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Full Length Research paper

Comparative studies on properties of amylases extracted from kilned and unkilned malted sorghum and corn

OYEWOLE O. I.[1]* and AGBOOLA F. K.[2]

[1]Department of Biochemistry, Osun State University, Osogbo, Nigeria.
[2]Department of Biochemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.

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This study investigated the activities of α-amylase, β-amylase and glucoamylase extracted from kilned and unkilned sorghum and corn. Dry grains of sorghum and corn were obtained, steeped and allowed to undergo malting at room temperature for 48 h. Part of the malted starch was kilned by taken into an oven at 50°C for 24 h while the other part was derootted by hand before further processing. α-amylase, β-amylase and glucoamylase were extracted from the kilned and unkilned malted cereals and assayed. Results obtained showed that sorghum is richer in amylases than corn. The activities of the three enzymes were higher in unkilned malt than kilned malt which indicates an appreciable loss in enzymes activities during kilning. The cereals are also rich in glucoamylase compared to the other two enzymes. All the enzymes have appreciable glucose yield on maltose substrate. These results demonstrated that sorghum and corn are good sources of amylases which are the basic enzymes required for hydrolysis of starch to glucose in many industrial processes most especially in brewing.

Key words: Amylase, sorghum, corn, kilned malt, unkilned malt.

INTRODUCTION

Amylases are the enzymes responsible for breaking down amylose (starch). There are three types of amylase namely: α-amylase, β-amylase and glucoamylase. α-amylase (endo-1, 4-α-D-glucan glucohydrolase, EC 3.2.1.1) is an extra cellular enzyme that randomly cleave the 1,4-α-D-glucosidic linkages between adjacent glucose units in the linear amylase chain. This endozyme split the substrate in the interior of the molecules. β-amylase (β-1, 4-glucan maltohydrolase, EC 3.2.1.2) is an exoacting enzyme that cleaves non-reducing chain ends of amylase, amylopectin and glycogen molecules. It hydrolyses alternate glycosidic linkages yielding glycoside linkages maltose. Glucoamylase (exo-1, 4-α-D-glucan glucano-hydrolase, EC 3.2.1.3) hydrolyses single glucose units from the non-reducing ends of amylase and amylopectin in a stepwise manner. Biologically active amylases extracted from plants source are among the most important enzymes and are of great significance in present-day biotechnology. They could be potentially useful in the pharmaceutical and fine-chemical industries if enzymes with suitable properties could be prepared. Interestingly, the first enzyme produced industrially was amylase from a fungal source in 1894 which was used as a pharmaceutical aid for the treatment of digestive disorders (Manners and Marshall, 1969). With the advent of new frontiers in biotechnology, the spectrum of amylase application has widened in many other fields such as clinical, medicinal and analytical chemistry as well as their widespread application in starch saccharification and in the textile, food, brewing and distilling industries (Alli et al., 1998).

Sorghum (*Sorghum bicolor*) is a cereal crop cultivated in warmer climates worldwide and utilized for grain, fiber and fodder (Hulse et al., 1980). Corn or maize (*Zea mays*) constitute a staple food in many regions of the world and is used in the preparation of corn flakes, corn meal, porridge, corn bread and other baked products

*Corresponding author. E-mail: ioluoye@yahoo.com. Tel: +2348066808560.

Table 1. Amylases activities from maltose curve.

	Sorghum		Corn	
Enzyme	**Unkilned**	**Kilned**	**Unkilned**	**Kilned**
α-amylase (mg maltose/ml/min)	1.20±0.07[a]	0.44±0.02[b]	0.52±0.03[c]	0.18±0.01[d]
β-amylase (mg maltose/ml/min)	2.10±0.12[a]	0.63±0.05[b]	0.94±0.05[c]	0.41±0.02[d]
Glucoamylase (mg maltose/ml/min)	2.78±0.38[a]	0.94±0.04[b]	1.22±0.05[c]	0.75±0.03[d]

Each value is a mean of 5 determinations ±SD. Values with different alphabetical superscript ([a, b, c, d]) along a row are statistically different at P<0.05.

(EtokAkpan, 1988). Starch from maize can also be made into plastics, fabrics adhesives and many other chemical products.

