academicJournals

Vol. 16(42), pp. 2031-2042, 18 October, 2017
DOI: 10.5897/AJB2017.16201
Article Number: F48723A66345
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Evaluation of crude preparations of *Saccharomyces cerevisiae* (ATCC 52712) pectolytic enzymes in cassava starch extraction: Effects of variety on yield and starch recovery rates

Japheth Kwame Agyepong[1*] and John Barimah[2]

[1]Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology, Private Mail Bag (P.M.B), Kumasi, Ghana.
[2]Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology, Private Mail Bag (P.M.B) Kumasi, Ghana.

Received 11 August, 2017; Accepted 29 September, 2017

Application of enzyme preparations has shown great potential in aiding extraction procedures. However, the focus has mostly been on single crop varieties, thereby limiting knowledge on the effects of enzyme technology to those (single) varieties studied. The present work compared the effects of various dosages of crude pectolytic enzymes from *Saccharomyces cerevisiae* (ATCC 52712) on yield and extraction rates of starch from the roots of five indigenous cassava varieties (*Nkabom*, *Afisiafi*, *Doku duade*, *Bankye hemaa* and *Esam bankye*). The study aimed to establish whether varietal differences (with respect to response of variety to the technology) existed and to establish which variety is best suited for the technology. Generally, application of the crude pectolytic enzymes with activity of about 4.91 U significantly (P<0.05) increased starch yield and recovery rates in all selected cassava varieties. However, optimization of both yield and recovery rate was dependent on an interplay of variety, enzyme dosage and holding time for enzyme action. An enzyme dosage of 0.02% was found as significant (P<0.05) for peak of starch yield in the *Esam bankye* and *Nkabom* varieties at 0.5 and 1 h holding time in the *Afisiafi* variety; 0.025% enzyme dosage at 0.5 h holding time was the optimum treatment combination for starch yield in both *Bankye hemaa* and *Doku duade* varieties. The study therefore showed that although application of pectolytic enzymes for starch extraction enhances yield, the technology is affected by varietal differences. Given the heavy dependence of most Ghanaian industries on starch, the technology if made available would greatly boost the productivity of these sectors at relatively lower cost.

Key words: Cassava varieties, crude pectinase, pectolytic enzyme dosage, polygalacturonase, submerged fermentation.

INTRODUCTION

Until recently, Ghana's economy has progressively thrived on the performance of her agricultural sector. An economic survey report indicates that in the year 2016, the sector contributed about 20% of the country's gross domestic product (GDP) (CIA, 2017). Of this, cassava is estimated to have contributed about 22% of agricultural

gross domestic product (AGDP) (Essabrah-Mensah, 2016) with production potential estimated to have increased, from the third quarter, by about 4% at the end of the year (FAO, 2016). The crop, roots and leaves are equally a staple and contributes immensely to dietary caloric intake and to industry. The cassava root, especially, finds application in many domestic and industrial circles. Its starch has many remarkable characteristics including high paste viscosity, high paste clarity and high freeze-thaw stability, which are advantageous to many industries (Chinma et al., 2013). The Food and Agriculture Organization (FAO) estimates that in the year 2015 about 8,405 tons of cassava flour and starch were traded globally and although Africa is touted the highest exporter of cassava tuber (exporting about 153,451 tons of global exports of 281,050 tons), only Nigeria contributed significantly (about 150 tons) to exports of cassava flour and starch from Africa (FAO, 2016). However, they also reported that global demand for cassava starch could increase especially as global and regional demands for alcohol, ethanol, starch and animal feed sectors, as well as their lucrative export markets continue to rise (FAO, 2016). In Ghana, the opening of an export-oriented starch factory near Accra in 2003 led to an "explosion" in farmers' demand for high-yielding, disease-resistant varieties to help feed the factory with raw materials (Business and Financial Times Newspaper, 2013). This factory, the Ayensu Starch Factory, established as part of the Presidential Special Initiative (PSI) on cassava, was projected to work at 70% installed capacity (Business and Financial Times Newspaper, 2013); however, its operations were fraught with challenges, key among which were power cuts, financial challenges and insufficiency of raw materials (Addo, 2013): the factory could therefore only operate at 20% of its installed capacity (Business and Financial Times Newspaper, 2013). An optimization of production parameters and a careful selection of variety could have helped boost production and enhance the revenue outlook for the African starch industry. Some work have been carried out on enzymatic extraction of starch from native crops in many places worldwide (Sit et al., 2015; Pinyo et al., 2016) and results from these studies have been very remarkable.

In Ghana, cassava is commonly used in starch production and attempts at improving extraction processes with enzymes have been with only single varieties, usually with the *Afisiafi* (Dzogbefia et al., 2008a, b), a variety which is also mealy. The aim of this study was therefore to establish if other cassava varieties would produce a similar (improved) response to starch recovery rates and yield when enzyme technology is applied. It was expected that varietal differences in cell wall biochemistry (especially regarding pectin content) would greatly influence the parameters being measured; this will help determine which variety is best suited for the technology. With this, we could also establish what varieties would be better suited for industrial starch production and which would be best for dietary consideration and thereby avoid undue competition between food and other industrial applications.

METHODOLOGY

Cassava, manioc or yucca (*Manihot esculenta* Crantz) is a perennial shrub of the New World which is currently the sixth world food crop for more than 500 million people in tropical and sub-tropical Africa, Asia and Latin America (El-Sharkawy, 2004). Taxonomically, the crop belongs to the family Euphorbiaceae and the genus *Manihot* which is known to have about 100 species among which, the *M. esculenta* Crantz is the only commercially cultivated species (Alves, 2002).

The mature plant grows to an average height of about 1 to 4 m (Alves, 2002). It is a monecious plant that is cross pollinated and seed propagated, leading to genetic segregation among the various species. As a crop, cassava is vegetatively propagated via stem cuttings.

Cassava was first introduced into Ghana by Portuguese slave ships from Brazil in 1750 (Safo-Kantanka, 2004). Since that time, much genetic variability has arisen mainly through accidental hybridization and spontaneous recombination between varieties. The current climax population, however, is a product of farmers' artificial selections (Fregene et al., 2003). Such varieties have been given names by farmers to demonstrate major attributes of the varieties. Hence, names such as 'Bankye-Broni' (DMA-001), 'Tu-gyabi-tuntum' (DMA-003), 'Bokentenma' (DMA-015), 'Nfiemenu-Bankye' (DMA-016), 'Kowoka' (DMA-009), 'Bankye-soja' (WCH-1), 'Ampe nkyere', 'Bankye-Ababaawa' (ASF-010) and many others, now commonly ascribed to local cassava varieties, are available on the Ghanaian market (Safo-Kantanka, 2004).

Morphology and uses of some cassava varieties in Ghana

Cassava cultivars can be distinguished by their morphological characteristics such as leaf size, colour and shape, branching habit, plant heights, colour of stem and petioles, tuber shape, time - to - maturity, yield and level of cyanogenic glycosides in the tuber and leaves (IITA, 1990). For example, the *Afisiafi* clone (*TMS 30572*) was introduced from IITA to Ghana in 1988 under the code *GC/88-07*. Its morphological characteristics include light green petiole, brownish grey mature stem and a light brown outer skin of tuber with cream inner skin. The variety can be grown in both major and minor seasons and is highly tolerant to major pests and diseases. Information on the uses and yield of some commonly grown cassava varieties in Ghana are as shown Table 1.

'Afisiafi' and 'Tek bankye' have peak flour yields of 23 and 22.4%, respectively, at 13 months after planting, while 'Abasafitaa' and 'Gblemoduade' had their peak flour yields increasing uniformly from 11 to 13 months after planting after which it decreased. 'Abasafitaa' and 'Tek bankye' were also reported to have flour yields increasing from 9 through 12 months after planting after

*Corresponding author. E-mail: japhethagyepong@yahoo.com.

Table 1. Features and uses of some cassava varieties grown in Ghana.

