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Thermal Stability of α-Amylase from Malted Jowar (*Sorghum bicolor*)

R. Siva Sai Kumar, Sridevi Annapurna Singh, and A. G. Appu Rao*

Department of Protein Chemistry and Technology, Central Food Technological Research Institute, Mysore 570 020, India

Malted cereals are rich sources of α-amylase, which catalyzes the random hydrolysis of internal α-(1−4)-glycosidic bonds of starch, leading to liquefaction. Amylases play a role in the predigestion of starch, leading to a reduction in the water absorption capacity of the cereal. Among the three cereal amylases (barley, ragi, and jowar), jowar amylase is found to be the most thermostable. The major amylase from malted jowar, a 47 kDa α-amylase, purified to homogeneity, is rich in β structure (∼60%) like other cereal amylases. T_m, the midpoint of thermal inactivation, is found to be 69.6 ± 0.3 °C. Thermal inactivation is found to follow first-order kinetics at pH 4.8, the pH optimum of the enzyme. Activation energy, E_a, is found to be 45.3 ± 0.2 kcal mol^{-1}. The activation enthalpy (ΔH^*), entropy (ΔS^*), and free energy change (ΔG^*) are calculated to be 44.6 ± 0.2 kcal mol^{-1}, 57.1 ± 0.3 cal mol^{-1} K^{-1}, and 25.2 ± 0.2 kcal mol^{-1}, respectively. The thermal stability of the enzyme in the presence of the commonly used food additives NaCl and sucrose has been studied. T_m is found to decrease to 66.3 ± 0.3, 58.1 ± 0.2, and 48.1 ± 0.5 °C, corresponding to the presence of 0.1, 0.5, and 1 M NaCl, respectively. Sucrose acts as a stabilizer; the T_m value is found to be 77.3 ± 0.3 °C compared to 69.6 ± 0.3 °C in the control.

KEYWORDS: ***Sorghum bicolor*; α-amylase; thermal stability; starch degradation; malted cereal**

INTRODUCTION

α-Amylase, an endoenzyme (EC 3.2.1.1, 1,4-D-glucan glucanohydrolase), catalyzes the random hydrolysis of internal α-(1−4)-glycosidic bonds of amylose, amylopectin, and other related polysaccharides, giving rise to low molecular weight oligosaccharides and glucose. The exact ratio of these saccharides depends on the source and the nature of the α-amylase used (*1*). These enzymes are widely distributed in microbes, plants, and animals. In addition to its biochemical interest, this enzyme has a number of important commercial applications in the sugar, brewing, alcohol, and textile industries (*2*).

Supplementary foods absorb a large quantity of water and increase their bulk when reconstituted using hot water attributable to their pregelatinized starch content. Malted cereals are a rich source of hydrolytic enzymes (amylases) and can be an integral part of supplementary foods. Amylases, in the supplementary food, predigest starch, leading to a decrease in water absorption capacity and an increase in nutrient density.

Sorghum/milo (jowar), a coarse tropical cereal, serves as a staple food for over 750 million people. Its ability to grow in areas that are low in moisture and fertility, coupled with photosynthetic efficiency, makes it an attractive alternative to other cereals (*3*). Malted sorghum, found to produce a thermostable α-amylase among cereal amylases, is addressed in this paper. The purpose of this work has been to screen cereals for thermostable α-amylases, which retain activity when hot water is added to supplementary foods to reconstitute them. The isolation, characterization, and thermal inactivation kinetics of the α-amylase from sorghum are reported for the first time.

* Corresponding author (telephone +91-821-2515 331; fax +91-821-2517 233; e-mail appu@cscftri.ren.nic.in).

MATERIALS AND METHODS

Materials. DEAE-cellulose, Sephadex G-75, acrylamide, bisacrylamide, TEMED, ammonium persulfate, protein molecular mass markers (SDS), 3,5-dinitrosalicylic acid, maltose, and maltooligosaccharides were obtained from Sigma Chemical Co., St.Louis, MO. All other chemicals used were of analytical/HPLC grade.

