Evaluation of beta amylase activity in selected Ghanaian varieties of sweet potato (*Ipomea batatas*).

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ABSTRACT

In order to select a suitable Ghanaian variety of sweet potato for use as a source of enzymes for the production of glucose syrups, four common varieties of sweet potatoes - Sauti, Santom pona, Faara and Okumkom - cultivated in two different agro-ecological zones of Ghana were evaluated for β-amylase activity. Results indicated that the pink-skinned varieties - Faara and Okumkom – exhibited a significantly higher β-amylase activity ($p<0.01$) than the yellow-skinned varieties – Sauti and Santom pona. The β-amylase activity of Sauti was significantly lower ($p<0.01$) than that of Santom pona. There was however no significant difference between the β-amylase activity of Faara and Okumkom. Sweet potatoes cultivated in the Forest zone showed significantly higher β-amylase activity ($p<0.01$) than those from the savannah zone. It is concluded that Faara and Okumkom varieties cultivated in the forest zone would be the most suitable varieties for use as a source of β-amylase for the hydrolysis of starchy materials in the production of glucose syrups.

Keywords: β-amylase, sweet potato, varieties.

INTRODUCTION

The three major amylolytic enzymes in storage sweet potato roots are α-amylase, β-amylase and starch phosphorylase (Hagenimana and Simard, 1994). β-amylase is however the most abundant of the three and constitutes about 5% of the total soluble protein of the tuberous root (Nakamura

et al., 1991). The importance of sweet potato as an important source of *β*-amylase has been documented by several workers (Chang *et al*., 1996; Cheong *et al.,* 1995; Takahata *et al*., 1994; Jiang *et al*., 1994; Hagenimana *et al.*, 1994a; Hagenimana *et al.*, 1994b; Toda *et al*., 1993). Sweet potato *β*-amylase was the first amylase to be obtained in crystalline form (Bernfeld, 1955). Unlike *α*-amylase, which is localised in the outer layers of the root, *β*-amylase is ubiquitously distributed throughout the root (Hagenimana *et al*., 1992). Cheong *et al*. (1995) reports that sweet potato *β*-amylase is a tetramer of identical subunits which are arranged to exhibit 222 molecular symmetry. The tetrameric nature of sweet potato *β*-amylase uniquely distinguishes it from other *β*-amylases, which are monomeric (Nakamura *et al*., 1991). The subunit of the enzyme is a single polypeptide consisting of 498 amino acid residues. It shows 50-60% identity in the amino acid sequence with those of *β*-amylases from soybean and barley, and about 25% identity with those of three bacterial *β*-amylases. The Glu 187 residue is believed to play an important role in catalysis, whilst the Cys 96 residue is important in the inactivation of enzyme activity by sulfhydryl reagents (Toda *et al*., 1993; Cheong *et al*., 1995). Different varieties of sweet potato have been shown to exhibit varying levels of *β*-amylase activity. According to Morrison *et al*. (1993) staple-type lines of sweet potato have higher levels of *β*-amylase synthesis than traditional-type lines. Whereas the staple-type lines (non-sweet) were showing *β*-amylase protein contents of between 361-374*μ*g/g of fresh root, the traditional-type lines (sweet) had levels in the range of 12-60*μ*g/g. Takahata *et al*. (1994) reports *β*-amylase activity ranges of between 600 and 1300 *μ*mol/min/g fresh weight for six sweet potato lines assessed. Working with two varieties of sweet potato, Chang *et al*. (1996) showed that *β*-amylase isolated from different varieties of sweet potato had different specific activities and kinetic constants. However the pH and temperature optima, pH stability and thermostability did not seem to vary significantly between varieties. Storage of sweet potatoes significantly increases the *β*-amylase activity up to 90 days of storage after which the activity declines. The peak period and the rate of increase however vary between varieties (Morrison *et al*., 1993). Several varieties of sweet potato are cultivated in Ghana for food uses. In order to exploit the *β*-amylase potential of the sweet potato for the hydrolysis of starchy materials it was considered necessary to identify the most suitable variety for use as a source of *β*-amylase. This study therefore sought to evaluate the *β*-amylase activity of the different sweet potato varieties.

MATERIALS AND METHODS

Materials and Diagnostic Kits

Four sweet potato varieties were harvested from the experimental farms of the Crops Research Institute, Kumasi, Ghana. The names of the varieties were Sauti, Santom Pona, Okumkom, and Faara. A diagnostic kit comprising p-nitrophenyl maltopentaoside (PNPG5) for β-amylase analysis was obtained from Megazyme International Ltd. in Ireland..

