ASSESSMENT OF THE HYDROLYTIC PERFORMANCE OF LOCALLY SOURCED CRUDE AMYLASES ON ROOT (CASSAVA & POTATO) AND CEREAL (MAIZE) STARCHES FOR SYRUP PRODUCTION

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ABSTRACT

Starches from cassava, potato and maize were hydrolysed by locally sourced crude amylases to assess and compare the performance of the enzymes in converting root and cereal starches to glucose syrup. Standard amylases (Sigma USA) served as the reference enzymes. Selected physical and sensory characteristics of the syrups produced were evaluated simultaneously with the syrups produced by the standard amylases. The crude amylases showed higher ($P \leq 0.05$) activity on maize starch than on cassava and potato starches. The hydrolytic performance of the crude amylases increased with increase in substrate concentration up to a maximum substrate concentration of 10%. The crude alpha amylase exhibited a dextrinization time of 2.5h, 2.75h and 3.0h for maize, cassava and potato starches and produced 13%, 12% and 11.8% glucose respectively. The crude gluco-amylase had maximum saccharification time of 72h for cassava and potato starches, 84h for maize and produced 24.37%, 21.8% and 21% glucose respectively. The crude amylases produced syrups in the type II category with Dextrose Equivalent (DE) of 47 and 50 from potato and cassava starches respectively, while syrup from maize starch fall within type III category with DE of 61. Potato starch syrup exhibited higher ($P \leq 0.05$) apparent viscosity and low ($P \leq 0.05$) mean score for colour and taste than cassava and maize syrups.

KEY WORDS: Amylases, Starches, Glucose syrups, Dextrinization, Saccharification

INTRODUCTION

Glucose syrups are sweetening agents that have been widely used in food and pharmaceutical industries. Syrups are often preferred to other sweetners because of some perculiar characteristics they exhibit. Codex Alimentarius defined glucose syrup as a purified concentrated aqueous solution of nutritive saccharide obtained from starch. This definition implies that glucose syrup can be produced from any starch source. Usually the choice of a starch source is influenced by the ease of availability and cost of separating the starch from other constituents of the starch source. The choice of starch for many years has been maize in developed countries. However in developing countries of the tropics like Nigeria where maize is the raw material for many industrial productions, starchy tubers like cassava which occur abundantly may be considered an alternative starch source.

Among the starchy tubers, cassava has high starch content that can be easily separated from other constituents and the extraction does not require highly sophisticated technology. Since according to Onabolu et al. (2003) Nigeria is the world's largest producer of cassava, there is a need to diversify the utilization of cassava and enhance its production.

Conversion of starch to glucose syrup can be achieved by acid hydrolysis, acid-enzyme hydrolysis or by exclusive enzyme hydrolysis (Howling and Jackson 1990). The hydrolytic method employed usually influence the composition of the syrup produced (Emil, 1992). Acid hydrolysis was the oldest method and Akobundu and Eke (1987) explored the conversion of starch to syrup by acid hydrolysis. Because there are limitations associated with acid converted syrups, exclusive enzyme hydrolysis has become the preferred method of starch conversion to glucose syrup (Berry and Alistan, 1990). Enzyme conversion produce high dextrose syrups with better functional characteristics than acid conversion method (Howling and Jackson, 1990). Rolle (2000) noted that enzyme catalysed processes offer considerable opportunities for diversifying the utility of raw agricultural material and by products like starch but they are considered expensive due probably to the prohibitive cost of highly purified enzymes.

Amylases are used in the enzyme conversion of starch to glucose syrup. The alpha amylases hydrolyse the $\alpha 1, 4$ bonds of the starch to produce dextrin and other high molecular weight saccharides of between 15 – 20 Dextrose Equivalent (DE) while gluco-amylase saccharifys the dextrin mixture by cleaving both the $\alpha 1, 4$ and $\alpha 1, 6$ linkages (Sanni et al., 1992).

Alpha amylases can be produced from bacteria or fungi. Bacterial α-amylases are more heat stable but the fungal α-amylases have higher saccharifying ability (Berry and Alistan, 1990). Gluco-amylases are produced from many fungi especially Aspergillus and Rhizopus species. However Abe et al. (1988) noted that most amylases from Aspergillus specie hydrolyse cereal starches but only few have been used for root and tuber starches.