Malted Cereals. Barley (*Hordeum jubatum*, commercial variety), ragi (*Eleusine coracana*, commercial variety), and jowar (*Sorghum bicolor* variety M-35-1) were purchased from a local market in Mysore, Karnataka, India. The seeds were cleaned and soaked in distilled water for 12 h. Thereafter, they were allowed to germinate for 60 h. After germination, the seeds were air-dried and vegetative growth portions were removed by gentle manual brushing. Seeds were powdered to a particle size of 355 μm. The powder was stored in an airtight container at 4 °C after defatting using hexane.

Determination of α-Amylase Activity. A modification of the procedure devised by Bernfeld (*4*) was used to determine enzyme activity. Gelatinized soluble starch, 2%, was incubated with enzyme appropriately diluted with acetate buffer (0.05 M, pH 4.8) having 13.6 mM calcium, for 5 min at 60 °C. The reaction was stopped by the addition of reagent (3,5-dinitrosalicylic acid). One unit of enzyme activity is defined as micromoles of maltose equivalent released per minute under the assay conditions. Specific activity is expressed as

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activity units per milligram of protein. The protein concentration of the purified enzyme was calculated by taking $E^{1\%}_{1cm}$ as 24.2 at 280 nm (*5*).

Purification of Sorghum Malt α-Amylase. α-Amylase was purified at 4 °C with modifications to the procedure reported earlier (*6*). Defatted sorghum malt was extracted with a 1:10 ratio of acetate buffer (0.05 M, pH 4.8) having 13.6 mM calcium ($CaCl_2 \cdot 2H_2O$) and centrifuged to remove coarse particles. This was subjected to ammonium sulfate precipitation (0−40%). After centrifugation, the precipitate was redissolved in Tris-HCl (0.1 M, pH 7.0) having 13.6 mM calcium, dialyzed, applied to a DEAE-cellulose column (20 × 1.5 cm, 35 mL), and eluted with the same buffer to eliminate contaminating proteins. Bound proteins were eluted with a buffer containing 0.3 M NaCl to recover amylase activity. The pooled, active fractions were precipitated by ammonium sulfate (0−70%). The precipitate was equilibrated in Tris-HCl (0.1 M, pH 7.0) having 13.6 mM calcium and 0.3 M NaCl and chromatographed on a gel filtration column (G-75 superfine, 135 × 1 cm, volume = 110 mL) using the same buffer. The active fractions were pooled and reprecipitated using ammonium sulfate (0−70%) to recover the active protein in pure form. Enzyme concentration was determined using $E^{1\%}_{1cm} = 24.2$ (*5*).

Homogeneity of the Enzyme Preparation. The homogeneity of the enzyme was checked by 12.5% SDS-PAGE (*7*). PAGE, under native conditions, was done without SDS and β-mercaptoethanol. The gels were stained for protein with silver nitrate (*8*), whereas enzyme activity was stained using iodine (*9*). Markers in the molecular mass range from 66 to 14.2 kDa were used to determine the molecular mass of α-amylase on SDS-PAGE.

Determination of Kinetic Constants. To determine the effect of substrate concentration on enzyme activity, substrate concentrations ranging from 0.26 to 2.1% were used. Kinetic constants, K_m and V_{max}, were calculated from Lineweaver−Burk plot.

pH Optimum. The activity of α-amylase was tested in different pH values in 0.05 M acetate buffer (3.5−6.0), Tris-HCl buffer (7.0−8.0), and borate buffer (9.0) with 2% gelatinized soluble starch as a substrate. All buffers contained 13.6 mM Ca^{2+}, unless otherwise stated. The relative activity at different pH values was calculated (taking the maximum activity obtained as 100%).

Temperature Optima. To determine the temperature optimum of sorghum malt α-amylase, activities were determined at a temperature range of 30−80 °C. The assay was carried out in the same manner as described under Determination of α-Amylase Activity.

