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Research

The use of Fungal Glucoamylase Enzyme for the Production of Glucose Syrup from Cassava Starch

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Abstract

Glucoamylase was obtained from the solid state fermentation of rice bran using Aspergillus niger. The enzyme extract was used in the hydrolysis of cassava starch to produce glucose syrup. The enzyme activity of the glucoamylase was 200 mg glucose/g/ml enzyme. Hydrolysis of starch was by acid hydrolysis and the use of enzyme. The glucose syrup obtained had a pH of 5.9, specific gravity 1.0033, total reducing sugars 30.4%, total solids 80% and dextrose equivalent 38%. The physical and chemical properties of the cassava glucose syrup were compared with two other tested samples.

Keywords: Glucoamylase, glucose syrup, cassava starch

Introduction

The need to expedite development and self reliance through optimal utilization of local raw materials has made the utilization of cassava for novelty foods and non foods imperative. More so, as Nigeria becomes the highest producer of cassava in the world market, (CBN report, 2000).

Traditionally, cassava is processed into fermented products and starch. The starch is mainly used in the textile industry, for warp sizing, cloth and felt finishing (IITA, 1990). However, starch has other economic potentials, for instance, as a raw material for glucose syrup production (Kent, 1983). Federal Office of Statistics Annual Report (2000) indicated millions of naira was spent on the importation of sugar syrups into Nigeria. Glucose syrups are essentially industrial sugars used in the manufacture of food products, and are mainly consumed in the confectionery industry (Dina and Akinrele, 1970).

Glucose, a C_6 sugar obtained by the bioconversion of starch molecules found readily in Cassava contains 65% moisture, 32-35% starch, 0.7-2.5% protein and 0-1.3% of fat (Kay, 1987). The starch molecule consists of glucose units which are linked by alpha 1, 4 glucosidic to form amylose chains while amylopectin shows a highly branched structure, with alpha 1-6 bonds, in addition to the alpha 1, 4 linkage. Hydrolysis of starch occurs when linkages between the anhydrous pyranose units are broken catalytically by using acid or enzyme catalysis. The alpha amylase and glucoamylase enzymes are known to catalyse the conversion of starch to glucose (Kent, 1983). The objective of this study is to cultivate glucoamylase enzyme by solid state fermentation using local substrates and use the enzyme to produce glucose syrup from cassava starch as an integrated cassava utilization proposal for local starch industries.

Materials and Methods

Cassava Starch

Fresh cassava tubers were peeled, washed, and grated aseptically. The grated cassava was mixed with water and then filtered. Settling of the starch was allowed to take place until the suspension forms a clear liquid.

Dewatering was carried out in a clean bag and the sample dried in the oven at 100°C for 24 hrs (Onabolu *et al*, 1998).

Production of Glucoamylase

Production of Glucoamylase was done using Solid State Fermentation Process: Aluminium tray containing rice bran was moistened with water and autoclaved at 121°C for 1 hour in a Hitachi KV4 autoclave. After cooling, the sterile rice bran was inoculated with spores of *Aspergillus niger* (strain obtained from Federal Institute of Industrial Research, Oshodi, Lagos). The tray was covered with cheese cloth and incubated for 5 days in a sterile cabinet at 25°C (Palmer, 1981). The enzyme was extracted after 5 days of incubation by flooding the seeded rice bran with water. The exudates was filtered with Whatman No.1 filter paper using a vacuum filtration technique and concentrated *in vacuo* at 60°C with a rotary evaporator. Glucoamylase activity was determined by modified methods of Pandey and Radhakinshnnan (1993). The reducing power of sugar formed was estimated by the Shaffter Somogyi method (1980). Enzyme activity was expressed as the amount of glucose formed per hour per ml enzyme.

Assay of Glucoamylase Activity

0.2ml of the enzyme was added to 5 ml of 4% starch solution in a test tube placed in a water bath and incubated at 60°C for 60 minutes. The hydrolysis reaction was terminated with 0.5ml sodium hydroxide (Na0H). 5 ml of Schaffer-Somogyi reagent was added, plugged with cotton wool placed in a boiling water bath for 15 minutes and cooled for 5 minutes. 2ml of potassium iodate solution and 3ml of 2N sulphuric acid respectively were carefully added down the side of the tube. The mixture was thoroughly mixed to dissolve the Copper II oxide and allowed to rest for 5 minutes. The contents were titrated with 0.05N Sodium thiosulphate using starch as indicator. The amount of glucose produced was determined from a table of standards.

Production of Glucose Syrup (acid/enzyme hydrolysis)

A 30% slurry of cassava starch using distilled water was adjusted to pH 2.0 with 1N HCl, heated to 60°C (liquefaction) and adjusted to pH 4.5 on cooling with sodium carbonate. 200µ/ml enzyme was added, incubated for 48 hours at 60°C (saccharification) and heated to 80°C to inactivate the enzyme. The hydrolysate was filtered using Whatman No. 1 filter paper in a Bucchner funnel. The solution was decolourised with activated charcoal and concentrated at 70°C to obtain syrup (Fig. 1). Glucoamylase produced from a strain of *Aspergillus niger* with activity of 200mg/h/ml enzyme was used.

