ENZYME HYDROLYSIS OF CASSAVA PEELS: TREATMENT BY AMYLOLYTIC AND CELLULOLYTIC ENZYMES

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ABSTRACT

Cassava peels provide a cheap non-food biomass waste that can be hydrolysed to simple sugars as a useful feedstock. Unlike most crop wastes, they have high starch content as well as lignocellulose. In this study an enzymatic treatment of cassava peels by various concentrations of amylase and glucoamylase is considered. Steam explosion pre-treatment reduced rates and yields of hydrolysis. Milled peels suspended at 10% w/v yielded a maximum reducing sugar of 0.41g (as glucose) per gram of peels. HPLC analysis showed that levels of soluble oligosaccharides remained low throughout. A pre-treatment with amylase at 95^{o}C slightly increased rates although final yield was the same. Additional treatment with cellulolytic enzymes increases the total hydrolysis yield to 0.61g (as glucose) per gram of peels representing 91% of the carbohydrate in cassava peels.

Keywords: Starch rich biomass; Pretreatment; Amylolytic and Cellulolytic Hydrolysis

1.1 INTRODUCTION

Biomass resources provide an excellent feedstock for both fuel and chemicals. The competition for food makes the use of waste and other non food crops a more attractive option for production of these valuable products. Most lignocellulose biomass conversion to simple sugars and subsequently to biofuels and other chemicals has been from substrates with lignin, cellulose and hemicellulose polymers. Very few biomass wastes have starch in addition to lignin, cellulose and hemicellulose. Cassava peels is one such waste. Potato peels, sorghum bran, yam peels are also other examples of starch rich lignocellulose biomass. Cassava (*Manihot esculata*) is a woody shrub extensively cultivated as an annual crop in tropical regions of the world for its edible starchy root. It is mainly consumed in Africa when converted to various food products by fermentation. Cassava peels are a by-product of cassava. The peel of the cassava is 1-4mm thick and accounts for 10-14% of the total dry matter of the root (Adegbola and Asaolu, 1986; Nartey and Moller, 1973). The United Nations Food and Agricultural Organization (FAO) report shows that Nigeria alone produced 38 million metric tonnes of cassava per annum as at 2004 while current reports from USAID/Market report show that Nigeria currently produces over 45 million metric tonnes of cassava per annum. The Nigerian Government Presidential Cassava Initiative in conjunction with the United Nations Industrial Development Organization developed a Cassava Master Plan in 2006 in which the projected cassava production is expected to reach 150 million metric tonnes per annum by 2020(FAO 2013). This would in turn generate over 15 million metric tonnes of cassava waste per annum. Currently, cassava waste which includes the peels, leaves and unused leftover stalks from the processing of cassava is used

as animal feed and also as manure in small farms in the rural area. Much of the waste is burnt or thrown away. The need to convert this waste into biofuel or other valuable products becomes necessary.

Differences in composition of cassava peels have been reported in literature. Bayitse et al 2015 reported that Cassava peels contain Starch 47.16%, Arabinose 2.35%, Xylose 2.31%, Lignin 1.92% with glucose reported at 83.41%. This report did not differentiate the amount of glucose obtained from starch, cellulose and hemicelluloses. Another analysis of the chemical composition of cassava peels indicates the following chemical composition: dry matter 86.5–94.5 %; organic matter 81.9–93.9 %; crude protein 4.1–6.5 %; hemicellulose and cellulose 34.4 %; and lignin 8.4 % (Kongkiattikajorn and Sornvoraweat 2011). This report did not state how much residual starch was available from the peels. Some studies have explored enzymatic hydrolysis of mixed cassava wastes, including peels and residues from starch processing. These studies also compared acid and alkaline hydrolysis with enzymatic hydrolysis of mixed cassava waste (Elechi et al 2016, Mohammed et al 2014, Srinorakutara et al, 2006; Yoonan and Kongkiattikajorn 2004). More recent studies on cassava peels (Bayitse et al 2015) focus on the optimization of cassava peels using mixtures of cellulase and beta-glucanase enzyme. Studies on pretreatment strategies involving the use of dilute sulphuric acid, methanol with catalyst (organosolv) and alkali prior to microbial enzymatic hydrolysis for the production of fermentable sugars for bioethanol production have also being reported (Nweke and Abiamere 2014).

This paper will investigate the effect of combining amylases and cellulases to produce maximum hydrolysis of the peels at higher cassava peel concentration. Studies of enzyme hydrolysis of cassava peels in literature (Bayitse et al 2015, Srinorakutara et al, 2006; Yoonan and Kongkiattikajorn 2004) have typically been carried out at low substrate

concentration (less than 2% w/v) however this study will look at cassava peels at higher concentrations (5%, 10% and 14% w/v). This paper will examine a process option where the peels are first treated with amylase and glucoamylase to digest the starch component before a subsequent treatment of resuspended cassava peels with cellulase and hemicellulase enzymes. It will also investigate the effect of Hot water and Steam explosion treatment on cassava peels, effect of pH as well as the effect of enzyme dosage. A HPLC analysis of the released sugars at different reaction times is also examined and compared with the reducing sugar yield. This is expected to show the sugar release patterns in starch hydrolysis.

The inexpensive nature of cassava peels as well as its abundance creates an opportunity for cassava exploitation in the production of sugar feedstock for biofuels, chemicals or other applications.

2.0 MATERIALS AND METHODS

2. 1 Substrate and Enzymes

Cassava peels were obtained from a local farm in Makurdi, Nigeria in September 2010. No information on the pedigree of the cassava plant is available. The cassava peel was soaked in water for 40 minutes. This was carried out to ensure an easier removal of the skin. Knives were used for peeling the cassava tubers. These were air and sun dried at about 30^0C stored and then transported to the United Kingdom. The peels were received at the University of Strathclyde Laboratory in October 2010. The peels were then stored in airtight 5L containers until use. The enzymes used were a generous gift from Novozymes A/S. Liquizyme SC DS; an alpha amylase with declared activity of 240 KNU-S/g from *Bacillus licheniformis*(KNU-S Alpha amylase Unit) and Spirizyme Fuel HS-A glucoamylase with

declared activity of 1425 AGU/g from *Aspergillus niger* (AGU; amyloglucosidase unit) were both used. Cellulase enzymes were also used for further cellulose treatment. Viscozyme Cassava R; a product with a declared activity of 100 FBG/g (where FBG is betaglucanase units) from *Aspergillus aculeatus* containing a mixture of hemicellulase, cellulase and xylanase was used. In this paper it will be referred to as cellulase R. Viscozyme Cassava C – a cellulase enzyme from *Trichoderma reesei* with a declared activity of 700 EGU/g (where EGU is endoglucanase unit) was also used. In this paper, this enzyme will be referred to as cellulase C.

2.1 Feedstock characterization

The physico-chemical characterization of the cassava peel was carried out at CIEMAT Madrid to determine the residual composition of the cassava peels. The feedstock characterisation assays were performed using the Laboratory Analytical Procedures developed by the United States National Renewable Energy Laboratory and methods developed by the Association of Official Analytical Chemistry (Sluiter et al, 2006a, 2006b, 2004c; Hames et al 2008). Determination of total solids in samples was done according to the laboratory analytical procedure for determination of total solids in biomass. Samples were dried at 105^0C overnight to eliminate water content. Extractive contents of the samples were determined using the laboratory analytical procedure for determination of extractives in biomass (Sluiter et al 2010).

