EFFECTS OF PROCESSING CONDITIONS ON HYDROLYSIS OF CASSAVA STARCH

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Abstract

Different procedures for hydrolyzing raw cassava starch were studied, namely: acid, acid-enzyme and enzyme-enzyme hydrolysis. The effects of temperature, initial cassava starch concentration, acid concentration and time on acid hydrolysis using dilute hydrochloric acid were investigated. In addition, the effect of initial cassava starch concentration and time on acid-enzyme hydrolysis with HCl/fungalmyl/amyloglucosidase and HCl/ termamyl/amyloglucosidase as well as the enzyme-enzyme hydrolysis with fungalmyl/amyloglucosidase and termamyl/amyloglucosidase were also investigated and the local optimum operating conditions and the yield from the three methods were compared. The results showed that in acid hydrolysis the local optimum operating conditions were: 15% cassava starch concentration, 0.5 M HCl, at 70 °C and 10 h operating time which produced 32.5 g/L reducing sugar concentration and dextrose equivalent (DE) of 22. The acid-enzyme hydrolysis with HCl/ fungalmyl/amyloglucosidase, using 30% initial cassava starch concentration and total effective operating time of 50 h, produced 72 g/L reducing sugar concentration and DE of 24.8 and was the better of the two acid-enzyme hydrolysis studied. The best procedure was the enzyme-enzyme hydrolysis with termamyl/amyloglucosidase using 30% initial cassava starch concentration, which produced 152 g/L reducing sugar concentration and DE of 50.9. The total effective operating time was 60 h.

Keywords: Cassava starch, hydrolysis, enzyme, dextrose equivalent.

1. Introduction

Apart from water, cassava tuber is predominantly composed of starch (Edwards, 1974; Grace, 1977). Starches are carbohydrate (or energy) storage materials in plants. Starch is a polymer where the individual units in the polymer are glucose. The two constituents of starch are amylose and amylopectin. Amylose, the linear fraction, consists entirely of long, unbranched chains containing a large number of glucose units. Amylopectin, the branched fraction, consists of similar glucose chains but there are frequent side branching of molecules (Reed, 1975). Hydrolysis of starch provides raw materials for many industries. It involves the cleavage of the glucosidic bond between the monomeric units (D-glucose) of starch. This is a reaction that is catalyzed by dilute acids (acid hydrolysis), enzymes (enzyme-enzyme hydrolysis) or acid and enzymes (acid-enzyme hydrolysis). There also exist microbial techniques of converting starch to the desired products (Omemu *et al.*, 2005).

Various works done on acid hydrolysis of starch favour the use of dilute hydrochloric acid because it acts mildly on starch with lesser amount of side products (Swanson and Cori, 1948; Thompson *et al.*, 1953). Sulphuric and phosphoric acids produce a lot of side products that ultimately inhibit the progress of hydrolysis and enhance the growth of microbes (Peppler and Perlman, 1980). The mechanism and kinetics of acid hydrolysis have been reported (Swanson and Cori, 1948; Bunton *et al.*, 1955).

Enzymes that are capable of catalyzing the hydrolysis of starch are widely distributed in nature. They can be found in the digestive secretions of animals and within the cell of most animals, plants and microorganisms. These enzymes have been isolated and purified and are sold in commercial quantities. Examples are α-amylase (commercial name: fungalmyl), heat stable α-amylase (commercial name: termamyl), β-amylase, and glucoamylase (commercial name: amyloglucosidase). Of the two components of starch, amylopectin presents the greater challenge to enzymatic hydrolysis. This is due to the residues involved in α-1,6 glucosidic branch points. Most hydrolytic enzymes are specific for α-1,4 glucosidic links, yet the α-1,6 glucosidic links must also be cleaved for complete hydrolysis of amylopectin to glucose. The mechanism and kinetics of enzyme hydrolysis have been reported (Mayer and Larner, 1959; Whistler and Paschall, 1965; Roberts, 1977). The three stages in the

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enzymatic hydrolysis of starch according to Chaplin (2001) are: gelatinisation-this involves the dissolution of the starch granules to form a viscous suspension; liquefaction-this involves partial hydrolysis of the starch with concomitant loss in viscosity; saccharification-this involves the production of glucose and maltose by further hydrolysis.

Raw materials such as fermentable sugars, maltose and glucose syrups are sought for by the fermentation, pharmaceutical and bakery industries in Nigeria. There is need to develop an efficient procedure for the production of fermentable sugars and glucose syrups from tubers (e.g. cassava, corn). Cassava (*Manihot esculenta* Crantz) also known as manioc, tapioca or yucca (Grace, 1977; Tonukari, 2004), is an important starchy staple of lowland tropics and a major source of food support of some of the poorest nations of the World (Onabolu *et al.*, 1998; Mba *et al.*, 2001). It is a shrubby, tropical perennial plant that originated in South America and introduced to Africa and Asia in the 16th century by Portuguese travelers (Alaux and Fauquet, 1990; Guthrie, 1990). The cassava produced in Africa makes up to 54% of the World production (FAO, 2003) and Nigeria is the leading producer in the World. Most of the cassava roots produced in Nigeria (Adewusi *et al.*, 1999) and other West African countries are converted into important staple food products like *gari*, *fufu* and *tui*. In spite of its importance, a large proportion of the tubers are lost annually due to ineffective storage techniques. In order to add value to cassava rather than exporting it in its raw form, this work investigates the hydrolysis of cassava starch with acid, acid-enzyme and enzyme-enzyme with a view of comparing the local optimum operating conditions and the yield from the three methods studied.

2. Materials and Methods

(a) Preparation of cassava starch

Fresh cassava tubers obtained from a private 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 starch.

