

*Full Length Research Paper*

## Effect of different hydrolysis methods on starch degradation

F. A. Aderibigbe<sup>1\*</sup> and L. A. Adejumo<sup>2</sup>

<sup>1</sup>Department of Chemical Sciences, Ondo State University of Science and Technology, Okitipupa, Ondo State, Nigeria.

<sup>2</sup>Department of Chemical Sciences, Osun State University, Osogbo, Nigeria.

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**Hydrolysis of cassava starch was carried out using different processing routes namely: malt extract; acid; combinations of  $\alpha$ -amylase and amyloglucosidase; combinations of acid,  $\alpha$ -amylase and amyloglucosidase and combinations of malt extract,  $\alpha$ -amylase and amyloglucosidase. The results of hydrolysis of all the five routes shows a wide degree of variance in their susceptibility to acid/enzyme hydrolysis in starch conversion of malt extract; acid; combinations of  $\alpha$ -amylase and amyloglucosidase; combinations of acid,  $\alpha$ -amylase and amyloglucosidase; combinations of malt extract,  $\alpha$ -amylase and amyloglucosidase with dextrose equivalent (DE) of 24.29; 33.33; 73.43; 61.29; 76.74 DE, respectively, except the combinations of  $\alpha$ -amylase and amyloglucosidase and combinations of malt extract,  $\alpha$ -amylase and amyloglucosidase that shows very closed range. The best result was obtained with hydrolysis of the combinations of malt extract,  $\alpha$ -amylase and amyloglucosidase which was observed to be more efficient than using any of the other routes in the present work.**

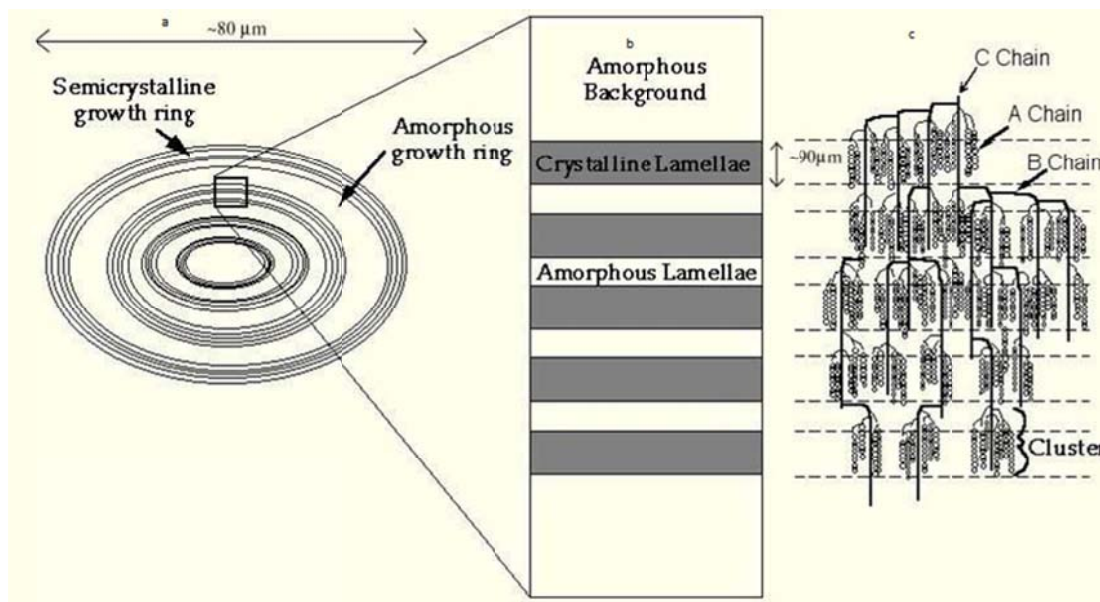
**Key words:** Degradation, routes,  $\alpha$ -amylase, glucoamylase, hydrolysis, dextrose equivalent (DE).