2.2 Milled, washed and dried peel (MWO)

Sun dried peels (40 g) were milled for 3 min in a Kenwood BL450 kitchen blender with a grinding mill attachment to give a powder (approximately 60-450 µm). Powder (10 g) was washed with 40 mL water, under rotation at 40 rpm for 10 min to remove soluble

contaminants. Tubes were then centrifuged at 2880 x g for four minutes and the supernatant discarded. Washing was repeated twice, and the final reducing sugar concentration in the supernatant was below 1 g glucose/L. The residue was then dried at 50^0C for 24 hours to give final moisture content below 10%. Sampling for analysis or hydrolysis was quite tricky to obtain representative samples. Batches of cassava peels that were milled at different times were mixed in 50ml centrifuge tubes. The centrifuge tubes were filled halfway and gently rolled at roughly 2 rotations per second to ensure even distribution of particles of different sizes. This method minimised the stratification of the powdered cassava peels. Sampling was found to be critical to results obtained as it was discovered that failure to proceed this way had an adverse effect on reproducibility of the hydrolysis rate and yield.

2.3 Hot Water Pre-treatment (Hydrothermal treatment) of cassava peels

A representative sample of the milled, washed and dried cassava peel was suspended in 0.05M sodium acetate buffer (pH values between 4 and 6) at a solid content of 10% in an Erlenmeyer flask. Amylase enzyme was added to the mixture and the flask weighed. The mixture was hydrothermally pretreated by heating to approximately 95^0C. After 2 hour incubation, the flask was weighed again and 0.05M sodium acetate buffer was added to make up for the lost water due to evaporation. The pH was then adjusted with NaOH, as the cassava peels are slightly acidic. After cooling, subsequent hydrolysis by glucoamylase was then carried out, as described below.

2. 4 Steam Explosion pre-treatment

The steam explosion of the cassava peels was done at CIEMAT Madrid and the machine used for the pre-treatment is made up of three units: a steam accumulator, a steam

explosion reactor and a discharge cyclone. The steam accumulator supplies steam at a temperature of 210°C to the steam explosion reactor. The steam explosion reactor is the chamber where the lignocellulosic biomass is compressed and suddenly de-pressurised. It consists of a 3" diameter stainless steel 316 vertical pipe, limited by two 3" diameter stainless steel 316 throttle valves. The input valve on the top of the chamber opens and closes by hand and is used to load the biomass as received in the reactor. The output valve on the bottom of the chamber opens by a triggering and spring device in less than 1 second. The mixture of steam and biomass is thus discharged violently, and passes through a pipe that carries it to the cyclone. The discharge cyclone is built of stainless steel 316. The peels were held at 210^{o}C for 5 min before decompression. The pretreated peels were then frozen and stored at -20^{o}C. Stored pretreated peels used for enzyme hydrolysis were thawed and were composed of pieces between 1 mm and 1 cm. Small samples from 3 or 4 different places were taken and mixed to make them more representative.

2.5 Enzymatic hydrolysis

After pre-treatment, 10%w/v (5g per 50 ml) and 14% (7g/50ml) w/v cassava peels were hydrolysed simultaneously or sequentially by amylase and glucoamylase enzymes. In all cases, hydrolysis was performed in duplicate and the results are presented as mean values. Tukey's Test is used for statistical analysis of data. The digestion was carried out in a 250ml Erlenmeyer flask covered with aluminium foil. 0.05M sodium acetate (50 ml) at different pH ranging from 4 to 6 was used as buffer. The experiments were carried out at an incubation temperature of 50^{0}C using a Grant GLS 400 Water bath incubator with shaker at 220 strokes per minute. The shaker provides a linear shaking motion. The reaction vessels were at a depth of 50mm and a stroke length of 18mm. Samples from the supernatant were then

analysed either by DNS assay or HPLC assay. The reaction was stopped by mixing with DNS reagent or 0.1M HCl for the HPLC analysis.

Further treatment by cellulase enzymes was also carried out. This process option involves washing the hydrolysed peels thrice with 0.05M sodium acetate and drying the peels in the oven at 40^0C for 24 hours. The peels are then resuspended for digestion by a cocktail of cellulase enzymes. Three different enzyme volumes of 50µL, 150µL and 300µL in 50 ml cassava peels suspension were used throughout for these experiments. This corresponds to 0.1%v/v, 0.3%v/v and 0.6%v/v respectively.

2.6 Estimation of Reducing Sugar by DNS assay

The dinitrosalicylic (DNS) colourimetric method (Miller, 1957) for the determination of glucose was used to assay the content of reducing sugar. The assay was read at 575nm using a Beckman Coulter DU 800 Uv-Vis Spectrophotometer and samples were diluted to contain between 0.1 and 1 mg glucose equivalent per ml, where the calibration curve is reasonably sensitive.

2.7 HPLC Analysis

HPLC was used to monitor the release of oligosaccharides as the reaction progresses. The Shodex Sugar KS 801 column separates by size exclusion and some specific interactions with sugars. A Waters 2695 HPLC system fitted with a Shodex guard column used the following conditions; Sample volume 10ul , Mobile Phase Hplc water, Flow rate 1 ml/min, Column Temperature 60^0C, Run time 15 minutes. Detection was by a Waters 410 refractive index detector.

Standards curves for glucose, maltose and cellobiose showed that the areas of chromatograms represented the mass concentration of standards and so for DP 3 and above, the mass concentration of maltotriose and maltotetrose were estimated using glucose as a standard. Oligosaccharides of DP 5 and above were not separated, and a molecular weight of 990.86g/mol (maltohexose) was used to estimate the molar concentration. This might not hold for maltodextrins as these are soluble in water up to DP 60 (Arantes and Saddler 2010), so the molar concentration of larger maltodextrins may be overestimated.

3. 0 RESULTS AND DISCUSSION

3.1 Chemical composition of Cassava peels

Table 1 here:

Compositional analysis carried out by CIEMAT Madrid gave the results in Table 1 above. It can be observed from the table that cassava peels have high starch content. Results also show that a combined cellulose and hemicellulose content of 32.3% agrees with those obtained in literature. Kongkiattikajorn and Sornvoraweat 2011 showed a combined value of 34.4% for cellulose and hemicellulose content. Table 1 also gives a breakdown of the hemicellulose content which agrees with results obtained by Bayitse et al 2015 who reported contents of 2.31% and 2.35% for Arabinose and Xylose respectively. However there were significant differences in the starch content obtained. Bayitse et al 2015 reports a starch content of 47.16% compared to 28% as shown in Table 1. This can be attributed to several factors; the process of peeling carried out on the cassava peel which leaves starch residues on the peels and the variety of cassava used for the experiments.

3. 2 Effect of steam explosion pre-treatment

Steam explosion is often used to improve the digestibility of lignocellulosic wastes. Amylase and glucoamylase hydrolysis carried out on cassava peels pretreated by steam explosion did not give a high reducing sugar yield compared to the milled cassava peels as shown in fig 1. Hydrolysis yields are shown by the conventional measure of reducing sugar as glucose as a percentage of substrate mass. Note that because of the addition of water, complete hydrolysis of pure starch or cellulose gives about 111% reducing sugar on this basis.

Fig 1 here

Fig 2 here

Cassava peels pretreated by steam explosion also gave lower reducing sugar yields at a slower rate when compared to milled cassava peels (Fig 1). It is likely that chemical degradation of carbohydrates during steam explosion contributes here. Glucose is known to decompose to hydroxymethylfurfural (HMF), 1, 6-anhydroglucose, levulinic acid, and formic acid at high temperatures (Corredor et al, 2007). It is possible that these sugar derivatives have inhibiting effect on the amylolytic enzymes. Starch degradation during steam explosion may also leave less to be hydrolysed enzymatically. Fig 2 however shows that treatment with cellulase enzymes on Cassava peels pretreated by steam explosion gave a higher reducing sugar yield compared to the milled cassava peels. Final reducing sugar yield for steam exploded peels is shown to be 37%. Earlier results from table 1 in section 1.1 above has total cellulose and hemicelluloses component to be 33% and so this higher hydrolysis yield might be attributed to the complete hydrolysis of the cellulose/hemicelluloses component. It might also involve the partial hydrolysis of the starch component especially

as steam explosion occurs at very high temperature which could gelatinise the starch therefore making its digestion easier. These results show that while steam explosion is effective for cellulose hydrolysis, it is ineffective for starch hydrolysis. Subsequent results presented in this paper do not use steam exploded material.