(b) Acid hydrolysis of raw cassava starch

Samples of 10 g and 12 g of raw cassava starch were made into slurry and cooked differently in 100 mL of 0.1 M HCl in two different 250 mL Erlenmeyer flasks. Hydrolysis was carried out in an incubator shaker at 250 rpm (the agitation for all the experiments) and at different temperatures of 40 °C, 50 °C, 60 °C and 70 °C. At each temperature, samples were withdrawn at the start of reaction and at regular time intervals of 1h for 12h. Each sample withdrawn was neutralized with 0.1 M NaOH and filtered. The filtrates were immediately analysed for reducing sugar concentration by DNS method (Miller, 1959). All the experiments in this work were carried out in triplicates and only the average values were reported. Similarly, five different samples of 15 g cassava starch were made into slurry and cooked each in 100 mL of 0.1, 0.2, 0.3, 0.4 and 0.5 M HCl in five different 250 mL Erlenmeyer flasks. Hydrolysis was carried out for each sample in an incubator shaker at 70 °C. The reducing sugar concentration was determined as previously stated.

(c) Acid-enzyme hydrolysis of raw cassava starch

Sample of 7.5 g and 15 g of cassava starch were made into slurry and cooked in 50 mL of 0.1 M HCl in four different 250 mL Erlenmeyer flasks. Hydrolysis was carried out for 20h in an incubator shaker at 70 °C. Samples were withdrawn at the start of reaction and at 5h intervals and neutralized with 0.1 M NaOH, filtered and analysed for reducing sugar concentration. The pH of the mixture was regulated by adding 50 mL of 0.05 M phosphate buffer solution (pH 5.3), prepared by method of Lurie (1975), to each of the hydrolysis mixture. After this, two of the Erlenmeyer flasks containing the reaction mixture were placed in an incubator shaker at 62 °C. When constant temperature was attained, fungalmyl (EC 3.2.1.1) (Chaplin, 2001) from *Aspergillus niger* was added (5 mL/kg of starch). The mixture was kept at this condition for 15h. During this period samples were withdrawn at 5h intervals, boiled to quench the reaction, filtered and the filtrate analysed for reducing sugar concentration. Then, the mixture was cooled and maintained at 60 °C. Amyloglucosidase AMG (EC 3.2.1.3) (Chaplin, 2001; Ayemor *et al.*, 2002), from *A. niger*, was added (5 mL/kg of starch) and the hydrolysis was carried out for another 50 h. Samples were withdrawn at 5 h intervals, boiled for 15 min to quench the reaction, filtered and analysed for reducing sugar concentration. The procedures described above were similarly carried out with the other two Erlenmeyer flasks containing the mixture. The mixtures were placed in an incubator shaker at 72 °C. When the mixture attained the constant temperature of 72 °C, termamyl (EC 3.2.1.1) (Chaplin, 2001), from *Bacillus Licheniformis*, was added (5 mL/kg of starch). The mixture was allowed to remain at this condition for 15h. During this period samples were withdrawn at 5h intervals, quenched filtered and analysed for reducing sugar concentration. The mixture was cooled and maintained at 60 °C and amyloglucosidase was added (5 mL/kg of starch). The hydrolysis was carried out for 50h. Samples were withdrawn at 5 h intervals and analysed for reducing sugar concentration.

(d) Enzyme-enzyme hydrolysis of raw cassava starch

Samples of 15 g and 30 g cassava starch were each made into slurry and cooked in 100 mL of 0.05 M

phosphate buffer solution (pH 5.3) (Lurie, 1975), in four different 250 mL Erlenmeyer flasks. Two of the flasks containing the mixtures of the different samples were placed in an incubator shaker at 62 °C. When constant temperature was attained, fungalmyl was added (5 mL/kg of starch) and the mixture kept at this condition for 35h. During this period samples were withdrawn at the start of reaction and at 5h intervals, quenched, filtered and analysed for reducing sugar concentration. After, the mixture was cooled and maintained at 60 °C, amyloglucosidase was then added (5 mL/kg of starch) and the hydrolysis was carried out for another 50h. Samples were withdrawn at 5h intervals and analysed for reducing sugar concentration. The procedure described above was similarly carried out by placing the other two Erlenmeyer flasks containing different samples in an incubator shaker at 72 °C. When the mixtures attained constant temperature of 72 °C, termamyl, was added (5 mL/ kg of starch) and the mixtures were kept at this temperature for 35h. Samples were withdrawn at the start of reaction and at 5h intervals and analysed for reducing sugar concentration. The temperature was brought down to 60 °C, amyloglucosidase was added (5 mL/kg of starch) and the hydrolysis completed as in the previous case.

(e) Determination of reducing sugar concentration

The reducing sugar content of the hydrolyzates was determined by the DNS method (Miller, 1959). However, the DNS Reagent was modified according to Mwesigye (1988).

Modified DNS Reagent

Two hundred grams of Rochelle salt (potassium sodium tartarate) were dissolved in 200 mL of distilled water. Ten grams of sodium hydroxide was also dissolved separately in 200 mL of distilled water in a 500 mL beaker. To the sodium hydroxide solution, 10 g of DNS (3,5- dinitrosalicylic acid) and 2.52 mL (2 g) of 80% (w/v) phenol, were added simultaneously. After stirring to complete dissolution, the mixture was added to the Rochelle salt solution. The resultant mixture was then made up to one liter with distilled water. This gave the stock of the modified DNS reagent containing 1% (w/v) DNS acid, 0.2% (w/v) phenol, 1% (w/v) sodium hydroxide and 20% (w/v) Rochelle salt (Mwesigye, 1988). The well mixed DNS reagent was then stored under refrigeration in an amber coloured bottle.

