetate or phosphate buffers of pH ranging from 4.0 to 8.0 at 30°C. The control was steeped in distilled water. Grains obtained from the steeping process were blotted to remove excess water or buffer and were spread in a malting chamber at 30°C. The grains were sprinkled with the respective buffers or with water used for the steeping process at 6 h intervals until the acrospire was about 1 cm in length (usually about 48 h). All the grains were uniformly germinated for the same length of time under these conditions. Germination was arrested by drying the green malt in a hot air oven (Fisher Isotemp oven model 175) for 24 h at 60°C to obtain the kilned malt. The moisture content of the kilned malt was 7.0–8.0%. Green malt was stored in tightly sealed cellophane in the freezer at −20°C. The pH that caused the highest amylase induction in this experiment was noted. In another experiment, grains were steeped in the buffer at this noted pH, but at different temperatures of 0, 20, 30, 40, 50 and 60°C before germination.

Sorghum α-amylase extraction

One hundred grams of the kilned and unkilned malt grains was ground to fine powder with a pestle and mortar and the α-amylase induced was extracted by preparing a 30% homogenate of the malted grains, using the respective buffers or water that were used for the induction process, except that the homogenization buffers contained 1 mM $CaCl_2$. A clear supernatant was obtained by centrifuging the resultant homogenate for 30 min at 10,000*g*. The crude supernatant of the malt that gave the highest α-amylase was purified as described below.

Enzyme assay

The number of reducing ends released upon starch hydrolysis by α-amylase was estimated using the modified method of Bernfeld *(6)*. A unit of α-amylase activity was defined as the amount of enzyme that liberated reducing sugar equivalent to 1 μg of D-glucose per min at 25°C under the standard assay conditions. Confirmatory α-amylase activity assay was carried out using a modified starch-iodine binding test of Xiao *et al. (7)*.

Determination of the protein concentration

Protein concentration was determined following the method of Bradford *(8)* with bovine serum albumin as the standard protein.

Enzyme purification

Purification of the crude extract by ion-exchange chromatography on CM-Sepharose. In a typical purification, 20 mL of clear supernatant was layered onto a 2.5 × 40 cm column of CM-Sepharose that had been equilibrated with 10 mM sodium phosphate buffer pH 6.5 containing 1 mM $CaCl_2$. Fractions of 5 mL were collected at a flow rate of 33 mL h^{-1}; bound proteins were eluted with a 0–1.0 M NaCl gradient. The enzyme activity in the fractions was measured by assaying each fraction for amylase activity. The protein profile was determined by measuring the absorbance of the fractions at 280 nm. Active fractions were pooled and lyophilized.

Gel filtration column chromatography. Post ion-exchange active fractions that had been lyophilized were re-dissolved and centrifuged to remove the insoluble precipitate, and 1.0 mL of this solution was applied on a 1.0 × 48 cm column of Bio gel P60 that had been equilibrated with a 10 mM sodium phosphate buffer pH 6.5 containing 1 mM $CaCl_2$. Fractions (5 mL) were collected at a flow rate of 16 mL h^{-1}. Active fractions were pooled and lyophilized for further use.

Determination of sub-unit molecular weight. The subunit molecular weight of the purified α-amylase was determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) according to the method of Laemmli *(9)* using a 4% stacking gel and a 12% separating gel.

Determination of kinetic parameters. The apparent kinetic parameters (Michaelis constant, K_m, and maximal velocity, V_{max}) were determined for the purified sorghum α-amylase by incubating aliquots of the enzyme extract at 30°C for 5 min with final concentrations of soluble starch between 0.1 and 1.0%, while keeping all other components constant. The amount of reducing sugar formed was measured and units of activity were calculated. The data obtained were analysed using a nonlinear regression analysis software of Dr Easterby (Hyper 32) of the University of Liverpool.

Effect of temperature. The effect of temperature on the activity of the purified α-amylase was quantified by incubating aliquots of the purified enzyme solution at 35, 40, 50, 60, 70, 80 and 90°C for 10 min. The residual activity of the enzyme was then determined following the procedure earlier described. The residual α-amylase activity was plotted against temperatures.