### INTRODUCTION

Starch is laid down in all higher plants in the form of bi-refrangent, and semi-crystalline granules. These granules are primarily composed of two glucose polymers, essentially linear amylose and highly branched amylopectin. The granule crystallinity is associated with the amylopectin component (Montgomery and Senti, 1958; Meyer, 1942). The currently accepted amylopectin structure involves short amylopectin chains forming double helices and associating into clusters (Robin et al., 1974a, 1975b). These clusters pack together to produce a structure of alternating crystalline and amorphous lamellar composition (Figure 1). Regions of amylopectin

double helix formation fall within the crystalline lamellae, whilst the amylopectin branch points lie in the amorphous lamellae. In the model detailed in Figure 1, the lamellae are oriented perpendicular to the helix axis. It has also been suggested that the lamellae may be inclined with respect to the helix axis (Oostergetel and Van Bruggen, 1987; Van Breemen et al., 1994). This possibility is not considered in this paper. For the amylopectin within the crystalline lamellae, three different types of crystalline structure, labelled A, B and C, are identified by wide angle X-ray scattering. A connection has been established between the crystalline structure and the length

\*Corresponding author. E-mail: [aderibigbe\\_fatai@yahoo.com](mailto:aderibigbe_fatai@yahoo.com). Tel: +2348033822123.



**Figure 1.** Schematic diagram of the structure of a starch granule. **(a)** Single granule comprising concentric rings, each containing stacks of amorphous and crystalline lamellae. **(b)** Amorphous and crystalline lamellae. **(c)** Chains of amylopectin arranged in a cluster structure (Jenkins and Donald, 1995).

of the amylopectin chains forming the clusters (labelled as A chains) (Hizukuri et al., 1983; Hizukuri, 1985).

Short A-chains are associated with A-type crystallinity, longer A-chains display B-type crystallinity, whilst intermediate-length A-chains show C-type crystallinity. The combined size of the crystalline plus amorphous lamellae is 9 nm (Jenkins et al., 1993) within the granule stacks of the amorphous and crystalline lamellae form radial rings (growth rings), around 120–400 nm thick (Yamaguchi et al., 1979), bounded by amorphous rings of a similar size. The precise structural role played by amylose is unclear. It is likely that a large portion is found within the amorphous growth ring, with only small amounts associated with the semi-crystalline growth ring 1. It has been suggested that some amylase co-crystallizes with amylopectin within the crystalline lamella (Blanshard, 1987; Kasemsuwan and Jane, 1994). Amylose may also form inclusion complexes with any lipids present internally within the starch granule.

Starch species exist with varying amylose and amylopectin contents. Naturally occurring starches typically have a range between 17% amylose (83% amylopectin) for tapioca and 28% amylose (72% amylopectin) for maize and wheat starch (Jenkins and Donald, 1995). Amylases are starch degrading enzymes. They are widely distributed in microbial, plant and animal kingdoms. They degrade starch and related polymers to yield products characteristic of individual amyolytic enzymes. Initially the term amylase was used originally to designate enzymes capable of hydrolyzing  $\alpha$ -1,4 - glucosidic bonds of amylose, amylopectin, glycogen and their degradation products (Bernfeld, 1955; Fisher and

Stein, 1960; Myrback and Neumuller, 1950). They act by hydrolyzing bonds between adjacent glucose units, yielding products characteristic of the particular enzyme involved. In recent years a number of new enzymes associated with degradation of starch and related polysaccharides structures have been detected and studied (Buonocore et al., 1976; Griffin and Fogarty, 1973).

The objective of this work was to carry out hydrolysis on cassava starch using different routes with a view to identifying optimal processing method. Considerable work on cassava starch hydrolysis has been done (Aderibigbe, 2005; Solomon et al., 2006; Aderibigbe, 2011; Anozie and Aderibigbe, 2011), suggested various hydrolytic procedures for starch hydrolysis, designed and fabricated a pilot plant for future starch conversion to fermentable sugars. The malt extract has essential hydrolytic actions of endogeneous enzymes, mainly amylases and proteases, which convert insoluble carbohydrates and proteins into fermentable sugars and amino acids (Aderibigbe et al., 2012a).  $\alpha$ -Amylase is used to pre-thin the gelatinized starch. Next, glucoamylase (amyloglucosidase) hydrolyzes  $\alpha$ -1,4 links releasing glucose molecules from the non-reducing end of the chain. The  $\alpha$ -1,6 branch links are also hydrolyzed but very much less rapidly.