MATERIALS AND METHODS

Collection of plant material

Dry grains of sorghum (*S. bicolor*) were obtained from Derivative Company Ltd. Lagos, while corn (*Z. mays*) was obtained from Ora Gada Market in Ogbomoso, Nigeria. The grains were screened to remove broken seed and other impurity.

Chemicals/reagents

Dinitrosalicylic acid, sodium hydrogen phosphate, ammonium sulphate, calcium chloride, soluble starch, disodium hydrogen phosphate, ethanol, phosphoric acid, bovine serum albumin (BSA), coomasie brilliant blue G-25, sodium potassium tartarate, anhydrous sodium carbonate, maltose, sodium hydroxide, sodium sulphate, cupric sulphate, sodium acetate and glacial acetic acid were obtained from British Drug House (BDH), Poole, England.

Equipments

Laboratory equipment used includes AB205 Meltler Toledo weighing balance made in Switzerland, LEC Grant Incubator along with Golden lamp made in USA. Double Beam UV spectrophotometer made in Cambridge, England and centrifuge model 800 D micro field instrument made in England with maximum speed of 4000 rpm.

Steeping and germination of grain samples

200 g of the grains were steeped for 48 h by soaking in distilled water at room temperature. The water was changed every 8 h to prevent microbial growth. After 48 h, the water was drained from the sample and the damp seeds spread out in a malting chamber to germinate for 48 h at room temperature. The germinated grains were divided into two parts. The first part was taken into an oven at 50°C for 24 h. This is a process called kilning while the other part (unkilned) was derootted by hand before further processing.

Extraction of amylases from kilned and unkilned malt

5g of the malt was homogenized with 3 volume of the respective homogenization buffer (0.1 M acetate buffer, pH 5.5 for α-amylase, 0.1 M phosphate buffer, pH 6.0 for β-amylase and 0.5 M acetate buffer, pH 4.5 for glucoamylase) and poured in a clean beaker. They were kept in the refrigerator for 1 h with intermittent stirring every 10 min. This was followed by centrifugation at 6000 rpm for 20 min to remove the debris. It was then filtered with two layers of cheese clothes to remove fat deposits (Anon, 1986). Assay for protein and enzyme activities were then carried out.

Enzyme assay

The method of EtokAkpan (1988) was employed in the determination of enzyme activities. Crude preparations of each enzyme were diluted 2:10. The reaction mixture contained 0.5 ml of 1% starch solution, 0.48 ml distilled water and 0.02 ml of the enzyme solution. 1 ml of freshly prepared colour reagent was added to the reaction mixture and boiled for 5 min in cold running water. The absorbance was taken at 470 nm. The procedure was repeated for all amylases using respective colour reagent. Maltose concentration was determined as described by Fix and Fix (1997) and protein was measured by the Bradford method (Metwally, 1998).

Statistical analysis

Data obtained were reported as mean of 5 replicates ±SD. Statistical significance was determined by using one way analysis of variance (ANOVA) followed by Duncan Multiple Range Test and differences were considered significant at P<0.05.

RESULTS

Tables 1, 2 and 3 show the activities of the amylases in kilned and unkilned malt extrapolated from protein, maltose and glucose standard curves respectively. From the results, sorghum is richer in amylases than corn. The results also revealed that activities of the three enzymes are higher in unkilned malt than in kilned malt with all the substrates. It can also be seen in the tables that glucoamylase has the highest activities among the three enzymes followed by α-amylase in both kilned and unkilned malt. The activities of the enzymes with glucose substrate are the highest compared to other substrate that is maltose and protein as can be seen in the tables.

DISCUSSION

From the results obtained for enzymes activities using

Table 2. Amylases activities from protein curve.

	Sorghum		Corn	
Enzyme	**Unkilned**	**Kilned**	**Unkilned**	**Kilned**
α-amylase (mg protein/ml/min)	0.072±0.003[a]	0.039±0.002[b]	0.049±0.002[c]	0.030±0.001[d]
β-amylase (mg protein/ml/min)	0.084±0.004[a]	0.041±0.001[b]	0.060±0.004[c]	0.038±0.002[b]
Glucoamylase (mg protein/ml/min)	0.160±0.009[a]	0.068±0.005[b]	0.133±0.008[c]	0.074±0.004[d]

Each value is a mean of 5 determinations ±SD. Values with different alphabetical superscript ([a, b, c, d]) along a row are statistically different at P<0.05.