Heat stability studies. Aliquots of the purified α-amylase were incubated at temperatures ranging from 35 to 70°C. Aliquots were withdrawn at intervals of 10 min for up to 90 min and assayed for residual activity. The residual activities were expressed as a percentage of the activity at 0 min, which was taken to be 100%. The percentage residual activity was plotted against time of incubation.

Effect of pH. The activity of the sorghum α-amylase was determined at various pH values ranging from 2.0 to 9.0 at intervals of 0.5 pH units. The different pH buffers were used in place of the standard assay buffer. The activity of the sorghum α-amylase was plotted against the different pH values.

Effect of metal ions. The effect of metal ions on the sorghum α-amylase activity was investigated by incubating the enzyme and substrate in the assay buffer at 30°C for 5 min in the presence of varying concentrations of the metal ions, dissolved in the assay buffer, together with the appropriate control without the metal ions. The metal ions were Na^+, K^+, Mn^{2+}, Cu^{2+} and Ca^{2+} and levels varied between 0.0 and 312 mM in the final assay mixture. The activity obtained was expressed as a percentage of the control, which was taken to be 100%.

Results

Induction of α-amylase

Visual inspection (not shown) of the green malts showed that those produced from the grains steeped in a 10 mM phosphate buffer pH 6.5 had more vegetative growth compared with the green malts obtained from grains steeped in buffers at other pH values. When the steeping temperatures were varied, the green malts from grains that had been steeped at 20°C in the 10 mM phosphate buffer, pH 6.5, showed more growth than those obtained from other steeping temperatures at this particular pH.

The α-amylase was extracted from both the green malt and the kilned malt at each of the different conditions. Figures 1–3 are a summary of the respective specific activities as a function of the varied parameters. Figure 1 shows the specific activity of the α-amylase of the crude supernatants obtained from green malt steeped in buffers, at the indicated pH values plotted against the respective pH of steeping. Figure 2 shows the specific activity of α-amylase of the crude supernatants obtained from kilned malt, steeped in buffers at the indicated pH values, plotted against the respective pH of steeping. Figure 3 shows the temperatures of the buffer that gave the highest α-amylase induction (10 mM phosphate, pH 6.5) and was further varied as indicated. The sorghum grains were steeped in the buffer at these temperatures, the malt obtained was kilned and the

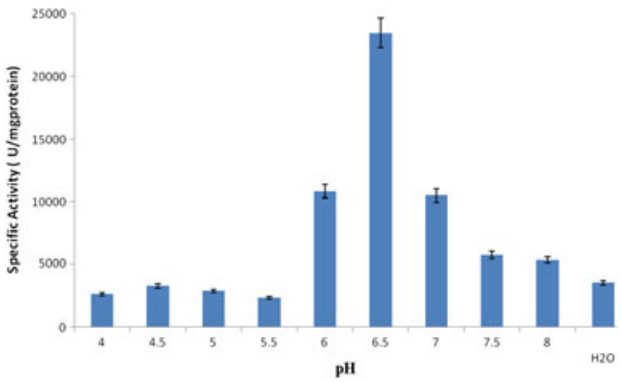

Figure 1. Profile of α-amylase activity as a function of pH from the green sorghum malt. The values shown are an average of three replicates ± SEM.

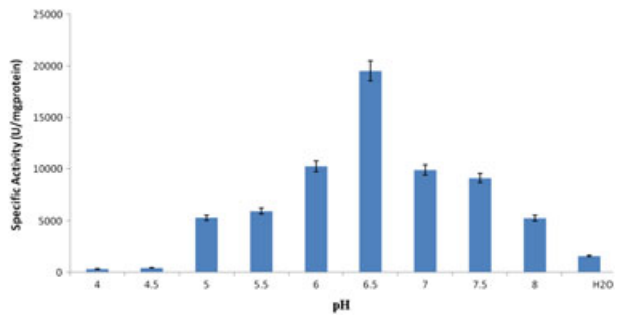

Figure 2. Profile of α-amylase activity as a function of pH from the kilned sorghum malt. The values shown are an average of three replicates ± SEM.