## MATERIALS AND METHODS

### Cassava flour preparation

Fresh cassava harvested from a farm in Ede, Osun State, Nigeria

were peeled and washed with tap water, chipped into small sizes and dried at 60°C for 48 h in a cabinet dryer. The dried chips were dry-milled and screened to produce the flour. The flour was hydrolysed using a pilot plant designed and fabricated after the previous laboratory experiment (Aderibigbe, 2005).

#### Malted sorghum preparation

Sorghum bicolor invers (L. meench) also obtained from Timi market in Ede, Osun State of Nigeria and malted (Ilori et al., 1990). The sorghum grains were cleaned, then steeped in 500 ppm formaldehyde solution for 18 h with replacement of the steep liquor at 6 h intervals (Solomon et al., 1994). The steeped grains were germinated in a germination chamber at  $28 \pm 2^\circ\text{C}$ , prior to germination; the chamber was disinfected with 1500 ppm formaldehyde solution. During germination the kernels were turned and wetted twice daily with steep liquor. The germination was carried out for 108 h that is, 4½ days after which it was terminated by kilning at  $48 \pm 20^\circ\text{C}$  and milled to pass through 600 µm screen.

#### Burtonized water preparation

One hundred milliliters of salt slurry (CaSO<sub>4</sub>, 235 g, CaCl<sub>2</sub>·H<sub>2</sub>O, 75.5 g, CaCl<sub>2</sub>·7H<sub>2</sub>O, 126 g, NaCl, 17 g, will be dissolved in 1 L of deionized water) will be added to 6.8 L of tap water to obtain the burtonized water (mineral water) (Briggs et al., 1986).

#### Cassava flour hydrolysis with malt extract

Samples of the sorghum malt of 17.5 kg weight were mixed with burtonized water to make 15 L, inside a hydrolyzer at different instances and heated to 50°C and held at that temperature, with constant stirring at 200 rpm, for 30 min. The residue sparged thrice with 5 L of burtonized water each time to extract the maximum amount of the malt enzymes. The enzyme liquor was kept at 50°C in a water bath. The residue was mixed with a seventeen and a half-kilograms (35% slurry) sample of cassava flour made to 20 L with burtonized water. The resulting mixtures were maintained at 50°C for 10 min. Thereafter, it was heated to 98°C, held at this temperature for another 10 min to gelatinize the starch, and then cooled to 64°C. The gelatinized starch slurry was then added to the extracted enzyme and the final mixture was kept at 64°C (the optimum temperature for β-amylase activity for a period of 1 h after which the temperature was raised to and maintained at 72°C (optimum temperature for α-amylase) activity for a further 1 h period with the pH varied from 4.5; 5.0, 5.5 to 6.0 to determine the optimum product level. Samples were withdrawn regularly and filtered after stopping the enzymatic activities by boiling for 15 min and analyzed for reducing sugar using Dinitrosalicylic method as described by Miller (1959).

The procedures described above for the case of malt to cassava flour ratio 1:1 were carried out in triplicates and only the average values were reported. The hydrolysis was similarly carried out with malt to cassava flour ratio 2:3 and only the average was reported.

#### Acid hydrolysis of cassava flour

A 17.5 kg cassava flour hydrolysis was dispatched into the hydrolyzer and conducted at different acid (HCL) concentrations of 0.05, 0.1, 0.2, 0.3 and 0.4 M and at temperature and agitation speed of 80°C and 250 rpm, respectively for a period of 180 min. Samples were drawn at regular time intervals and analyzed for reducing sugar using Dinitrosalicylic method as described by Miller (1959). The hydrolysis was carried out in triplicates and only the average values are reported.

