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Thermal Stability of β-Amylase Activity and Sugar Profile of Sweet-Potato Varieties during Processing

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Abstract

Sweet-potato root is a good source of β-amylase for food applications. β-amylase catalyses the conversion of soluble starch to sugars thus increasing the free sugar composition and sweetness of processed sweet-potato products. This study was undertaken to determine effects of temperature, time and their interactions on thermal stability of β-amylase and sugar profile of sweet-potato roots. Moreover, percentage variability of temperature, time, genotype and interactions on β-amylase was assessed. Temperature, genotype, time, and genotype x temperature interaction accounted for 50%, 26%, 11% and 8% of the variation respectively. Increasing temperature and heating time generally reduced β-amylase activity and enhanced maltose formation. Heating sweet-potato roots up to 75°C reserve relatively higher percentage of the endogenous amylases required for starch conversion and free sugar formation in the tempered product. The relatively high β-amylolytic potential of Santom Pona makes it a good raw material for the brewery and sugar syrup industry.

Keywords: β-amylase; Temperature; Genotype; Sweet-potato roots; Thermal stability

Introduction

Amylases are of fundamental importance to processing and eating qualities of sweet-potato roots [1,2]. Literature is replete with information on the presence and role of amylases in sweet-potato roots [1,3,4]. There are three major types of amylases in sweet-potato root; α-amylase, β-amylase and starch phosphorylase. However β-amylase is the most abundant [5,6] and important during processing [7]. Sweet-potato flour with high β-amylolytic activity has been successfully used to increase wort extract of sorghum beer [8]. Utilisation of native amylases in intact roots involves heating the roots to gelatinise the starch fraction, and activate the amylases for starch degradation to maltose and limit dextrins [4,9,10]. Rapid heating of sweet-potato mashes to 70°C may be optimal for starch conversion and subsequent ethanol production [6]. Effect of temperature on β-amylase activity of six sweet-potato lines of varying maltose content has been investigated and reported [6]. However the percentage variability of the temperature, time and more especially interactions with β-amylase activity were not fully investigated. This current study therefore sought to examine the impact of time of heating, concentrations of key sugars including sucrose, the predominant sugar in raw roots, and interactions during processing of four different sweet-potato varieties. The presence and synergistic effect of α-amylase, at relatively higher temperature, in sugar formation was also investigated. Such information would be useful in controlling free sugar composition during processing of sweet-potato roots.

Methodology

Experimental design

A factorial design with three replications was used for all the experimental runs. The main factors were genotype, temperature, and heating time. Variables measured included β-amylase activity, α-amylase activity and sugars (sucrose, maltose, glucose and fructose). The experimental levels were 4, 3 and 4 for the genotype, temperature and time respectively.

Materials

Four sweet-potato varieties *viz.*, Ligri, Santom Pona, Hi-Starch and Apomuden with varying dry matter content, and yield (Table 1) were supplied by the Council for Scientific and Industry Research (CSIR)-Crops Research Institute (CRI), Fumesua, Ghana. They were planted at the experimental field of CRI in Ohawu, Volta region of Ghana. The site falls within the coastal savannah agro-ecological zone. The roots were harvested at four months and stored under room conditions (25-27°C) for a week prior to analysis.

Sweetpotato variety	Dry matter content (%)	Yield (t/ha)
Apomuden	21.9	30
Santom Pona	34.4	17
Ligri	34	18.4
Hi-Starch	40	18

Table 1: Dry matter content, and yield of the four sweet-potato varieties.