The objective of this study was to assess the hydrolytic performance of locally sourced crude amylases on root starches (cassava and potato) and compare the performance with that on cereal (maize)

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starch. The study was prompted by a desire to profitably utilize cassava starch that is discarded as a waste product in the routine processing of cassava into garri.

MATERIALS AND METHODS

Aspergillus niger was procured from the Department of Microbiology, University of Nigeria, Nsukka. Corn and potato starches were procured from a commercial stock while cassava starch was prepared in the laboratory. All chemicals used were of analytical grade and were procured from a chemical store.

Cultivation of *Aspergillus niger* for alpha amylase preparation

A submerged medium containing 20% corn starch, 2.5% corn steep liquor in 100ml 0.05M acetate buffer pH 6, autoclaved (210^0C) for 1.5h was prepared. The fungus *Aspergillus niger* was cultivated in the medium and incubated for 120h at 30^0C with rotary shaking at 100rpm for 40h. After incubation, the enzyme was harvested by filtration and contrifugation at 10,000 x G for 30min at 4^0C as described by Dharmisthiti et al. (1986). The crude α-amylase was precipitated from the supernatant with 50% ammonium sulphate, washed with distilled water (x 2) dialysed for 12h against 0.01M phosphate buffer pH 6 as described by Bollag and Edelstein (1991) and kept at 5^0C until needed for use.

Cultivation of *Aspergillus niger* for Gluco-amylase Preparation

A similar medium as described for α-amylase was prepared, liquefied with α-amylase, adjusted to pH 4.5 and autoclaved (121^0C) for 1.5h. The fungus *Aspergillus niger* was inoculated into the medium. Fermentation was done for 5 days at 30^0C with rotary shaking (100rpm) for 40h. Drop in pH was controlled with ammonia solution. The enzyme was harvested by filteration and centrifugation (10,000 x G) as described by Darmisthti et al. (1986). The harvested crude gluco amylase was dialysed for 12h in 0.01M phosphate buffer pH 6 and stored at 5^0C until needed for use.

Preparation of Cassava Starch

Freshly harvested cassava tubers were washed, peeled, cut and dipped in 0.05M Sodium bisulphite solution for 20min (Radley, 1976). Grating was done manually using a grater of 25 saws per 2.5cm at 0.5cm distances on the rotor. The cassava mash was slurried in 0.05M Sodium bisulphite (pH 7.0) solution and sieved through 150μm and 325μm sieves sequentially. The sedimented starch was recovered after decantation and washed three times with acidified water (0.01M Hcl, pH 6.5). The starch was dewatered, dried, analysed for chemical composition and used as substrate for amylolysis.

Determination of the crude enzyme activity

Activity of the crude amylases was determined by the method of Dharmasthiti et al. (1986). A reaction mixture containing 5ml of 1% pregelatinized starch, 1ml of 0.1M acetate buffer (pH 6) and 1ml of deionised water was inoculated with 1ml of the crude enzyme extract. The mixture was incubated for 10min at 40^0C. The concentration of reducing sugar (glucose) in the reaction mixture after incubation was determined by the dinitro-

salicylic acid (DNS) method described by Miller (1972). One unit of amylase was defined as the amount of enzyme releasing one microgramme of reducing sugar per minute. The activity was calculated with the expression:

$$\text{Amylase activity} = \frac{\text{Volume of enzyme used (ml)}}{\text{Equivalent of glucose } (\mu g)} \text{ x Time (min)}$$

Effect of starch concentration on the hydrolytic performance of locally sourced crude amylases:

Different concentrations (2, 4 --- 20%) of acetate buffered (0.01M, pH 6) gelatinized cassava, maize and potato starches were prepared and incubated with 1ml of the crude enzyme at 40^0C for 10min. After incubation, the glucose content of each reaction mixture was determined by the DNS method described by Miller (1972).

Determination of Dextrinization and Saccharification Time

Five milliliters (5ml) of 10% concentration of each starch sample (pre-gelatinised) was incubated with 1ml of alpha amylase for times varying from 1 to 4h for liquifaction.