#### Cassava flour hydrolysis with α-amylase and glucoamylase

A 17.5 kg (35 % slurry) cassava flour was dispatched into the hydrolyzer and cooked at 98°C for about 10 min so as to gelatinize the starch and a quantity of termamyl heat stable α-amylase from *Bacillus licheniformis* (equivalent to 0.5 mL/kg starch) was added and allowed to remain at this condition for 5 mn to thin out. It was cooled down to 72°C for another 25 min. The mixture was further cooled down to 60°C when a quantity of amyloglucosidase (equivalent to 0.5 mL/kg starch) was also added and allowed to remain at this condition for 2 h while the pH varied from 4.5; 5.0, 5.5 to 6.0 to determine the optimum product level. Samples were withdrawn regularly and filtered after stopping the enzymatic activities by boiling for 15 min and analyzed for reducing sugar using Dinitrosalicylic method as described by Miller (1959). These procedures were carried out in triplicate and only the average values are reported.

#### Cassava flour hydrolysis with acid, α-amylase and glucoamylase

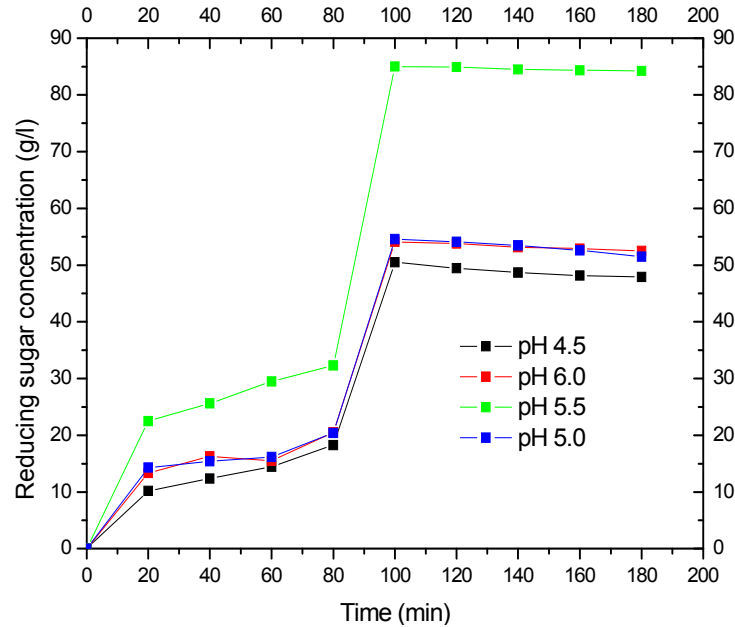
A 17.5 kg cassava flour hydrolysis was dispatched into the hydrolyzer and the analysis was conducted at acid (HCL) concentrations of 0.4 M and as in "malted sorghum preparation" section followed by procedure of "Burtonized water preparation" section of this work. Samples were withdrawn regularly and filtered after stopping the enzymatic activities by boiling for 15 min and analyzed for reducing sugar using Dinitrosalicylic method as described by Miller (1959). The procedures were carried out in triplicate and only the average values are reported.