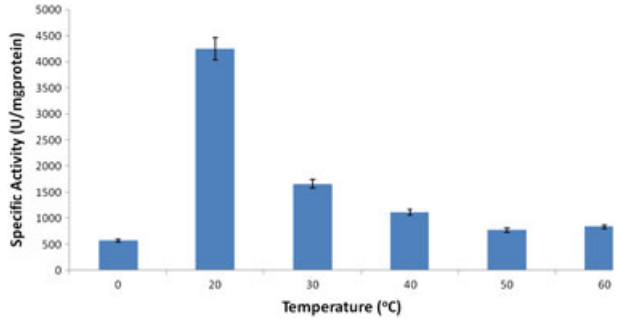

Figure 3. Effect of temperature on the induction of α-amylase in the sorghum malt at pH 6.5.

α-amylase extracted following the procedure described previously. The α-amylase activity and protein concentration of each malt extract were determined and the specific activity obtained was plotted against the respective steeping temperature.

Green malt obtained from grains steeped at 20°C in phosphate buffer pH 6.5, termed the optimum condition, contained the highest amylase activity compared with other conditions employed, thus showing a correlation between visual inspection and actual amylase induction. The specific activity of α-amylase extracted from malts produced at the optimum condition was 23,400 U mg^{-1} protein. To ensure that the amylase produced under the optimum condition had similar characteristics to what was usually obtained using the traditional approach, the amylase induced under the optimal condition was purified and thereafter characterized.

Ion exchange chromatography

A typical elution profile of the crude homogenate of sorghum α-amylase on CM-Sepharose CL-6B ion exchange chromatography is shown in Fig. 4. The crude extract of α-amylase from the unkilned malted sorghum, produced under the optimized steeping condition (phosphate buffer pH 6.5 at 20°C) with a specific activity of 4246 U mg^{-1} protein, was applied to a CM-Sepharose column. Three main activity peaks were obtained, one before a 0–1.0 M NaCl gradient and the other two after the start of the gradient. The major peak, denoted peak B, was purified further.

Gel filtration chromatography

The post ion–exchange fraction (peak B) was freeze-dried. The lyophilized precipitate thus obtained was re-dissolved in the appropriate buffer and layered onto a Biogel P60 gel filtration column. Fractions with activity were pooled for further characterization. An earlier attempt was made to purify the enzyme on a Sephadex G-100 column. This resulted in nonspecific interaction of the α-amylase with the resin and subsequent digestion of the dextran. The purification summary is shown in Table 1.

Molecular weight and kinetic constants

A single protein band was obtained for the sorghum α-amylase. The α-amylase band corresponded to that of lysozyme with a molecular weight of 14.5 kDa and thus the molecular weight

Table 1. Summary of the purification of crude α-amylase obtained from green malt produced under optimized conditions

Purification step		Total volume (mL)	Protein concentration (mg mL^{-1})	Enzyme activity (U mL^{-1} min^{-1})	Total protein (mg)	Total activity (U)	Specific activity (U mg^{-1})	Yield (%)	Purification fold
Crude enzyme		83	0.59	1710	49.0	141,500	2890	100	1
Ion exchange on CM-Sepharose CL-6B	A	14	0.06	257	0.88	3,598	4,080	3.0	2
	B	62	0.04	414	2.23	25,670	11,500	18	4
	C	58	0.06	173	3.31	10,030	3,040	7.0	2
Bio- gel P60	a	6.0	3.86	2,160	23.2	12,980	560	9.0	19
	b	4.0	2.98	2,550	11.9	10,220	857	7.0	31
	c	3.0	2.96	2,570	8.88	7,700	867	5.0	31

The crude fractions were separated into three peaks named A, B and C on ion exchange chromatography. Each of these peaks was separately pooled and further purified by gel filtration, producing corresponding peaks labelled a, b and c.

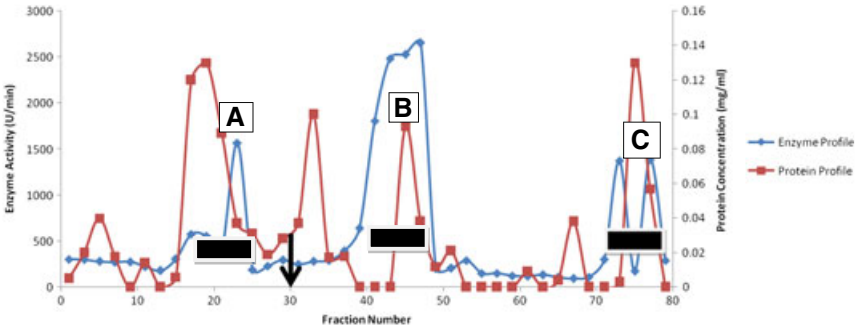

Figure 4. Elution profile of the crude extract of α-amylase obtained from green malted sorghum on a CM-Sepharose ion-exchange column.

