Nigerian Journal of Pharmaceutical Research
Vol. 9 No 1 pp. 23 - 29 (February 2011)

BIOSYNTHESIS AND CHARACTERIZATION OF GLUCOSE SYRUP DERIVED FROM *MANIHOT DULCIS* TMS 4(2) 1423 STARCH

[1]S. C. Umerie*., [2]I. C. Uzochukwu and [1]E. J. Maduike-Okwuchukwu

[1]*Department of Production Technology, Faculty of Engineering and Technology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.*
[2]*Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.*

Abstract
Glucose syrup was produced from untreated and acid-treated starch obtained from low cyanide cassava species, *Manihot dulcis* TMS 4(2)1423 (sweet cassava), by enzymatic hydrolysis. The starch for biosynthesis was processed from freshly harvested tubers using the standard traditional method. Acid-treated starch was obtained by treatment of the derived starch with 0.1M HCl. While dextrinization was achieved with the aid of α-amylase, saccharification was achieved with the aid of glycoamylase and fungamyl enzymes. Granular size, moisture content and iodine tests were determined for both the untreated and acid-treated starch samples using standard methods. In order to characterize the prepared glucose syrups, Fehling test, Biuret test, glucose concentration (GC), dextrose equivalent (DE), relative density (RD), pH, viscosity and surface tension (ST) were determined. Granular size range of 7 – 15 µm and moisture content of 12 % were determined for the untreated starch sample. The acid-treated starch had granular size range of 5 – 12 µm and moisture content of 8 %. Both starches had positive iodine test results. Glucose syrup derived from untreated starch afforded GC of 52.6 mmol/litre, DE of 52.30, RD of 1.0736, pH of 2.30, viscosity of 2.70 centipoise, percentage yield of 1.51 and ST of 46.45 dynes/cm. Glucose syrup derived from acid-treated starch had GC of 56.2 mmol/litre, DE of 58.60, RD of 1.1702, pH of 2.50, viscosity of 4.30 centipoise, percentage yield of 1.72 and ST of 48.57 dynes/cm. While the Fehling tests were positive, the Biuret tests were negative for both syrups. Acid-treated *Manihot dulcis* TMS 4(2)1423 starch was recommended for the commercial production of glucose syrup.

Keywords: Glucose syrup, cassava starch, biosynthesis, *Mannihot dulcis*

INTRODUCTION

Starch, a polymer of glucose joined together by glycosidic bonds, is the major storage polysaccharide of higher plants (Bello-Perez *et al*, 2002). It is widely distribution in nature and has been used since early time, not only as food, but also as a useful product in various practical and industrial applications (Zhang *et al*, 2007; Trease and Evans, 1978).

Treatment of raw starches can impart new properties, improve some of the inherent properties or repress and modify some of their other properties. These treatments may involve the use of heat, acid, alkali, oxidizing chemicals or other chemical agents that may result in the introduction of new chemical groups and/or changes in the size, shape, and structure of the starch molecules. Partial hydrolysis of starch with acid, for example, weakens the granule structure, resulting in thin-boiling

starches with lowered hot and cold paste viscosities (Glicksman, 1969; Parmerter, 1969).

Starch degradation products are mainly used as basic carbohydrate sweeteners in the food industry. They are obtained from starch that has been hydrolyzed to form water-soluble products ranging from maltodextrins to syrups and crystalline glucose (commercially known as dextrose). Although starch hydrolysis is traditionally accomplished by mineral acid catalysis, biotechnological advances have led to the use of enzymes, which now allows for the controlled production of a variety of glucose and glucose-based products (i.e., maltodextrins). Essentially, five groups of enzymes are involved in the hydrolysis of starch. The endo- and exo-amylases have activity primarily on the alpha 1, 4 linkages, whereas the debranching enzymes act exclusively on the alpha 1, 6 linkages. A fourth group of enzymes, the isomerases act on glucose to transform it to fructose (Guilbot and Mercier, 1984). The cyclodextrin glycosyl transferase group degrades starch by catalyzing cyclization and disproportion reactions (Nielsen, 1991).