Separation and Identification of Products by HPLC. To determine the mode of action of the enzyme, the degree of polymerization (DP) of the products was determined by HPLC (*10*). One hundred milliliters of soluble maltodextrin (30% in 0.05 M acetate buffer, pH 4.8, having 13.6 mM calcium and 0.02% sodium azide) was incubated with 5400 units of α-amylase for different time intervals (2, 6, 12, 24, and 72 h) at 60 °C. At the end of the incubation time, 10 mL of reaction mixture was drawn and 3 volumes of absolute ethanol added to stop the reaction. The mixture was kept for 6 h at 4 °C for precipitation and settling of the undigested maltodextrin. The precipitate was separated by centrifugation at 10 000 rpm and discarded. The supernatant was concentrated by vacuum evaporation as reported. The concentrated products were dissolved in ultrapure water, filtered through a 0.22 μm membrane, and 20 μL was injected and analyzed by HPLC on a μBondapak NH_2 column (3.9 × 300 mm, 10 μm) using an acetonitrile/water solvent system (70:30) at a flow rate of 1 mL/min. The amounts of oligosaccharides (DP1−DP7) were quantified by peak integration, with standards detected using a refractive index detector. Glucose (DP1), maltose (DP2), maltotriose (DP3), maltotetraose (DP4), maltopentaose (DP5), maltohexaose (DP6), and maltoheptaose (DP7) were used as standards.

Fluorescence Spectra. Fluorescence measurements were carried out using a Shimadzu RF-5000 spectrofluorometer attached to a constant-temperature circulating water bath. Excitation and emission slit widths were set at 5 and 5 nm, respectively, using a 10 mm path length cuvette in 0.05 M acetate buffer, pH 4.8, containing 13.6 mM calcium. The protein concentration used was less than 0.1 OD at 280 nm to minimize inner filter effects.

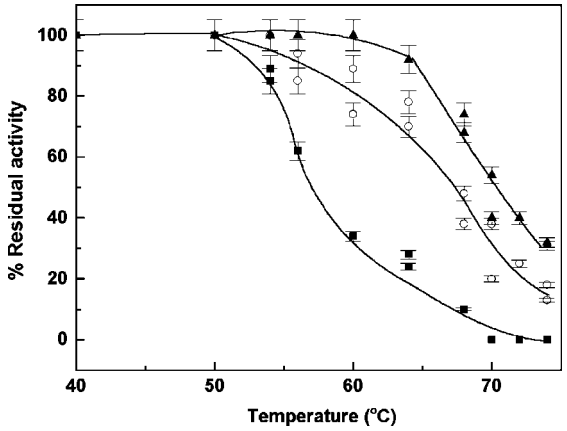

Figure 1. Thermal stability of cereal amylases: barley (■); ragi (○); Jowar (▲). Samples in 0.05 M acetate buffer containing 13.6 mM Ca^{2+}, pH 4.8, were incubated at the test temperature for 15 min, cooled rapidly to 4 °C, and assayed for residual activity at 40 °C.

Circular Dichroism (CD) Measurements. CD spectra were recorded on a Jasco J-810 spectropolarimeter calibrated with *d*-(10) camphor sulfonic acid, ammonium salt, using protein concentrations of 0.094 and 0.83 mg/mL, for far-UV and near-UV regions, respectively. All of the enzyme solutions were prepared in acetate buffer (0.05 M, pH 4.8) containing 13.6 mM calcium, unless indicated otherwise. Molar ellipticity values were expressed as mean residue ellipticity using a residue mass of 110 g mol^{-1}. Far-UV CD was recorded in the range of 190−260 nm using a 1 mm path length cell, whereas near-UV CD (240−320 nm) was recorded using a 10 mm cell. The scan speed was 10 nm/min using a bandwidth of 1 nm. An average of three runs was recorded. The secondary structure of α-amylase was analyzed using the computer program of Yang et al. (*11*).

Thermal Stability. The enzyme was incubated at different temperatures in the range of 30−85 °C for 15 min. Enzyme aliquots were immediately cooled in an ice bath and assayed for activity as described under enzyme assay. T_m, the midpoint of thermal inactivation, was calculated as the temperature at which 50% loss of activity occurred under the test conditions. Inactivation kinetics was carried out by incubating the enzyme at the test temperature. Aliquots of the enzyme were drawn at different time intervals and cooled to 4 °C, and residual activity was assayed. Activation energy (E_a), enthalpy (ΔH^*), entropy (ΔS^*), and free energy change (ΔG^*) were obtained from the Arrhenius plot.