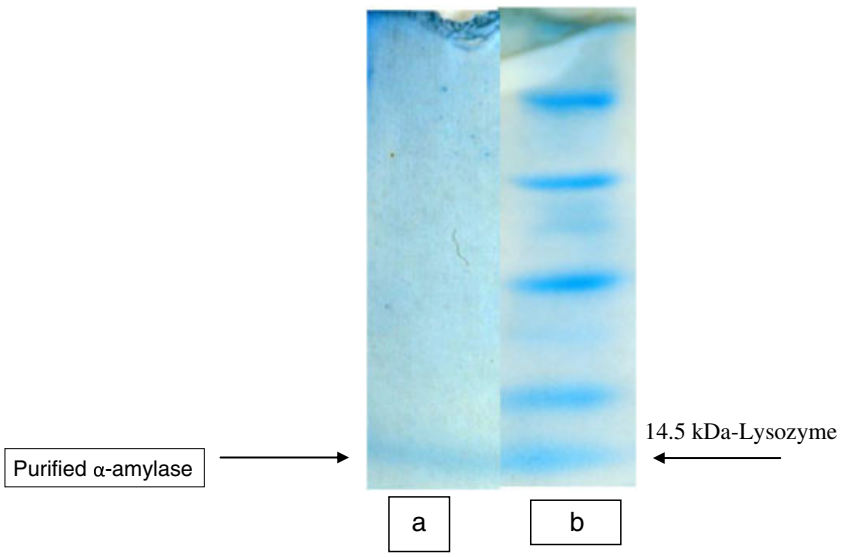

Figure 5. SDS–PAGE of molecular weight standard marker proteins together with the purified sorghum α-amylase. Lane a is the purified main isoform of sorghum α-amylase. Lane b contains the standard marker proteins consisting of from the top, myosin (200 kDa), β-galactosidase (119 kDa), serum albumin (66 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), trypsin inhibitor (20 kDa) and lysozyme (14.5 kDa).

was estimated to be 14.5 kDa (Fig. 5). The computer-estimated apparent K_m and V_{max}, from the nonlinear regression analysis of the kinetic data for the α-amylase, was 1.092 ± 0.05 mg mL^{-1} and 5248 ± 1.981 U mg^{-1} protein, respectively.

Effect of substrate concentration on the activity of purified sorghum α-amylase

The effect of substrate concentration on the activity of purified sorghum α-amylase was tested at a starch concentration

between 0 and 10 mg mL^{-1} in the final assay mixture. Figure 6 is a summary of the results obtained. There was a potent inhibition of the activity above a starch concentration of 6.4 mg mL^{-1}. At a substrate concentration of 10 mg mL^{-1}, the activity was completely abolished.

Effect of temperature on the activity of sorghum α-amylase

The summary of the effect of temperature on the purified α-amylase is presented in Fig. 7(A). The profile was obtained by incubating the enzyme with the substrate for 10 min at the indicated temperatures. The residual activity obtained was plotted against the respective temperature of the assay. The activity increased gradually from 35°C to the optimum temperature of 60°C. Beyond this optimum temperature, the activity gradually declined, and at 85°C there was no activity detected.

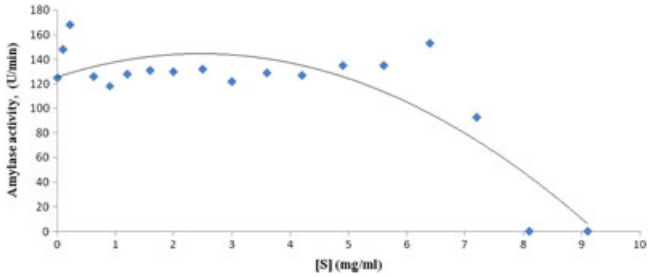

Figure 6. Effect of substrate on the activity of purified sorghum α-amylase.