The manufacture of dextrose feed stock from starch may involve successive enzymatic steps of action of a thermostable α-amylase (starch liquefaction), the addition of amyloglucosidase (further saccharification) and the hydrolysis of oligosaccharides or dextrins to low-molecular weight sugars such as glucose, maltose or a mixture of these and their by-products (MacAllister, 1979). Dextrose is used in the preparation of dextrose injection (Atherden, 2006). Maltodextrins, in contrast, consists of D-glucose units linked primarily by alpha 1, 4 bonds (Austin and Pierpoint, 1998) and with a dextrose equivalent (DE) lower than 20. Maltodextrins are commercially distributed as white powders or concentrated solutions.

Although maize starch has been traditionally used in the manufacture of glucose syrups, attention is gradually shifting to non-conventional sources of starch such as banana, amaranth, potato and sorghum (Abraham *et al*, 1988). Since cassava is one of the largest produced crops in Nigeria (IFAD, 2005), the study attempts the biosynthesis of glucose syrups from a locally available low cyanide cassava species and characterizes the physicochemical properties of the syrups produced from untreated and acid-treated starch.

MATERIALS AND METHODOLOGY

Plant material

Freshly harvested tubers of *Manihot dulcis* (Family, Euphorbiaceae) or "sweet cassava" TMS 4(2)1423 (locally referred to as "rogwo") with little or no cyanide content (<1.00 ppm) were obtained in June 2010 from the Nnobi outstation of Anambra State Agricultural Development Programme, Anambra State, Nigeria.

Commercial enzymes

The "NOVO" commercial liquid enzyme preparations were gifts from Life Breweries Ltd., Onitsha, Nigeria. Three enzymes were obtained and used undiluted: "Termamyl 120L" (*Bacillus licheniformis* α-amylase), of density 1.2 gml^{-1} and activity 120 $KNUg^{-1}$ (1 kg NOVO α-amylase unit, KNU, being the amount of enzyme which breaks down 5.26 g starch h^{-1} at NOVO's conditions); ``Amyloglucosidase NOVO (AMG)´´ glucoamylase), of density 1.2 gmL^{-1} and activity 1 AGU (one amyloglucosidase unit) being the amount of enzyme which breaks down one micro-mole of maltose per minute

at NOVO's standard assay procedure; "Fungamyl" (*Aspergillus oryzae* α-amylase), of approximate density 1.25 gmL^{-1} and activity 1 FAU (one Fungal alpha-Amylase Unit) being the amount of enzyme which breaks down 5.26 of starch h^{-1} at NOVO's conditions. (NOVO Enzymes, Denmark).

Extraction of starch

Freshly harvested *M. dulcis* tubers were peeled, washed and milled to a slurry with an electric milling machine. The resulting pulp was sieved through a 200-mesh sieve cloth and the starch suspension allowed to settle for about 6 hours. Water was decanted off and the resulting starch cake dried, pulverized and stored for use.

Preparation of acid-treated starch

Starch (200 g) was mixed with one litre of 0.1M HCl, heated and maintained at 60 ^{o}C for 2 hours in a water bath. The starch was washed thoroughly with water, filtered, dried, pulverized manually and stored for use.

Characterization of untreated and acid-treated starch

The granular size of untreated and acid-treated cassava starch 24 measured for the iodine-stained suspensions of granules at 10 x and 100 x magnification using binocular microscope and a Neubauer counting chamber. The method of the Association of Official Analytical Chemists was used for the determination of the moisture content (AOAC, 1975). The gelatinization temperature of 5 % starch suspension was determined by the method of Umerie *et al*., 1997. Iodine test was carried out by mixing the starch powder (about 0.1g) with one drop of iodine solution and observing the colour produced.