Variety	Year released	Maturity period (months)	Mean root yield (T/ha)	Total dry matter (%)	Uses	CMD Resistance
Afisiafi	1993	12-15	28-35	32	Starch, flour, gari	Tolerant
Abasafitaa	1993	12-15	29-35	35	Starch, flour, gari	Tolerant
Tekbankye	1997	12-15	30-40	30	fufu, ampesi, gari	Susceptible
Dokuduade	2005	12	35-40	30	Starch, gari	Resistant
Agbelifia	2005	12	40-45	33	Starch, gari	Resistant
Essam bankye	2005	12	40-50	35	Flour, gari	Resistant
Bankyehemaa	2005	9-12	40-50	32	Flour, gari, fufu	Resistant
Capevars bankye	2005	9-12	30-35	30	Flour, gari, fufu, starch	Resistant
Bankye botan	2005	12-15	25-30	28	Flour, gari, starch	Tolerant
Eskamaye	2005	15-18	16-23	25	Tuo, konkonte	Tolerant
Filindiakong	2005	15-18	16-20	28	Tuo, konkonte	Tolerant
Nyerikobga	2005	15-18	17-29	30	Tuo, konkonte	Tolerant
Nkabom	2005	12-15	28-32	32	Starch, fufu	Tolerant
IFAD	2005	12-15	30-35	30	Starch, fufu	Tolerant
Ampong	2010	12	40-50	36	Flour, Starch, fufu	Resistant
Broni Bankye	2010	12	40-45	33	Flour, bakery products	Resistant
Sika bankye	2010	12	40-45	36	Flour, Starch	Tolerant
Otuhia	2010	12	35-40	39	Flour, Starch	Resistant

RTIMP Ghana, 2014.

which flour yield fell. The starch yield of flour, its solubility, ash content and pasting characteristics were all reported (Apea-Bah et al., 2007) to be significantly affected by variety.

With regards to physicochemical dynamics of the extracted starch with age, Sriroth et al. (1999) reported that age of the root and environmental conditions at harvest influence granule structure and hydration properties and that starches extracted from cassava roots harvested at different times were characterized by unique starch granule structure and function.

Cassava cultivation

Cassava is propagated using stem cuttings. The cuttings, usually cut into 20 to 25 cm long, can be planted in a slanting or angular position (45°) by burying in the soil with one-third of the cuttings above the soil surface and ensuring that lateral buds point upwards. This is where the cuttings sprout. Conventionally, it is recommended that the cuttings are planted at a spacing of 1 × 1 m on the crest of ridges or mounds. This will give a plant population of 10,000 stands/ha. Vertical or angular planting is recommended in areas of high rainfall. Horizontal planting is better in dry areas. Generally, farmers plant by hand and it takes 8 to 10 persons to plant 1 ha in 1 day.

The optimum age when the starch and dry matter yields are highest is 9 to 12 months after planting, depending on the variety and the climate. Some varieties mature in 15 to 18 months. Extended cold season may delay the maturity of cassava. Harvesting too early results in a low yield, while delayed harvesting could reduce yield.

Cassava roots are harvested when they are mature to have accumulated enough starch but have not yet become fibrous. The root is harvested by cutting the plant stem at about 30 to 50 cm above the ground and then gently pulling the residual stem to lift the roots out of the soil. This is to avoid bruising the tubers as this could hasten deterioration of the root especially during storage. Harvesting is done when the soil is wet and loose.

Plant materials

Fresh local cassava (*M. esculenta* Cranz) varieties *Afisiafi*, *Esam bankye*, *Bankyehemaa* and *Nkabom* and *Doku duade* harvested at nine months after planting (MAP) were obtained under a running project at the Department of Agriculture Engineering, K.N.U.S.T. All varieties were planted on the same field of the Agriculture Research Station (at *Anwomaso-Domeabra*, Kumasi) and had been subjected to similar edaphic and climatic conditions.

Cell culture and enzyme production

Prior to enzyme production, *S. cerevisiae* (ATCC 52712) cells were propagated and subcultured in malt extract broth (M.E.B.) and agar (M.E.A.) slants to obtain pure cultures. A loop full of pure culture from M.E.A. slant was inoculated in 100 ml malt extract broth and incubated for 3 days at 28°C. During the period, light absorbance (at 540 nm) of the culture in MEB and cell enumeration (on malt extract agar using pour plate technique) were taken at 12 h intervals. The values obtained were used to derive a standard calibration curve for cell density. 4 ml of M.E.B. culture (density 6.32 × 10^{12} per 100 ml) was then transferred into 100 ml of 1% pectin medium– formulated based on a modification of the method used by Ranganna (1986) and cultured for 8 days at 28°C. During this period, the concentration of crude protein (enzyme) was monitored and cell density was estimated daily at 540 nm using spectrophotometry vis-à-vis the standard (calibration) plots for cells density obtained; crude protein (enzymes) were obtained by centrifugation at a speed of 3600 g for 10 min at a temperature held

at 4°C by careful aseptic recovery of the supernatant with 1000µL micropipette into sterile boiling tubes.

Pectolytic enzyme assay

Assaying for polygalacturonase (pectolytic enzyme) activity of the crude extract was carried out using the method described by Ranganna (1986). One unit of polygalacturonase (PGase) activity was as defined by Jayani et al. (2005). They defined one unit (U) of PGase activity as the amount of enzyme that liberates 1 µmol/ml/min of D-galacturonic acid from pectic substances under standard assay conditions.

Amylase enzyme assay

This was based on modification of the method described by Bernfield (1955). A 1% starch solution was slightly warmed in 100 ml sodium acetate buffer (0.1 M, pH 4.7). The extraction buffer was 1 M K_2HPO_4, pH 6.5. 1 ml of 1% starch and 1 ml of the crude enzyme extract were incubated at 27°C for 15 min. At the end of the incubation period, the reaction was stopped by the addition of 2 ml of dinitrosalicylic acid reagent and the resulting solution heated in a boiling water bath for 5min. While the test tubes and its content were warm, 1 ml of 40% potassium sodium tartrate solution was added and the content cooled under running tap water. The volume was then made up to 10 ml by the addition of 6 ml water. Spectrophotometric measurement (absorbance) was read at 560 nm. However, for the control, the reaction between the 1% starch and crude enzyme extracted was terminated at zero time. The amount of the reducing sugars formed was calculated from a standard graph prepared from known concentrations (10 to 100 mg) of maltose.

Determination of moisture content in the cassava pulp

Moisture content was determined using gravimetric methods described by Association of Official Analytical Chemists (AOAC, 1990).

Preparation of cassava mash for starch extraction

The procedure used for starch extraction from each variety is as shown in the flow chart (Figure 1). The methodology was to simulate starch extraction processes of traditional starch producers; however the method was slightly improved by submerged fermentation of the cassava mash with the crude enzymes.

Determination of starch (suspension) flow rate

Immediately the holding time for each mash was up, the enzyme-cassava mash mixture was emptied on to a cotton (cheese) cloth lining a funnel with a diameter of about 9 cm. The open end of the funnel (from which the starch milk was to drain) was inserted into a 250 ml measuring cylinder prior to pouring the mash unto the cheese cloth. Volume of each starch suspension obtained at 15 s intervals was recorded for 3 min (180 s).

Statistical analysis

Data were analysed using the completely randomized design (CRD). All data were subjected to ANOVA and significant differences were tested using the Duncan's new multiple range test. For all parameters measured, the statistical software used was the *SigmaPlot* for Windows Ver. 11 by Systat Software Inc.® (2008).

RESULTS AND DISCUSSION

Moisture content of cassava pulp

Cassava pulp from the various varieties had moisture contents that were significantly different. Moisture contents recorded were similar to values reported in some literature (Morgan and Choct, 2016). The *Esam bankye* variety had the least moisture content and the *Afisiafi* had the highest amount of moisture in its pulp. This information could prove helpful in the selection of variety as low moisture varieties have high dry matter content (which includes starch).