3.3 Effect of Change in Concentration

3. 3. 1 Effect of Peel Concentrations

Substrate concentrations of 10%w/v cassava peels and 14% w/v cassava peels gave similar reducing sugar yields at same enzyme concentrations as shown in Fig 3 below. With 5% w/v peels both rates and yields of hydrolysis were lower. This trend was also observed for cellulolytic hydrolysis. Previous studies have reported the use of much lower peel concentrations for hydrolysis. Bayitse et al 2015 reports 0.20g of cassava peels in 10ml of acetate buffer representing 2% w/v for cellulose hydrolysis while Yoonan and Kongkiattikajorn 2004 used 1.5%w/v cassava peels. Results obtained for amylase and amyloglucosidase enzyme treatment by Yoonan and Kongkiattikajorn 2004 shows that 29.89% reducing sugar was obtained when compared to 40.11% in figure 3 below. The variation can be attributed to the differences in residual starch content in cassava peels.

Fig 3 here

Subsequent experiments shown in this paper were carried out with 10%w/v cassava peels. Hydrolysis experiments carried out at 14%w/v gave less reproducible hydrolysis yield when compared to 10%w/v during the first one hour of the reaction. The viscosity of the cassava peel suspension at 14%w/v might have been responsible for the low reproducibility

observed, as sampling becomes more difficult. As reaction progresses, the suspension becomes less viscous making it easier for more effective mixing and sampling.

3. 3. 2 Effect of Enzyme Concentration

Fig 4 here

Fig 4 shows that enzyme concentrations of 0.3%v/v of amylase and glucoamylase gave maximum reducing sugar yield in 24 hours. The reaction progress with 0.6%v/v enzymes is probably not significantly different. 0.1%v/v enzyme concentration gave a slower reaction and lower reducing sugar yield. Similar results were obtained by Yoonan and Kongkiattikajorn 2004 when they carried out hydrolysis of cassava peels at three different enzyme concentrations and results showed optimal reducing sugar yields of 30% for starch hydrolysis and 35% for cellulase hydrolysis of cassava peels (Yoonan and Kongkiattikajorn 2004). Bayitse et al 2015 varied enzyme concentration for cellulase and hemicellulase enzymes for cassava peels and obtained maximum reducing sugar of 69%.

3.4 Oligosaccharide intermediates

An analysis of the oligosaccharides released as the reaction continues was also done and compared with the reducing sugar assay. Fig 5 shows that most of the reducing sugar in the supernatant is free glucose, even at short reaction times. The total molar concentration of reducing sugars analysed by HPLC is also consistent with the results from the DNS assay. The oligosaccharides do account for a higher fraction of the mass in the supernatant, because of their higher molecular mass. Fig 6 shows more detail of how the oligosaccharide concentrations change with time. Overall, it appears that once oligosaccharides are solubilised, they tend to be fairly rapidly hydrolysed to glucose.

Fig 5 here

Fig 6 here

3. 5 Total yield and enzyme action on cellulose

The reducing sugar yield of about 41% for amylolytic hydrolysis of cassava peels is however more than might be expected from the compositional analysis of cassava peels in Table 1. A starch content of 28% would correspond to a reducing sugar yield of 31% on complete hydrolysis. Analysis of cassava peels used the Megazyme Total Starch assay procedure (amyloglucosidase/α-amylase method) listed as AOAC method 996.11 using HPLC for glucose analysis. The discrepancy observed seems too large to reflect sampling error alone. It is possible that the standard assay may not digest all starch in the peels. Another possibility is that non starch components of the cassava peels were also hydrolysed. To investigate this, the amylase and glucoamylase enzyme preparations were used to treat Whatman filter paper no 1.

Fig 7 here

Fig. 7 above shows that hydrolysis was observed, suggesting that these enzymes might have had a hydrolysing effect on the cellulose fraction of the cassava peels. The action on filter paper may be due to cellulolytic enzymes present in the preparations, rather than the amylases themselves. The over 40% reducing sugar yield does make it likely that there is complete digestion of the starch portion of the cassava peels.

3.5 Pretreatment with hot water and amylase

Hot water treatment with simultaneous amylase action was also investigated. It was combined with enzyme hydrolysis carried out at the optimum conditions for both amylase and glucoamylase enzymes.

Fig 8 here

Fig 9 here

Fig 10 here

Fig 8 shows that sequential treatment with amylase at 95^0C for 2 hours followed by glucoamylase treatment for a further 22 hours did not yield any significant advantage in final reducing sugar yield over the combined treatment with amylase and glucoamylase at 50^0C. Fig 8 also show that hydrolysis at pH 5 and 6 didn't show any significant difference in hydrolysis yield, while it is clearly lower at pH 4. However, Fig 9 shows that the hotwater and amylase treatment brings about an increase in the hydrolysis rate in the period after glucoamylase addition, and it is possible that the reaction is brought to completion before 24 hours.

Fig 10 emphasises the first 2 hours of the reaction, and shows that reducing sugars are actually produced slightly more quickly with both enzymes at 50^oC, rather than with amylase alone at 95^oC. Fig 10 also shows that reactions at pH 4 are clearly slowest, while pH 6 is slightly faster than pH 5.

3.6 Consecutive Hydrolysis: Additional Hydrolysis treatment with Cellulase Enzymes

This paper also considers an additional process step that involves washing out the sugars after starch digestion and re-suspending the cassava peels for a subsequent cellulolytic treatment of the peels. This process step is expected to maximize the digestion of both starchy and cellulose parts of the peels. Fig 11 and Fig 12 shows that the final reducing sugar yield obtained is 61.5% for amylase treatment followed by a subsequent treatment with cellulases and 62.5% for amylase treatment followed by a hot water treatment and cellulase

enzyme treatment(amylase-HW-C n R). This process involves washing the peels with 0.05M sodium acetate after the initial amylase treatment and then drying the peels in an oven at 40^{o}C for 24 hours, this resulted in a slowing down of the cellulolytic reaction as shown in 11 as it is believed that drying substrates causes a collapse of the walls making adsorption of enzymes more difficult (Scallan 1974) However what is interesting to note is that 62% of reducing sugar yield represents about 91% of carbohydrate conversion of the peels. Comparison with maximum sugars released for combined amylolytic and cellulolytic hydrolysis of cassava peels shows 50% reducing sugar yield for enzyme hydrolysis and 59.9% reducing sugar yield for sulphuric acid hydrolysis (Yoonan and Kongkiattikajorn 2004). The hydrolysis method adopted by these researchers did not involve a re-suspension of the cassava peels after an initial hydrolysis by either amylolytic or cellulolytic enzymes.

Fig 11 here

Fig 12 here

This process step maximizes the digestion of carbohydrate in cassava peels although it is cumbersome as it requires washing, separating the hydrolysed sugars and drying for 24 hours in an oven.

4.0 CONCLUSION

The goal of this research is to develop a strategy for maximum enzymatic degradation of starch rich cassava peels. Initial Amylolytic treatment of cassava peels showed carbohydrate conversion of 41% whereas hydrolysis by cellulase and a cocktail of hemicellulase enzymes gave yields of 31%. A combined treatment of amylolytic treatment with a subsequent treatment of resuspended peels with cellulase enzymes releases high yields of monosaccharides with 91% conversion of carbohydrates from cassava peels.

