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Laboratory scale production of glucose syrup by the enzymatic hydrolysis of starch made from maize, millet and sorghum

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ABSTRACT: Glucose syrup production from maize, millet and sorghum starch was investigated. The starch extracted from yellow maize after steeping for 72hrs and purified by sedimentation method had the greatest yield of 86.71% (4.34 ± 0.37 g) followed by the starch made from millet and sorghum with a yield of 65.94% (3.30 ± 0.25 g) and 64.71% (3.23 ± 0.09 g), respectively. The lowest gelatinization onset temperature of $59.25 \pm 0.90^\circ\text{C}$ was observed with sorghum starch. The glucose recovered with pure amyloglucosidase from *Rhizopus* mold produced 17.15 ± 0.10 mg/ml reducing sugar from yellow maize starch after 10 minutes. Millet and sorghum starch followed with a yield of 15.79 ± 0.20 mg/ml and 11.32 ± 0.26 mg/ml in 10 minutes, respectively. Liquid glucose produced with the pure amyloglucosidase revealed a dextrose equivalent of $78.28 \pm 0.57\%$, $73.50 \pm 0.66\%$ and $65.66 \pm 0.61\%$ for sorghum, yellow maize and millet, respectively. Sorghum and yellow maize starch exhibited good potential as substrates for glucose syrup production.

Key words: Glucose syrup; Enzymatic hydrolysis; Amyloglucosidase; Starch.

Introduction

Starch, the raw material required for the production of low molecular weight products (glucose/dextrose, maltose, maltotriose and dextrin) is widely applied in sugar, spirits, textile as well as brewing (Selmi *et al.*, 2000). Starch is found in the endosperm of cereal grains (Stare and McWilliam, 1977), roots and tubers of crops (Fox and Cameron 1982; Omemu *et al.*, 2004).

The conversion of starch to various sweeteners is achieved through a chemical (acid) or an enzymatic process. The use of enzymes however has more advantages to the former (Yankov *et al.*, 1986) due to the formation of undesirably coloured and flavoured breakdown products, and the process appears to be totally random which is not influenced by the presence of α -1, 6-glucosidic linkages and its difficulty to control (Chaplin, 2004).

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The industrial processing of starch to glucose, maltose and dextrin involves gelatinization, liquefaction and saccharification processes (Hall, 2001) using amylolytic enzymes from microbial and plant sources (Robyt and Ackerman, 1955). Carbohydrate based agricultural products like starch tubers and cereal occur abundantly in most developing countries of the tropics (Okolo *et al.*, 1995 and Anthony, 1996). Cereal grains such as maize, millet and sorghum are important staple foods found in the diet of the people within the Northeastern Nigeria. These cereals are widely cultivated within the subregion, and to a larger extent, the country with an aggregate annual production of 23.9 million tonnes in year 2003 (FAO Reviews, 2006). However, despite their importance, a large proportion of these cereals are lost yearly due to non-availability of appropriate technology and industry to harness these into various useful products such as glucose syrup, maltose syrup, high fructose corn syrup and maltodextrins.

Glucose, an important industrial product of starch hydrolysis finds application as bulk sweetener in the food (Rothwell, 1981) pharmaceutical (Aboje, 2007) and confectionary industry (Fox and Cameron, 1982). The production of glucose, maltose and dextrins from starch of maize (Sutherland *et al.*, 1986), banana (Igoe, 1989; Bello-Perez *et al.*, 2002) cassava (Aboje, 2007) and sweet potato (Omemu *et al.*, 2004) has been well documented in many parts of the world. However, production of these important products of starch hydrolysis in Nigeria has been largely obtained from starch of tubers such as cassava whose cultivation is in large scale in the Southern part of the country (Aboje, 2007).

This study is aimed at the production of glucose syrup by the enzymatic hydrolysis of Maize, Millet and Sorghum starch.

Materials and Methods

Maize (yellow), Millet (SOSAT-C88) and Sorghum (chakalari white) was obtained from the Lake Chad Research Institute, Maiduguri. Borno state, Nigeria.