Enzyme activities of the crude enzyme extract

A polygalacturonase (pectolytic enzyme) activity of 4.91 U with specific activity (calculated per minute) of 4.291 U/mg protein was recorded in the crude enzyme preparation. Bali (2003) however reported a pectinase activity of 210.37 nmoles/ml/60 min suggesting that the crude enzyme preparation had a rather high activity. Endogenous amylase activity of 0.293 U/ml (with a specific activity of 0.257 U/mg) was also recorded from the extract. This value is also higher than the 0.16 and 0.09 U/mg (for amylase activity and specific activity, respectively) reported by Dzogbefia et al. (2008a). This also suggests a high possibility of amylolysis occurring during stages of cassava mash incubation with the enzyme.

Effects of enzyme dosage on starch yield and reaction time

Starch content from each mash was generally lower than those reported in most literature (Aldana and Quintero, 2013; Roslimi et al., 2016). This is probably due to differences in methodology used in estimating starch content. However, since all the varieties were exposed to the same experimental and edaphic conditions, it is possible to compare and appreciate the contributions of variety to the process.

At very low enzyme dosages (between 10 and 20 mg/200 g of mash) improvement in yield was not significantly different from that of the control ($P>0.001$) (Figures 2 to 6). Significant yields were however recorded at higher dosages (between 30 and 50 mg/200 g of mash) in all varieties with optimum yields being recorded in the 40 and 50 mg dosages. The *Doku duade* and *Bankyehemaa* varieties recorded their highest yields with the 50 mg/ml crude protein per 200 g mash (0.025%)

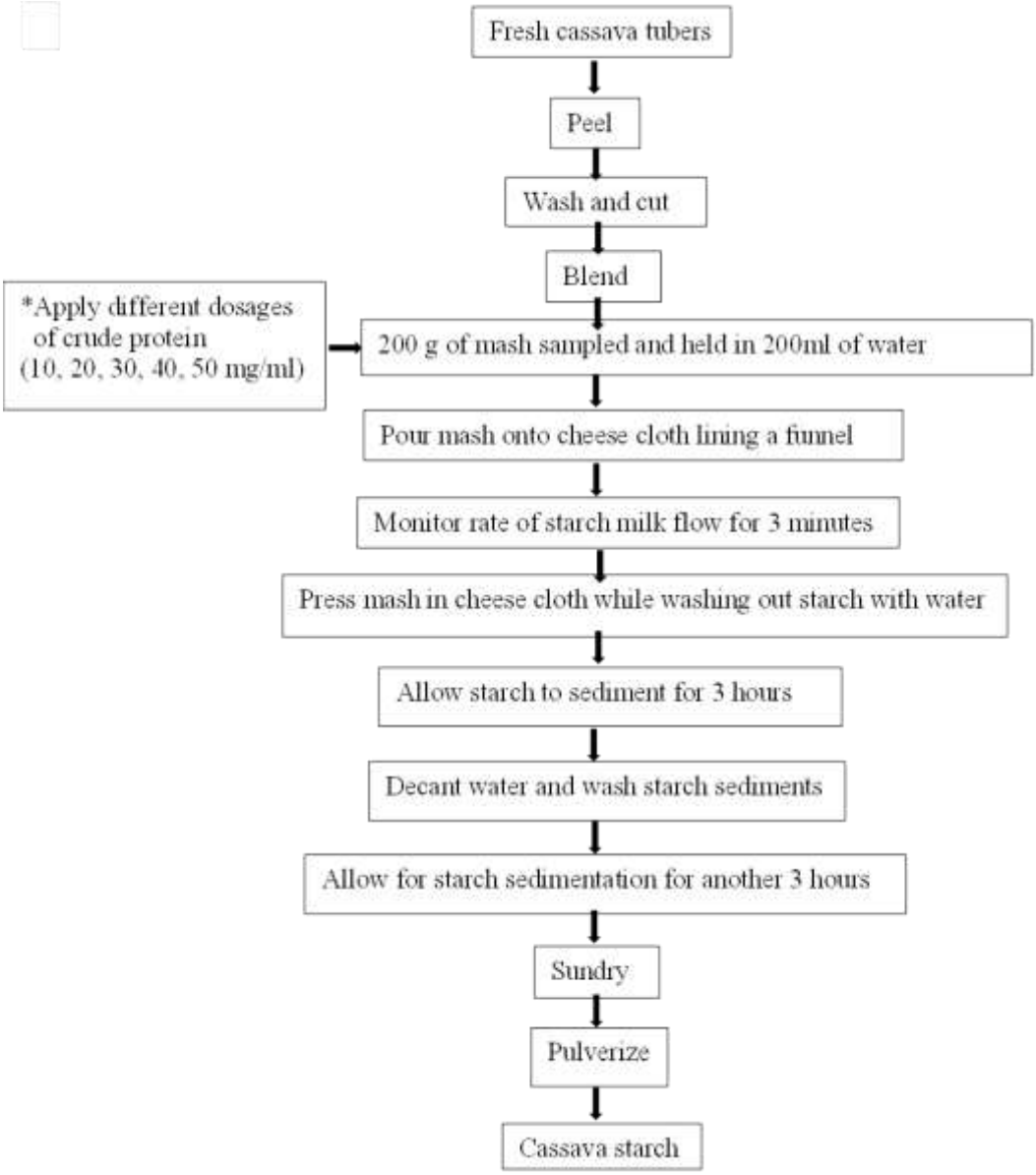

Figure 1. Flow chart of procedure used in enzyme-assisted starch extraction. * Control samples did not require this step.

dosage; all the other varieties (*Afisiafi*, *Nkabom* and *Esam bankye*) gave their (most significant) highest starch yield with the 40 mg/ml per 200 g mash (0.02%) dosage. Gummadi and Panda (2003) mentioned substrate inhibition kinetics in PGase activity. The enzyme has an alternative (allosteric) site to which the same substrate binds to inhibit substrate binding at the active site and enzyme activity only increases at higher enzyme dosages.

Although, quantitative data on the pectin content of cassava root is rather scanty, pectin content of some root tubers have been estimated to range from 0.2 to 2.5 g per 100 g of mash (Schoeninger et al., 2000). At high PGase dosages, varieties with high pectin content would present high PGase activity. Additionally, differences in the amounts of mineral ions in the pectin framework could also influence the results observed. Pectin, a polyanionic molecule, naturally binds divalent ions like Ca^{2+}, Mg^{2+}, Cu^{2+} and Zn^{2+} as part of its framework. These ions, at high concentrations have been reported to be rather inhibitory to PGase activity (Vázquez et al., 1986). This suggests that varieties that sequester high mineral ions would subject PGase to higher levels of inhibition at long holding times as more of these ions are released. However, pectate lyase (PL), whose isoforms have been reported (O'Neill et al., 2001) to require Ca^{2+} in its activity could take over pectolysis. Thus, any further pectolytic activity to release granular starch will depend on what