A 10%w/v cassava peel concentration is the optimum cassava peel concentration because it guarantees an easier mixing of the enzymes and substrate compared to less than 2% w/v cassava peels concentration that have been used in literature. Enzyme dosages showed that enzyme concentrations of 0.3%v/v gave maximum reducing sugars in 24 hours.

The study also shows that steam explosion pretreatment is not a good pretreatment strategy for starch treatment as it destroys the starch in cassava peels or inhibits the amylolytic enzymes however Hot water treatment was shown to increase hydrolysis rate for starch treatment.

HPLC analysis also showed that once oligosaccharides are released, there are rapidly solubilized into free glucose even at short reaction times.

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FIGURE CAPTIONS

Fig 1 Comparison of the hydrolysis of 10%w/v (5g/50ml) cassava peels pretreated by steam explosion (SE) and milling (MWO-Milled, Washed and Oven dried). Hydrolysis experiment was carried out in 0.05M sodium acetate buffer at 50^0C and pH 5. 0.3%v/v (150µL/50mL) amylase and 0.3%v/v (150µL/50mL) glucoamylase enzymes were used. Differences between the pre-treatments were significant at 95% level up to 1 hour, and 99% level beyond.

Fig 2 Progress curve of the enzymatic hydrolysis of 10%w/v(5g/50ml) cassava peels in 0.05M sodium acetate buffer at pH 5 with 0.3% v/v(150µL/50mL) each of cellulase C and cellulose R at temperature 50^0C. MWO – milled peels; SE – steam exploded peels. Differences between the pre-treatments were significant at 99% level, except for 0.083 hours (95% level) and 24 hours (NS).

Fig 3 Comparison of the enzymatic hydrolysis of 10%w/v (5g/50ml) and 14%w/v (7g/50ml) milled cassava peels in 0.05M sodium acetate buffer at pH 5 and 50^0C for 48 hours at different enzyme concentrations. Both amylase and glucoamylase solutions were added at 0.1%v/v, 0.3%v/v or 0.6%v/v (50µL/50mL, 150µL/50mL, 300µL/50mL). Differences between 0.1% and higher enzyme concentrations were significant at 99% for both peel concentrations.

Fig 4 Progress curves of the enzymatic hydrolysis of 10%w/v(5g/50ml) cassava peels in 0.05M sodium acetate buffer at pH 5 with amylase and glucoamylase and at temperature 50^0C. Differences between 0.1% and higher enzyme concentrations were significant at 99%. Some differences between 0.3% and 0.6% enzyme were calculated as significant at between 90 and 99%, but others were not.

Fig 5 Concentration of oligosaccharides during enzyme hydrolysis of 10%w/v (5g/50ml) milled cassava peels by 0.3% v/v(150µL/50mL) amylase and glucoamylase at pH 5 and at temperature 50^0C

Fig 6 Hydrolysis products found by HPLC, compared with DNS assay for 10%w/v(5g/50ml) milled cassava peels at 0.3%v/v (150µL/50mL) enzyme concentration in 0.05M sodium acetate buffer at pH 5 and at temperature 50^0C

Fig 7 Enzyme hydrolysis of 5%w/v (2.5g/50ml) Whatman filter paper by 0.3%v/v (150µL/50mL) amylase and 0.3%v/v (150µL/50mL) glucoamylase in 0.05M sodium acetate buffer at pH 5 and 50^0C

Fig 8 Final reducing sugar yield after an initial 2 hours of amylase treatment at different pH and subsequent treatment with glucoamylase for a further 22 hours. Control experiment represents amylase +glucoamylase at pH 5 without HW. All 3 comparisons of different pH values for amylase treatment were significant at 99% level. The final reducing sugar at pH 4 was significantly lower (99% level) than all 3 other cases. The final value at pH 6 was significantly higher than pH 5 (95% level) or the control (99% level).

Fig 9 Comparison of the progress curve of the enzymatic hydrolysis of 10%w/v(5g/50ml) cassava peels in 0.05M sodium acetate buffer with amylase and glucoamylase at 0.3v/v(150µL/50mL) enzyme concentration using different pre-treatment strategies. All reactions at pH 5. MWO is the control reaction with milled peel at 50^oC. HW refers to treatment with amylase at 95^oCfor the first 2 hours, the vessel was then allowed to cool to 50^oC and then a subsequent addition of glucoamylase for a further 22 hours. The treatments were calculated to be significantly different (99% level) at all times between 0.083 and 6 hours, except at 1 hour (95% level), despite the cross-over in progress curves.

Fig 10 Progress curve of the enzymatic hydrolysis of 10%w/v (5g/50ml) cassava peels in 0.05M sodium acetate buffer over 2 hours. With amylase at 95^0C or both enzymes at 50^oC. Most differences between conditions were significant at 99% level between 0.5 and 2 hours, except for one case at 95% level.

Fig 11 Progress curve of the overall enzymatic hydrolysis of 10%w/v (5g/50ml) cassava peels in 0.05M sodium acetate buffer. The cassava peels were first treated with 0.3%v/v (150µL/50mL) amylase and glucoamylase for 24 hours at temperature 50^0C. Sugars were then washed out and the residue resuspended in 0.05M sodium acetate buffer with 0.3%v/v(150µL/50mL) of cassava C and cassava R. (Amylase-C n R) and with hot water (Amylase-Hw-C n R) at 50^oC for a further 48 hours. All at pH 5. The only significant differences (95% level) between conditions were at 48 and 72 hours.

Fig 12 Progress curve of the enzymatic hydrolysis of 10%w/v (5g/50ml) resuspended cassava peels in 0.05M sodium acetate buffer. The cassava peels were first treated with 0.3%v/v (150µL/50mL) Amylase and glucoamylase for 24 hours at temperature 50^0C. The residue was then washed and oven dried after which a second treatment involved treating the peels with 0.3%v/v (150µL/50mL) of cassava C and cassava R. (Amylase-C n R) and with hot water (Amylase-Hw-C n R) at 50^oC for a further 70 hours. All at pH 5. % reducing sugar is based on original mass of cassava peels. All differences between enzymes were significant at 99% level, except for the point at 1 hour.

FIGURES

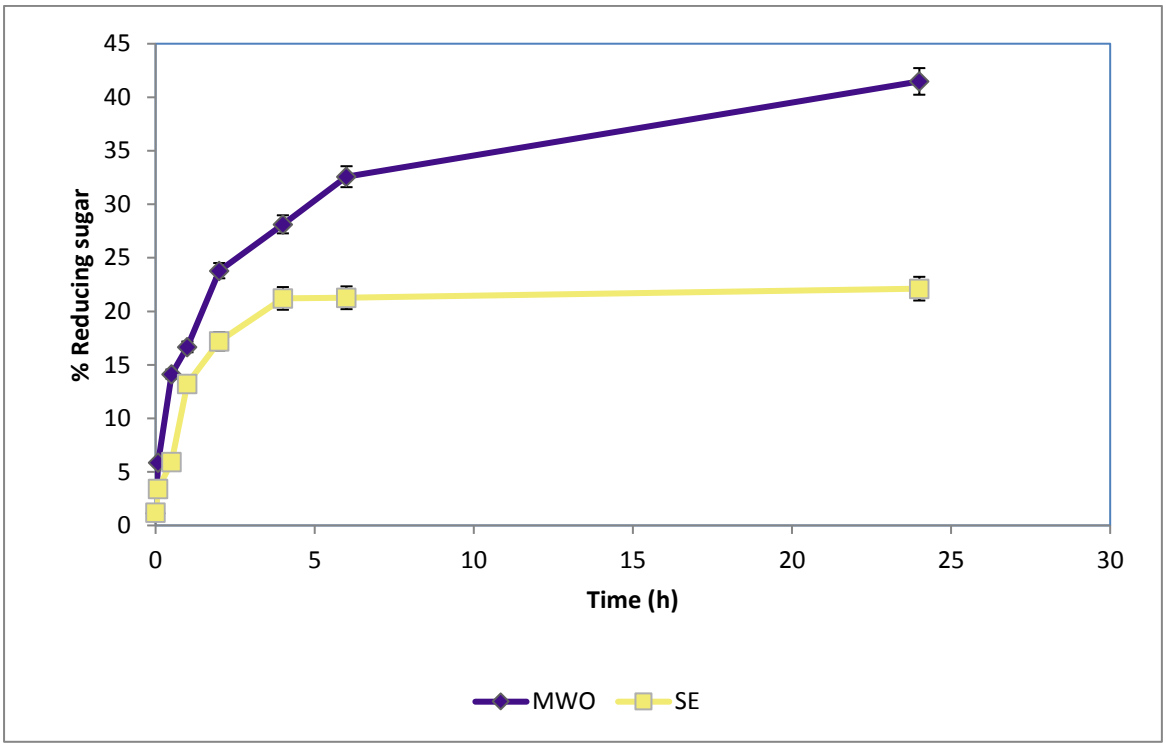

Fig 1:

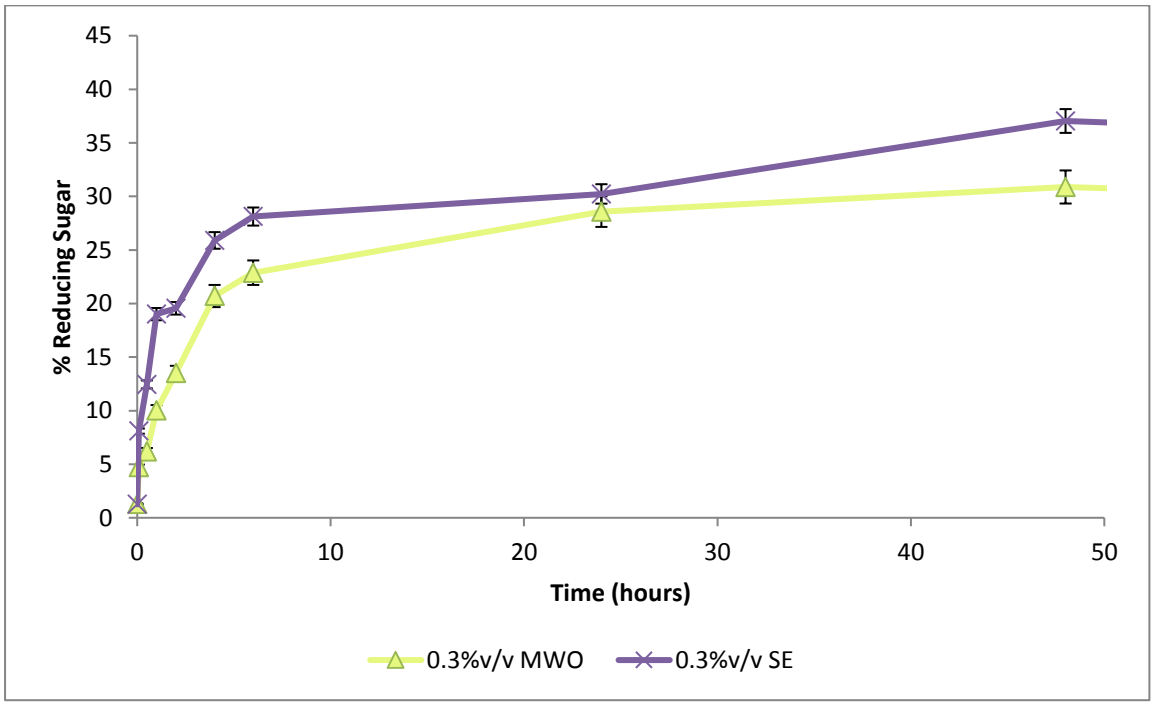

Fig 2:

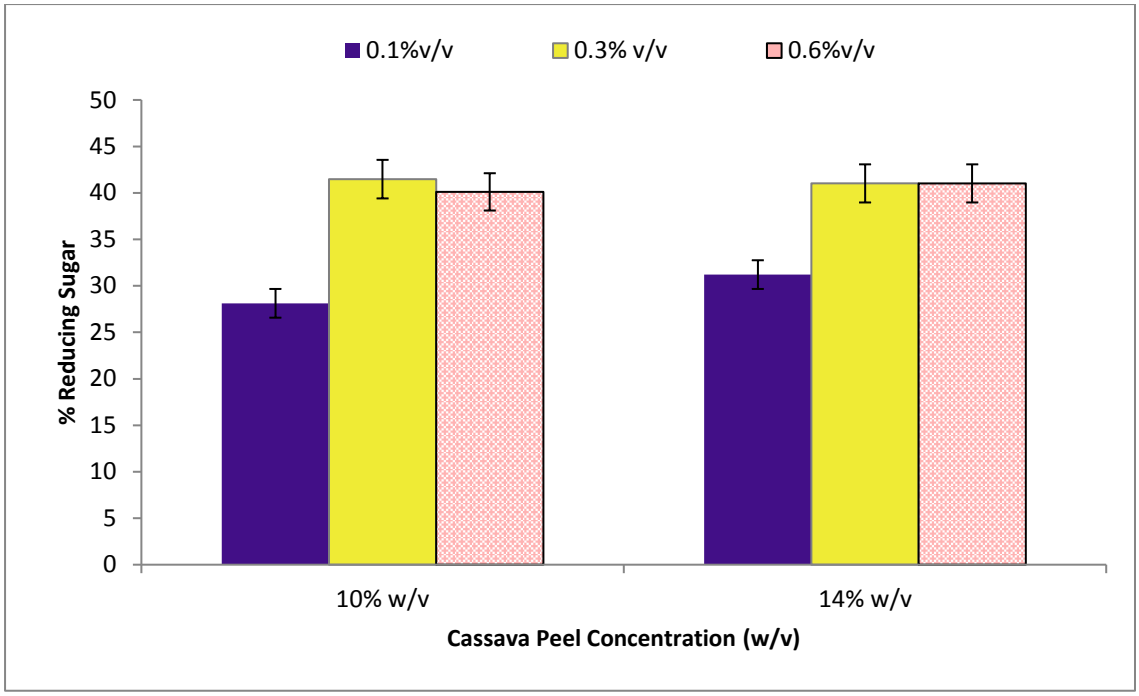
0.1%v/v
0.3% v/v
0.6%v/v
50
45
40
35
30
25
20
15
10
5
0
% Reducing Sugar
10% w/v
14% w/v
Cassava Peel Concentration (w/v)