Methods

Four medium-sized intact roots of each variety were washed, rinsed and air-dried (25-27°C). The roots were then quartered, rinsed in de-ionised water and dried with paper towel. Each quarter was sliced along its longitudinal axis into 1.0 cm thickness and composite samples within each variety divided into 13 sub-samples of 50 g each. One sub-sample was denoted as raw. The rest were tightly wrapped with aluminum foil and separated into four groups. Each group from the varieties were placed in a force-air temperature controlled oven (Genlab MINI/50/DKG) and heated at 65°C, 75°C and 85°C for 0, 10, 20 and 40 min. Termination of heating process was aided by submerging the heated sweet-potato root samples into ice bath for 20 min. The samples were peeled and frozen at -25°C to -28°C and freeze dried (YK-118/50, Taiwan) for 72 h. The dried samples were milled using Thomas Scientific laboratory Miller (model 3383 L70) and sieve through 40 mesh receiver (Thomas scientific, cat no. 3383N20).

Amylase extraction and determination

Extraction of amylases: To 0.5 g of freeze-dried and milled sweet-potato root sample was added 5 mL of 1 M Tris/HCL buffer (pH 8.0) containing disodium EDTA (20 mM) and cysteine HCL (100 mM). Amylase was extracted for a maximum of 1 h with intermittent vortexing at room temperature. The extracts were obtained by centrifuging the mixture in a bench centrifuge (800 D, China) at 4,000 r/min for 10 min. The supernatant was decanted into another labelled centrifuge tube. Approximately 0.1 mL of the supernatant was added to 2.0 mL dilution buffer (MES buffer, pH 6.2) and was used for total β-amylase assay. For α-amylase, 1.5 mL of sodium malate (1 M, pH 5.4) together with sodium chloride (1M), calcium chloride (40 mM) and sodium azide (0.1%) was added [11,12].

Amylase assay: Amylase extracts (0.1 mL) of each sample was dispensed to 15mL polypropylene tubes and pre-incubated for 5 min at 50°C and 60°C for β and α-amylase respectively together with the substrates; p-Nitrophenyl β-Maltotrioside (PNP β-G3) - for β - amylase and Block p-nitrophenyl Maltoheptaoside (BPNPG7) - for α-amylase. To each tube containing the amylase extracts, 0.1 mL of the substrate solution was added, stirred and incubated at 50°C and 60°C for exactly 10 min from time of addition. 1.5 mL of stopping reagents (1% Trizma base.) of pH 11.0 was added and stirred. The absorbance of the reaction mixtures and the reagent blanks were read at 400 nm. One unit of activity was defined as the amount of enzyme required, in the presence of excess β-glucosidase (for β-amylase) and α-glucosidase (for α-amylase), to release one micromole of p-nitrophenol from PNPβ-G3 or BPNPG7 in one minute under the defined assay conditions and termed a Betamyl-3 and Ceralpha Unit correspondingly. Betamyl-3 unit was converted to International unit (U/g) using a factor of 193.9. Ceralpha unit was also expressed as International unit (U/g) on starch substrate with a factor of 4.1 [11,12].

Sugars determination

Freeze-dried and milled sweet-potato samples were sent to the Quality Plant Product Laboratory (Department of Crop Science, University of Gottingen, Germany) for sugars analysis using High Performance Liquid Chromatography (HPLC). Water extract of the freeze-dried samples (0.1 g in 100 mL) was incubated in a water bath at 60°C for 1 h and treated with 0.2 mL Carrez I and Carrez II solution to remove proteins. The mixture was purified by centrifugation (Sorvall RC-5B Refrigated Superspeed, GMI, Ramsay, USA) for 10 min at 10000 rpm. Sugars were determined from the membrane-filtered supernatant (pores size 0.45 µm). Glucose, fructose, sucrose, and maltose were separated using a LiChrospher 100 NH_2 (5 µm) 4 × 4 mm pre-column in combination with a LiChrospher 100 NH2 (5 µm) 4 × 250 mm separation column (Merck KGaA, Darmstadt, Germany) and an acetonitrile: pure water solution (80:20 v/v) as mobile phase at a flow rate of 1.0 mL min^{-1} at 20°C and an injection volume of 20 µL. Sugars were detected with a Knauer differential refractometer 198.00 (Knauer, Berlin, Germany).