(f) Calculation of Dextrose Equivalent

The Dextrose Equivalent (or percentage conversion of substrate) was calculated using the relationship:

$$\text{D.E.} = \left(\frac{\text{reducing sugar concentration (g/L)}}{\text{total initial substrate concentration (g/L)}} \right) \times 100$$

3. Results and Discussion

(*a*) Acid hydrolysis

The results obtained during acid hydrolysis of raw cassava starch are presented in Figs. 1-3. The variation of reducing sugar concentration with time and temperature using 10% initial cassava starch concentration and 0.1 M HCl is presented in Fig. 1. It was observed that the reducing sugar concentration increased with temperature and time but leveled off after 10h to a plateau. The maximum reducing sugar concentration was about 20 g/L at 70 °C. The increase in temperature probably caused slight swelling of the dispersed cassava starch granules thereby enhancing more random attack by the acid on the glucosidic bonds. Hydrolysis of sweet potato starch up to 164 °C has been reported (Azhar and Hamdy, 1981). Higher temperatures were not explored in this study because of equipment limitation. Fig. 2 shows the variation of reducing sugar concentration with time and temperature using 12% initial cassava starch concentration and 0.1 M HCl. It was observed that the reducing sugar concentration increased with temperature and time but leveled off after 8h. It was further observed that increase in starch concentration brought the final values of reducing sugar concentration to about the same values despite the differences in temperature. However, increase in initial starch concentration reduced the dextrose equivalent from 20.7% to 19.1% (Figs. 1 & 2). This could have been due to the reduction in the susceptibility of the acid to the glucosidic bonds in the starch granules; hence, fewer bonds were cleaved.

Fig. 3 shows the variation of reducing sugar concentration with acid concentration using 15% initial cassava starch concentration at 70 °C. It was observed that reducing sugar concentration increased with acid concentration from 0.1 M to 0.5 M HCl but the curves leveled off after about 9h of hydrolysis. The dextrose equivalent at 0.5 M HCl was 22% and at 0.1 M HCl it was 14.3%.

(*b*) Acid-enzyme hydrolysis

Observations made during acid-enzyme hydrolysis of raw cassava starch are displayed in Figs. 4 and 5. The variations of reducing sugar concentration with time using 15% and 30% initial cassava starch concentrations, and HCl/fungalmyl/ amyloglucosidase is shown in Fig. 4. There was an exponential increase in the reducing sugar concentration within the first 40h of hydrolysis, after which there was no further significant increase. It was observed that the reducing sugar concentration leveled off after 60h of hydrolysis. When the initial cassava starch concentration of 15% was used, a yield of about 32.5 g/L was obtained while using initial cassava starch concentration of 30% gave a yield of about 72 g/L. The dextrose equivalents in

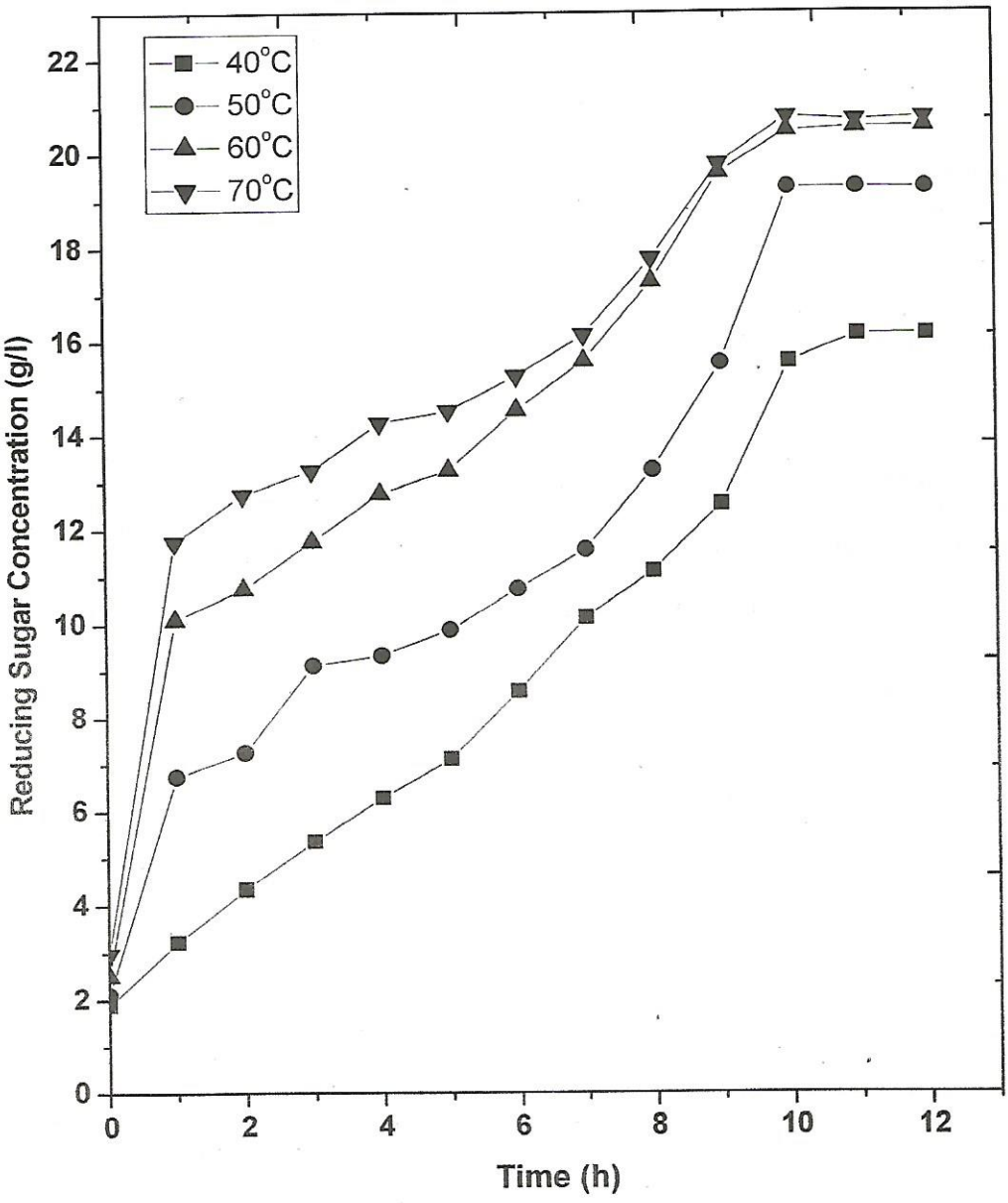


Fig. 1: Variation of Reducing Sugar Concentration with Time at different Temperatures and 0.1 M Acid Concentration using 10% Cassava Starch Slurry.