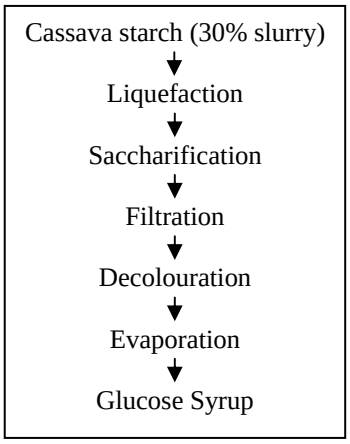

Fig. 1: Flow diagram for production of Glucose syrup

Dextrinisation

25% cassava starch slurry with pH adjusted to 1.9 using hydrochloric acid was heated under continuous stirring. It was subsequently sterilized at 15 psi for 20 minutes.

Saccharification

The liquefied starch was cooled to 60°C, pH adjusted to 4.5 with sodium carbonate and 1% glucoamylase added. The resulting hydrolysate was incubated at 60°C for 48 hours and heated to 80°C to inactivate the enzyme.

Glucose Syrup

The glucose solution obtained from the saccharification process was filtered through two layers of Whatman No.1 filter paper in a Buckner funnel connected to a vacuum pump after passing through activated charcoal. The extract was concentrated by evaporation using a heating mantle at 70°C to obtain glucose syrup.

Complete Acid Hydrolysis

25% starch slurry was liquefied using hydrochloric acid with pH 1.9. The mixture was heated and sterilized at 121°C for 50 minutes before the reaction was stopped with sodium carbonate to neutralize the acid and decolourised with activated charcoal.

Chemical and Physical Analyis of Glucose Syrup

Glucose syrup yield was determined by measuring the volume of syrup produced, per unit weight of cassava starch. The pH was measured with pye Unican pH meter, standardized with buffer pH 4.0. The total sol-

ids, specific gravity and the ash content were determined according to AOAC (1980). Reducing sugar level was determined by the Shaffer-Somogyi (1980) and the dextrose equivalent by Ranken (1984) methods. The reftractometer reading was determined and expressed in degree Brix at 25°C. All analyses were carried out in duplicates.

Results and Discussion

The average glucoamylase activity is shown in Table 1. The use of glucoamylase for hydrolysis of cassava starch gave glucose syrup of which the chemical and physical properties are indicated in Table 2.

Table1:Influence of enzyme dosage on Glucoamylase activity

Sample	Enzyme dosage %	Enzyme activity (mg/glucose/h/ml enzyme)
1	0.5	188
2	1.0	198
3	1.5	200
4	2.0	198

Table 2: Chemical and physical analysis of enzyme- and acid- converted glucose syrup

Properties	Enzyme converted	Acid converted
Syrup Yield (%)	46	42
pH	6.0	5.0
Total solids (%)	80	78
Sugar (°Brix) (25°C)	78	76
Specific gravity (20°C)	1.003	1.003

The syrup obtained has a higher total solid content and sugar level compared with that obtained from acid hydrolysis. This indicates proper saccharification. The syrup yield was slightly more in the enzyme hydrolysed cassava starch. Acid hydrolysis may degrade starch partially which makes the process less effective. The glucoamylase enzyme attacks the starch molecule at the reducing end of the polypeptide chain, and is able to hydrolyse the alpha 1-6 linkages. In an enzyme-enzyme hydrolysis, the amylase could be used for liquefaction instead of the acid used in this study, but it attacks only the 1-4 linkages (Kent, 1983). The glucoamylase attacks both alpha 1-4 and alpha 1, 6 linkages although at different rates (Kent, 1983; Schwardt, 1990). The extent of hydrolysis and nature of the product according to Palmer (1981) depends on the particular method used. This method seems convenient because of the availability of the fungal glucoamylase enzyme.

Table 3 contains the properties of cassava glucose syrup and the two brands of commercial glucose syrup. The three samples differ in pH, dextrose equivalent (DE) and sugar levels but the specific gravity and total solids are non significantly different ($P<0.05$). Since the production process of the two commercial samples tested are not specified, the differences may be attributed to the raw materials used and processes of production. DE, indicating the degree of starch hydrolysis, is not necessarily related to the actual glucose content of the syrup (Kent, 1983). However, it is directly related to the reducing power of all sugars present (Ranken, 1984). Glucose containing syrups are generally categorized by their degree of conversion as measured by the amount of reducing sugar present (Schenck, 1989). According to classification by Schenck (1989), cassava glucose syrup obtained falls into type I (DE 20-30). Glucose syrup 'brand A' tested falls into type IV (DE > 73) and glucose syrup 'brand B' falls into type II (DE 38-58). The total solid content of the three samples are non-significantly different and fall within acceptable range (Schenck, 1989; Ranken *et al*., 1997).

Table 3: Comparison of cassava glucose syrup and other commercial glucose available in the market

Properties	Lab. Produced cassava glucose	Commercial glucose	
		A	B
pH	6.0	3.8	6.7
Specific gravity	1.0	1.0	1.0
Sugar (°Brix) (25°C)	78	81.4	78
Total solid	80	80	79
DE %	38	76	48
Ash	1.6	2.8	2.4

A: Becham B: Glaxo

Conclusion

The study showed that the glucoamylase enzyme produced was successfully used in the hydrolysis of cassava starch to produce glucose syrup. The method of hydrolysis used gives cassava glucose syrup type I which could be of Industrial application. As Nigeria becomes the worlds' largest producer of cassava, it is important that this crop be utilized maximally for products of economic importance in order to boost the economy.

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