Fig 3

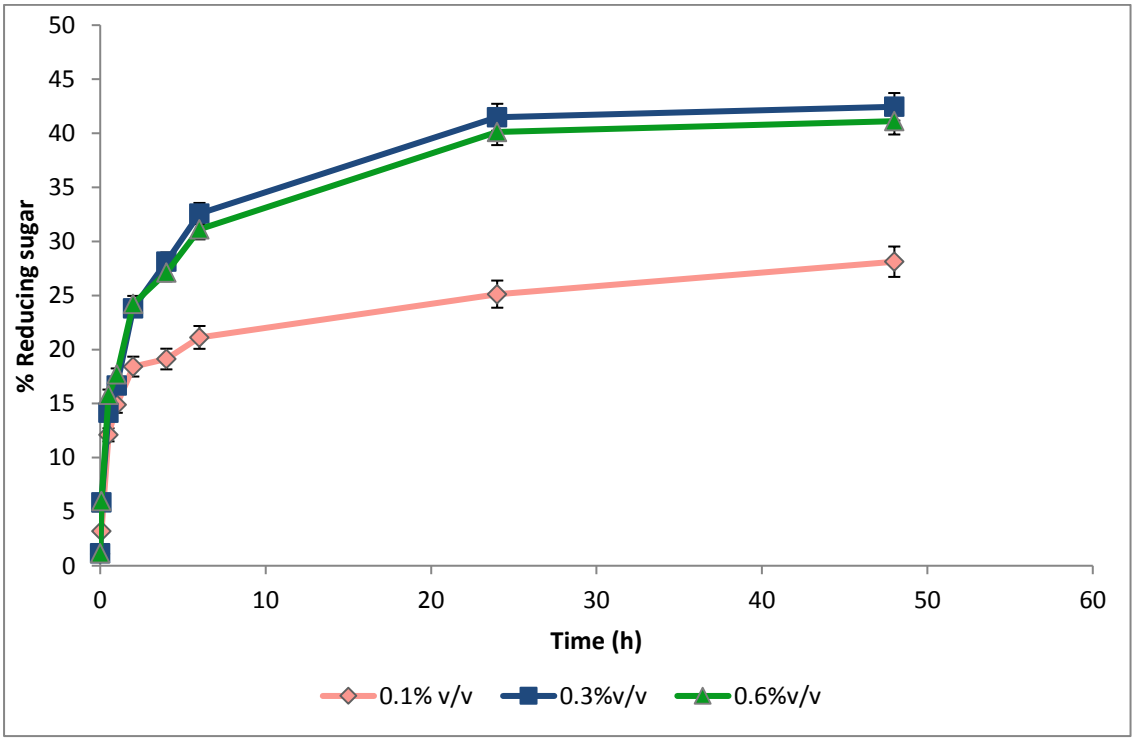
50
45
40
35
30
25
20
15
10
5
0
% Reducing sugar
0
10
20
30
40
50
60
Time (h)
0.1% v/v
0.3%v/v
0.6%v/v

Fig 4*:*

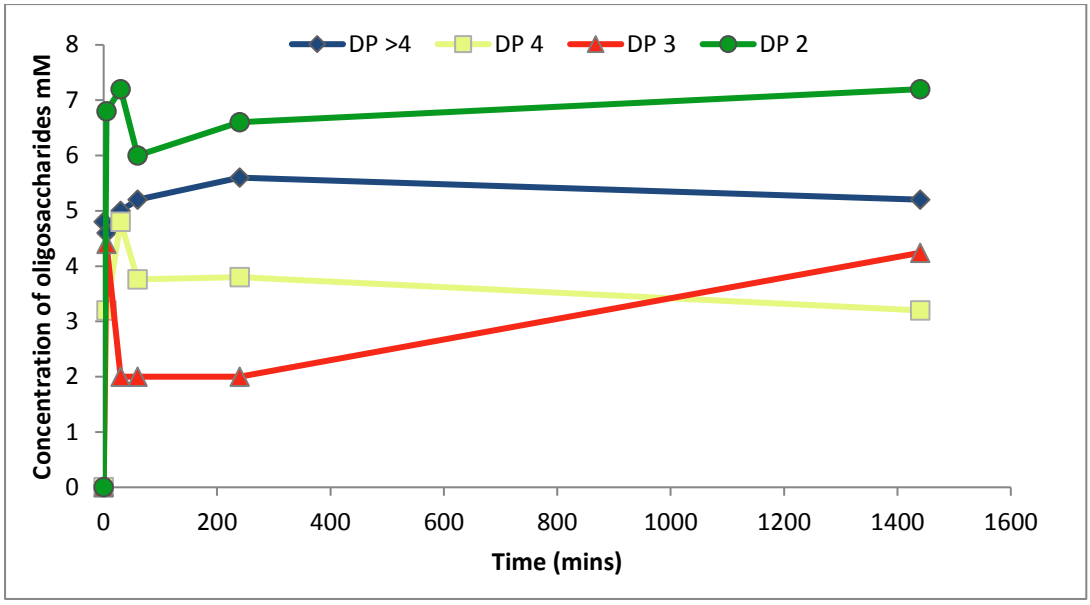
DP >4
DP 4
DP 3
DP 2
Concentration of oligosaccharides mM
Time (mins)
0
1
2
3
4
5
6
7
8
0
200
400
600
800
1000
1200
1400
1600

Fig 5

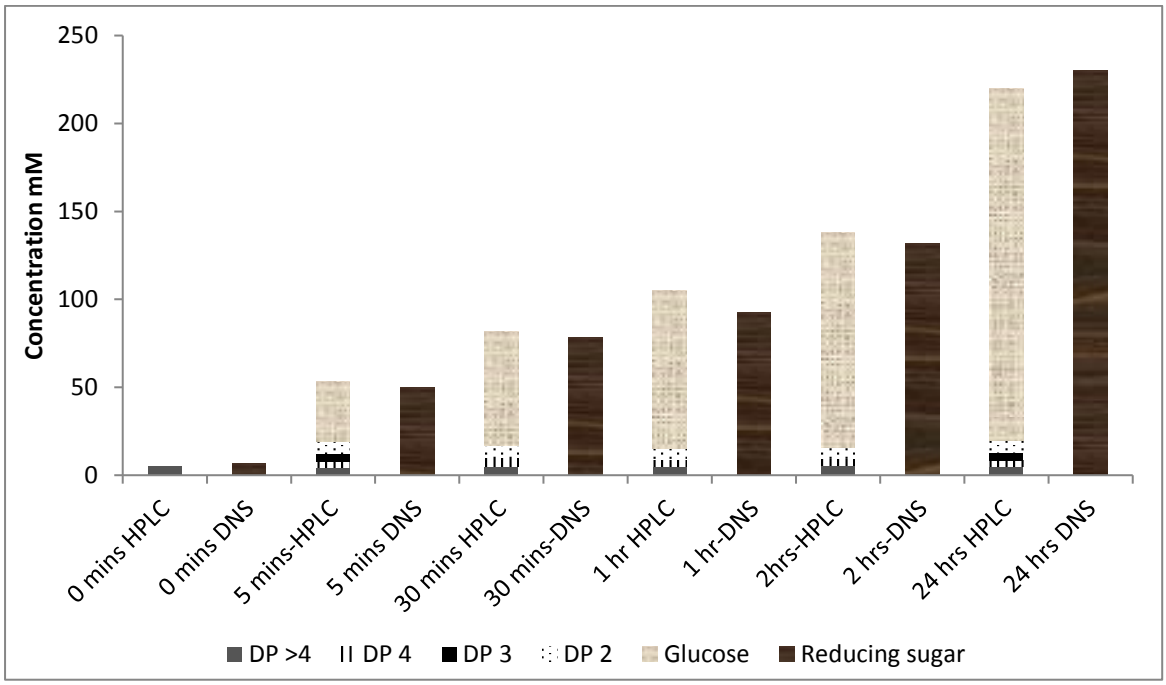
Concentration mM
250
200
150
100
50
0
0 mins HPLC
0 mins DNS
5 mins-HPLC
5 mins DNS
30 mins HPLC
30 mins-DNS
1 hr HPLC
1 hr-DNS
2hrs-HPLC
2 hrs-DNS
24 hrs HPLC
24 hrs DNS
DP >4
DP 4
DP 3
DP 2
Glucose
Reducing sugar