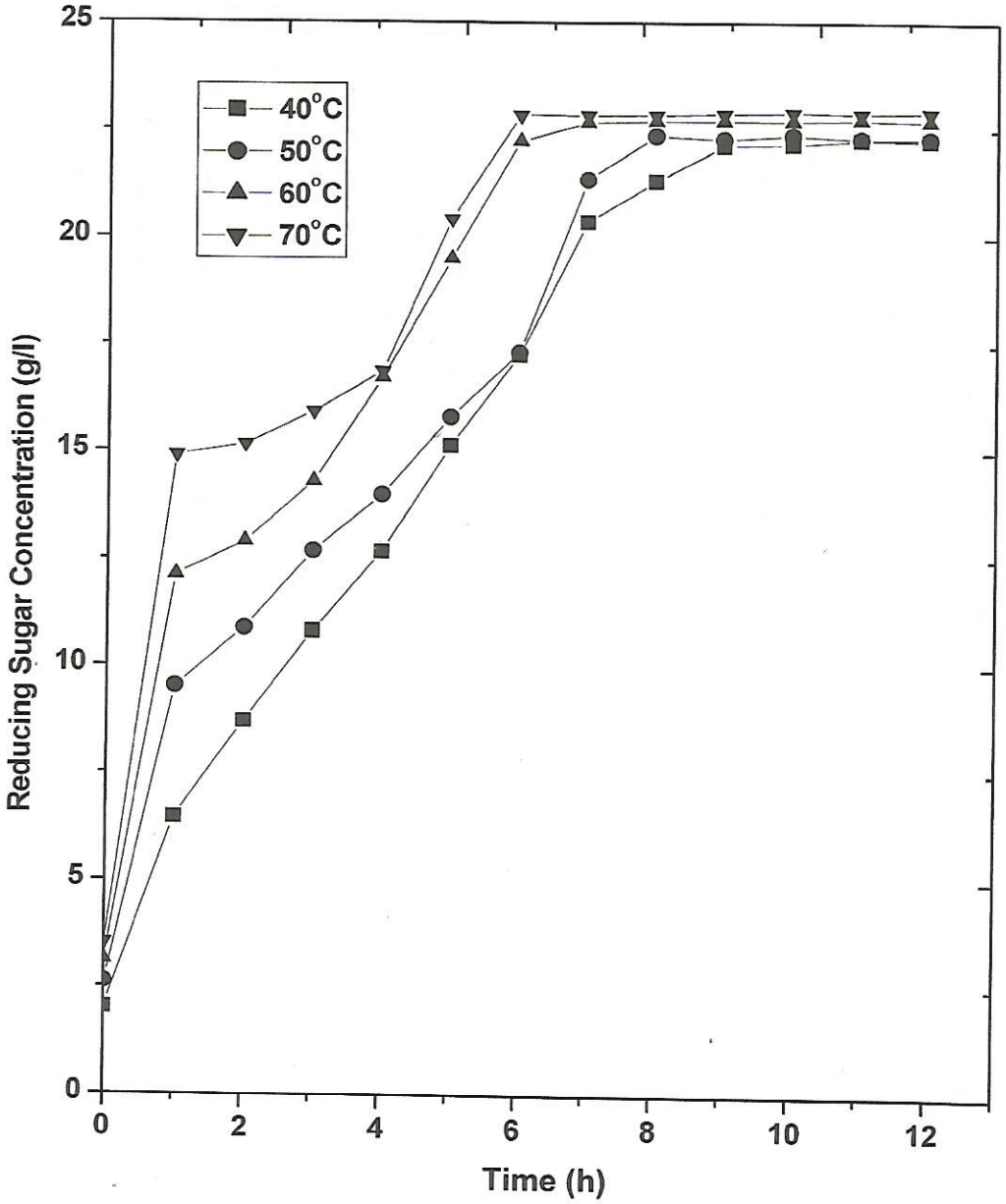


Fig. 2: Variation of Reducing Sugar Concentration with Time at different Temperatures and 0.1 M Acid Concentration using 12% Cassava Starch Slurry.