Similar volumes (5ml) of liquefied 10% concentration of the starches were incubated with 1ml of Gluco-amylase for 12 to 96h for saccharification. Following liquifaction and saccharification, the glucose content of each samples hydrolysate was determined (Miller, 1972).

Production of Syrup

A 5ml volume of 10% concentration of each starch sample was inoculated with 1ml of the crude α-amylase and dextrinized for 3h. Following dextrinization, the dextrin mixtures were heated to 100^0C adjusted to pH 5, inoculated with 1ml Gluco-amylase and incubated for 72h at 40^0C to enable saccharification. The glucose content of each saccharified sample was determined (Miller, 1972).

Analyses

The unconverted and converted starches were analysed for selected, chemical, physical, and sensory characteristics.

All the starches were analysed for moisture, ash, crude protein and fat using standard AOAC (1990) method. Total carbohydrate was determined by the method described by Diamond and Denman (1973) while crude fibre was determined by the AACC method (1983).

Residual cyanide in the cassava starch and syrup was determined by direct potentiometric method using linamarase prepared from cassava cortex as described by Cooke (1978). Each reaction mixture containing 1g of sample, 32ml of distilled water, 1ml of 1.0M sodium Hydroxide and 3ml of enzyme linamarase was incubated for 15min after which the cyanide content was read from the ion analyser as described by Rao and Hahn (1984).

Viscosity

The apparent viscosity of the syrups was determined on 50ml of each syrup sample at 30^0C using the universal torsion viscometer.

Total Solids

Total solid was determined by evaporating 2g of each syrup to dryness on a boiling water bath and then completing the drying in a hot air oven at 55^0C for 3h to constant weight. Total solid was calculated as the percentage of the weight difference between the wet and dried samples.

pH

pH was determined on 1ml of each syrup sample at 25^0C using a Crison 414 pH metre.

Dextrose Equivalent (DE)

Reducing sugar in 0.1g of each sample syrup was extracted with hot 80% ethanol and evaporated to dryness on a water bath at 80^0C. The extract was rehydrated with 10ml distilled water. A 0.3ml volume of the extract diluted to 3ml was added, 3ml of DNS reagent and boiled for 20min. Absorbance of the developed colour was determined at 550nm and used in calculating the Dextrose Equivalent (DE) as described by Moreton and Gibson (1980).

Sensory analysis

A 25 member panel evaluated the syrups for colour and taste on a 7 point scale where 1 denotes very poor and 7 denotes very good.

Statistical analysis

Data were analysed by one way analysis of variance (ANOVA). Means were separated with Duncan's New Multiple Range Test using the SPSS package version 10.

RESULTS AND DISCUSSION

Table 1 shows the chemical composition of the starches used as substrates for the crude locally sourced amylases. Maize starch showed more than 3 fold higher ($P \leq 0.05$) crude protein, fat and fibre and lower ($P \leq 0.05$) carbohydrate than cassava and potato starches. The relatively higher carbohydrate content of cassava seem to suggest that cassava may have an advantage over the other sources. The composition observed in this study compares with the values reported by Radley (1976) and Norman (1981).

Cassava starch used in this study had a residual cyanide content of 0.80ppm which is much lower than the safe level permitted in cassava (20mg/kg) product garri (NIS, 1997).

Effect of starch source on the crude amylase activity

Table 2 shows the effect of starch source on the crude amylase activity. Significant ($P \leq 0.05$) differences were observed in the activity of the crude enzymes on the different starches. Activity of the crude α amylase differed ($P \leq 0.05$) with the source of starch. The crude enzymes exhibited highest ($P \leq 0.05$) activity on maize starch and lowest ($P \leq 0.05$) activity on potato starch. Glucoamylase activity followed a similar trend but its activity on cassava and potato starches did not differ ($P > 0.05$) statistically. Okolo et al. (1995) had also reported higher susceptibility of maize starch to amylase activity than cassava starch. The lower activity of the crude enzymes on cassava starch compared to maize starch was attributed to the lower amylose (17%) content in cassava starch. The digestibility of starches by amylases is known to be influenced by the amylose content of the starch (Smith, 1982). Bhat et al. (1983) also noted in addition that the susceptibility of starches to amylases depends on the degree of polymerization and the presence of non-reducing ends on the starch surface. However higher levels (60 – 75%) of amylose has been reported to also resist amylolysis (Paramahans and Tharanathan, 1982). The crude enzymes' low activity on potato starch despite its reported higher (24%) level of amylose compared to cassava starch could not be easily explained. However the fact that the standard amylases (sigma, USA) also exhibited similar relatively low activity on potato starch seem to suggest that potato starch was resistant to amylolysis. Taniguchi et al. (1982) and Okolo et al. (1995) had also observed the relative indigestibility of potato derived starch compared to other starches.