Fig 6

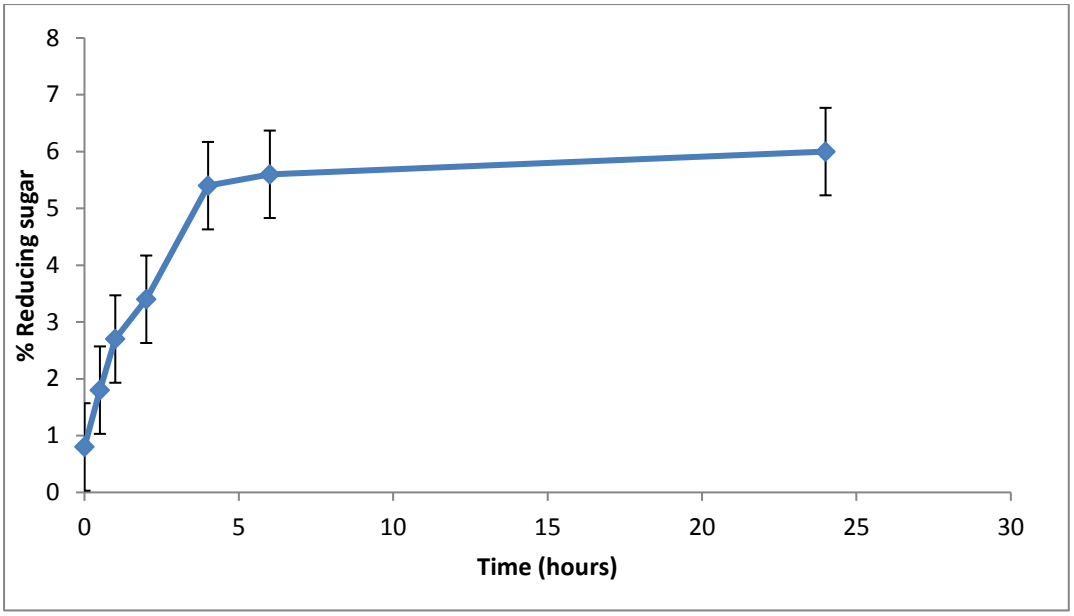
% Reducing sugar
Time (hours)
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30

Fig 7

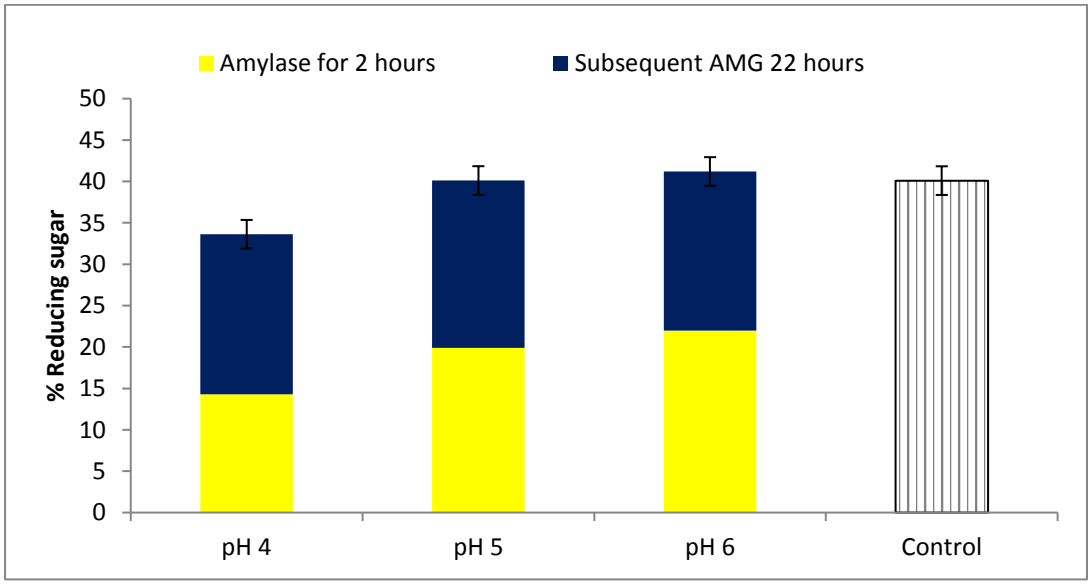
Amylase for 2 hours
Subsequent AMG 22 hours
% Reducing sugar
0
5
10
15
20
25
30
35
40
45
50
pH 4
pH 5
pH 6
Control

Fig 8

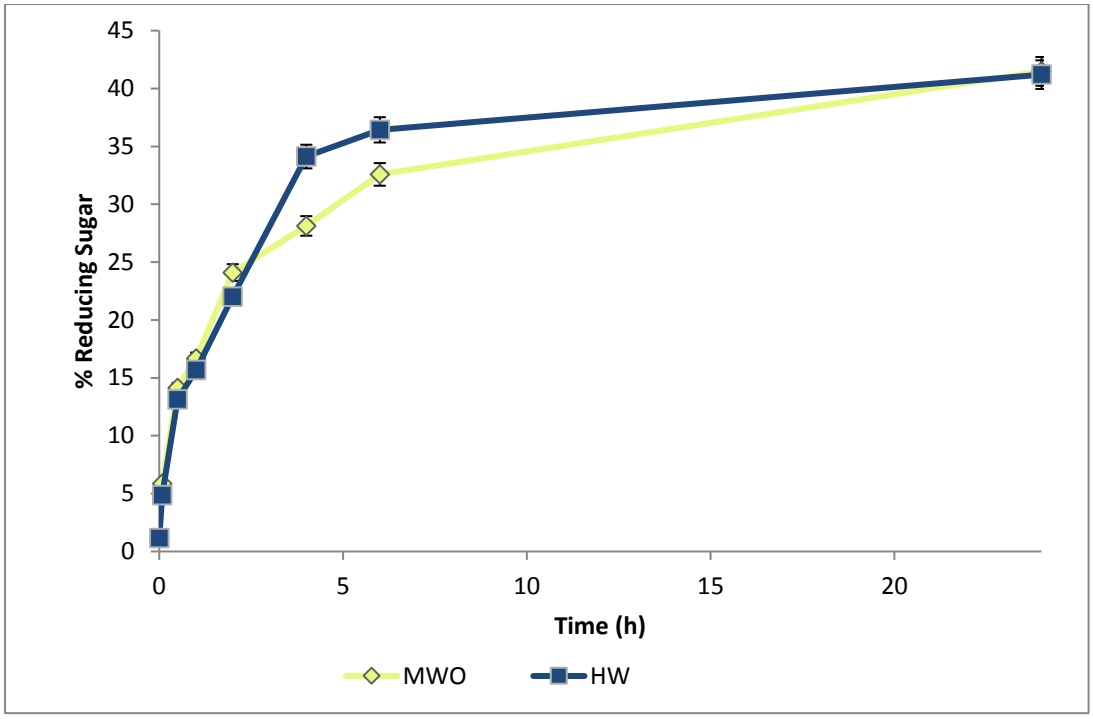
45
40
35
30
25
20
15
10
5
0
% Reducing Sugar
0
5
10
15
20
Time (h)
MWO
HW