#### Cassava flour hydrolysis with malt extract, α-amylase and glucoamylase

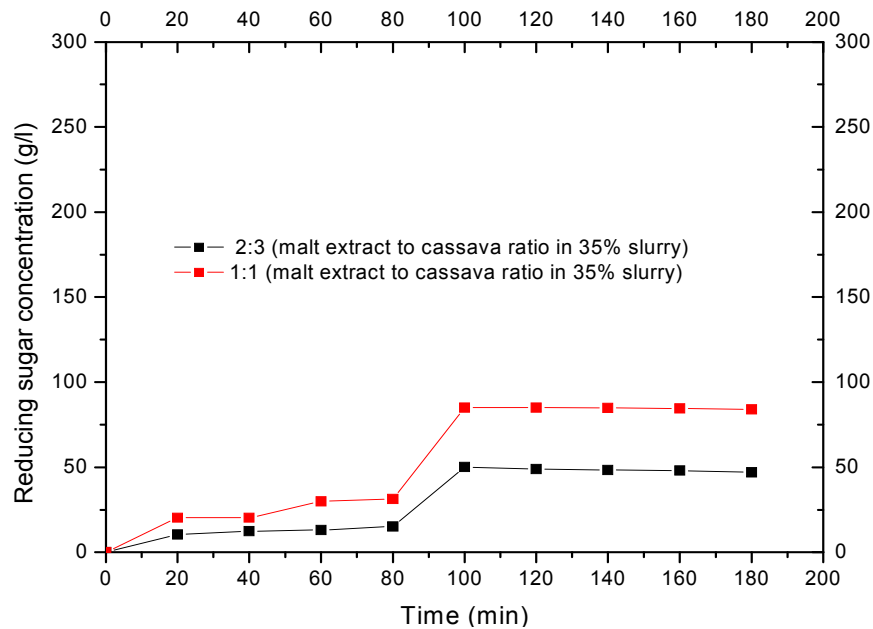
A 17.5 kg (35% slurry) cassava flour was dispatched into the hydrolyzer and cooked at 98°C for about 10 min so as to gelatinize the starch and hydrolyze it with malt extract, α-amylase and glucoamylase as carried out in "cassava flour preparation" section and "Burtonized water preparation" section of this work. Similarly pH was varied from 4.5; 5.0, 5.5 to 6.0 to determine the optimum product level. Samples were withdrawn regularly and filtered after stopping the enzymatic activities by boiling for 15 min and analyzed for reducing sugar using Dinitrosalicylic method as described by Miller (1959). These procedures were carried out in triplicate and only the average values are reported.

## RESULTS AND DISCUSSION

The variation of reducing sugar concentration with time and pH using combinations of malt to cassava starch ratio 1:1 in 35% slurries is presented in Figure 2. It was observed that the reducing sugar concentration increased from pH 4.5 to 5.5 and dropped as pH increased to 6.0 but levelled off after 100 min. The maximum reducing sugar was about 85 g/L at pH 5.5. Figure 3 presents variation of reducing sugar concentration with time and malt to cassava starch ratios 1:1; 2:3 at pH 5.5 in 35% slurries respectively. It was observed that combinations with ratio 1:1 gave a better conversion than ratio 2:3 which was just about 50 dextrose equivalent (DE) compared with the yield of ratio 1:1. This could probably be possible because it contained enough malt extract to



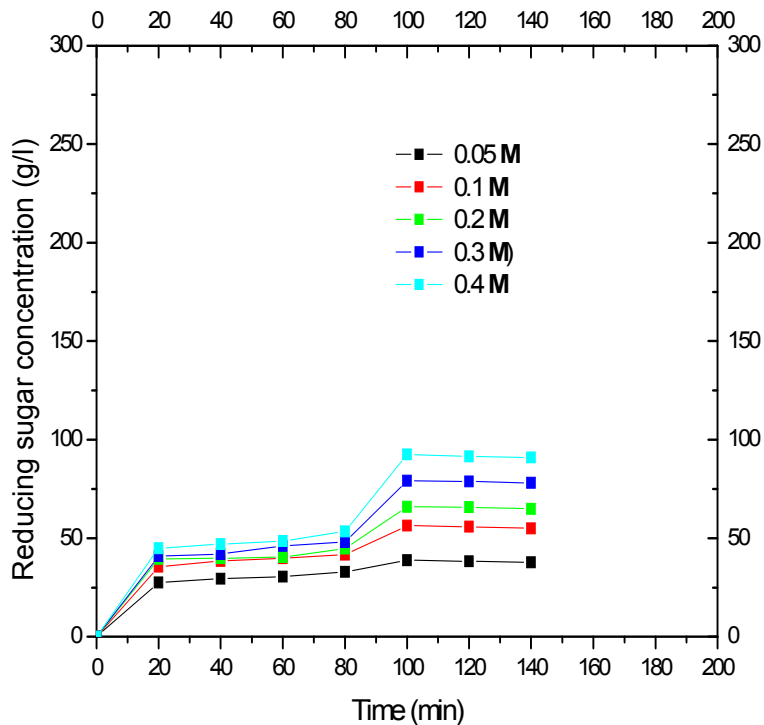
**Figure 2.** Variation of reducing sugar concentration with time in malt extract hydrolysis with cassava starch basis of 350 g/L.