Table 1: Proximate composition of cereal (maize) and tuber (cassava and potato) starches used as substrates for the crude amylases.

Starch source	Moisture (%)	Crude protein (%)	Crude fat (%)	Carbohydrate (%)	Fibre (%)	Total ash (%)
Cassava	13.25 ± 0.1^b	0.5 ± 0.1^a	0.20 ± 0.5^a	84.3 ± 0.2^b	0.0 ± 0.01^a	0.2 ± 0.1^a
Maize	12.0 ± 0.1^a	9.0 ± 0.5^c	4.0 ± 0.1^b	72.0 ± 0.1^a	1.95 ± 0.1^c	1.0 ± 0.1^b
Potato	12.50 ± 0.1^{ab}	2.07 ± 0.5^b	0.35 ± 0.5^b	83.8 ± 0.01^b	0.25 ± 0.1^b	1.0 ± 0.02^b

Values are mean of triplicate determinations $\pm$ S.D.
Mean on the same column bearing different superscript differ significantly ($P \leq 0.05$)

Table 2: Effect of starch source on amylase activity

	Crude amylases		Purified standard amylases	
Starch source	α-amylase (unit/ml)	Gluco-amylase (unit/ml)	α-amylase (unit/ml)	gluco amylase (unit/ml)
Cassava	0.20 ± 0.01[b]	0.17 ± 0.01[b]	0.07 ± 0.01[ab]	0.025 ± 0.01[b]
Maize	0.15 ± 0.00[a]	0.10 ± 0.05[a]	0.05 ± 0.01[a]	0.022 ± 0.00[a]
Potato	0.26 ± 0.01[c]	0.22 ± 0.10[b]	0.09 ± 0.01[ab]	0.028 ± 0.00[c]

Values are mean of triplication determinations ± S.D.
Means on the same column bearing different superscript differ significantly ($P \leq 0.05$)

Effect of Starch Concentration

The effect of starch concentration on the hydrolytic performance of the crude amylases is shown in Fig 1. The hydrolytic ability of the crude amylases increased with increase in the concentration of the substrates up to a maximum concentration of 10% for all the starches. After attaining the maximum concentration, a sharp and significant ($P \leq 0.05$) decline in the glucose produced was observed in all the starches. The decline was attributed to a possible saturation of the active sites of the enzymes with substrates such that increasing the starch concentration without a corresponding increase in enzyme concentration to replenish/augment the amount used up already to produce glucose did not increase glucose production.
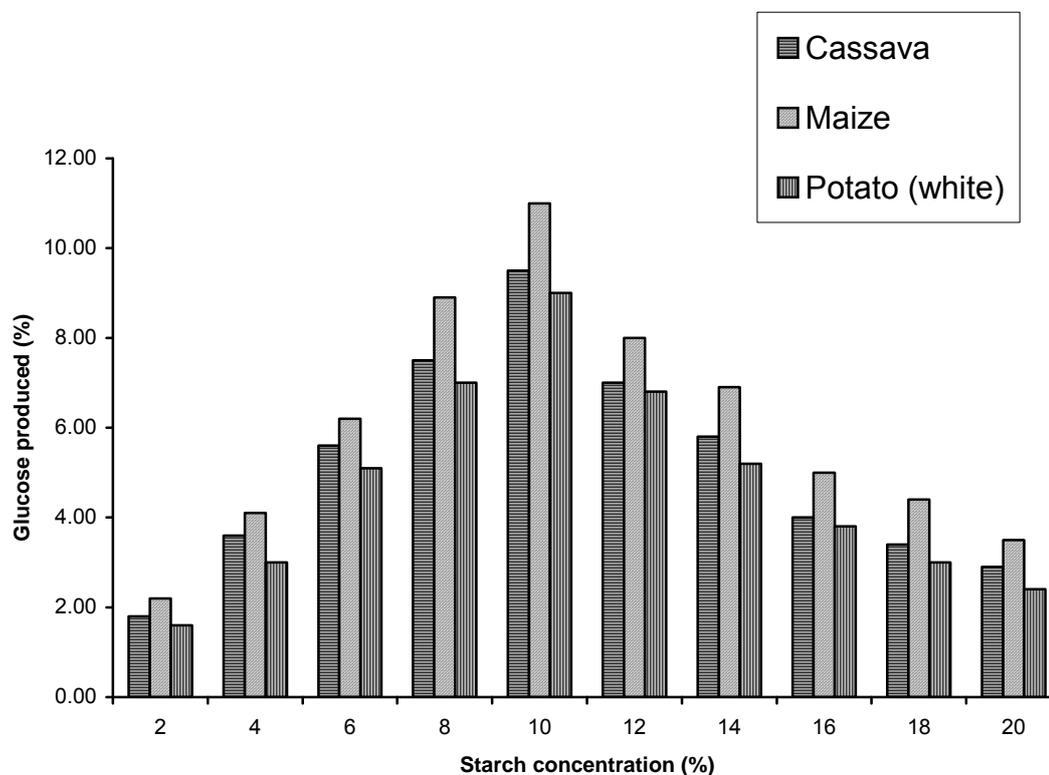
Fig 1: Effect of starch concentration on the hydrolytic performance of the crude amylases at 40°C