RESULTS

Thermal Stability of Cereal Amylases. Amylase activities have been extracted from germinated barley, ragi, and jowar in 0.05 M acetate buffer, pH 4.8. Residual activities of the enzyme, incubated in the range of 40−75 °C for 15 min, are shown in **Figure 1**. Amylase from malted jowar is found to be the most thermostable (**Figure 1**), with a T_m of 70 ± 0.5 °C compared to barley (T_m = 57 ± 0.6 °C) and ragi amylase (T_m = 67 ± 0.3 °C). Hence, the major amylase from germinated jowar has been further purified and characterized.

Extraction and Purification of Sorghum Malt α-Amylase. α-Amylase is present in trace amounts in ungerminated sorghum but increases greatly during germination (~10 times). Amylase has been extracted from sorghum malt, precipitated by ammonium sulfate, and fractionated using a DEAE-cellulose column as given under Materials and Methods. This step helped to remove large amounts of unbound and contaminating proteins. Bound proteins have been eluted with 0.3 M sodium chloride with a recovery of 19.3 and a purification of 18.1-fold (**Table 1**). The active fractions have been pooled, concentrated, and loaded on a G-75 superfine column to recover the pure protein with a purification factor of 24.7 and a specific activity of 2741

Table 1. Purification and Recovery of α-Amylase from Malted Sorghum

	total activity (units)	total protein (mg)	specific activity (units/mg of protein)	purification factor	overall yield (%)
crude	139320	1257	111	1	100
0–40% precipitation	36438	61.3	594	5.3	26.1
DEAE-cellulose chromatography	27000	13.4	2014	18.1	19.3
Sephadex G-75	23850	8.7	2741	24.7	17.1

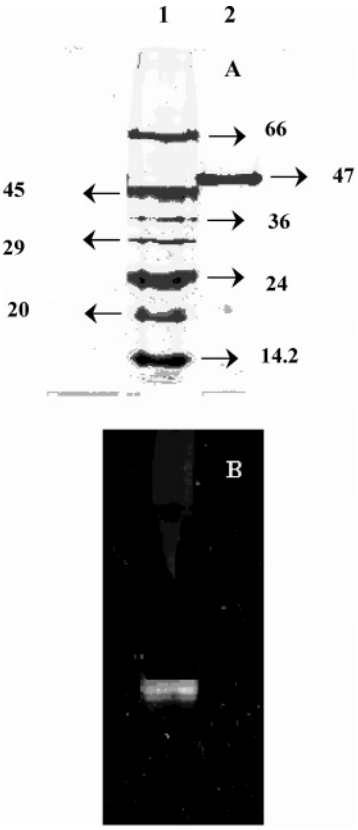

Figure 2. (**A**) SDS-PAGE of sorghum α-amylase: lane 1, molecular mass markers (Sigma), 14.2–66 kDa; lane 2, jowar α-amylase. (**B**) PAGE of purified α-amylase from malted jowar: activity staining.

units/mg. The overall yield of amylase activity from germinated jowar is 17.1%. The homogeneity of the preparation has been checked by SDS-PAGE (**Figure 2A**) and activity staining (**Figure 2B**). The enzyme appears to move as a single band, indicating the absence of hetero-subunits. The molecular mass of the protein is determined to be 47 kDa by SDS-PAGE.

It is difficult to quantitate the content of α-amylase in sorghum because of the presence of different isomers with different specific activities. However, an attempt has been made by measuring the relative proportions of different proteins extracted in the medium and correlating with activity stain of α-amylase. It was estimated to be 1.3% of the total proteins in sorghum.

Enzyme Characteristics. *Determination of K_m and V_{max}.* The apparent Michaelis constant (K_m) and V_{max} for sorghum α-amylase were determined for gelatinized starch to be 1.55% and 6535 μmol/min/mg of protein, respectively.

pH and Temperature Optimum of Sorghum Amylase. The enzyme was active over a wide pH range with a pH optimum lying between 4.5 and 5.0, similar to other cereal amylases. Sorghum α-amylase was found to have a temperature optimum of 60 ± 0.5 °C, which is higher than that reported for ragi (*12*), barley (*13*), wheat (*14*), pearl millet (*15*), and immature barley (*16*).