Table 3. Amylases activities from glucose curve.

	Sorghum		Corn	
Enzyme	**Unkilned**	**Kilned**	**Unkilned**	**Kilned**
α-amylase (mg glucose/ml/min)	1.64±0.11[a]	0.72±0.06[b]	1.17±0.08[c]	0.52±0.04[d]
β-amylase (mg glucose/ml/min)	2.23±0.13[a]	1.14±0.08[b]	1.96±0.14[c]	0.98±0.05[d]
Glucoamylase (mg glucose/ml/min)	9.90±2.42[a]	5.65±1.60[b]	5.20±1.48[b]	2.20±0.14[c]

Each value is a mean of 5 determinations ±SD. Values with different alphabetical superscript ([a, b, c, d]) along a row are statistically different at P<0.05.

different substrates, all the three enzymes have higher activities in unkilned malt compared to kilned malt. This might be due to loss of enzyme activities during kilning. Kilning involve heat treatment which can have adverse effect on enzyme activities. Enzymes are protein and when subjected to high temperature beyond the optimum, they are denatured due to loss in conformation (Yun and Matheson, 1990). It is very important that maximum activity of the enzymes is retained during the preparation as enzymes for industrial use are sold on the basis of overall activity (Pandey, 1992). The reduction can also be as a result of inactivation by the buffer solution employed during the preparation of the kilned cereals. Amylases from sorghum and corn have been reported to be reversibly unfolded by chemical denaturants (EtokAkpan and Palmer, 1990). Enzyme inactivation can be caused by heat, proteolysis, sub-optimal pH, oxidation, denaturants, irreversible inhibitors and loss of cofactors or coenzymes (Palmer et al., 1989). Proteolytic activities can also be responsible for the observed reduction in activities in kilned malt. Proteolysis is most likely to occur in the early stages of extraction and purification when the proteases responsible for protein turnover in living cells are still present. In their native conformations, enzymes have highly structured domains which are resistant to attack by proteases because many of the peptide bonds are mechanically inaccessible and because many proteases are highly specific. Therefore, it is important to keep enzyme preparations cold to maintain their native conformation and slow any protease action that may occur (Aniche and Palmer, 1990). The key to maintaining enzyme activity is the maintenance of their conformation so as to prevent unfolding, aggregation and changes in the covalent structure (Glennie and Wright, 1986). From the results obtained, it can also be seen that kilned and unkilned sorghum and corn are richer in glucoamylase than the other. This is followed by β-amylase.

It has been reported that glucoamylase and β-amylase are usually of plant origin while α-amylase is obtained mostly in microorganism (EtokAkpan and Palmer, 1990). The reason might also be because glucoamylase has higher optimum temperature (60°C) than β-amylase (55°C) and α-amylase (45°C) meaning that it can withstand heat treatment than the other two enzymes (EtokAkpan, 1988). The differences in activities recorded for the three enzymes with the substrates arise probably because they have different cleaving points on the substrate. Alpha amylases cleave maltose units internally bringing little change in sweetness and large decrease in viscosity. β-amylase is an exoacting enzyme that cleave maltose units from the ends to bring large change in sweetness and little decrease in viscosity (Sen et al., 1997). Glucoamylase hydrolyses single glucose units from the non-reducing ends of amylose and amylopectin in a stepwise manner bringing large change in sweetness and little decrease in viscosity (Metwally, 1998).

Conclusion

Results obtained in this study showed that sorghum and corn are good sources of alpha-amylase, beta-amylase and glucoamylase. The enzymes are present in appreciable quantities in these cereals and can be extracted for industrial use. It is also evident from this study that appreciable amount of these enzymes were

lost during the process of kilning which involve drying. Therefore, heat processing should be avoided in order to obtain maximum yield of amylases from this plant source. Further kinetic study is required on the extracted enzymes to fully understand their properties before embarking on large scale production of the enzymes from this source.

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