Effect of reaction time on dextrinization

There were marginal variations in the reaction time required by the crude α-amylase to attain maximum dextrinization on the different starches (Fig 2). The crude α-amylase exhibited maximum dextrinization time at 2.5h, 2.75h and 3h for maize, cassava and potato starches respectively to produce 14%, 13% and 12% glucose. Increasing the reaction time for all the starches did not increase dextrinization nor the concentration of glucose produced. Aunstrup (1985) reported a maximum dextrinization time of 3h for bacteria α-amylase. The purified standard α-amylase also showed maximum dextrinization time at 3h with all the starches but it produced higher concentrations of glucose from the starches (35%, 34% and 33.6% glucose from maize, cassava and potato starches respectively) than the crude α-amylases. The observed variation in dextrinization time was attributed to differences in the susceptibility of the starches to amylolysis due to differences in starch composition (Bhat et al., 1983).

Effect of reaction time on saccharification

Figure 3 shows the effect of reaction time on the saccharification performance of the crude gluco-amylase. There were gradual but significant ($P \leq 0.05$) increases in the concentration of glucose produced by

the crude gluco-amylase with increase in saccharification time up to a maximum time of 72h (40^{0}C) for cassava and potato starches while maize starch showed maximum saccharification at 84h. The crude gluco-amylase produced significantly (P $\leq$ 0.05) differing concentrations of glucose (24.37%, 21.8% and 21% from maize, cassava and potato respectively) at their maximum saccharification time. Although the purified standard enzyme showed similar maximum saccharification time (72h) with the crude enzyme on cassava and potato starches, it produced significantly (P $\leq$ 0.05) higher concentration of glucose (55%, 52% and 51% from maize, cassava and potato respectively) from all the starches compared to the crude enzyme. The