Experimental Design

This experiment was designed to determine whether varietal differences, and agro-ecological factors affect the development of β-amylase in sweet potatoes. Four varieties of sweet potatoes were investigated in two agro-ecological zones. Soil samples from the two agro-ecological zones were analysed to provide an understanding of any possible differences that may be observed in the β-amylase levels in samples from the two zones.

Cultivation, Sampling and Sample Preparation of Sweet Potatoes

Four varieties of sweet potato were cultivated in two ecologically different locations (Kumasi, in the forest zone; Okyereko, in the coastal savannah) in Ghana. Sampling of the sweet potatoes started three months after planting, and continued at two-week intervals for two months. The sampled potatoes were washed, sliced and treated with 1% Sodium metabisulphite solution before drying at 40^{o}C for 15 hours. After drying the chips were milled, screened through a 500μm mesh and stored at 5^{o}C prior to enzyme assay.

β-Amylase Assay

The assay procedure was based on the method of Mathewson and Seabourn (1983) and McLeary and Codd (1989). It employs a high purity α-glucosidase and p-nitrophenyl-α-D-maltopentaose (PNPG5) as substrate. The level of α-glucosidase used ensures maximum sensitivity of the assay. On hydrolysis of p-nitrophenyl maltopentaoside to maltose and p-nitrophenyl maltotrioside by β-amylase, the nitrophenyl trioside is immediately cleaved to glucose and free p-nitrophenol by the α-glucosidase present in the substrate mixture. Thus the rate of release of p-nitrophenol relates directly to the rate of release of maltose by β-amylase. The samples (0.5g) were extracted with 5.0ml of Buffer A of pH 8.0 (prepared from Trizma Base

{Tris[hydroxymethyl]aminomethane}, Sodium EDTA and Cysteine), over a one hour period and then centrifuged and diluted 1250 times with Buffer B (100mM sodium maleate solution of pH 6.2). Substrate and the enzyme extract (0.2 ml each) were pre-incubated and reacted with each other at 40^{o}C for exactly 10 minutes. The reaction was stopped by the addition of 1% Trizma base (3.0 ml.) and the absorbance read at 410 nm. A reaction blank was prepared by adding 3.0 ml of the stopping reagent to the pre-equilibrated substrate before the addition of the enzyme extract. Beta amylase activity was appropriately calculated from the absorbance values.

Soil Analysis

In order to explain the effects of the different agro-ecological conditions on the beta amylase development the soil from the different ecological zones were analysed for selected parameters.

Electrical Conductivity and pH

Twenty (20) grams of air dry soil was shaken with 50ml of water at 20^{o}C for 2hrs. The electrical conductivity and pH of the extract were respectively determined with a conductivity meter and a pH meter (Hesse, 1971).

Exchangeable Acidity

Fourty (40) grams of air dry soiil was extracted with 100ml of 1M KCl solution and filtered. Twenty (20) millilitres of the extract was titrated with 0.02M NaOH solution using phenolphthalein as indicator. Total exchangeable acidity was calculated from the amount of base used (Hesse, 1971).

Organic Matter and Organic Carbon

One (1) gram of air dry soil was suspended in 10ml of 0.17M $K_2Cr_2O_7$ solution. The suspension was digested in 20ml conc. H_2SO_4 for 30mins. Twenty (20)ml. Of water was added and allowed to cool. Ten (10) ml of H_3PO_4 (85%) was then added and immediately titrated with 1M $FeSO_4$ solution using 3ml of diphenylamine-4-sulphonic acid barium salt as indicator. The differnce between the titre value and that of a blank determination was used to calculate the percentage carbon (%C). The percentage organic matter was obtained by multiplying the %C by a conversion factor of 1.724, on the premise that 58% of organic matter is carbon (Hesse, 1971).

Available Phosphorus

Two hundred (200) grams of air dry soil was extracted with an extraction medium (mixture of 1M HCl and 1M NH_4F) for one minute. Two (2) ml of the extract was mixed with 8ml boric acid (0.5%) and 2ml of a mixed reagent (ammonium molybdate solution and potassium antimonyl tartarate). After 30 min the absorbance of the solution was measured at 880nm and the milligrams phosphate read from a standard curve (Bray, 1945).