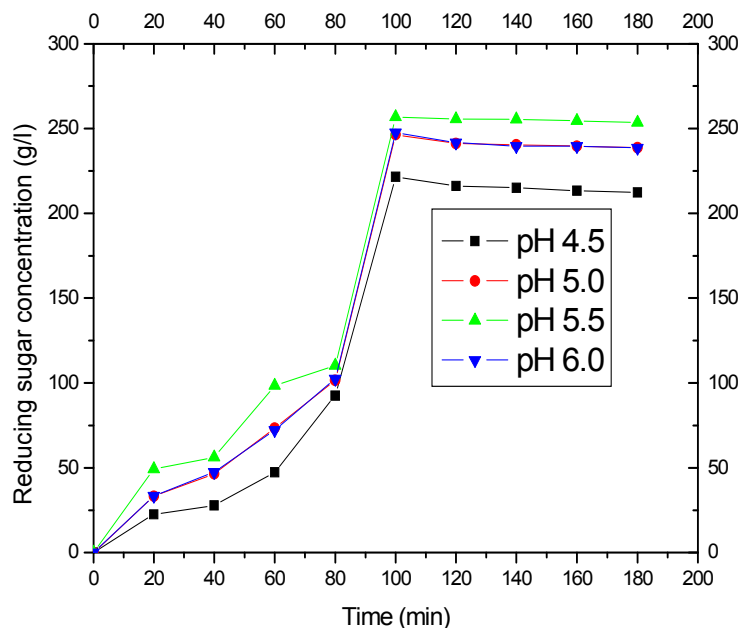
**Figure 3.** Variation of reducing sugar concentration with time in malt extract hydrolysis with different ratio of combination of malt to cassava at the optimum pH with cassava starch basis of 350 g/L.

hydrolyse the starch than 2:3 ratio combinations. The reducing sugar concentration increased with time over a period of study for the two cases but levelled off after 100 min. Figure 4 shows variation of reducing sugar concentration with acid concentration at 80°C. It was

observed that the reducing sugar concentration increased with acid concentration from 0.05 to 0.4 M HCL but the curves levelled off after 100 min of hydrolysis. The DE at 0.4 M HCL was 51 and at 0.05 M HCL it was 19. In Figure 5 there were tremendous improvements in the



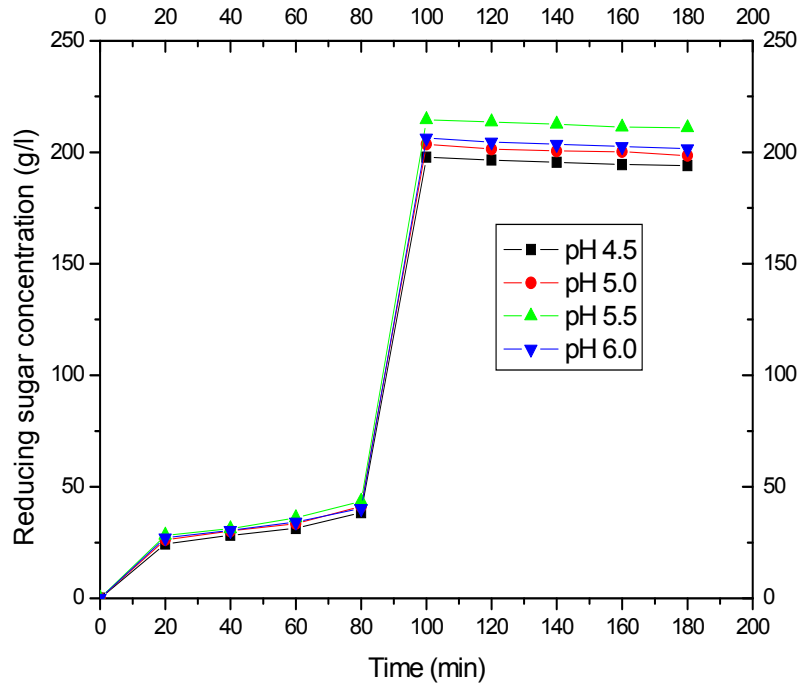
**Figure 4.** Variation of reducing sugar concentration with time in acid (HCl) hydrolysis with cassava starch basis of 350 g/L.