Fig 9

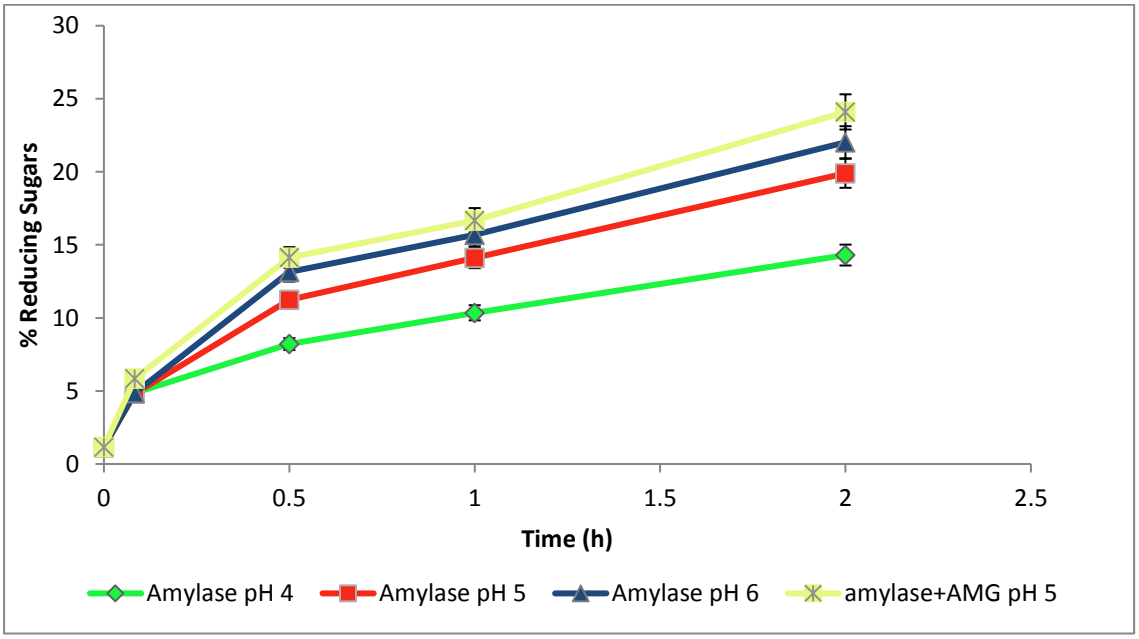
30
25
20
15
10
5
0
% Reducing Sugars
0
0.5
1
1.5
2
2.5
Time (h)
Amylase pH 4
Amylase pH 5
Amylase pH 6
amylase+AMG pH 5

Fig 10

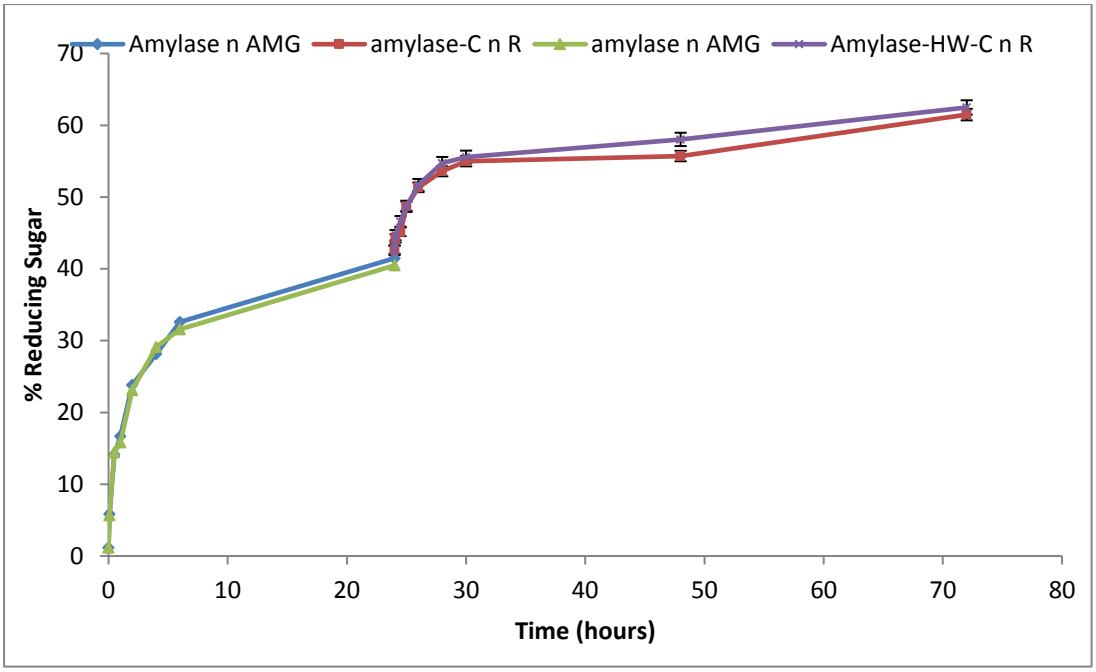
Amylase n AMG
amylase-C n R
amylase n AMG
Amylase-HW-C n R
% Reducing Sugar
Time (hours)
0
10
20
30
40
50
60
70
80

Fig 11

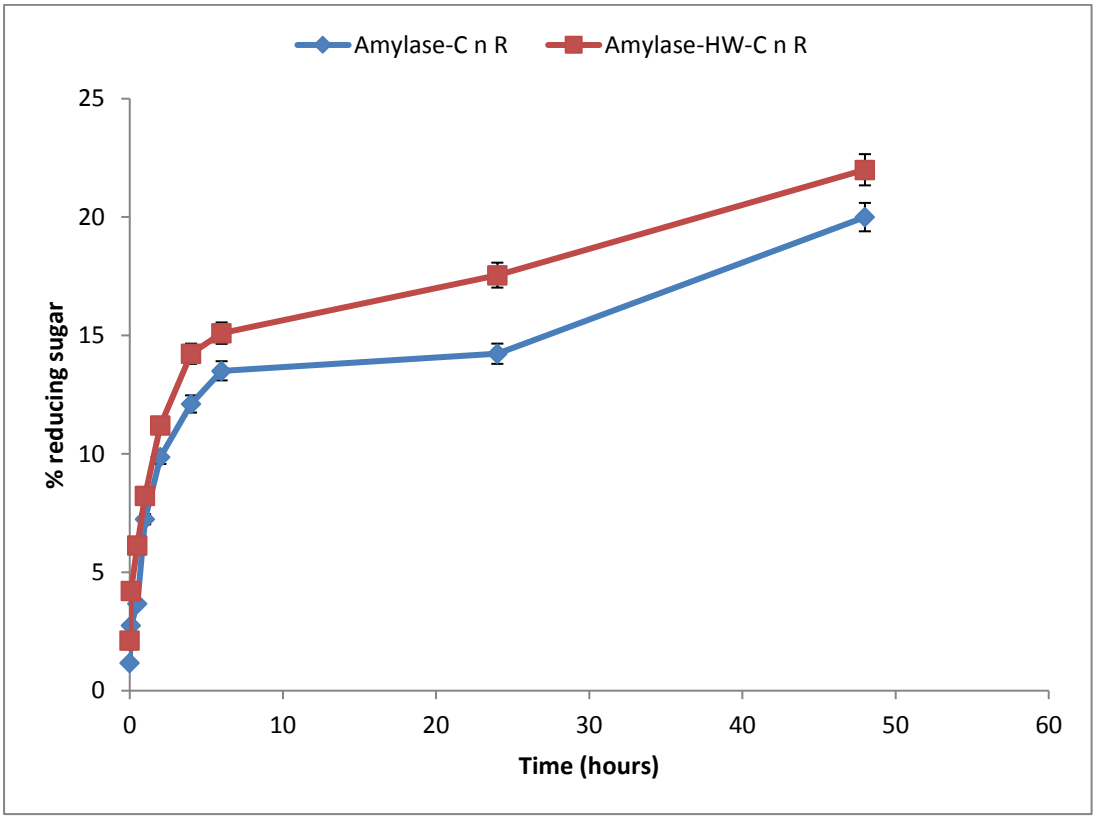
Amylase-C n R
Amylase-HW-C n R
% reducing sugar
Time (hours)
0
5
10
15
20
25
30
40
50
60

Fig 12

	Mean	Standard Deviation
Starch	28.0	1.4
Cellulose	23.9	0.9
Total Hemicellulose =9.4 *Xylan(4.1);Galactan(3.0);Arabinan(1.8); Mannan(0.5)*	9.4	0.8
Lignin • **Acid insoluble lignin** • **Acid soluble lignin**	 22.9 1.1	 1.1 0.1
Ash	7.4	0.2
Extractives	5.3	0.1
Acetyl groups	0.4	0.1
Others	1.6	
Total	100	

Table 1*:Table showing cassava peel composition.*