Results and Discussion

Variability of temperature, time and interactions on β-amylase activity of sweet-potatoes

The percentage variability due to temperature, time, genotype and their interactions on β-amylase activity during processing of sweet-potatoes is presented in Table 2. Temperature and genotype accounted for the largest proportion of the experimental variance of 50.37% and 25.94% respectively. Heating time contributed 11.25% while genotype by temperature interaction accounted for 7.95%. Temperature by time interaction and genotype by time interaction account for 2.49% and 1.44% respectively. The combined interaction effect of all the factors was also significant and explained 0.56% of the variance observed.

Source of Variation	+Variance (%)	F Value
Temperature (Temp)	50.37**	40775.6
Genotype (GT)	25.94**	20996.9
Time	11.25**	9103.84
GT*Temp	7.95**	6433.85
Temp*Time	2.49**	2021.17
GT*Time	1.44**	1167.02
GT*Temp*Time	0.56**	452.29

Table 2: Source of variation and percentage variance of genotype, temperature and time on β-amylase activity of sweet-potato root [**Significant at p<0.05; +Calculated from sum of squares].

These current findings correspond well with earlier reports that temperature has profound effect of β-amylase activity during processing [6]. It decreased the activity of beta amylase when six sweet-potato lines of varying maltose content were heated [4,6]. However, the percentage contributions from each of the factors (temperature, genotype and time) and their interaction have not been evaluated prior to this current work. The results of this study imply that thermal stability of β-amylase in sweet-potato roots is also significantly determined by the interaction between variety and the processing conditions including temperature and time.

Thermal stability rate of β-amylase activity in four sweet-potato varieties

Rates of thermal stability of β-amylase activity in four sweet-potato varieties profiled in the current study are presented in Figure 1 (A, B and C). Temperature-time combined treatment generally had a negative effect on β-amylase activities in all the varieties. Activity generally decreased when temperature and heating time were

increased. The effect was more profound at 75°C and 85°C for longer holding times (40 min), where all the varieties lost nearly all of their β-amylase activity. β-amylase activities were relatively stable at 65°C throughout the holding times. Wide variation was however observed among the varieties at the onset of the heating regime at 65°C.

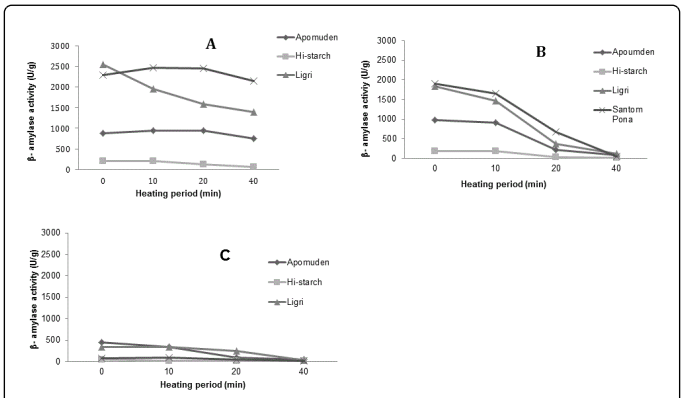

Figure 1: Changes in β-amylase activity during temperature-time heating regimes of four sweetpotato varieties: Santom Pona; Ligri; Apomuden; Hi-Starch. A - 65°C; B - 75°C; and C - 85°C. LSD=22.07.