The Fehling test was also carried out for the untreated and acid-treated cassava starches. Five millilitres of a mixture of equal parts of Fehling's solution 1 and 2 were added to 5 ml of the respective starch solutions in water. The mixture was heated in a water bath for 5 minutes. A brick red precipitate at the bottom of the test tube indicates the presence of reducing sugar.

Preparation of glucose from untreated and acid-treated starch

Glucose syrups were prepared from untreated and acid-treated starch by the modified method of White and Kennedy, 1988. Sixty grams of the untreated starch was weighed and mixed with 150 ml of distilled water in a 1 litre beaker, stirred to form a slurry and the pH adjusted to 6.2 by the drop-wise addition of 5 % NaOH. The starch slurry was heated to 100 ^{0}C for 10 minutes in a water bath. Two hundred milligrams of calcium chloride solution and 1.44 ml of Termamyl enzyme (α – amylase enzyme) were added in order to stabilize the enzyme. The preparation was allowed to stay at 100 ^{0}C for 10 minutes in a water bath. The temperature of the water bath was reset to 90 ^{0}C for 2 hours to achieve complete liquefaction of the starch. The liquefied starch was then cooled to 60 ^{0}C and the pH adjusted to 4.3 with concentrated hydrochloric acid. Amyloglucosidase (1.44 ml) enzyme and Fungamyl enzyme (150 mg) were added to the liquefied starch. The solution was maintained at 60 ^{0}C until it was fully saccharified. After saccharification, the glucose syrup so produced was heated up to 75 ^{0}C, and maintained for 5 minutes in order to inactivate the enzymes. The iodine test was performed at intervals to ascertain the completion of hydrolysis of starch before mashing off. The resulting glucose syrup was first cooled to 30 ^{0}C

and then to 4 ^{0}C to allow the non-hydrolysed materials to settle at the bottom of the beaker. Filtration of the upper layer into a flask using No 1 Whatman filter paper afforded the final glucose syrup. This procedure was repeated for acid-treated starch and the resultant glucose syrup samples stored in a refrigerator prior to subsequent analysis.

Characterization of glucose syrups prepared from untreated and acid-treated starch

The relative densities of the prepared glucose syrups were determined by the method of Moss, 1980. The Biuret and Fehling tests were used to determine the presence or absence of protein and glucose in the syrups respectively (Plummer, 1987). The concentrations of the glucose in the syrups were obtained with a glucometer and their percentage yields subsequently calculated. The DE of both glucose syups were determined spectophotometrically. Surface tension of the syrups were evaluated using Du Nouy surface tensiometer and the average values obtained corrected based on water as the reference (Beckett and Stenlake, 2004). The viscosities of the glucose syrups were evaluated with Ostwald's viscometer and values obtained from $_{25}$ appropriate calculations with water as the reference (Atherden, 2006).

RESULTS AND DISCUSSION

The use of enzyme-enzyme conversion technique on gelatinized cassava starch was fundamental in the production of glucose syrup as the enzyme effected the hydrolysis of starch to yield glucose. The white colour and moisture contents of the untreated and acid-treated starches indicated the high purity of the prepared starches and the absence of extraneous materials (Umerie *et al.*, 1997). The iodine test for the untreated and acid-treated starch samples showed blue-black colour, confirming the samples to be starch indeed. The gelatinization temperature range (Table 1) obtained for the starches were similar to those of sago and arrowroot starches (Yetti *et al*., 2007). Heating the untreated starch resulted in more viscous gel relative to the acid–treated starch indicating higher stability of the untreated starch granules against disintegration during cooking (Yetti *et al*., 2007).

The granular structure of the acid – treated cassava starch appeared mostly round in shape and conformed more with those of cereal grains (3-30 μm) than those of tubers such as potato (10-100 μm) (Umerie *et al*., 1997). The arrangement of the starch granules showed that granules of the untreated starch were closely packed together while that of the acid-treated starch showed sparse clusters of few and countable granules. The sparse granular arrangement of the acid-treated cassava starch is expected to increase its susceptibility to enzymatic hydrolysis affording improved glucose yield (Parmerter, 1969).