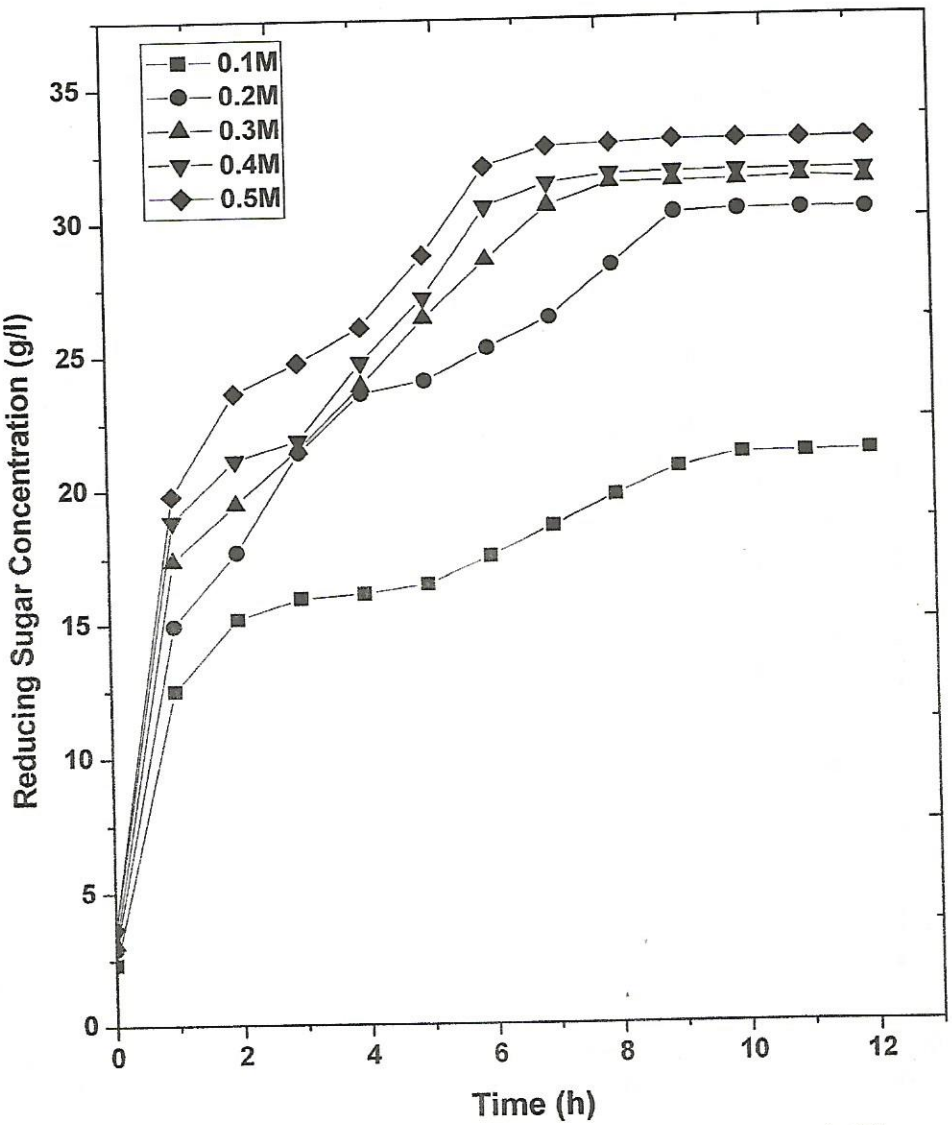


Fig. 3: Variation of Reducing Sugar Concentration with Time at different Acid Concentrations and at 70°C Temperature using 15% Cassava Starch Slurry.

both cases of initial cassava starch concentrations of 15% and 30% were 21.6% and 24.8%, respectively.

Fig. 5 shows the variations of reducing sugar concentration with time using 15% and 30% initial cassava starch concentrations in acid-enzyme hydrolysis with HCl/termamyl/amyloglucosidase. Fig. 4 is similar in pattern to Fig. 5. The yields obtained using 15% and 30% initial cassava starch concentrations were 22.5 g/L and 52 g/L, respectively. The dextrose equivalents were also 15.2% and 17.5%, respectively. Comparison of the dextrose equivalents in case where fungalmyl were used (Fig. 4) with that of termamyl (Fig. 5), showed that the dextrose equivalents in HCl/fungalmyl/amyloglucosidase system was higher than that in HCl/termamyl/amyloglucosidase system. This could have been due to the fact that fungalmyl is more favoured in acid medium than termamyl (Fogarty and Kelly, 1980).

Three stages of hydrolysis reactions were observed in Figs. 4 and 5. The effective time for the first stage hydrolysis (acid hydrolysis) was 15 h, after which the reaction leveled off to a plateau. Similarly, the effective times for the second and third stage hydrolysis with enzymes were 10 and 25h, respectively. Therefore, the total effective time for the three stage hydrolysis was 50h.

(*c*) Enzyme-enzyme hydrolysis

Fig. 6 shows the variations of reducing sugar concentration with time using 15% and 30% initial cassava starch concentrations, in enzyme-enzyme hydrolysis with fungalmyl/amyloglucosidase. The reducing sugar concentration leveled off to a plateau after 70 h of hydrolysis. The yields of 64 g/L and 148 g/L were obtained using 15% and 30% initial cassava starch concentrations, respectively. The dextrose equivalents were also 42.8% and 49.2%, respectively.

Fig. 7 shows the variations of reducing sugar concentration with time using 15% and 30% initial cassava starch concentration, in enzyme-enzyme hydrolysis with termamyl/amyloglucosidase. The yields in terms of the reducing sugar concentration were 66 g/L and 153 g/L and the dextrose equivalents were 44.2% and 50.9%, respectively. Comparison of the results in Fig. 6 with the one in Fig. 7 showed that the pair of enzymes, termamyl/amyloglucosidase, performed better than the pair of fungalmyl/amyloglucosidase. This could be due to the fact that the medium was tending towards neutrality where termamyl was more favoured, as termamyl is stable between pH of 4.8 and 6.5 while fungalmyl is stable between pH of 4 and 5 (Fogarty and Kelly, 1980).

Two stages of hydrolysis reactions were observed in Figs. 6 and 7. The effective time for the first stage hydrolysis was 25h after which the reaction leveled off to a plateau. Also the effective time for the second stage hydrolysis was 35h. Therefore, the total effective time for the two hydrolysis reactions was 60h.

(*d*) Dextrose Equivalents

The summary of the dextrose equivalents calculated from the bench-scale hydrolysis in this study are presented in Table 1. In acid hydrolysis, increased initial cassava starch concentration resulted in increased reducing sugar concentration but reduced dextrose equivalents at low acid concentration of 0.1 M HCl. The dextrose equivalent was increased at higher acid concentration of 0.5 M HCl. Therefore, both high concentrations of cassava starch and acid are favoured in acid hydrolysis, although the purity of the product was not investigated. The acid attack on starch is more random and to the core of the starch molecules which may lead to side products and impurities whereas enzyme-enzyme hydrolysis of starch occurs without producing any inimical by-products. In acid-enzyme and enzyme-enzyme hydrolysis, increased initial cassava starch concentration resulted in increased reducing sugar concentration and dextrose equivalents. It has been reported that it is necessary to gelatinize and liquefy slurry with 30-40% dry matter in order to make starch susceptible to enzymatic break down (Priest, 1984).