disparity in activity and concentration of glucose produced was attributed to the fact that the locally sourced amylases were not purified. However a partially purified gluco-amylase from *Aspergillus niger* was reported to produce 11.5% and 10.8% glucose from finger millet and groundnut starches respectively after 100h saccharification time at 37^{0}C (Tharanathan et al., 1980). Differences in starch composition and the presence of contaminants like protein and fat usually affect starch susceptibility to saccharification. Bhat et al. (1983) suggested that the presence of small α-amylase in gluco-amylase enhances gluco-amylase activity.
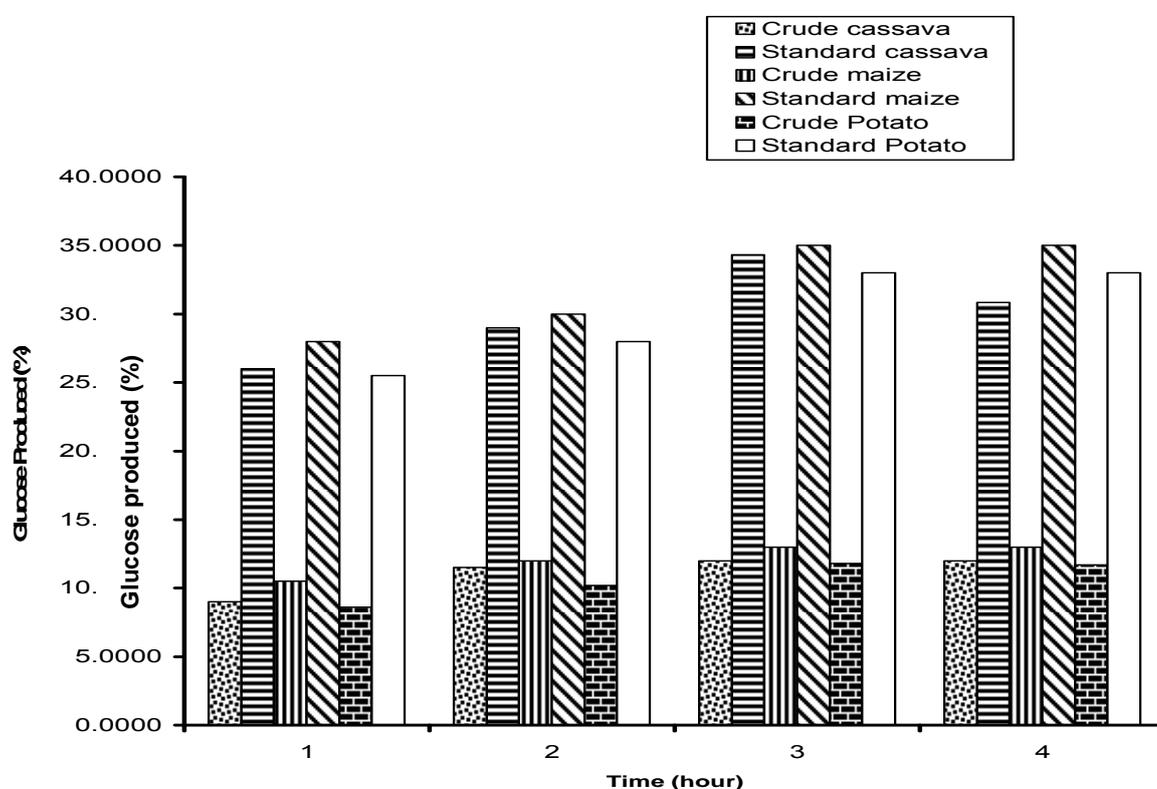
Fig. 2: Effect of Time on dextrinization of Crude and
Standard a-amylase
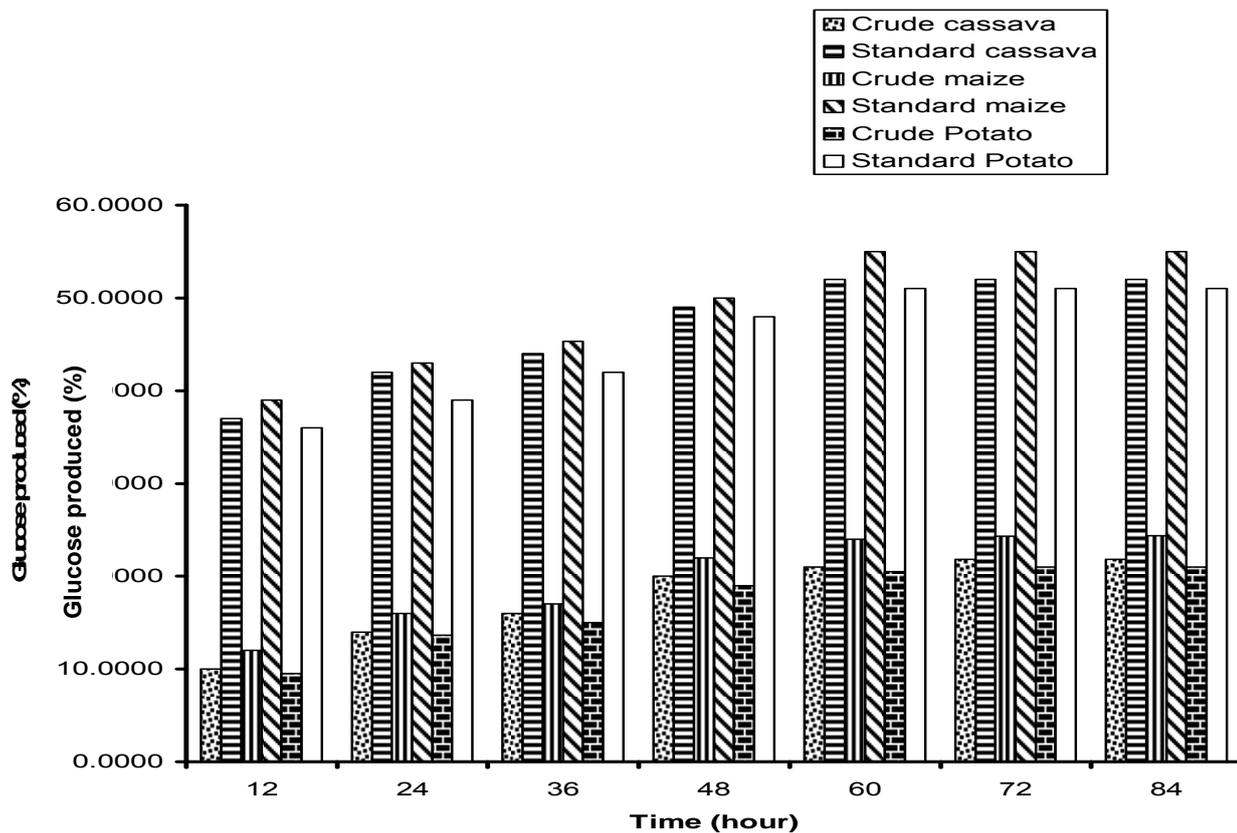
Fig. 3: Effect of Time on Saccharification of Crude and Standard Gluco amylase