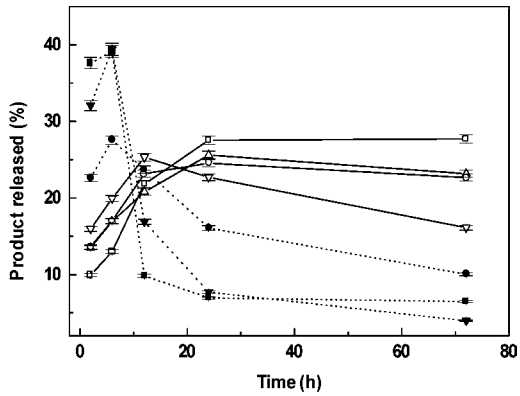

Figure 3. Product distribution curves in the hydrolysis of maltodextrin catalyzed by α-amylase. Hydrolysis was carried out at pH 4.8 and 60 °C for different time intervals; products were concentrated and analyzed by HPLC on a Waters μBondapak NH_2 column (3.9 × 300 mm, 10 μm) using an acetonitrile/water solvent system (70:30) at a flow rate of 1 mL/min as given under Materials and Methods. The amounts of products (DP1–DP7) released were quantified by peak integration, using standards for refractive index detection: (△) glucose; (□) maltose; (○) maltotriose; (▽) maltotetraose; (●) maltopentaose; (▼) maltohexaose; (■) maltoheptaose.

Product Identification by HPLC. Products of enzymatic hydrolysis of maltodextrin were identified using oligomers of maltose as standards. Glucose and oligosaccharides, varying in their DP ranging from 2 to 7, released during the course of the reaction were trapped at different intervals of time by stopping the reaction with ethanol. Higher oligosaccharides (DP 5−7) were the major products released in the early stages of the hydrolysis (2−6 h) (**Figure 3**). By 24 h, there was an increase in the concentrations of low molecular weight oligosaccharides (1−4) with a corresponding decrease in the higher oligosaccharides (>4). The final products of the reaction were maltose, glucose, and maltotriose.

Spectral Properties. The enzyme in acetate buffer (0.05 M, pH 4.8) with 13.6 mM Ca^{2+} exhibited an intrinsic fluorescence when excited at 280 nm. The enzyme exhibited an emission maximum at 346 nm, indicating that the tryptophan residues were in a fairly exposed environment (data not shown).

The near-UV CD spectra of the enzyme revealed minima at 284 and 272 nm and a maximum at 250 nm with shoulders at 263 nm (negative band) and 255 nm (positive band) (**Figure 4A**). The far-UV CD showed a minimum at 213 nm, indicating predominantly β structure (**Figure 4B**). The analysis of CD data shows a helix content of 19%, β structure of 48%, β turn of 12%, and aperiodic of 21%.

Measurement of Thermal Inactivation Parameters. Thermal inactivation kinetics of the enzyme was determined by incubation of the enzyme at the test temperature for various intervals of time. The thermal inactivation was found to follow first-order kinetics, indicating the existence of a single inactivation mechanism. The time at which loss of activity reached 50% was taken as the experimental half-life for the enzyme, and the