4. Conclusion

It was found that in acid hydrolysis, increase in temperature, initial concentration of cassava starch and acid concentration resulted in increase in reducing sugar concentration and dextrose equivalent. In both acid-enzyme and enzyme-enzyme hydrolysis, it was also found that increase in initial concentration of cassava starch resulted in increase in reducing sugar concentration and dextrose equivalent. In acid-enzyme hydrolysis, the combination of HCl/fungalmyl/amyloglucosidase performed better than the combination of HCl/termamyl/amyloglucosidase. In enzyme-enzyme hydrolysis, the combination of termamyl/amyloglucosidase performed better than the combination of fungalmyl/amyloglucosidase. Overall, the enzyme-enzyme system of termamyl/amyloglucosidase gave the best performance. The optimum sequence of operation in acid-enzyme hydrolysis was found to be 15h for acid, 10h for fungalmyl or termamyl and 25h for amyloglucosidase. The optimum sequence of operation in enzyme-enzyme hydrolysis was found to be 15h for fungalmyl or termamyl and 35h for amyloglucosidase.

Table 1: Dextrose Equivalent of various Cassava Starch in Acid, Acid-Enzyme, and Enzyme-Enzyme Hydrolysis

Cassava Starch Concentration	Dextrose Equivalent (DE)					
	Bench Scale Behaviour					
	Acid (HCl)		Acid-Enzyme		Enzyme-Enzyme	
	0.1 M	0.5 M	HCl/Fungal myl/AMG	HCl/Termal myl/AMG	Fungal myl/AMG	Termal myl/AMG
10%	20.7	-	-	-	-	-
12%	19.1	-	-	-	-	-
15%	14.3	22	21.6	15.2	42.8	44.2
30%	-	-	24.8	17.5	49.2	50.9

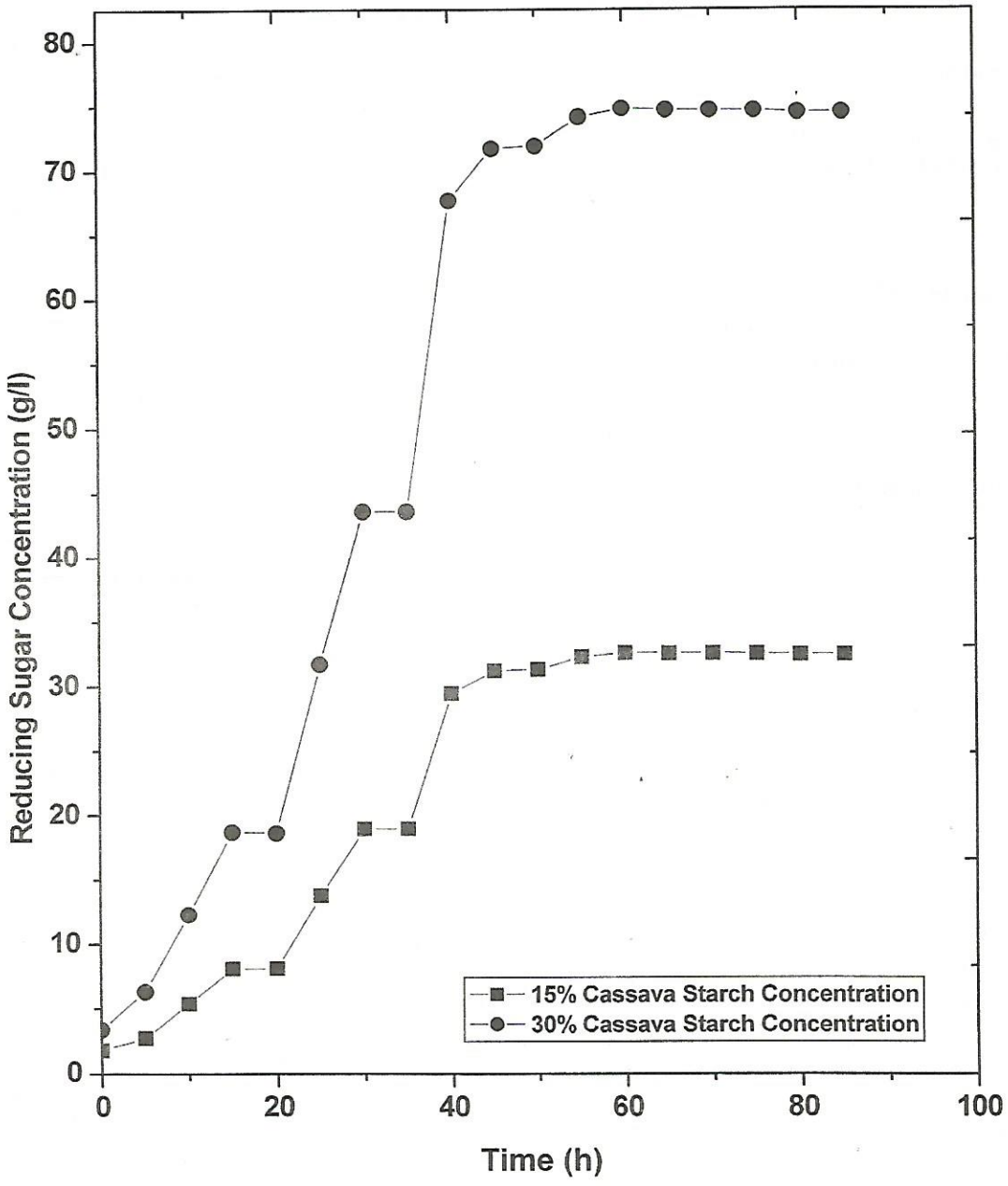


Fig. 4: Variation of Reducing Sugar Concentration with Time in Acid-Enzyme Hydrolysis (HCl/fungalmyl/amyloglucosidase)