Selected Physical and Sensory Properties of the Syrup

Table 3 shows selected characteristics of the syrups produced by the crude amylases and the purified standard amylases. Syrups produced with the crude amylases showed significantly higher (P $\leq$ 0.05) apparent viscosity than the syrups produced with the standard purified enzymes except cassava syrup which had comparable apparent viscosity (P > 0.05) with potato starch syrup from the standard purified enzymes. This disparity in viscosity was attributed to differences in the activity of the enzymes and the degree of resistance to digestion exhibited by the starches. Potato starch that showed high resistance to hydrolysis had high proportion of unhydrolysed saccharides of higher molecular weight and these influenced the viscosity of the resulting syrup. Generally viscosity of syrups is affected by the amount of higher molecular weight saccharides which has not been converted to lower molecular weight sugars, dextrose and maltose in the syrup (Howling and Jackson, 1990). However the viscosity of syrups obtained in this study is within the range for enzyme converted syrups in type II and type III category. Expectedly, the more saccharified syrups from the standard purified enzymes showed lower apparent viscosity.

Table 3: Selected physical and Sensory properties of the syrup

Physical properties	Crude Amylases Converted syrups			Refined standard amylases Converted syrups		
	Cassava	Maize	Potato	Cassava	Maize	Potato
Viscosity (CP)	380 ± 0.0^c	330 ± 0.1^b	420 ± 0.0^d	300 ± 0.2^a	300 ± 0.0^a	380 ± 0.0^c
Total solids (%)	48 ± 1.0^d	47 ± 0.2^c	49.7 ± 0.6^e	42.5 ± 0.03^b	41.3 ± 0.01^a	43 ± 0.0^b
pH	5.2 ± 0.1^a	5.5 ± 0.0^b	5.7 ± 0.0^c	5.1 ± 0.05^a	5.6 ± 0.05^{bc}	5.7 ± 0.5^c
Cyanide (ppm)	0.8 ± 0.1	ND	ND	0.8 ± 0.1	ND	ND
Dextrose Equivalent	50 ± 0.2^b	61 ± 0.3^c	47 ± 0.1^a	95 ± 0.1^e	99 ± 0.0^f	89 ± 0.1^d
Sensory properties						
Colour	4.92 ± 0.64^b	5.96 ± 0.61^{cd}	4.56 ± 0.58^a	6.12 ± 0.60^d	6.48 ± 0.51^e	5.75 ± 0.53^c
Taste	5.48 ± 0.51^b	5.92 ± 0.40^c	4.88 ± 0.44^a	6.32 ± 0.48^d	6.68 ± 0.48^e	5.68 ± 0.56^{bc}