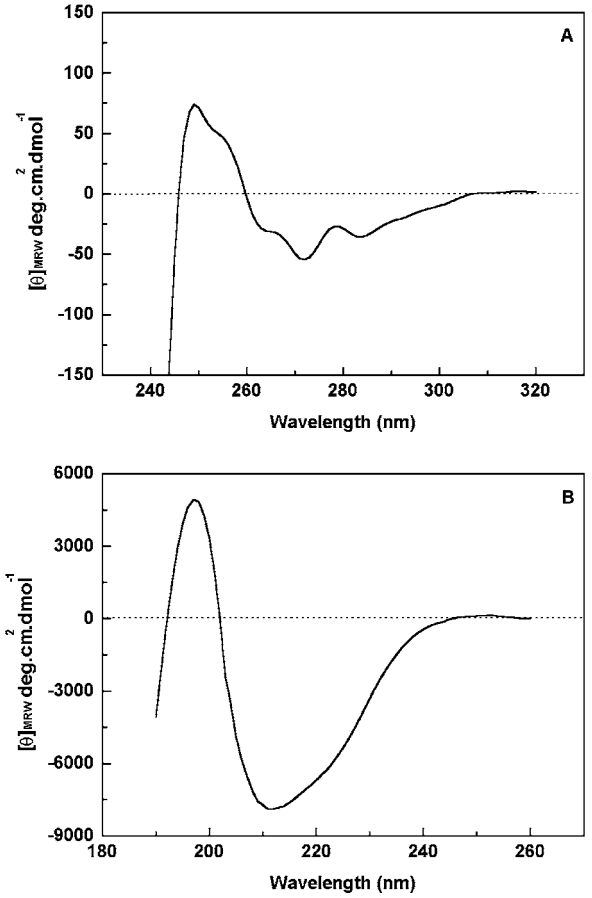

Figure 4. CD spectra of jowar α-amylase: (**A**) near-UV; (**B**) far-UV. Protein concentration used for near-UV CD was 0.83 mg/mL, and 0.094 mg/mL was used for far-UV CD. The spectra were run in the ranges of 320–240 nm (for near-UV CD) and 260–190 nm (for far-UV CD) at a scan speed of 10 nm/min in a cell with a path length of 1 cm (near-UV CD) or 1 mm (far-UV). An average of three runs was taken. A mean residue weight of 110 was considered for calculating mean residue ellipticity.

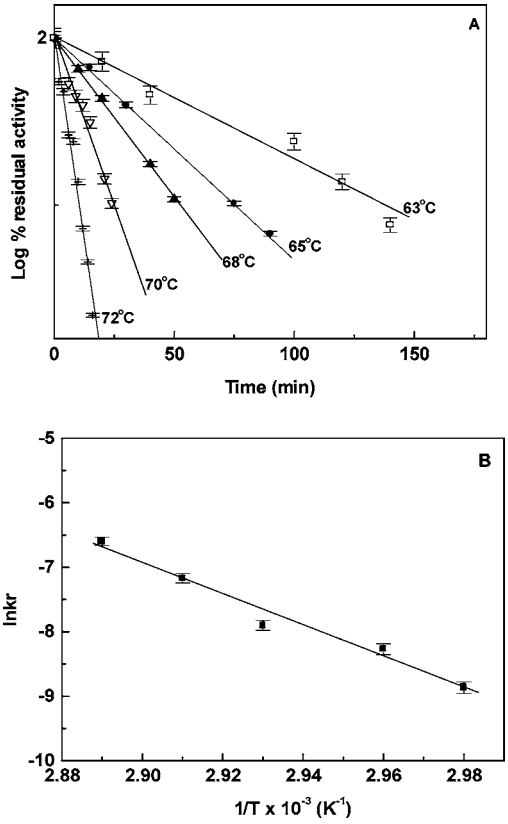

Figure 5. (**A**) Thermal inactivation profile of jowar α-amylase. Enzyme (4.93×10^{-3} mg/mL) was incubated at 63 °C (□), 65 °C (●), 68 °C (▲), 70 °C (▽), and 72 °C (+). Aliquots were drawn at different intervals of time and cooled to 4 °C, and the remaining activity was measured under standard assay conditions. (**B**) Arrhenius plot for the thermal inactivation of jowar α-amylase. The slope of the plot equals $-E_a/R$.

rate constant was determined (**Figure 5A**). From the rate constants at different temperatures, an Arrhenius plot was constructed and thermodynamic parameters were estimated. E_a (activation energy) was determined to be 45.3 ± 0.2 kcal mol^{-1} from the slope of the Arrhenius plot (**Figure 5B**). The activation enthalpy (ΔH^*), entropy (ΔS^*), and free energy change (ΔG^*) were calculated to be 44.6 ± 0.2 kcal mol^{-1}, 57.1 ± 0.3 cal mol^{-1} K^{-1}, and 25.2 ± 0.2 kcal mol^{-1}, respectively.

Midpoint of Thermal Inactivation, T_m. The thermal stability of α-amylase was tested by incubating the enzyme for 15 min at different temperatures (30−85 °C) and measuring the residual activities. The enzyme was stable up to 60 °C with a T_m of 69.6 ± 0.3 °C.

Effect of Salts and Sugars on Thermal Stability of α-Amylase from Sorghum. Addition of NaCl decreased the thermal stability of α-amylase. The T_m of α-amylase decreased to 66.3 ± 0.3, 58.1 ± 0.2, and 48.1 ± 0.5 °C in the presence of 0.1, 0.5, and 1 M NaCl, respectively (**Figure 6A**).