The glucose syrups from untreated and acid-treated starches were light brown and pale yellow (amber) in colour respectively. The extent of conversion of cassava starch to glucose is typically quantified by dextrose equivalent (DE), which is roughly the fraction of the glycosidic bonds in the starch that have been broken down. The DE values obtained in both the untreated and acid-treated starches were higher than the minimum recommended standard (>20) for a glucose syrup (Bello-Perez *et al.*, 2002). The DE values for the glucose syrups (Table 2) placed them among the medium converted syrup of DE 45-58 (Kooi, 1965), which are used mainly in brewing industries. The higher converted glucose syrups having DE $\geq$ 65 are used in bread

industries while the lower converted glucose syrups have DE ≤ 45 are used in confectionary industries. Medium converted DE syrups are known to be relatively sweet (Alkonis, 1977), the sweetness being due to its fructose content of about 15-42 % (White and Kennedy, 1988).

The pH values determined for both glucose syrups as shown in Table 2 were similar to each other (Bello-Perez *et al*., 2002) and fell within the specification of 2.0-5.5. Acidic pH of glucose syrups prevents alkaline degradation and possible decolouration especially during storage (Peckham, 1955). Viscosity of glucose syrup is a function of DE, temperature and solid content. Viscosity also increases with increase in syrup concentration. Expectedly, the prepared glucose syrup from the acid-treated starch has a higher viscosity than the syrup obtained from the untreated starch sample. This higher viscosity is attributable to its higher DE and GC. Concentration achieved during the processing may also account for viscosity differences (Kertnz, 1965).

Both glucose syrups derived from the untreated and acid-treated starches had no protein content. Protein concentrations ranging from

Table 1: Physicochemical properties of acid-treat and untreated starch

Parameter	**Untreated starch**	**Acid-treated starch**
Moisture content (%)	12.00	8.00
Colour	White	White
Gelatinization temp (^{o}C)	68-72	69-73
Iodine test	Blue black	Blue black
pH of slurry	2.7	2.3
Gel nature	Cloudy and more viscous	Less cloudy & less viscous
Size of granules (μm)	7-15	5-12

Table 2: Physicochemical properties of the glucose syrups from untreated and acid-treated starch samples

Parameter	Glucose syrup form untreated starch	Glucose syrup from acid-treated starch
Volume (ml)	96	102
Percentage yield (%)	1.51	1.72
Dextrose equivalent	52.30	58.60
Relative density	1.0736	1.1702
Baume density (^{0}Bé)	9.8924	20.9877
pH	2.30	2.50
Viscosity (centipose)	2.704377	4.305982
Surface tension (mN/m)	48.56542×10^{-3}	48.56542×10^{-3}
Protein content (%)	Nil	Nil
Taste	Less sweet	Sweet
Colour	Light brown	Amber
Glucose concentration (mmol/litre)	52.6	56.2

0.03 to 0.08 % are expected from glucose syrups derived from sweet cassava (Harris, 1971). The complete absence of protein in the glucose syrups derived from the TMS 4(2) 1423 cassava species may be attributed to the low protein content of the species used for the preparation.

CONCLUSION

The acid-treated starch afforded glucose syrup of higher DE than the untreated starch. The DE value of the derived glucose places it among the medium-converted glucose syrups. Physicochemical parameters of the glucose syrup derived from acid-treated starch compare favourably with literature values. Acid-treated starch derived from TMS 4(2) 1423 cassava species is recommended for the commercial biosynthesis of glucose syrup.

REFERENCES

Abraham E.T., Krishnaswamy .C. and Remakrishna, S.V. (1988). Hydrolytic Depolymerization of Starch. *Raw materials*, 40:387-392.

Alkonis, J.J. (1977). Confectioneries. In: McGraw-Hill Encyclopedia of Food and Agriculture. McGraw-Hill Book Company New Delhi, pp 295-297.