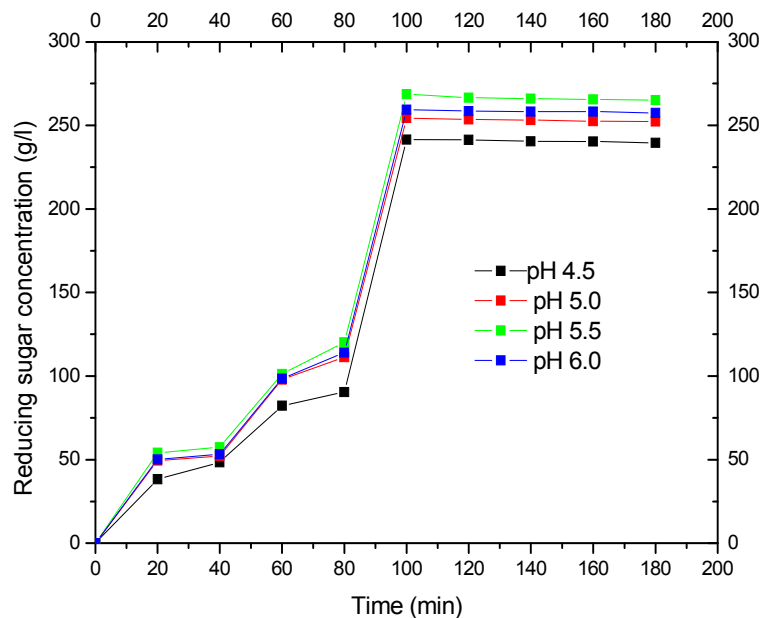
**Figure 5.** Variation of reducing sugar concentration with time in  $\alpha$ -amylase and amyloglucosidase hydrolysis with cassava starch basis of 350 g/L.

starch conversion to a DE of 73.43 with the combinations of amylase and amyloglucosidase in the hydrolysis at pH 5.5. When acid was added to amylase and amyloglucosidase, the conversion drop to 61.29 DE which is

significant as shown in Figure 6. Figure 7 further enhanced the starch conversion with the addition of malt extract to both amylase and amyloglucosidase to give overall best conversion of 76.74 DE. However, Figures 2



**Figure 6.** Variation of reducing sugar concentration with time in acid,  $\alpha$ -amylase and amyloglucosidase hydrolysis with cassava starch basis of 350 g/L.



**Figure 7.** Variation of reducing sugar concentration with time in malt extract,  $\alpha$ -amylase and amyloglucosidase hydrolysis with cassava starch basis of 350 g/L.

to 7 compared reducing sugar concentration with time in malt extract, acid; combinations of  $\alpha$ -amylase and amyloglucosidase; combinations of acid,  $\alpha$ -amylase and amyloglucosidase; combinations of malt extract,  $\alpha$ -amylase and amyloglucosidase media hydrolysis,

respectively. It was observed that malt extract; acid; combinations of amylase and amyloglucosidase; combinations of acid,  $\alpha$ -amylase and amyloglucosidase; and combinations of malt extract,  $\alpha$ -amylase and amyloglucosidase media gave about 24.29; 33.33; 73.43;

**Table 1.** Comparison of the DE at the Optimum Conditions in all the methods of hydrolysis

Medium	DE
Malt extract	24.29
Acid	33.33
amylase + amyloglucosidase	73.43
Acid +amylase + amyloglucosidase	61.29
Malt extract + amylase + amyloglucosidase	76.74

DE, Dextrose equivalent.

61.29 and 76.74 DE, respectively. Combinations of amylase and amyloglucosidase and combinations of malt extract,  $\alpha$ -amylase and amyloglucosidase media gave best yields with 73.34 and 76.74 DE, respectively. It was also observed that malt extract enhanced production of reducing sugar concentration and with a purer product compared with acid conversion.