Sucrose was a stabilizer of α-amylase. The T_m in the presence of 1 M sucrose shifted from 69.6 ± 0.3 to 77.3 ± 0.3 °C (**Figure 6B**).

DISCUSSION

It is well-known that germination of cereals causes a marked increase in their amylase activities (*17*). The chief function of starch-splitting enzymes in the resting grain is one of saccharification, but an additional property is imparted by germination and is referred to as liquefaction. This results in the conversion of thick starch pastes into thin watery liquids, making it easier to consume the food. Liquefaction also results in the food absorbing less water, rendering it more "nutrient dense".

One of the objectives of this work was to find a suitable and thermostable source of α-amylase for addition to supplementary foods in an effort to enhance their nutrient density. Thermal stability of α-amylase is a desirable feature as the supplementary food is reconstituted with hot water/milk before consumption. Some of the α-amylases are derived from cereals such as barley (*13*), rye (*18*), wheat (*14*), and ragi (*12*).

In the present study, cereal amylase activities from barley, ragi, and jowar with respect to thermal stability have been studied. Jowar amylase has better thermal stability compared to other cereal sources with a T_m of 70 ± 0.5 °C. Malted sorghum (jowar) is also shown to have ∼10 times greater amylase activity compared to ungerminated grain. Thus, isolation and characterization of the pure amylase are important so that future work to understand the basis for thermal stability is possible.

α-Amylase has been purified from malted sorghum to homogeneity with a molecular mass of 47 kDa. The molecular masses of most cereal α-amylases range from 42 to 46 kDa. The α-amylases from wheat (*14*) and ragi (*12*) have molecular masses of 42 and 47 kDa, respectively. However, there are exceptions wherein the molecular mass ranged from 20 to 57 kDa in the case of amylases isolated from wheat (*14*) and barley (*13*).

Table 2. Summary of α-Amylase Characteristics from Different Cereal Sources

source		specific activity (units/mg of protein)	pH optimum	temp optimum (°C)	mol mass (kDa) SDS-PAGE	K_m	V_{max} (μmol of maltose/ mg/min)
malted sorghum		2741	4.5–5.0	60 ± 0.5	47	1.55%	6535
malted ragi[a]	α_1	1500	5.0	45		0.59%	2381
	α_2	974	5.5	50	47	1.1%	1111
	α_3	1773	5.0	45		0.53%	2778
immature barley[b]		–[g]	5.5	45–50	–	–	–
malted barley[c]	α_1	–	5.5	55	52	–	–
	α_2						
malted wheat[d]	α_1	1480	5.5	55	41.5–42.5	-	-
	α_2	1300	5.7				
	α_3	1510	5.5				
immature wheat[e]	α_1	–	3.6–5.75	–	52–54	2.5×10^{-4} g/mL	–
	α_2	–	3.6–5.75			5.3×10^{-4} g/mL	
	α_3	–	3.6–5.75			2.3×10^{-4} g/mL	
pearl millet[f]		–	4.4–4.8	55	22–53	–	–

[a] Reference *12*. [b] Reference *16*. [c] Reference *13*. [d] Reference *14*. [e] Reference *22*. [f] Reference *15*. [g] –, not given.