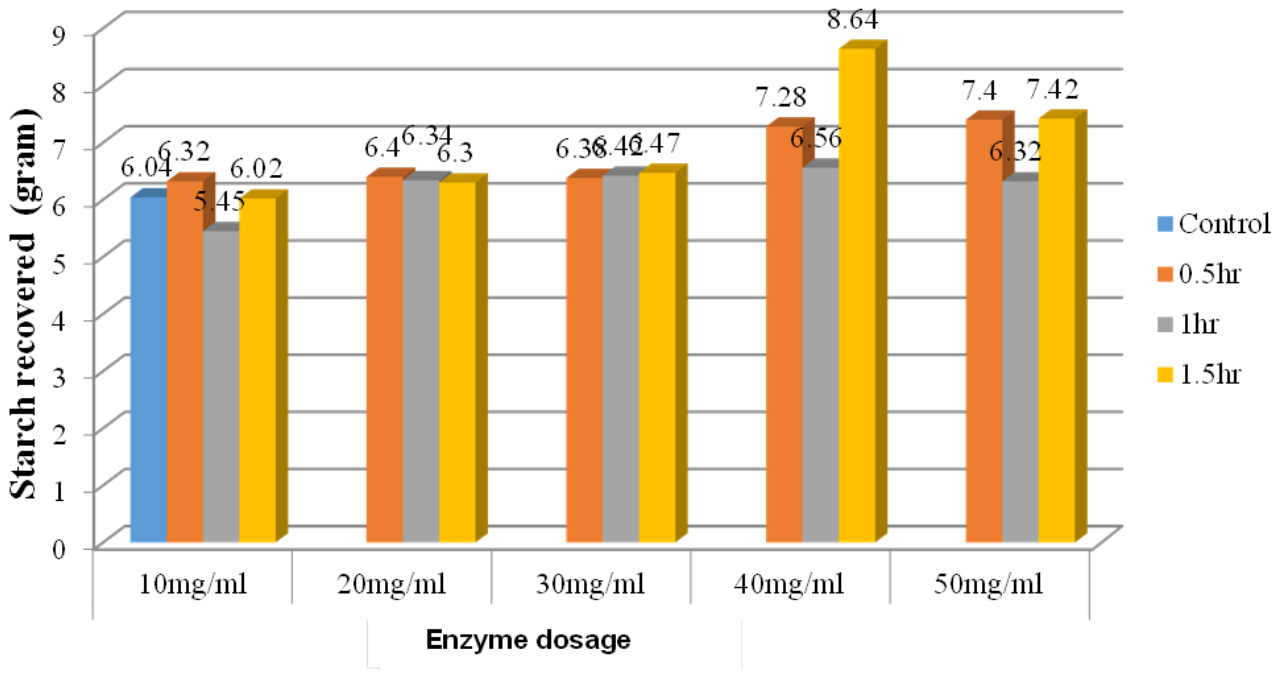

Figure 2. Effects of enzyme dosages on yield in the *Esam Bankye* variety.

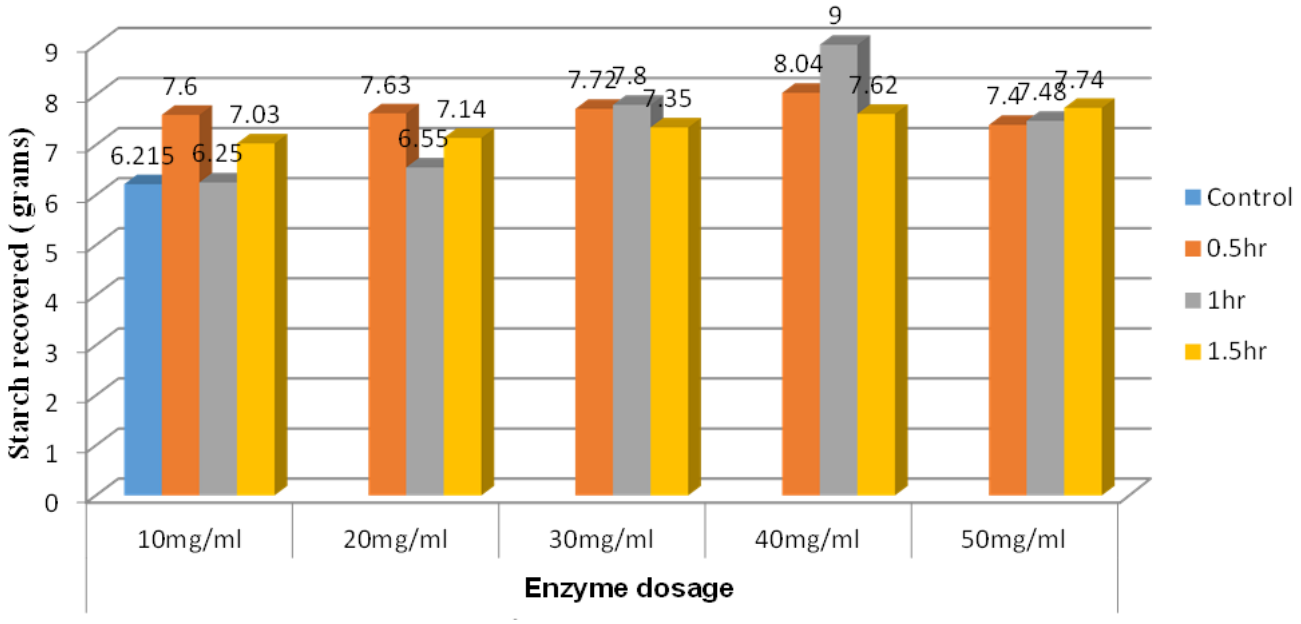

Figure 3. Effects of enzmye Dosages on Yield in the *Afisiafi* variety.

fraction of calcium ions are available to PL. Apparently, the use of PGase activity (in crude protein preparations)alone to estimate enzymatic response of variety to starch extraction presents a rather complex situation. It is however shown that longer holding times, due to the presence of endogenous amylase in the enzyme preparation, could have deleterious effects on the starch by converting it into limit dextrins (which includes glucose) (Miguel et al., 2013), maltose and maltotriose (Li et al., 2017); this affects the original functional properties of the starch, especially those related to starch granule structure and amylose content

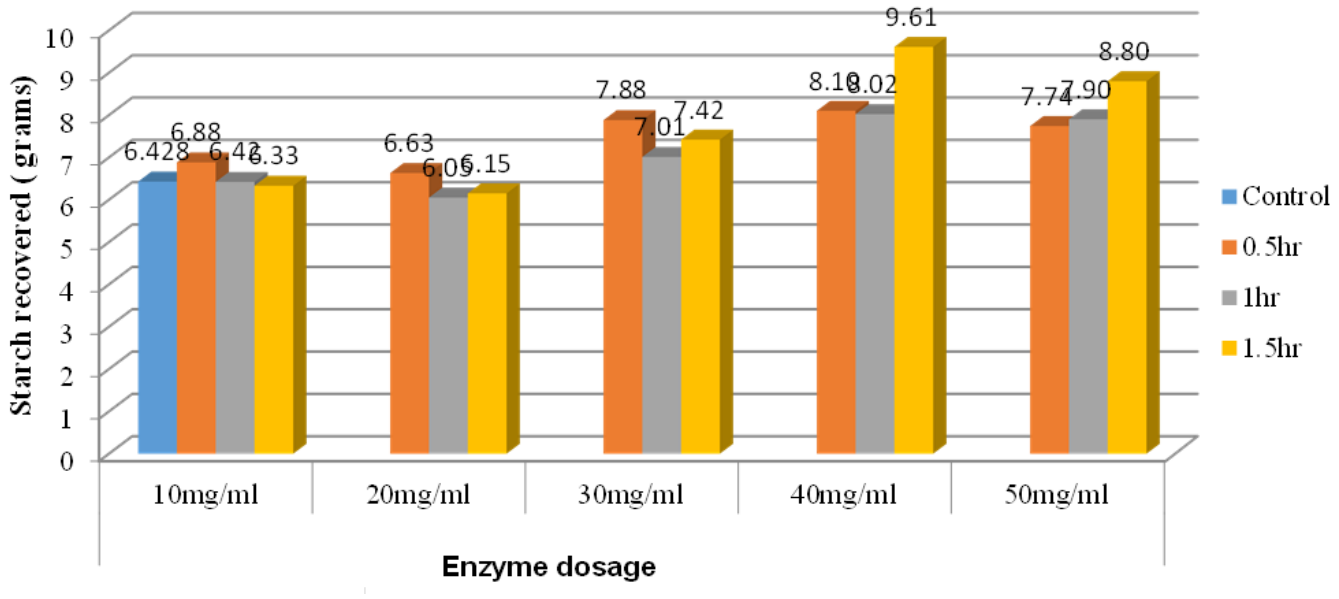

Figure 4. Effects of enzyme dosages on yield in the *Nkabom* variety.