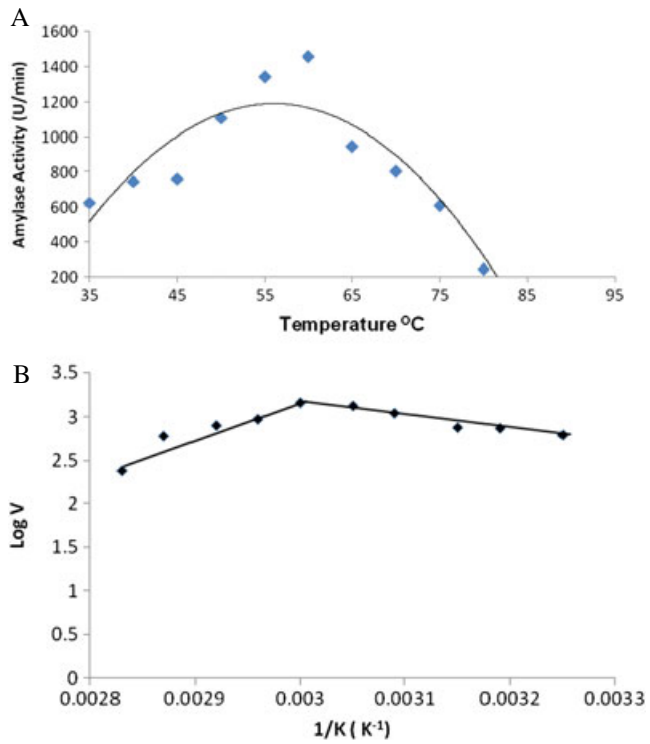

Figure 7. Effect of temperature on the activity of purified sorghum α-amylase. (A) Residual activity at each of the indicated temperatures. (B) Arrhenius plot for the determination of the activation energy (E_a) of purified sorghum malt α-amylase under the optimized conditions.

The activation energy (E_a) for the purified sorghum α-amylase-catalysed hydrolysis of starch was estimated from a plot of log V_{max} against K^{-1} (Fig. 7B). A value of 6.2 kcal K^{-1} mol^{-1} was obtained.

Heat stability studies

At an incubation temperature of 35°C, about 40% of the activity was lost after 90 min of incubation. The enzyme retained more than half of its activity at an incubation temperature of 60°C for 30 min. However, about 70% activity was lost upon 40 min incubation at 70°C and by 60 min, all the activity had been lost (Table 2).

Effect of pH on the activity of sorghum α-amylase

The effect of pH on the sorghum α-amylase activity was studied by assaying the enzyme for activity after incubation of the substrate with the enzyme for 5 min at a pH range from 2.5 to 9.5. There was no activity at pH 2.5–3.5 and at pH of 8.5–9.5. The optimum activity was obtained at pH 6.5 (Fig. 8). The activity at pH 5.5, 6 and 7 was more than 70% of the optimum activity.

Effect of metal ions on sorghum α-amylase activity

The effect of metal ions on the activity of purified sorghum α-amylase was investigated by assaying for activity in the presence of various concentrations of the metal ions in the assay

Table 2. Summary of the heat stability characteristics of sorghum α-amylase obtained under optimized conditions

	Residual activity (%)			
Time (min)	35°C	50°C	60°C	70°C
0	100	100	100	100
2	98	94	78	78
5	87	80	77	60
10	85	72	65	56
20	80	65	63	56
30	76	60	59	51
40	72	58	52	27
60	69	47	44	0
90	60	42	0	0

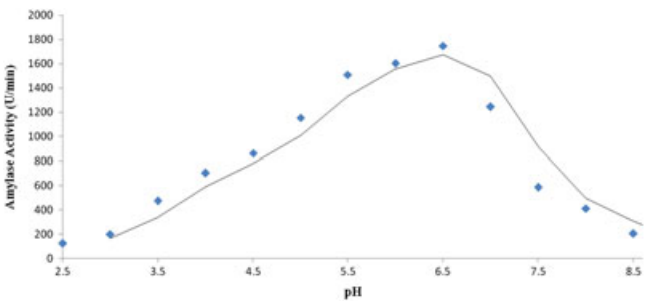

Figure 8. Effect of pH on the activity of purified sorghum α-amylase.

mixture. The concentration of the metal ions (Na^+, K^+, Mn^{2+}, Cu^{2+} and Ca^{2+}) was varied up to 312 mM. Of the metal ions examined, only Cu^{2+} inhibited the enzyme. The enzyme lost about 40% activity at a $CuSO_4$ concentration of 312 mM in the assay mixture. Na^+ and K^+ had no effect on the activity, whereas $CaCl_2$ activated the enzyme, the extent of activation being proportional to the concentration of the metal ion in the assay mixture (Table 3). There was a rapid increase in α-amylase activity up to 80 mM of $MnCl_2$ concentration, followed thereafter by a reduction in activity.