Sample Preparation

Extraction of Starch from Maize, Millet and Sorghum

Extraction and quantitative determination of maize, millet and sorghum starch was done as described by White *et al.*, (1990) modified by Krieger *et al.*, (1997). Five (5g) each of the cleaned yellow maize, millet and sorghum grains were steeped in 30mL of 1% sodium metabisulfite solution at ambient temperature for 24h, 48h and 72h. This was followed by manual removal of the pericarp and germ. Each of the separated endosperm was placed in a 50mL centrifuge tube with 10mL distilled water and homogenized using a vortex type tissue homogenizer (Ultra Turrax, 170W, 20000 rpm) at 5000 x g for 2 minutes. The homogenized slurry was filtered using a muslin cloth with several washes until the wash water became clear with a total volume of 500mL. The starch slurry was allowed to sediment and the supernatant drained. Each of the three starches was rinsed with 250mL of distilled water, drained twice and the sediment air dried.

Starch Yield

The dry matter recovered from the sedimentation procedure may contain trace amount of protein, fibre and other residues. The various starch yields were determined as described by Ji *et al.*, (2004).

$$\% \text{ yield} = \frac{\text{Dry weight of Starch Recovered from Extraction}}{\text{Dry weight of whole grains (5g)}} \times 100$$

Hydrolysis of Gelatinized Starch using Amyloglucosidase

As described by Bello - Perez *et al.*, (2002). 30-40% of each starch was gelatinized by cooking in boiling water bath for 5-8 minutes. The temperature was cooled to 55°C, followed by incubation with 0.001 % (w/v) phosphate - buffered amyloglucosidase (pH 4.6) for 4 hr. Activity of the enzyme is 21,000 units per gram (1 unit liberate 1mL reducing sugar in 3 minutes). Afterwards, the solution was cooled down to 4°C in an iced water bath and centrifuged

at 11000 x g for 30 minutes. The supernatant was clarified by stirring with activated charcoal 10% (w/v) at 55C for 30 minutes. After centrifugation the DE was determined and the syrup was concentrated to 40% solid by evaporation.

DE = (Reducing sugar) (100) (total solids)

Reducing Sugar Assay

Reducing sugar was estimated by the method of Omemu *et al.*, (2004). An aliquot (1mL) of the crude enzyme was incubated for 3 minutes at ambient temperature with 1mL each of the substrate solutions. The enzyme reaction was interrupted by the addition of 2mL dinitrosalicylic acid reagent. The test tube was heated for 5 minutes in boiling water and then cooled under running tap water. After the addition of 20mL water, the optical density of the solution containing the brown reduction product was determined photometrically at 540nm by means of Corning colorimeter (253) and a blank was prepared in the same manner without enzyme. A calibration curve established with glucose was used to convert the colorimeter reading into milligram of glucose or maltose.

Proximate Analysis

Analyses of the moisture, carbohydrate, protein, and ash content of maize, millet, sorghum and their respective syrup were performed using standard methods (AOAC, 2004).

Rheological properties of glucose syrup

This was determined as described by Finney, 1973 and Nkama *et al.*, (2000). The glucose syrup produced was tested for its viscosity using Brookfield viscometer (Model RT). Spindle number 7 of the Brookfield viscometer was used. It was inserted into the various syrups in 600ml beaker at room temperature. The shear rate was taken at different revolution of 10, 20, and 100 revolution per minute. The viscosity was read in centipoises.

Statistical Analysis

The data obtained from this study was subjected to statistical analyses using SPSS and randomized block design using SPSS version 13 and mini tab version 11 where SPSS fails to address the problem. Where the statistical differences where significant multiple comparison was further employed using the Duncan Multiple Range Test (DMRT). Means and standard errors were also computed.

Results and Discussion

Proximate Composition of Cereals

Proximate composition of the three cereal grains (Table 1) showed significant differences ($P < 0.05$) in moisture, carbohydrate, protein and crude fiber contents. While millet exhibited significantly lower moisture content compared to yellow maize and sorghum, it had significantly higher carbohydrate content than the two grains. The protein contents differed significantly between the three cereals with sorghum having the highest value followed by yellow maize and then millet. The crude fiber content was significantly lower in sorghum as compared to yellow maize and millet. Despite these differences between the three cereals, they showed no significant differences in terms of fat and ash contents. The differences observed between the three cereals might be as result of varietal differences or the physicochemical properties of the cereals. The results obtained for the total carbohydrate and crude fiber contents are higher than those reported by Leung, 1968 but within the range of those reported by Nkama *et al.*, (2000), Modu *et al.*, (2005) and Modu *et al* (2010)