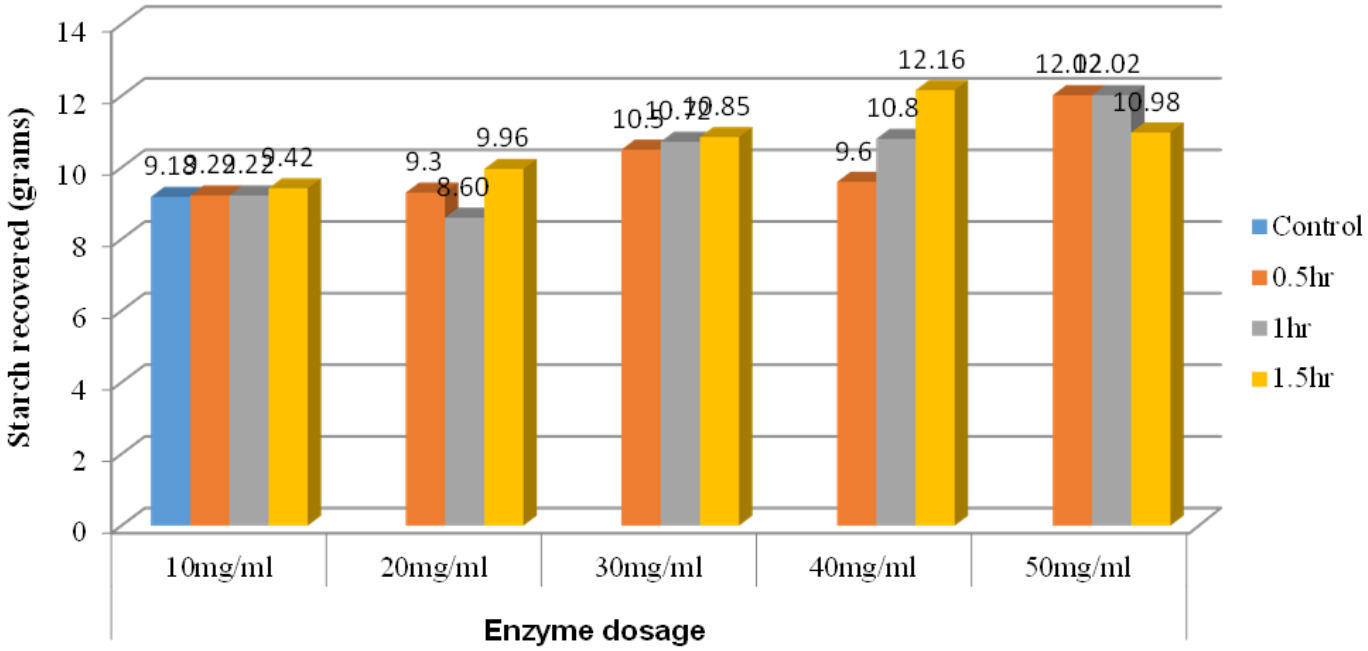

Figure 5. Effects of enzyme dosages on yield in the *Bankye hemaa* variety.

which ultimately determine the swelling, solubility and pasting properties of starch (Wang et al., 2015). Additionally, longer holding times affect the stability of pectinase enzymes (Vatanparast et al., 2014) and impact profits negatively as turnover time is increased. Hence if apparent (improved) yields at a given dosage and longer times are not so different from those obtained at higher dosages with shorter time requirements, then it will be economically prudent to adopt the latter. For these reasons, although, the *Esam bankye* recorded highest starch yield (about 43%) with the 0.02% enzyme dosage vs. 1.5 h holding time combination (Figure 2 and Table 2), the 0.02% vs. 0.5 h dosage combination could be adopted (starch yield of about 20.53% over the control).

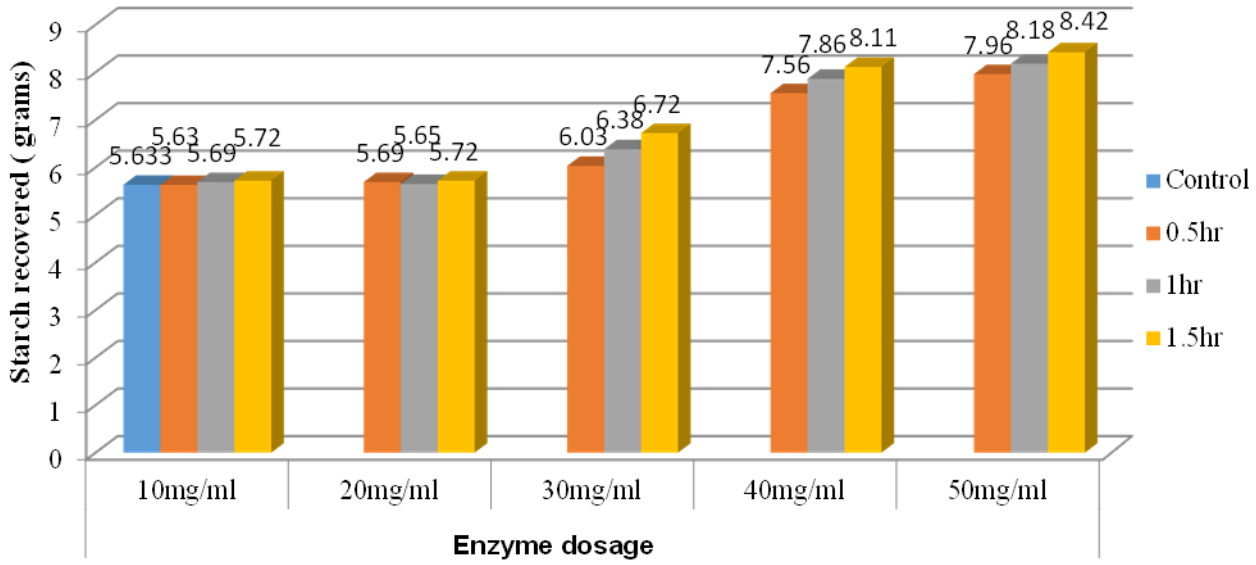

Figure 6. Effects of enzyme dosages on yield in the *Doku duade* variety.

Table 2. Moisture content of cassava root pulp from the various cassava varieties.

Cassava variety	Moisture content (%)
Esam bankye	55. 25 ± 0.589[a]
Doku duade	57.414 ± 1.360[b]
Afisiafi	65.857 ± 0.862[c]
Nkabom	61.197 ± 1.817[d]
Bankye hemaa	58.492 ± 0.699[b]

Different letters in parenthesis show significant (P<0.05) differences.

An optimal treatment combination (0.02% vs. 0.5 h) in the *Nkabom* gave an enhanced yield of 26.15%, while in the *Afisiafi* variety, an optimal holding time of 1 h at the 0.02% enzyme dosage gave an enhanced yield of about 45% which was significantly (P<0.001) higher than that obtained (about 29.36%) at the 0.5 h holding time. Dzogbefia et al. (2008a) gave similar reports of the 0.02% enzyme treatment being the most effective for starch extraction in this variety. However, they obtained significantly higher yields at the 0.5 h holding time with this dosage. These differences could be attributed to differences in activity of the enzyme (necessitating a longer holding time for optimal activity), difference in the age of the variety as well as varying environmental conditions under which the variety was cultivated (which affect the total biomass composition). The *Doku duade* and *Bankye hemaa*, however, required a treatment combination of 0.025% (50 mg/ml of crude protein in 200 g mash) dosage at 0.5 h (holding time) to optimize yield (about 41.46 and 30.91%, respectively).

Quantitative measurements of polygalacturonase (PGase) activity on samples using 0.5 g of mash from each variety gave an activity of 0.121 U/ml on the *Bankye hemaa* variety; 0.137 U/ml on the *Doku duade* variety; 0.181 U/ml on the *Afisiafi* variety; 0.067 U/ml on the *Esam bankye* variety and 0.094 U/ml on the *Nkabom* variety. This pattern of activities seems to agree with the trend for starch yield obtained due to the enzyme treatment (Table 3). Hence, the responses observed are largely due to the different amounts and/or type of fiber materials (including pectic substances) present (Moelants et al., 2014). Apparently, pectic substances from especially, the *Nkabom* and the *Esam bankye* varieties were low in their polygalacturonan (PG) contents which probably explain why these varieties required relatively lower dosages (0.02%) and shorter holding times for maximum activity.