Discussion

This study shows that, by varying the conditions under which the sorghum grains are steeped, malts with a higher α-amylase content could be produced. In the green malt, there was a little difference in the quantity of α-amylase induced at pH 4, 4.5, 5 and 5.5 compared with that of water, which is traditionally used as the steeping medium. The specific activity of the α-amylase in extracts obtained from malt steeped in water was 2900 U mg^{-1} protein. However, the activity rose to 23,400 U mg^{-1} protein at pH of 6.5, which is about eight times higher than the overall average obtained for water, or other steeping media at pH 4–5.5.

To further study whether a temperature change would enhance α-amylase induction, steeping at a pH of 6.5 was performed at 0, 20, 30, 40, 50 and 60°C. As mentioned above, malts obtained from grains steeped in buffer at 20°C contained more α-amylase than the malts produced at other temperatures. For preservation, colour and aroma development, malts are usually kilned. The green malts produced at these optimized conditions were kilned to determine whether the induced α-amylase would still be stable. Kilning was performed in a hot air oven at 60°C, following a previously established procedure *(3,5)*. The α-amylase induced was found to be quite stable, although as expected, part of the activity was lost owing to heat inactivation. Malts produced at the lower pH values (pH 4 and 4.5) were more affected by kilning than malts produced at the optimum pH. Visual assessments also showed that more vegetative growth had occurred at pH 6.5, than when water or other buffers were used as the steeping medium.

It has been previously reported *(2,10)* that the α-amylase of malts produced from sorghum grains have many isozyme forms. Three main isoforms were reported by Adewale and Oladejo *(5)*. To determine whether the isoforms were present or whether new forms had developed as a result of the optimized conditions, green malt α-amylase of the malt produced under the optimized condition was extracted. The crude extract, which was further purified and fractionated by ion exchange chromatography, showed three distinct peaks, which were assumed to correspond to the different isoforms reported earlier. Omar *(11)* obtained three peaks upon ion exchange purification of α-amylase from barley *Hordeum vulgare*. Subbarao *et al. (12)* also obtained three distinct peaks from partially purified α-amylase from the scutellum and aleurone layer of maize seeds. Moreover, Nirmala and Muralikrishna *(13)* reported three peaks using DEAE-Sephacel chromatography of finger millet α-amylases.

Table 3. Effect of metal ions on sorghum α-amylase activity

	Residual activity (%)				
Concentration (mM)	NaCl	KCl	$MnCl_2$	$CuSO_4$	$CaCl_2$
0	100	100	100	100	100
20	103	115	135	93	117
40	107	112	153	89	121
80	106	109	163	86	134
120	106	109	160	86	160
160	109	107	147	81	162
200	106	109	147	73	163
240	107	105	148	67	163
312	100	103	105	58	165

Size-exclusion chromatography on Biogel P60, permitted further purification of the enzyme, even though there was evidence of interaction. This type of interaction with acrylamide material suggests that the α-amylase may have some nonprotein part arising from *in-vivo* post-translational activity (for example phosphorylation or glycosylation of some amino acid residues). This had also been previously suggested by Adewale and Oladejo *(5)* and will be further investigated. Nevertheless, a single isoform obtained on CM-Sepharose was resolved into three peaks using Biogel P60 gel filtration chromatography. However, whether the three peaks were due to nonspecific interactions with the column material is not clear. The three peaks had identical properties during characterization. SDS–PAGE of the post gel-filtration column gave a single band on slab gels. The molecular weight was estimated to be 14,500 Da.