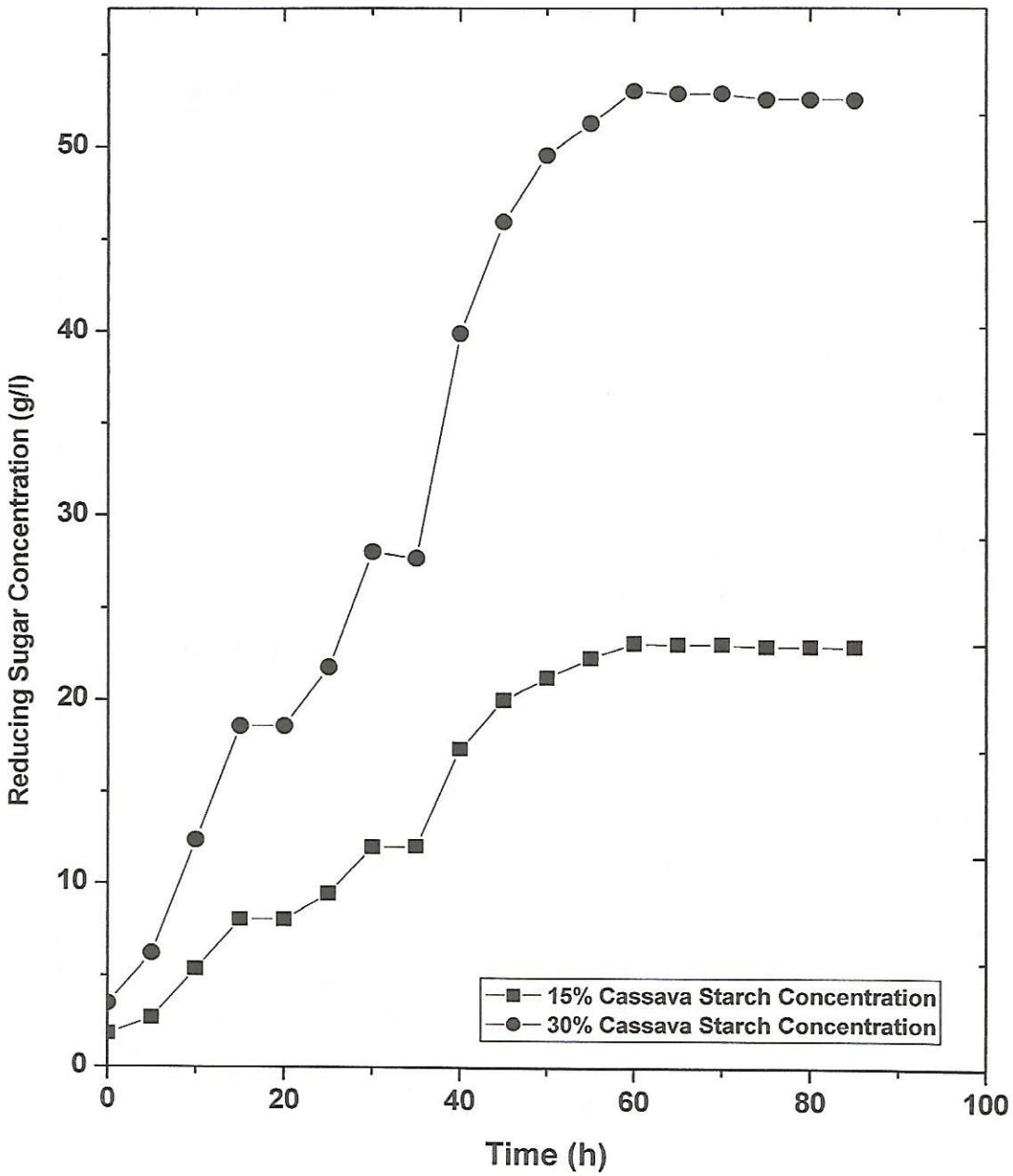


Fig. 5: Variation of Reducing Sugar Concentration with Time in Acid-Enzyme Hydrolysis (HCl/termamyl/amyloglucosidase)