Table 1: Proximate composition of Maize, Millet and Sorghum

	Ash	Moisture	Carbohydrate	Protein	Crude fibre	Fat
Yellow maize	1.00 ± 0.05	6.70 ± 0.10	93.17 ± 0.15	6.77 ± 0.06	15.50 ± 0.10	2.50 ± 0.05
Millet	1.00 ± 0.02	5.30 ± 0.10 ^a	94.70 ± 0.20 ^a	6.40 ± 0.10 ^b	19.50 ± 0.20 ^b	2.00 ± 0.01
Sorghum	1.00 ± 0.06	6.60 ± 0.02	93.27 ± 0.23	6.97 ± 0.02 ^c	14.43 ± 0.12 ^c	2.00 ± 0.02

Values are presented as Mean ± Standard Deviation of four determinations.

Mean values with different superscripts along a column are statistically different (P< 0.05)

Effect of Steeping Time on Starch Yield

Starch yield increased as the steeping time increased from zero to 24 hrs up to 48 hrs for all the three cereals. However from 48hrs to 72 hrs a decrease was observed in the yield of millet starch (Table 2). Steeping time > 48hrs resulted in higher starch yield with lower protein content. Wang and Johnson, 1992 reported a similar pattern of results. Possibilities might be as a result of unbroken particles from homogenization, which might result to the decreased starch yield in millet. It might again be due to the separation method adopted (sedimentation). The heavy starch fraction settled to the bottom of the beaker and the lighter protein fraction remained suspended in the water, thus lost in the top water in the process of decanting. The effectiveness of this technique has been reported by other workers like Gausman *et al.*, (1952); Biss and Cogan (1988); Steinke and Johnson (1991), Ji *et al.*, 2004 and Modu *et al* (2010).

Table 2: Effect of steeping time on starch yield

Variety	Time		
	24hrs	48hrs	72hrs
Yellow maize	3.79 ± 0.47 ^c	4.19 ± 0.37 ^c	4.34 ± 0.37 ^c
Millet	3.74 ± 0.31 ^c	3.87 ± 0.19 ^c	3.30 ± 0.25 ^b
Sorghum	3.07 ± 0.12 ^a	3.16 ± 0.13 ^a	3.23 ± 0.09 ^a

Values are presented as Mean ± Standard Deviation of four determinations.

Mean values with different superscripts along a column are statistically different (P< 0.05)

Effect of Temperature on the Cereal Starches

The onset gelatinization temperatures for the three cereal starches (Table 3) differed significantly (P<0.01). Millet starch showed significantly (P<0.05) higher onset (67.25 ± 0.96°C) and peak (71.25 ± 0.96°C) gelatinization temperatures compared to those of yellow maize and sorghum. The differences obtained for both onset and peak gelatinization temperatures of the three cereals may be related to the steeping time. It has been reported that starch from kernels steeped for 48 hours or more had greater onset gelatinization temperature and a narrower gelatinization temperature range than starch from kernels steeped for only 24 hours (Ji *et al.*, 2004). The likelihood of the starch undergoing annealing and thereby decreasing swelling power and solubility, and consequently delayed gelatinization had been reported (Krueger *et al.*, 1987; Fisher and Thompson, 1997). Krieger *et al.*, (1997) had also studied the annealing of commercial corn and observed that annealing narrowed the gelatinization temperature range and

increased peak temperature. The possibility that the starch used in this study may also have undergone some level of annealing with steeping time as observed by other workers, thus cannot be ruled out in this present study.

Hydrolysis of the gelatinized starch with the commercial enzyme amyloglucosidase (Figure 1) showed an optimal glucose concentration within 10 minutes of reaction time and significant decreases in glucose concentrations (starch hydrolysis) were observed for all the three cereal starches as the reaction time increased from 10 minutes to 60 minutes. This appears to suggest that starch hydrolysis by the commercial amyloglucosidase may be limited to a time range of 10 – 30 minutes.