The results of the effect of substrate (starch) on sorghum α-amylase obtained under the optimized conditions at varying substrate concentrations up to 9 mg mL^{-1} in the final assay mixture showed that there was potent substrate inhibition above 6.4 mg mL^{-1} of starch. At lower concentrations of the substrate, the kinetic parameters, K_m and V_{max} were estimated to be 1.092 ± 0.05 mg mL^{-1} and 5248 ± 1.981 U mg^{-1} protein, respectively, by nonlinear regression. These values are in the range of values reported by other authors who had employed traditional methods for α-amylase induction. Saleh *et al. (14)* reported a K_m of 1.1 mg mL^{-1} for starch. The immediate implication of the low K_m value is that the enzyme would be easily saturated with substrate and would have to be used at a low substrate concentration *(5)*. This is further reinforced by the fact that high substrate concentration is a potent inhibitor of this enzyme.

The study established that the optimum temperature of activity of the purified amylase was 60°C, while the optimum pH was 6.5. There was a gradual loss of activity on either side of the optimum temperature and pH. There was no activity at 80°C and minimal activity at 35–40°C. Also, no activity was detected at pH 9.0 and minimal activity was detected at pH 2.5–3.5. The enzyme was quite stable between 35 and 50°C. It retained about 50% activity at 70°C for 30 min and 30% activity at 70°C for 40 min. At 60°C, activities of 60 and 50% were retained on incubation for 30 and 60 min, respectively. Since sorghum starch gelatinizes at high temperatures, it follows that the purified amylase obtained in this study could not be applied to sorghum mashes, before or during gelatinization, in order to liquefy the gelatinized starch.

However, there may be applications for mash or starch obtained from other sources that have low gelatinization temperatures. Moreover, the α-amylase could be used for sorghum mashes if the gelatinized starch was cooled down to the optimum temperature of activity of this amylase. The

disadvantage of this method would be that it could increase the cost of production and increase the process time when applied, for example in the production of brewing wort.

It is well known that $CaCl_2$ is an activator of grain α-amylase *(10,13)*. This was confirmed in the current study. At the maximum $CaCl_2$ concentration employed, an approximately 1.7-fold increase in activity was noted compared with the control containing no salt. A $CaCl_2$ concentration between 120 and 312 mM $CaCl_2$ resulted in similar activities.

$MnCl_2$ was a potent activator, up to 80 mM, in the assay mixture. Beyond this concentration, the activation effect decreased, but the α-amylase activity was still higher than that in the control that had no addition. Metal ions activate enzyme activity by assisting in the anchoring of substrate to the enzyme, in stabilizing charges and transition state and in relay of charges. Activation of α-amylase activity by metal ions in this study might be due to some of these reasons.

$CuSO_4$ was a mild inhibitor and the inhibitory activity was less pronounced up to 120 mM, when about 10% of the activity was lost. At a concentration of 312 mM, 58% of the activity was retained.

Chloride of sodium or potassium had no observable effect on the activity of the purified sorghum α-amylase in this study. This observation is consistent with previous reports on grain α-amylase obtained from malts produced using a traditional approach. Nirmala and Muralikrishna *(13)* showed that Na^+ and K^+ had no effect on the α-amylase from millet (*Eleusine coracana*), and also reported that $CaCl_2$ was a potent activator.

The apparent lack of sensitivity of the sorghum grain α-amylase to high salt concentrations may be advantageous when deployed for commercial starch conversion processes. Salts are often added to enhance flavour or for stability of other proteins or enzymes. It follows that sorghum grain α-amylase can function efficiently under these conditions and that the stability can even be further enhanced by the addition of $CaCl_2$.

Conclusions

This study shows an approximate 8-fold increase in α-amylase activity by producing sorghum malt from grains steeped in 10 mM phosphate buffer pH 6.5 at 20°C, compared with water, which has traditionally been employed. The appearance of the resulting malt was comparable to the control (water-steeped malt) even after the kilning process. The molecular properties of the purified α-amylase obtained under these optimized conditions were similar to the reports of other workers who employed traditional methods for grain steeping.

Pending further stability studies, sorghum malt α-amylase obtained in this study could find applications as an additive in laundry detergents, as a fruit juice clarifier and in the paper industry.

Further work is needed to enhance the stability of the sorghum malt α-amylase. Such work may involve immobilization of the enzyme on a suitable insoluble support medium, or chemical modifications such as phosphorylation, acetylation or succinylation of some side chains may be employed.

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