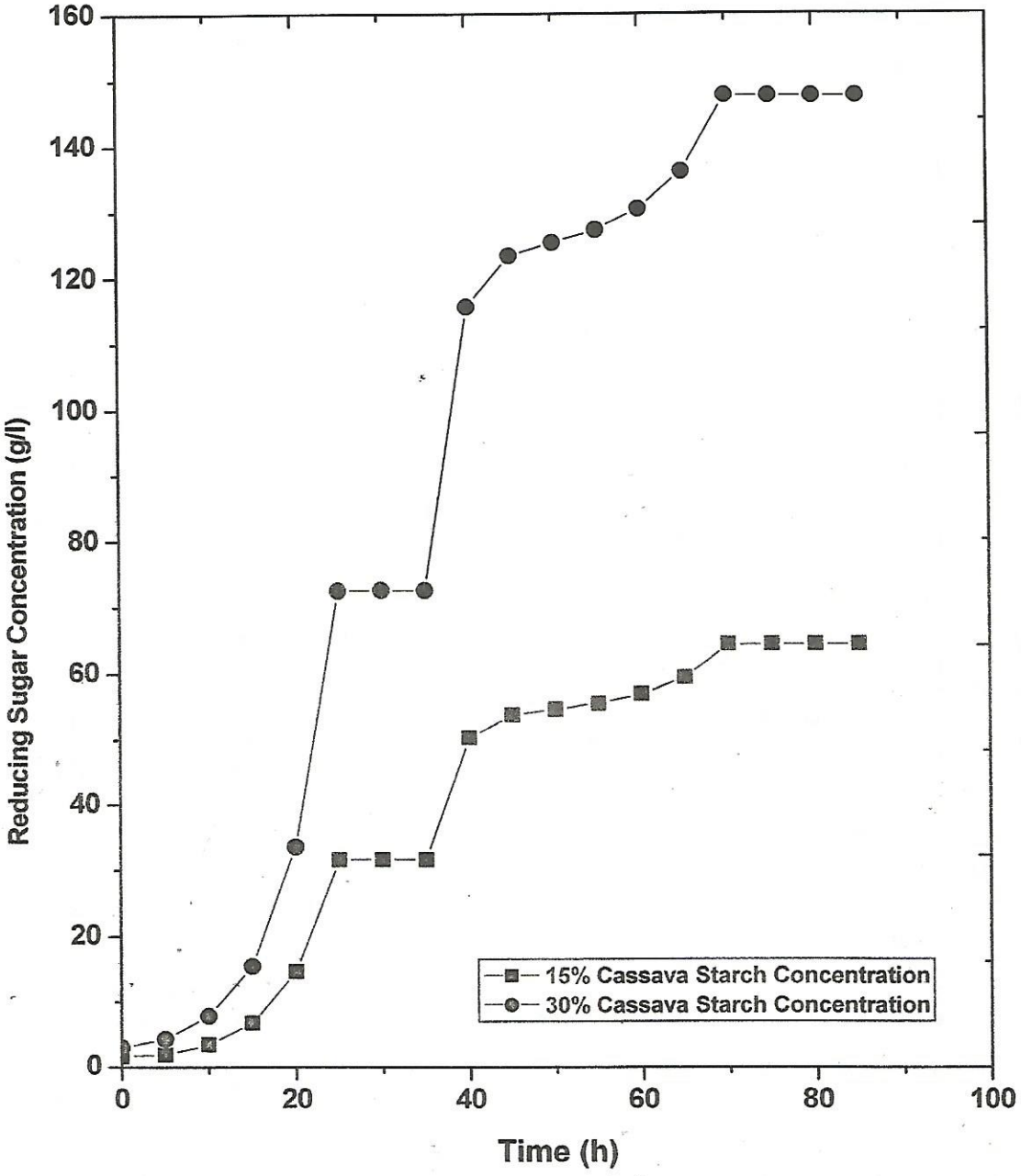


Fig. 6: Variation of Reducing Sugar Concentration with Time in Enzyme-Enzyme Hydrolysis (fungalmyl/amyloglucosidase)

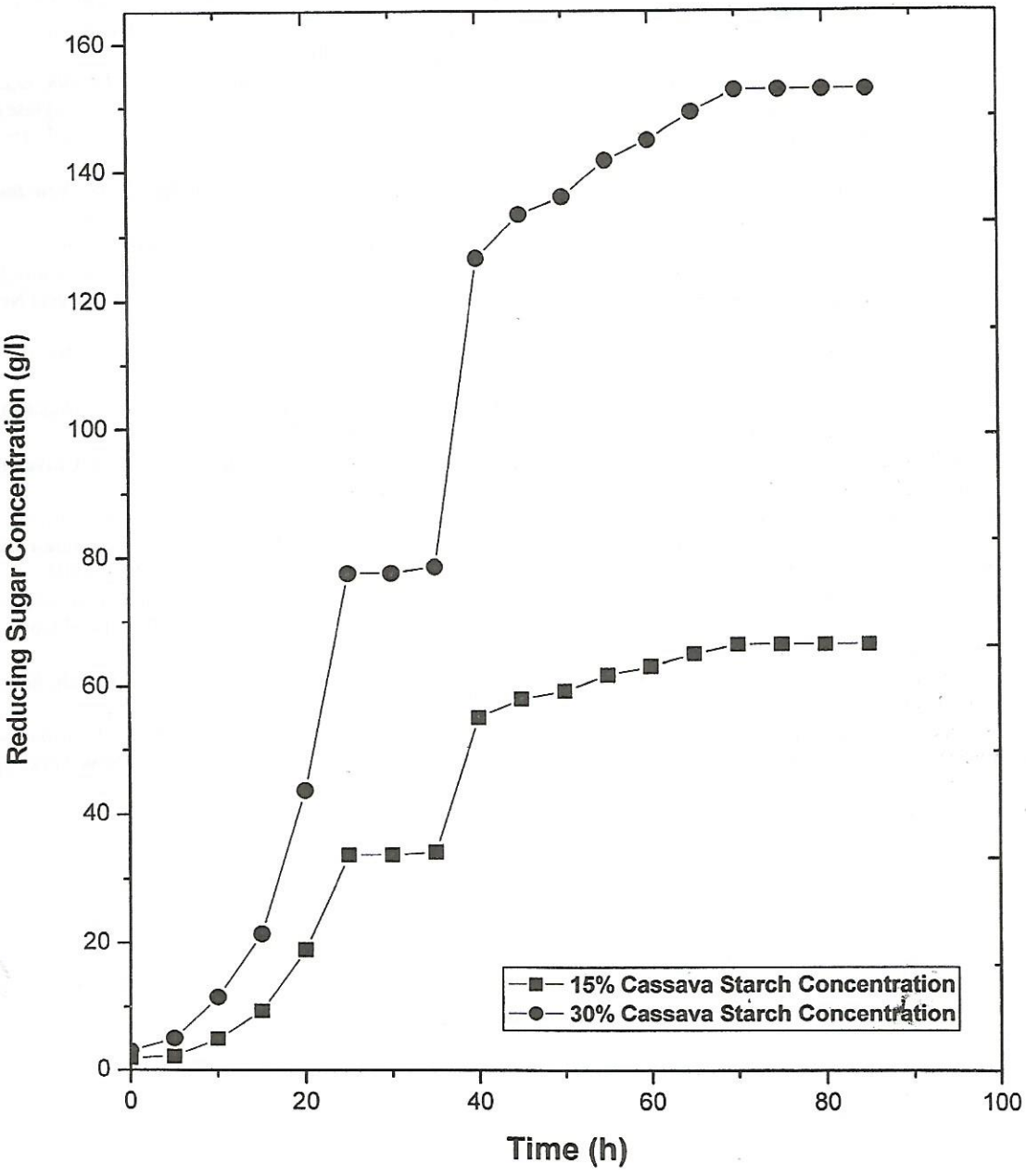


Fig. 7: Variation of Reducing Sugar Concentration with Time in Enzyme-Enzyme Hydrolysis (termamyl/amyloglucosidase)

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