Table 3: Gelatinization temperatures of the cereal starches

Variety	Temperature			
	Initial/Onset (T_i °C)	Peak (T_p °C)	Final (T_f °C)	Range ($T_f - T_i$ °C)
Yellow maize	63.00 ± 1.42^a	69.25 ± 0.50^a	73.00 ± 0.00^a	10.00
Millet	67.25 ± 0.96^b	71.25 ± 0.96^b	77.75 ± 1.26^b	10.50
Sorghum	59.25 ± 0.90^c	$70.00 \pm 0.50^{a,b}$	75.00 ± 0.82^c	15.75

Values are presented as Mean $\pm$ Standard Deviation of four determinations.

Mean values with different superscript along a column are statistically different ($P < 0.05$)

Hydrolysis of Gelatinized Starch with Commercial Amyloglucosidase

The results of hydrolysis of the gelatinized cereal starch with the commercial amyloglucosidase are presented in Figure 1. For all the three cereals, the optimal glucose concentration (starch hydrolysis) was obtained at 10 minutes reaction time. The glucose concentration (starch hydrolysis) increased significantly ($P < 0.05$) at the 10 minutes reaction time compared to the values obtained at 5 minutes of reaction. Also, significant ($P < 0.05$) decreases in glucose concentrations (starch hydrolysis) were observed for all the three cereal starches as the reaction time increased from 10 minutes to 60 minutes. Infact, for yellow maize and millet starch, the glucose concentrations were both zero suggesting little or no hydrolysis at all. This shows that starch hydrolysis by the commercial amyloglucosidase does not go beyond 30 minutes. However, for sorghum, although there was a sharp and significant decrease in glucose concentration as the time increased from 10 minutes to 25 minutes, a significant rise in glucose concentrations i.e. 7.15 ± 0.10 and 8.68 ± 0.12 mg/ml at 30 and 60 minutes respectively were observed. For all the cereal starch, at all the reaction times, the amount of reducing sugar released were significantly different from each other except for the yellow maize and millet starch which showed values of zero mg/ml at 60 minutes.