Available Nitrogen

The Kjeldahl metjhod was employed. The air dry soil sample (0.5g) was digested at 30^{o}C with 10ml conc. H_2SO_4 in the presence of selenium catalyst for 30min. The digest was diluted to 50ml with water and an aliquote was mixed with 30ml NaOH (30%) and distilled into a mixture of 1% boric acid and a mixed indicator solution containing methyl red and bromocresol green. The distillate was titrated against .002M $KH(IO_3)_2$ solution. A blank was prepared with boric acid and indictor solution only. The difference between the titre values of the blank and the sample was used to calculate the percentage nitrogen (%N) (Hesse, 1971)

Total Exchangeable Bases (TEB)

Five (5) grams of air dry soil was thoroughly mixed with 25g sand and leached with 100ml of a 1:1 mixture of 1M Ammonium acetate solution and ethanol . Total exchangeable bases was obtained by determining the amount of potassium, sodoium, calcium and magnesium in the leacheate and summing these up. Potassium and sodium were determined flame photometrically by comparing the intensities of radiation emitted by K and Na atoms in the leacheate with a standard series of KCl and NaCl solutions respectively. The standard solutions were prepared with a 1:1 mixture of ammonium acetate and ethanol. Calcium and magnesium were determined using atomic absorption spectroscopy by comparing the absorbances of a series of standard Cacl2 and $MgCl_2$ solutions prepared in situ from HCl, $CaCO_3$ and $MgSO_4.7H_2O$. (Blakemore, et al., 1972)

Cation Exchange Capacity (CEC) and % Base Saturation (%BS)

Five (5) grams of air dry soil was thoroughly mixed with 25g sand and leached with 100ml of 1M Ammonium acetate solution. The sodium in the leacheate was detrmined flame photometrically and expressed as CEC. A standard curve was prepared using a series of standard solutions of NaCl in Ammonium acetate against which the sodium in the leacheate was

compared. Percentage base saturation was calculated as the ratio of TEB to CEC and expressed as a perecentage (Blakemore, et al., 1972)

RESULTS AND DISCUSSION

In Figure 1 the variations in β-amylase activity between four varieties of sweet potatoes, and also between the two agro-ecological zones is graphically displayed. Table 1 shows the characteristics of the soils in the two agro-ecological zones where the sweet potatoes were cultivated.

Table 1. Characteristics of soils from the Forest and Savannah Zones

Parameters	Characteristics as per specified zone	
	Forest Zone	Savannah Zone
pH	5.83	5.47
Electrical Condictivity (ms/cm)	0.03	0.04
% Nitrogen	0.28	0.13
Available Phosphorus (mg/kg)	7.67	14.99
Available Potassium (mg/kg)	115.00	92.00
% Organic Carbon	2.24	0.95
% Organic Matter	3.86	1.63
Total Exchangeable Bases (mg/kg)	3.60	3.28
Exchangeable Acidity (mg/kg)	0.15	0.20
Cation exchange Capacity	3.75	4.81
% Base Saturation	96.00	95.84

The variety *Okumkom* showed the highest level of enzyme activity amongst all the varieties. There is however no significant difference between the β-amylase activity of this variety and that of the variety *Faara*. The literature will be reviewed to find out if there has been any established relationship between the skin colour of sweet potatoes and their physico-chemical and functional characteristics that have a bearing on enzyme activity. The sweet potatoes from the forest zone had significantly higher β-amylase activity than those from the savannah zone. This difference may be attributable to the either the weather conditions or the soil conditions. An assessment of the nutrient and physico-chemical characteristics of the soils from the two agro-ecological zones indicate that the principal difference between the two soils is in the

nitrogen, potassium, phosphorus, carbon and organic matter contents of the soils. The soil from the forest zone is richer in nitrogen, potassium, carbon and organic matter whilst that from the savannah zone is richer only in the phosphorus content (Table 1). With respect to the other parameters the two soils don't differ much. Enzymes, and for that matter β-amylases are protein compounds whose building blocks comprise principally of carbon and nitrogen (Lehninger, 1981). The higher nitrogen and carbon contents of the soil from the forest zone implies a higher capacity for the synthesis of proteins and consequently β-amylase, hence the higher levels observed in the sweet potatoes from the forest zone. Studies by Yoneyama *et al.* (1997) also showed that at the same level of fertilizer application or even when no fertilizer is applied the nitrogen uptake by sweet potatoes varies between varieties, and this could possibly explain why within the same agro-ecological zone (i.e. under similar soil conditions) the variety, Okumkom exhibited a higher β-amylase activity than Faara, both being pink-skinned varieties anyway.

SUMMARY AND CONCLUSION

The pink-skinned varieties of sweet potatoes, namely Faara and Okumkom are better sources of β-amylase and can therefore be best exploited for the production of maltose syrups. Cultivating these in the forest zone would further enhance their β-amylase activity and be most useful for the intended purpose of hydrolysing starchy materials. The application of nitrogen fertilizer and the use of organic manure could also possibly enhance β-amylase levels in growing sweet potatoes.

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FIGURE 1. VARIATIONS IN BETA AMYLASE ACTIVITY IN SWEET POTATO VARIEITIES FROM TWO DIFFERENT AGRO-ECOLOGICAL ZONES