It is however noteworthy that despite improvements in the yield due to enzyme treatment, a key determinant of choosing a variety would be the variety's innate starch content and physico-chemical properties of its starches. Comparing the controls (Figures 2 to 6), the *Bankye hemaa* variety produced the highest starch yield from its mashes and with enzyme treatment, seconded the *Doku duade* variety in terms of yield improvements (Table 3). This suggests that the variety will be the most suitable choice for enzyme-assisted starch extraction.

Effects of enzyme dosage on flow pattern and starch milk recovery

Generally, all the varieties displayed typical parabolic flow

Table 3. Summary of percentage increase in starch yield due to enzyme treatment at optimal dosage and holding times.

Cassava variety	Optimum treatment combination (dosage/holding time)	Increase in yield (%)
Nkabom	0.020% vs. 0.5 h	26.15 ± 7.367
Afisiafi	0.020% vs. 1.0 h	45.10 ± 11.877
Esam bankye	0.020% vs. 0.5 h	20.53 ± 11.409
Doku duade	0.025% vs. 0.5 h	41.46 ± 5.165
Bankye hemaa	0.025% vs. 0.5 h	30.91 ± 4.648

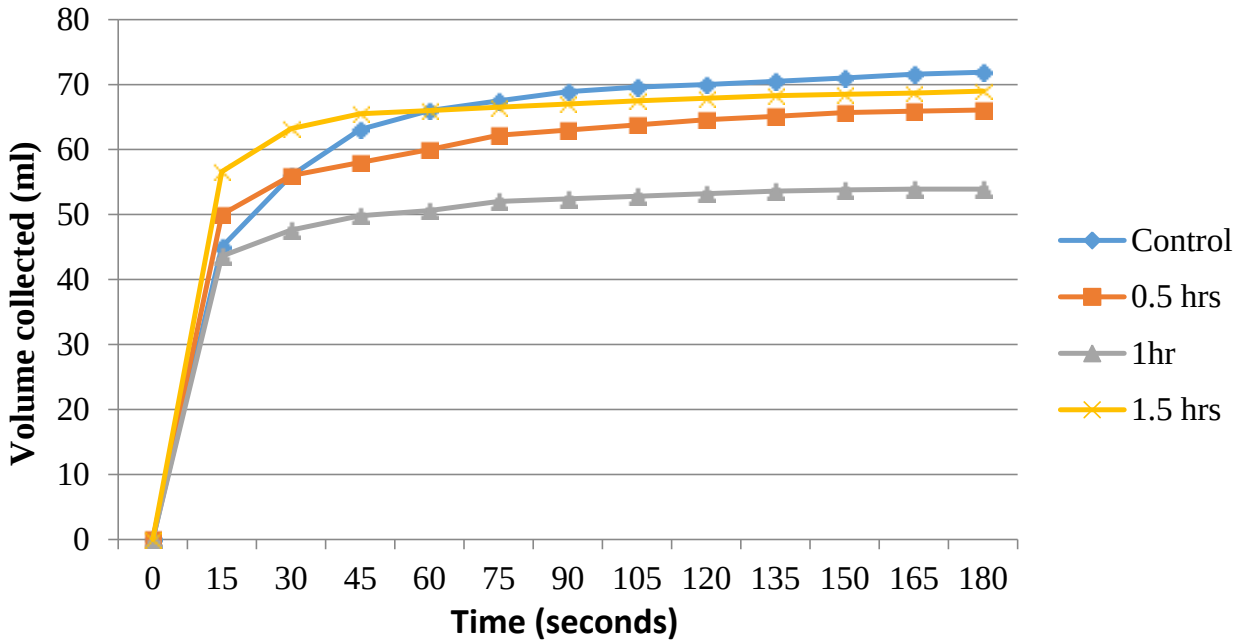

Figure 7. *Esam bankye* starch milk flow pattern at all holding times of its optimum enzyme dosage (40 mg/ml crude protein in 200 g mash, 0.02%).

patterns at all dosages and holding times with rates of starch milk recovery, after enzyme treatment, generally being higher when compared with their controls within the first 15 s of flow (Figures 7 to 11). This suggests that technology allows the pressing (Figure 1) of the mashes to be carried out earlier. The only exception to this general trend however was the *Esam bankye* variety where milk recovery for the control (as compared to enzyme treated samples) was faster.

Mashes from the *Bankye hemaa* and *Doku duade* varieties had their viscosities greatly reduced by the technology as these recorded the highest improvement in flow (Table 4). This observation pattern agrees with the trend of pectolytic activities on the mash (cited earlier).

CONCLUSION AND RECOMMENDATION

Optimization of starch extraction from cassava with crude pectolytic enzymes from *S. cerevisiae* was found to be dependent on variety as well as the dosage-holding time treatment combinations adopted. Generally, enzyme dosages of 0.02% (for the *Nkabom*, *Afisiafi* and *Esam bankye*) and 0.025% (for the *Doku duade* and *Bankyehemaa*) were required to optimize yield for starch recovery. This was related to the varying composition of pectic materials in the root mashes as activity of the crude enzyme preparation on the mashes varied. However, application of the technology generally enhanced starch yield and recovery in all varieties with the highest starch yield (due to enzyme treatment) being recorded in the *Afisiafi* and the greatest recovery rates being recorded in the *Bankyehemaa* variety.

However, the high (dry) biomass, susceptibility to pectolytic (enzyme) activity, high starch content (even without enzyme treatment) and low holding time requirements for enzyme-assisted yield optimization

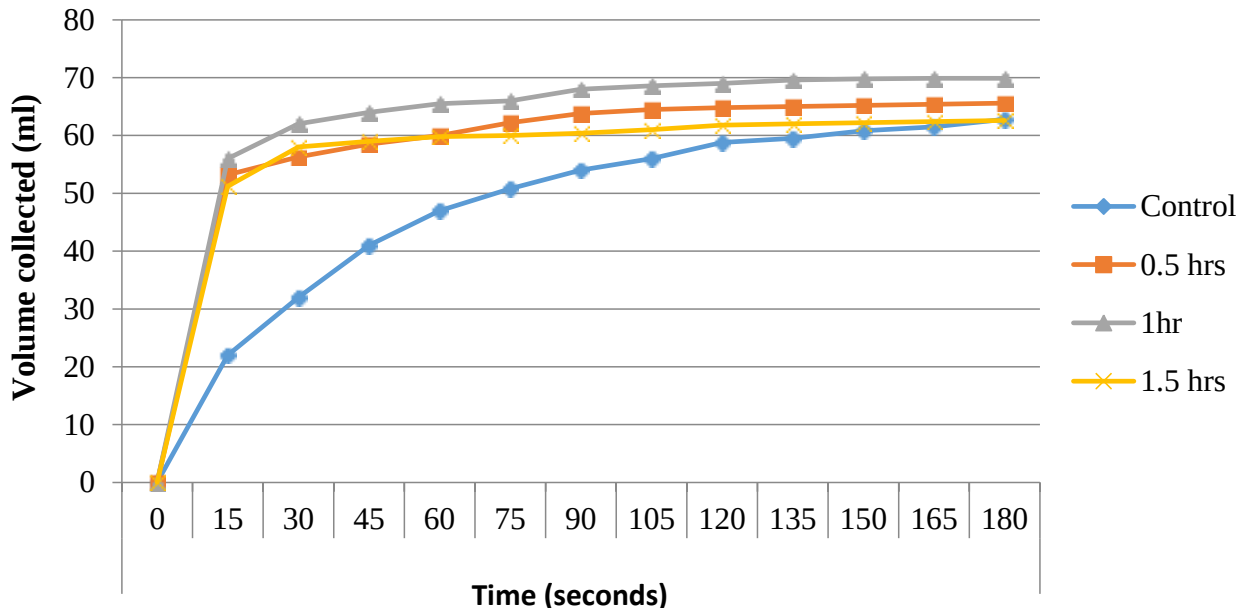

Figure 8. *Afisiafi* starch milk flow pattern at all holding times of its enzyme dosage optimum (40 mg/ml crude protein in 200 g mash, 0.02%).