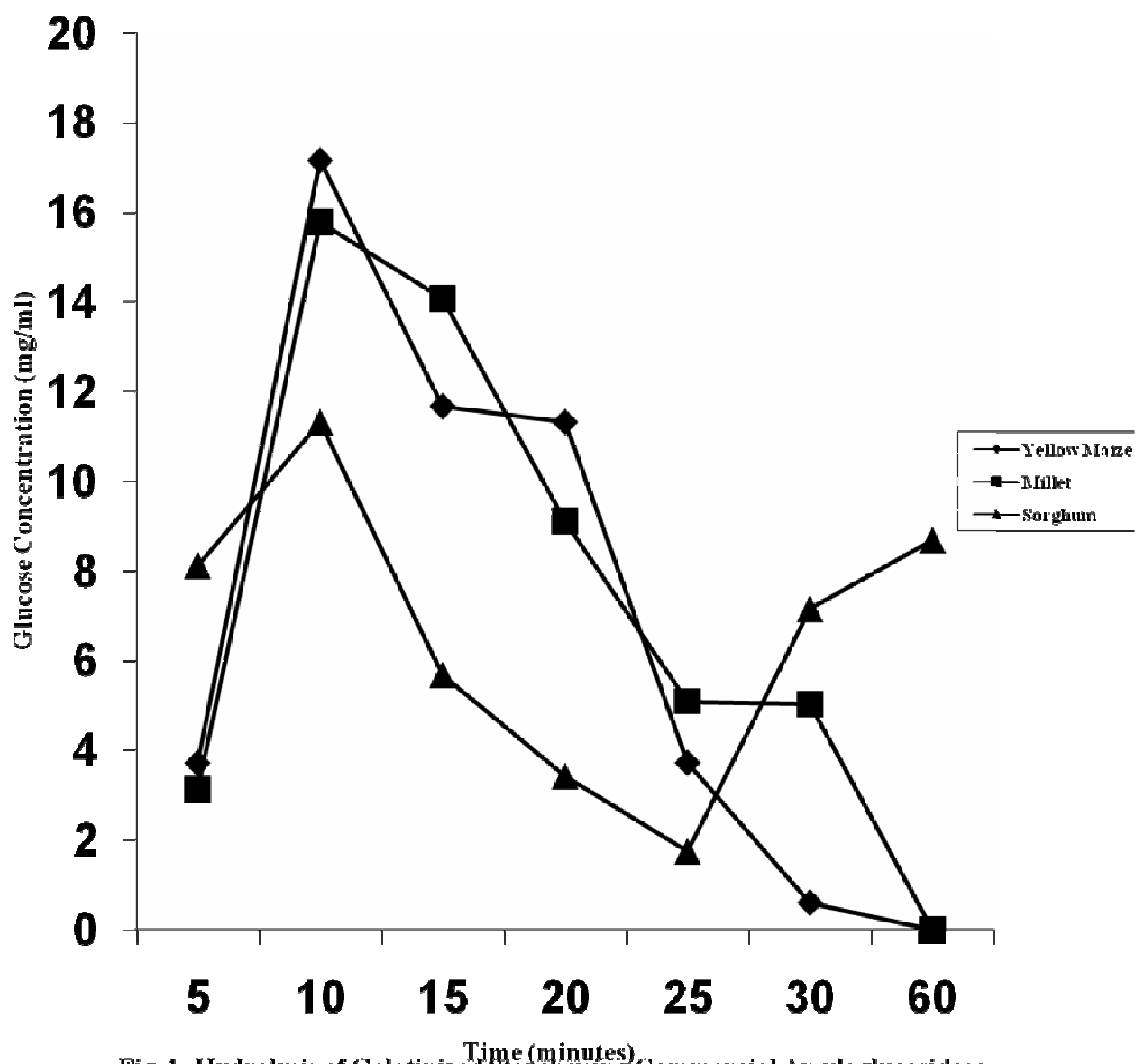


Fig. 1: Hydrolysis of Gelatinized Starch using Commercial Amyloglucosidase

Physicochemical Properties of Glucose Syrup

The physicochemical properties of the cereal glucose syrup presented in Table 4 shows that the product made from millet and sorghum starch had significantly ($P < 0.05$) higher ash content than that of yellow maize. There were also significant differences ($P < 0.05$) in the mean percentage total solids for the three glucose syrups. The mean percentage total solids was found to be higher in sorghum glucose syrup (51.63 ± 0.36) followed by yellow maize

(50.0 ± 0.40) and. Millet glucose syrup had the least with mean total solid (45.0 ± 0.30) The minimum total solids for this kind of product is >70 as reported by Pancoast and Junk (1980).

The values obtained in this study are far below 70 and may be ascribed to the use of only one type of enzyme for the starch hydrolysis. The dextrose equivalent which is the reducing power of the syrup revealed that the glucose syrup from sorghum had the highest mean value of 78.28 ± 0.57 as compared to that from yellow maize and millet with a DE of 73.50 ± 0.66 and 65.66 ± 0.61 respectively. The minimum recommended DE for glucose syrup as reported by Pancoast and Junk 1980 is >20. Asaratnam *et al.*, (1998) had reported DE values of 50-70 in sugar syrups obtained from cornstarch hydrolysis. Therefore from these results obtained in this study, it shows that sorghum glucose syrup is high DE syrup. The viscosity was reported for the various varieties at 50 rpm in centipoise. Sorghum was most viscous with a tinge yellow color but clear. The syrup from yellow maize had mean viscosity of 4160 Cp and was colorless and clear. Millet had a viscosity of 6400Cp but the color was like the sorghum syrup. The percentage-sulfated ash reported was in line with the results obtained from the untreated cereals.

Table 4: Physicochemical properties of Glucose Syrup

Variety	Sulfate ash (%)	Total solids (%)	Moisture content (%)	Viscosity (Cp)	DE (%)	Dry Matter (%)	Colour
Yellow maize	1.00 ± 0.15 ^a	50.00 ± 0.40 ^a	57.03 ± 0.20	41.60 ± 0.05 ^a	73.50 ± 0.66 ^a	42.98 ± 0.24	Colourless clear
Millet	1.50 ± 0.10	45.00 ± 0.30 ^b	62.80 ± 0.25 ^a	64.00 ± 0.03	65.66 ± 0.61 ^b	37.20 ± 0.20 ^a	Tinge yellow clear
Sorghum	1.38 ± 0.20	51.63 ± 0.36 ^c	57.23 ± 0.24	64.00 ± 0.40	78.28 ± 0.57 ^c	42.73 ± 0.22	Tinge yellow clear

Values are presented as Mean ± Standard Deviation of four determinations.

Mean values with different superscripts along a column are statistically different (P< 0.05)

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