Aderibigbe (2005) and Solomon et al. (2006) reported 21.67% conversion in 0.5 M dilute acid after 10 h, this is less by 11.66% to what is obtained in this work at the acid concentration of 0.4 M and at 80°C and 100 min. Azhar and Hamdy (1981) revealed from their work on acid hydrolysis of sweet potato that a maximum DE of 83 was reached after 18 min of hydrolysis using 0.1 M, HCl heated at 154°C. However, when the acid level was increased to 1.0 M, a rapid decrease in DE of 60 was noted within 6 min at 154°C followed by a marked decline to 42.5 DE after 12 min. Miller and Cantor (1952) reported that D-glucose formed during starch hydrolysis by acid was hydrated to 5-hydroxy-methyl furfural as the main product and lesser amount of hydroxyl-methyl furan. The acid conversion is higher and faster than malt extract conversion because the acid reaction went directly into the inner part of the starch granules while malt extract acted slowly at peripheral of the starch granules given purer products. The combinations of malt extract, amylase and amyloglucosidase medium gave the best overall yield compared with other processing routes adopted in this work.

Nebesyn (1990) reported a DE of 36.60 after 4 h of hydrolysis using 8 mg/mL of maltogenase. Ayernor et al. (2002) reported DE of 53.81 and 54.04 in combination of 8% w/v of rice malt extract with 300-unit/mL AMG and 10% w/v of rice malt extract with 200-unit/mL AMG after 2 h of hydrolysis. Nebesyn (1990) reported a DE of 22.60 after 4 h of hydrolysis with fungamyl  $\alpha$ -amylase and a DE of 33.60 after 4 h of hydrolysis using fungamyl  $\alpha$ -amylase with amyloglucosidase. There are a number of enzymes involved in the complete hydrolysis of starch to yield very high levels of sugars and each of these enzymes contribute only to some extent to the final yield and the type of sugars produced.

Table 1 compares the DE obtained in all the processing routes. The success of the combinations of malt extract

with  $\alpha$ -amylase and amyloglucosidase over other methods could be due to enzyme type,  $\alpha$ -amylase and  $\beta$ -amylase present in malt extract which was able to hydrolyse  $\alpha$ -1, 4 bonds and bypassed  $\alpha$ -1, 6 linkages (endo-acting amylases) and hydrolyse  $\alpha$ -1, 4 bonds and cannot bypassed  $\alpha$ -1, 6 linkages (exo-acting amylases), respectively in amylopectin and related polysaccharides of the starch and amyloglucosidase activity which is also exo-acting enzyme and  $\beta$ -amylase, which rapidly break starch down starting from non-reducing end to release glucose and increase the yield of sugars. These enzymes are also weakly hydrolytic towards  $\alpha$ -1, linkages, a fact of commercial importance since this activity permits the production of high glucose syrup (Tucker and Woods, 1991). Greater activity towards these linkages can be achieved by addition of more enzymes. However, this can result in the unwanted side reaction termed "reversion", in which the glucose molecules produced repolymerise to form isomaltose, and hence the final yield of sugars is lowered.

Selecting appropriate enzyme dosages and combination is important to achieving the desired sugar yield. Niagam and Singh (1995) reported that with a careful balance of the ratio of amyloglucosidase to  $\alpha$ -amylase, high - glucose syrup (30 – 50 % glucose, 30 – 40 % maltose) or high – maltose syrup (30 – 50 % maltose, 6 – 10 % glucose) could be achieved.

## Conclusion

Malt extract has successfully improved reducing sugar production by combining it with  $\alpha$ -amylase and amyloglucosidase from the traditional methods of hydrolysis used in the past. The success of the combinations of malt extract with  $\alpha$ -amylase and amyloglucosidase over other methods could be due to enzyme type,  $\alpha$ -amylase and  $\beta$ -amylase present in malt extract which was able to hydrolyse  $\alpha$ -1, 4 bonds and bypassed  $\alpha$ -1, 6 linkages (endo-acting amylases) and hydrolyse  $\alpha$ -1, 4 bonds and cannot bypassed  $\alpha$ -1, 6 linkages (exo-acting amylases) respectively in amylopectin and related polysaccharides of the starch and amyloglucosidase activity which is also exo-acting enzyme and  $\beta$ -amylase, which rapidly break starch down starting from non-reducing end to release glucose and increase the yield of sugars.

## Conflict of interests

The authors have not declared any conflict of interest.

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