Values are means of triplicate determination $\pm$ S.D.
Mean on the same row with different superscript differ significantly (P $\leq$ 0.05)
ND = Not detected

Total solid

Total solids content also exhibited a similar pattern as the viscosity reflecting the level of conversion (Table 3). Potato starch syrup showed higher ($P \leq 0.05$) total solid content than the maize and cassava starch syrups which also exhibited significantly ($P \leq 0.05$) higher total solids content than the syrups converted by the standard purified amylases. The degree of starch conversion is known to be inversely proportional to the total undissolved solid content. The total solid content observed in this study compares with the values (50 – 55%) reported by Radley (1976).

pH

The syrups showed acid pH range of 5.1 to 5.7 and compares slightly with a range of 5 – 6 reported for refined maltose from tapioca starch (Anon, 1980). Akobundu and Eke (1987) reported a pH of 4.78 for acid converted cassava syrup. Higher pH values are usually not recommended for syrups because of possible alkaline degradation and discolouration particularly during storage.

Cyanide

Cassava syrup from the crude and standard amylases showed residual cyanide content (0.8ppm) that is within the safe limit (2.0ppm). Akobundu and Eke (1987) reported a higher (1.7ppm) residual cyanide level in acid converted cassava syrup.

Dextrose Equivalent (DE)

Syrups produced by the locally sourced crude amylases showed Dextrose Equivalent (DE) values of 50, 61 and 47 for cassava, maize and potato syrups. Based on the DE, cassava and potato syrups are within the type II category while maize syrup is within the type III category of syrups (Aunstrup, 1979). Dextrose Equivalent (DE) is the basic guide to the different types of glucose syrup, it expresses the proportion of reducing sugar present in the syrup calculated as dextrose (Stansell, 1993). The purified standard amylases produced syrups with higher DE from cassava (95) maize (99) and potato (89) starches that can be classed under type IV category. The relatively lower DE value of syrups from the crude amylases is due to the fact that the extracts were not purified because the amount of reducing sugar produced and hence the DE increases with increased amylase activity (Dharmasthiti et al., 1986). However the class of syrups produced by the crude amylases also has application in food and confectionery industries as sweetners, humectants and bodying agents (Shallenberger, 1975).

Sensory Quality

Significant ($P \leq 0.05$) differences in sensory quality scores were observed among the syrups produced by the crude enzymes and between the syrups from the crude and purified standard enzymes (Table 3). Maize starch syrups from both enzymes (crude and standard) had higher ($P \leq 0.05$) taste scores than the cassava and potato starch syrups. Potato starch syrups from both crude and standard enzymes showed the lowest ($P \leq 0.05$) mean scores for colour and taste. Sweetness of syrups is related to the Dextrose Equivalent value so the sensory rating agrees closely with the observed DE values of the syrups.

Syrups produced by the standard amylases had good light brown colour that differed ($P \leq 0.05$) from the colour of syrups from the crude enzyme except the standard enzyme potato syrup which did not differ statistically ($P > 0.05$) from the crude enzyme maize syrup. Syrups from the crude amylases had lower ($P \leq 0.05$) mean score for colour than the standard amylase syrups. Among the crude amylase syrups, potato syrup had the lowest ($P \leq 0.05$) mean colour score. However, the sensory rating indicated good consumer acceptance since no mean score was below the average score of 3.5.

It is evident from this study that locally sourced crude amylases can be used to convert cassava starch to food grade syrup. Interestingly too, the crude amylases exhibited good activity on both cereal (maize) and tuber (cassava) starches, although the cereal starch showed higher susceptibility to hydrolysis and produced higher concentration of glucose than the tuber starches, however the amount of glucose produced from cassava starch by the crude amylases was quite appreciable. Based on the composition and characteristics of the syrups produced by the crude amylases, they can be used as bodying agents and humectants in some food systems. The crude amylases can therefore be used on cassava processing wastes to produce valuable industrial raw materials.

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