Santom Pona and Ligri varieties had the highest amylase activity whilst Apomuden and Hi-Starch showed the moderate and the lowest activities respectively. The differences were maintained throughout the heating times except Ligri, which drop from first to second rank after 40 min of heating. The high amylase potential and retention rate of Santom Pona make it a good alternative source of amylase for food applications, which employ relatively mild processing temperature. At 75°C, beta amylase activity decreased steadily with time in all the varieties. Hi-Starch completely lost its activity whilst Apomuden, Ligri and Santom Pona retained 30% to 50% of its activity after 20 min of heating. All the varieties lost most of their activity after 40 min of heating. Beta-amylase activities were greatly reduced (less than 500 U/g) at the beginning of heating at 85°C. Only Apomuden and Ligri retained some activity and maintained the activity for 10 min and 20 min respectively. Activities in Santom Pona and Hi-Starch varieties were not detected even at the beginning of the 85°C treatment. From the study, Ligri and Apomuden retained some activity even at 85°C and can hence withstand high temperatures during mashing and liquefaction processes. It should be noted that gelatinization, a rate determining step in starch conversion, occurs at temperatures ≥ 60°C 18,21, hence raw materials selected for brewing and syrup production should have the ability to retain high amyase activity during heating.

Variability of temperature, genotype and interactions on sugar profile of sweet-potato roots

Influence of temperature, genotype and their interaction on sugar profile (sucrose, maltose, glucose and fructose) of sweet-potato roots during heating is showed in Table 3. Genotypic influence was the most intense and accounted for 99.71%, 99.01 and 97.84% of the variance observed in glucose, fructose and sucrose content respectively. The lowest effect was recorded in maltose (54.89%). Conversely, effect of temperature was highest in maltose (31.56%) formation. The rest of the sugars had less than 1.50% of the variance resulting from temperature. Moreover, the interaction effect was highest for maltose content (13.56%) and least for fructose (0.27%). Sucrose and fructose recorded 1.08% and 0.83% respectively.

Source of variation	+Variance (%)			
	Sucrose	Maltose	Glucose	Fructose
Genotype	97.84**	54.89**	99.71**	99.01**
Temperature	1.09**	31.56**	0.22**	0.91**
Genotype x Temperature	1.08**	13.56**	0.83**	0.27**

Table 3: Source of variation and percentage variance of sugars during heating of sweet-potato roots. [+Percentage variance was calculated from sum of squares; **Significant at p=0.0001]

The results indicate that except for maltose content, sugars in sweet-potatoes are greatly determined by the genomic makeup of the variety and do not substantially response to temperature during heating. Hence, the final concentration of these sugars (sucrose, glucose and fructose) is at least 97% proportional to the initial concentrations during processing of sweet-potato roots. Variation in endogenous sugars (sucrose, glucose and fructose) have been reported to be minimal after cooking [13-16] though their concentrations during heating have not been widely investigated prior to this studies. Fructose, glucose and sucrose levels in Jewel sweet-potato cultivar were not affected by baking temperature 20. Variation in maltose content, however, has been extensively investigated [9,13,14,16]. Reasons has been largely assigned to the increased in temperature, which activates the hydrolytic ability of native amylases found in the root resulting in increased of maltose content in the final product [4,15].

Effect of temperature on sugar profile of four sweet-potato varieties

Variation in sugar profile of the four sweet-potato varieties during heating is presented in Figures 2 and 3.

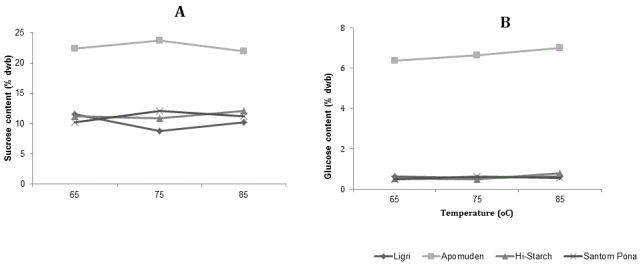

Figure 2: Changes in sucrose (A) and glucose (B) contents during heating of four sweet-potato varieties. LSD=0.07.

Sucrose, glucose and fructose concentrations in all the varieties were relatively stable throughout the heating period. Interaction effect was also minimal except at 75°C for sucrose contents. In all, Apomuden variety recorded the highest content of sucrose, fructose and glucose contents at 65°C and maintained the levels during the heating process. Effect of temperature was highly remarkable on maltose content, an observation confirming early conclusion that maltose content is greatly dependent of temperature (Table 3). Maltose, which was barely absent

in all the varieties prior to heating, increased to 6.76% in Apomuden for instance at 75°C.