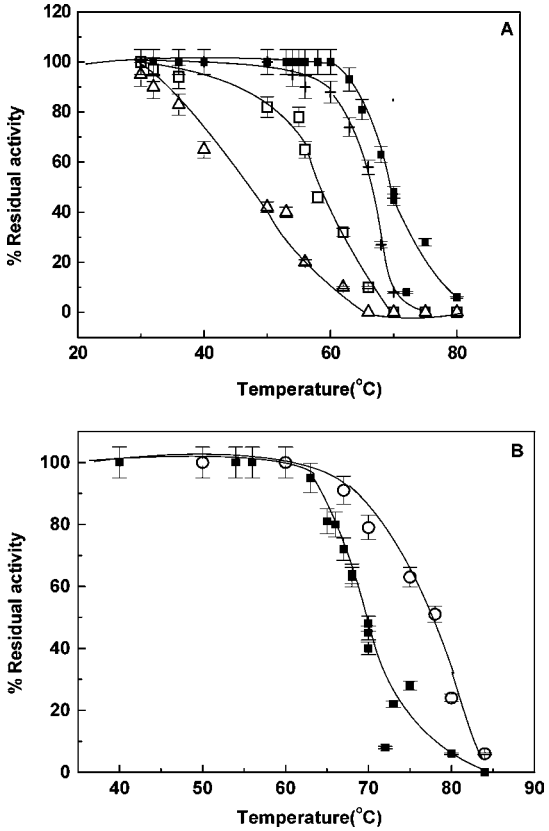

Figure 6. Thermal stability of α-amylase in the presence of additives: (**A**) effect of NaCl on thermal stability of α-amylase [enzyme concentration (4.9×10^{-3} mg/mL) with no salt (■), 0.1 M NaCl (+), 0.5 M NaCl (□), and 1 M NaCl (△)]; (**B**) effect of sucrose on thermal stability of α-amylase [enzyme concentration (4.9×10^{-3} mg/mL) with no salt (■) and 1 M sucrose (○), incubated for 15 min at the test temperature]. Samples were cooled to 4 °C, and residual activity was assayed as described under Enzyme Activity Assay.

In the present study, K_m and V_{max} values for gelatinized starch were found to be 1.55% and 6535 μmol/min/mg, respectively. K_m values for ragi α_3-amylase were found to be in the range of 0.53−1.0% for cereal starches, and the V_{max} values were found to be in the range of 2381−2778 units/mg/min (*12*). α-Amylase was found to have predominant β-structure (∼60%) and α-helical content of 19%. The CD spectra of Ca^{2+}-depleted and Ca^{2+}-saturated molecules of barley α-amylase were reported (*19*). The α-helical content of both forms was estimated to be 27%. Ca^{2+} depletion decreased the mean residue ellipticity between 200 and 208 nm, indicating an increase in the random structure emphasizing the role for Ca^{2+} in stabilizing the structure of the enzyme.

α-Amylase from sorghum was active over a wide pH range with a pH optimum of 4.5−5.0, akin to other cereal amylases. Optimum pH values for pearl millet (*15*) and malted barley (*13*) were reported to be 4.4−4.8 and 5.5, respectively. In the present study, α-amylase was found to have a temperature optimum of 60 ± 0.5 °C, which is higher than that reported for ragi (*12*), barley (*13*), wheat (*14*), pearl millet (*15*), and immature barley (*16*). A summary of enzyme characteristics from different plant sources is given in **Table 2**.

Many factors such as the purity of the preparation and the presence of calcium as well as substrate and other stabilizers can affect the thermal stability of the α-amylase. The stabilizing effect of substrate can be attributed to the presence of small amounts of Ca^{2+} present as impurities in the starch, and this can stabilize conformation of the enzyme in a more rigid and stable form against denaturing conditions (*20*). To determine the activation energy of the reaction catalyzed by α-amylase, an Arrhenius plot has been made covering the temperature range of 63−72 °C. The value of E_a, obtained from the slope of the line, is 45.3 ± 0.2 kcal mol^{-1}. The activation enthalpy (ΔH^*), entropy (ΔS^*), and free energy change (ΔG^*) are calculated to be 44.6 ± 0.2 kcal mol^{-1}, 57.1 ± 0.3 cal mol^{-1} K^{-1}, and 25.2 ± 0.2 kcal mol^{-1}, respectively. The values obtained were comparable to ΔH^*, ΔS^*, and ΔG^* values (46.1 kcal mol^{-1}, 55.6 cal mol^{-1} K^{-1}, and 26.0 kcal mol^{-1}) of a thermophilic bacterium (*21*).

NaCl and sucrose are added to supplementary foods to modify and improve the acceptability of the food. Sucrose is found to have a stabilizing effect on the enzyme, whereas NaCl is found to destabilize the enzyme activity without significant change in the enzyme structure.

Malted jowar could become a commercially viable source of α-amylase for viscosity reduction and to increase the nutrient density of supplementary foods. The thermal stability of the enzyme could be an added advantage.

ABBREVIATIONS USED

SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; K_m, Michaelis constant; V_{max}, maximum velocity; CD, circular dichroism; DP, degree of polymerization; UV, ultraviolet; TEMED, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine; NaCl, sodium chloride; HCl, hydrochloric acid.

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