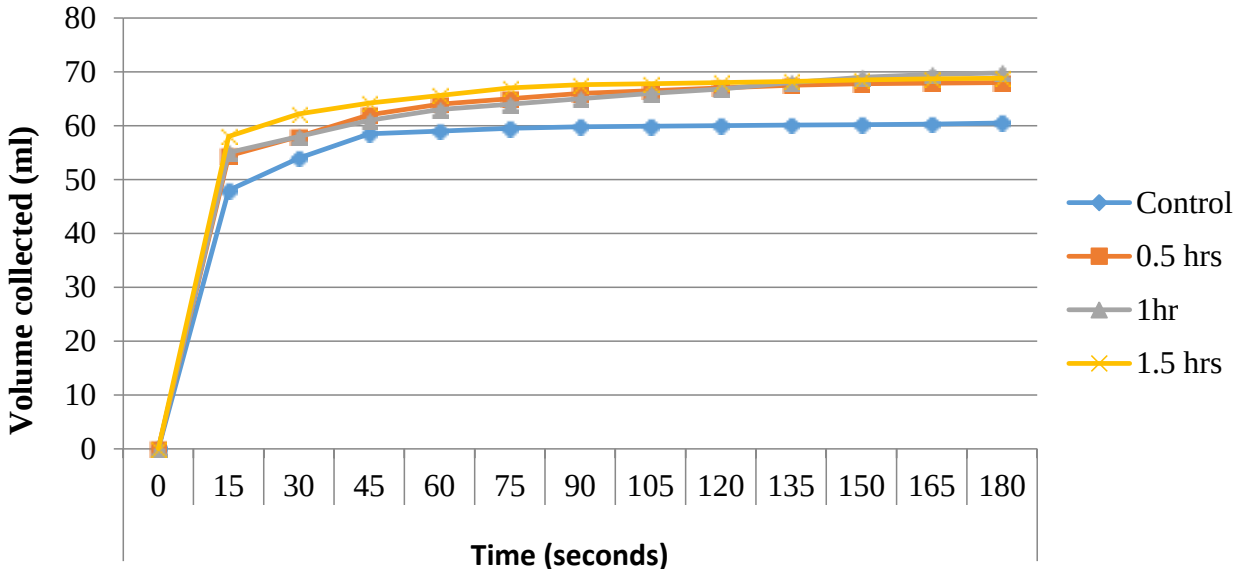

Figure 9. *Nkabom* variety's starch milk flow pattern at all holding times of its enzyme dosage optimum (40 mg/ml crude protein in 200 g mash, 0.02%).

altogether confirm that the *Bankyehemaa* variety is best suited for enzyme-assisted starch extraction. Its low root (pulp) moisture content also suggest that farmers would be transporting a lot more (dry) biomass from their farms and their pulp will probably dry faster for storage; results of both yield and starch milk recovery rates in the *Esam bankye* variety was rather inconsistent hence utilization of the variety for crude (pectolytic) enzyme-based cassava starch extraction might be discouraging.

Although, high dosages (as with the 25 mg/ml per 100 g mash in this work) of crude pectolytic enzyme and longer retention time (1hour and 1.5 hours) have been reported to significantly affect starch biochemistry by causing extensive amylolysis, the very high starch yields obtained from these dosages cannot be overlooked. Such dosages could be employed in the production of

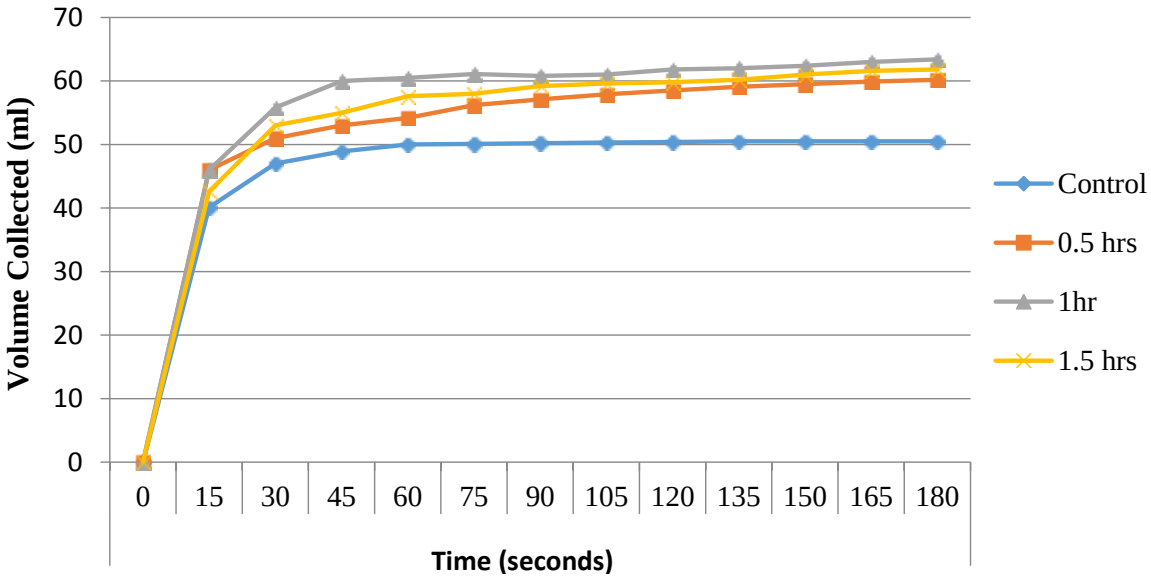

Figure 10. *Bankye hemaa*'s variety starch milk flow pattern at all holding times of its enzyme dosage optimum (50 mg/ml crude protein in 200 g mash, 0.025%).

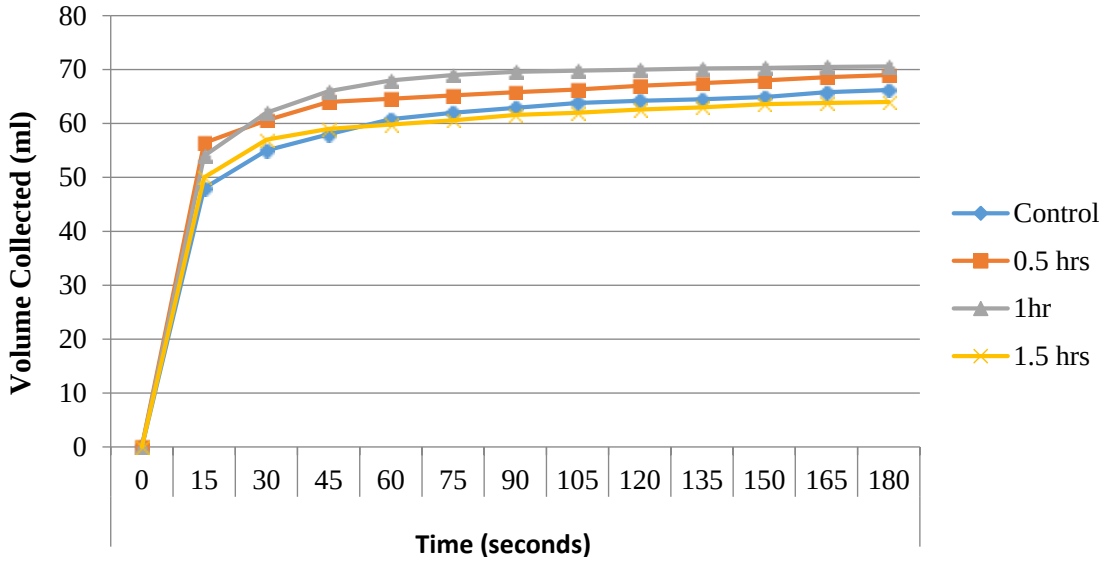

Figure 11. *Doku duade* variety's starch milk flow pattern at all holding times of its enzyme dosage optimum (50 mg/ml crude protein in 200 g mash, 0.025%).