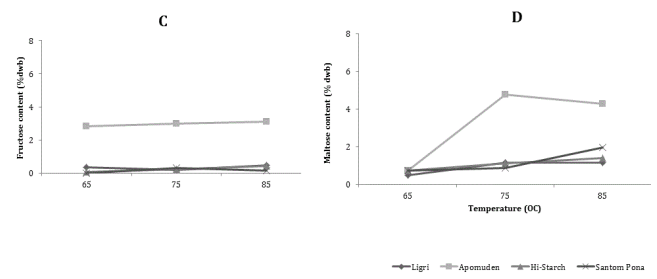

Figure 3: Changes in fructose (C) and maltose (D) contents during heating of four sweet-potato varieties. LSD=0.07.

Maltose content in Santom Pona began to increase substantially after 75°C. The presence and stability of beta amylase activity detected initially accounted for the variation in maltose content. Nevertheless, it should be noted that the optimum temperature of β-amylase falls below 75°C; hence the difference in maltose content at higher temperatures may be due to the activity of α-amylase [6,17]. Alpha amylase, which is relatively heat stable with optimum temperature of 71°C, rapidly degrades starch to lower molecular weight dextrins which are simultaneously hydrolysed into maltose by β-amylase [5,18].

Thermal stability of α-amylase activity was therefore assessed in order to ascertain its presence and synergistic contribution to β-amylase in sugar formation. The results revealed that the sweet-potato varieties also contained α-amylase (Figure 4).

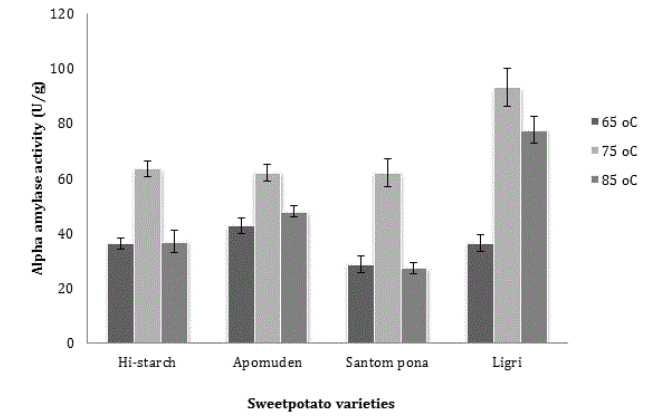

Figure 4: Alpha amylase activity of four sweet-potato varieties during heating at 65°C, 75°C and 85°C. Error bars represent standard deviations. LSD=2.31.

Alpha-amylase activity increased from 65°C to 75°C and declined at 85°C in all the varieties. Apomuden and Hi-Starch had the highest activity at 65°C while Santom Pona had the lowest. The activities were however not significantly different among the varieties at 75°C except Ligri. Ligri exhibited the highest activity of 90 U/g at 75°C, and also retained 75% of the activity at 85°C. Alpha-amylase had a higher optimum temperature (75°C) than β-amylase (65°C) demonstrating its ability to break down starch molecules at relatively higher temperature during processing. Variation in maltose content in this study hence resulted from the hydrolytic activity both α- and β-amylases.

Conclusion

The outcome of the study establishes that stability and hydrolytic power of β-amylase in sweet-potato roots is significantly influenced by the genetic composition of the variety, temperature, period of heating and the interaction between the amylase and processing conditions. Except maltose, final concentration of sugars was virtually directly proportional to the initial levels. Sucrose remained the major sugar after heating up to 85°C, though maltose content increased drastically. The sweet-potato varieties contained substantial amount of α-amylase and it presence may have enhanced the conversion of starch into maltose at higher temperatures.

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