AOAC (1975). Association of Official Analytical Chemists. Official Methods of Analysis, 12th edn, Washington D.C.

Atherden, L.M. (2006) Viscosity In: Bentley and Driver's Textbook of Pharmaceutical Chemistry, 8th edn: Oxford University Press, New Delhi, India, pp 14-18.

Austin, C.L. and Pierpont, D.J. (1998). The Role of Starch-Derived Ingredients in Beverage Applications. *Cereal Food World*, 43:748-752.

Beckett, A.H. and Stenlake, J.B. (2004). General Physical Methods. In: Practical Pharmaceutical Chemistry, 4th edn – Part 2. CBS Publishers and Distributors, New Delhi, India. Pp 9-51.

Bello-Perez L.A., Sanchez L, Sanchez-Hernand L, Moreno-Damian E, and Toro Vazquez J.F. (2002). Laboratory Scale Production of maltdextrins and Glucose Syrup from Banana Starch. *Food Technology Acta Scientific Venezolana*, 53: 44-48.

Glicksman, M. (1969). Starches. In: Gum Technology in the Food Industry Academic Press Inc., San Diego.

Guilbot; A. and Mercier. C. (1984). Starch. In: The Polysaccharides Aspinall, O. editor; Academic Press, New York, pp 209-282.

Harris, J.O. (1971). Economics of Wort Production, Modern Brewing Technology. Macmillan Press, Ltd, London.

IFAD (2005). A country case study towards a global strategy for cassava development Department of Agriculture, Federal Ministry of Agriculture and Natural Resources, Nigeria.

Kertnz, Z.I (1965). Food additives. Encyclopedia of Chemical Technology 2nd ed. Vol. 6 John Wiley and Sons. Inc. New York.

Kooi, (1965). Dextrose and starch syrups. Encyclopedia of Chemical Technology 2nd ed. Vol. 6. John Wiley Sons Inc. 919-936.

MacAllister R.V. (1979). Nutritive sweeteners made from starch, *Advances in carbohydrate Chemistry and Biochemistry*, 36: 15-56.

Moss, GL (1980) Practical Physics, 2nd ed Heinemann, London.

Nielson, H.K. (1991). Novel Bacteridytic Enzymes and Cyclodextrin Glycosyltransferase for the Food Industry. *Food Technology*. 45(1): 102-104.

Parmerter, S.M. (1969). Starch. In: Kirk-Othmer Encyclopedia of Chemical Technology, 2nd edn., Vol. 18, Standen, A. (editor). John Wiley, New York. pp 676-691.

Peckham. G.T. and Ira, G.D. (1955). Advances in Chemistry. Graves Macmillan Publishing Co. Inc. New York 12 - 43.

Plummer, D.T. (1987). An Introduction to Practical Biochemistry, 3rd edn, McGraw-Hill Book Company (UK) Ltd., England.

Trease, G.E. and Evans, W.C. (1978). Pharmacognosy, 11th edn. Bailliere Tindall, London.

Umerie, S. C., Obi, N. A. N. and Okafor, E. O. (1997) Isolation and characterization of starch from *Cyperus esculentus* tubers, *Bioresources Technology*, 62:63- 65.

White T.A. and Kennedy, J.F. (1988). Biotechnology of Bioactive Derivatives. Oxford University Press, New York pp 651-654.

Yetti, M., Nazamid, B.S., Roselina, K. and Abudlkarim, S.M. (2007). Improvement of Glucose Production by Raw Starch Degrading Enzyme Utilizing Acid-Treated Sago Starch as Substrate. *ASEAN Food Journal* 14(2): 83-90.

Zang Y.H., Evans B.R., Mielenz J.R., Hopkins R.C., Adams M.W. (2007). "High-yield hydrogen production from starch and water by a synthetic enzymatic pathway" (http://www.plosone.org/article). Retrieved 3rd June, 2010.