Table 4. Rates of starch milk flow from treated and untreated starch mashes of the cassava varieties.

Cassava variety	Average flow rate (ml/s) within the first 15 s		Percentage increase starch milk flow
	Control	Treated	
Afisiafi	2.5 ± 0.047	4.3 ±0.095	73.3 ± 0.555
Nkabom	2.7±0.094	3.2±0.000	17.1± 4.030
Bankye hemaa	2.2±0.047	3.9±0.047	78.5±6.091
Doku duade	1.8 ± 0.047	3.1± 0.000	73.7 ± 4.658
Esam bankye	1.8±0.047	1.7±0.047	-10.9±0.282

starches that find application in the food industry as this will enhance sweetness and digestibility of the starches.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Addo E (2013). Logistical Constraints collapse Ayensu Starch again. Available at https://www.ghanaweb.com/GhanaHomePage/business/Logistical-Constraints-Collapse-Ayensu-Starch-AGAIN-263892.

Aldana S, Quintero F (2013). Physicochemical Characterization of two cassava (*Manihot esculenta* Crantz) starches and flours. Scientia Agroalimentaria 1 (2013):19-25.

Alves AAC (2002). Cassava: Biology, production and utilization, CAB International, Brazil. pp. 63-89.

Association of Official Analytical Chemists (AOAC) (1990). Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists. Washington DC.

Apea-Bah FB, Oduro I, Ellis WO, and Safo-Kantanka O. (2007). Time of harvesting and its effects on quality of flour from four cassava varieties, Proceeedings of the 13th ISTRC Symposium, Pp. 248-259.

Business and Financial Times Newspaper (BFT) (2013). Ayensu Starch Factory resumes production. Available at https://www.ghanaweb.com/GhanaHomePage/business/Ayensu-starch-factory-resumes-production-267048

Bali R (2003). Isolation of extracellular fungal pectinolytic enzymes and extraction of pectin using kinnow waste as substrate. A Thesis submitted in Partial Fulfillment of the requirement for the Award of Master of Science (Biotechnology), Deemed University,Patiala,Punjab, India. In: Food Innovation Asia Conference 2010: Indigenous Food Research and Development to Global Market, June 17-18, 2010, BITEC, Bangkok, Thailand. Available at http://www.agri.ubu.ac.th/news/fiac/data/oral_session7.pdf.

Bernfield P (1955). Amylases, Alpha and Beta. Methods Enzymol. 1:149-158.

Central Intelligence Agency (CIA) (2017). World Fact Book. Available at https://www.cia.gov/library/publications/the-world-factbook/geos/print_gh.html.

Chinma CE, Ariahu CC, Abu JO (2013). Chemical composition, functional and pasting properties of cassava starch and soy protein concentrate blends. J. Food Sci. Technol. 50(6):1179-1185.

Dzogbefia VP, Ofosu GA, Oldham JH (2008a). Evaluation of locally produced *Saccharomyces cerevisiae* pectinase enzyme for industrial extraction of starch from cassava in Ghana. Sci. Res. Essays 3(8):365-369.

Dzogbefia VP, Ofosu GA, Oldham JH (2008b). Physicochemical and pasting properties of cassava starch extracted with the aid of pectinase enzymes produced from *Saccharomyces cerevisiae* ATCC52712, Sci. Res. Essays 3(9):406-409.

El-Sharkawy MA (2004). Cassava Biology and Physiology. Plant Mol. Biol. 56 (4):481-501.

Essabrah-Mensah E (2016). Cassava – Crop of the Decade. Business and Financial Times Available at http://thebftonline.com/business/agribusiness/18923/cassava-crop-of-the-decade-.html.

Food and Agricultural Organization (FAO) of the United Nations (2016), Food Outlook Biannual Report on Global Food Markets. P 36. http://www.fao.org/3/a-i7343e.pdf

Fregene MA, Suraez M, Mkumbira J, Kulembeka H, Ndedya E, Kulaya A, Mitchel S, Gullberg U, Rosling H, Dixon AG, Dean R, Kresovich S (2003). Simple sequence repeat marker diversity in cassava landraces: genetic diversity and differentiation in an asexually propagated crop. Theor. Appl. Genet. 107(6):1083-1093.

Gummadi SN, Panda T. (2003). Analysis of Kinetic Data of Pectinases with Substrate Inhibition. J. Microbiol. Biotechnol. 13(3):332-337

Jayani RS, Saxena S, Gupta R. (2005). Microbial pectinolytic enzymes: A review. Process Biochem. 40(9):2931-2944.

Li J, Zhou W, Francisco P, Wong R, Zhang D, Smith SM (2017). Inhibition of *Arabidopsis* chloroplast β-amylase BAM3 by maltotriose suggests a mechanism for the control of transitory leaf starch mobilization. PLoS One 12(2):e0172504.

Miguel ÂSM, Martins-Meyer TS, Costa Figueiredo ÉVD, Lobo BWP, Dellamora-Ortiz GM (2013). Enzymes in Bakery: Current and Future Trends. Food Ind. 14:288-321.

Moelants KRN, Cardinaels R, Buggenhout SV, Loey AMV, Moldenaers P, Hendrickx ME (2014). A Review on the Relationships between Processing, Food Structure, and Rheological Properties of Plant-Tissue-Based Food Suspensions. Compr. Rev. Food Sci. Food Saf. 13(3):241-260.

Morgan NK, Choct M (2016). Review Article Cassava: Nutrient composition and nutritive value in poultry diets. Anim. Nutr. 2(4):253-261.

O'Neill MA, Darvill AG, Albersheim P (2001). Pectic substances. In: Encyclopedia of Life Sciences. Web pub: www.els.net . Macmillan Reference Ltd., London.

Pinyo J, Luangpituksa P, Suphantharika M, Hansawasdi C, Wongsagonsup R (2016). Effect of enzymatic pretreatment on the extraction yield and physicochemical properties of sago starch**.** Starch - Stärke 68(1-2):47-56.

Ranganna S (1986). Handbook of analysis and quality control for fruit and vegetable products. Tata Me Graw- Hill Publishing Company, New Delhi. P 1112.

Roslimi DI, Herman, Sofyanti N, Chaniago M, Restiani R, Novita L. (2016). Characteristics of 22 cassava (*Manihot esculenta* Crantz) genotypes from Riau Province, Indonesia. Sabrao J. Breed Genet. 48(2):110-119.

Safo-Kantanka O (2004). Development of cassava varieties through the exploitation of variability in local germplasm: A Presentation at the 6th Scientific Meeting of the Cassava Biotechnology Network (CBN VI), CIAT, Cali, Columbia, 8th-14th March, 2004.

Schoeninger MJ, Bunn HT, Murray SS, Marlett JA (2000). Composition of Tubers Used by Hadza Foragers of Tanzania. J. Food Composit. Anal. 13:1-11.

Sit N, Deka SC, Misra S (2015). Optimization of starch isolation from taro using combination of enzymes and comparison of properties of starches isolated by enzymatic and conventional methods. J. Food Sci. Technol. 52(7):4324-4332.

Sriroth K, Santisopasri V, Petchalanuwat C, Kurotjanawong K, Piyachomkwan K, Oates CG (1999). Cassava starch granule structure–functional properties: influence of time and conditions at harvest on four cultivars of cassava starch, Carbohydr Polym., 38(2):161-170.

Vatanparast M, Hosseininaveh V, Ghadamyari M, Sajjadian SM (2014). Plant Cell Wall Degrading Enzymes, Pectinase and Cellulase, in the Digestive System of the Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). Plant Protect. Sci. 50(4):190-198.

Vázquez C, Martínez MJ, Lahoz R, Reyes F (1986). Effect of calcium and other metal ions on pectic activities from autolysed cultures of *Alternaria alternate.* FEMS Microbiol Lett. 37(2):227-230.

Wang S, Li C, Copeland L, Niu Q, Wang S (2015). Starch Retrogradation: A comprehensive review. Compr. Rev. Food Sci. Food Saf. 14(5):568-585.