

Full Length Research Paper

Physicochemical properties of starches extracted from local cassava varieties with the aid of crude pectolytic enzymes from *Saccharomyces cerevisiae* (ATCC 52712)

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Starches extracted from root mashes of the *Nkabom*, *Esam Bankye*, *Bankyehemaa*, *Doku Duade* and *Afisiafi* cassava varieties, with the aid of (crude) pectolytic enzymes from *Saccharomyces cerevisiae* pectolytic were analysed to compare effects of (pectolytic) enzyme technology on the physicochemical properties of the extracted starches. This was to help establish the extent to which varietal differences affect application of the technology and to inform the possible domestic or industrial application of the ensuing starches. Enzyme treatment generally did not affect the protein, fiber and ash content of the starches. However, it significantly increased moisture content, starch granule sizes, water binding capacity and swelling power of the starches in most of the varieties; pH of the extruded starches were also significantly decreased and the starches' colour was also significantly made lighter by the technology. Despite the general trends observed, the technology was found to impact physicochemical properties of some varieties more than others. The work therefore showed that the technology is variety-sensitive and could influence starch utility.

Key words: Amylolysis, cassava varieties, crude pectolytic enzymes, endogenous amylase, starch physicochemical properties.

INTRODUCTION

In many parts of the world, enzyme application in industry has become common. The food, pharmaceutical, nutraceutical and medical sectors are all profoundly impacted by enzyme use. As of 2013, global market for enzymes was estimated at 4.8 billion dollars and demand is expected to rise to about 7.1 billion dollars in the year 2018; this comes to a compound annual growth of about

8.1% between the said periods (<https://www.bccresearch.com/marketresearch/biotechnology/enzymes-industrial-applications-bio030h.html>).

In many parts of Africa, enzyme technology is a rather nascent novelty; it is applied, in some cases, in total oblivion in traditional food processing (such as in the fermentation of cassava chips and dough to produce

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foods like *kokonte* and *agbelima* respectively). Biocatalysts which serve as a source of enzymes for such (fermentation) processes are applied either submerged in a (nutrient) medium or incubated on a solid substrate. Very little documentation is however available on how such processing methods impact the physicochemical properties of the resulting food substances. Starch, like enzymes, is an important commodity whose industrial applications are diverse - ranging from the adhesive and textile to food and medical sectors. In Ghana, starch is recovered from root crops such as yam, cassava and potatoes. Cassava is however the crop of choice for starch production and different varieties are available for dietary and industrial considerations. This presents a challenge with regards to utility as certain varieties could be heavily depended on because of their appealing qualities for food or their inherent starch yield. The *Afisiafi* cassava variety, for example, has been cited as good for both starch production and for indigenous foods like *kokonte* (dried fermented chips), *agbelima* (tubers milled into dough) and *gari* (grated, fermented sieved and fried mash) (<http://rtimpkknowledgecenter.blogspot.com/2014/07/improved-cassava-varieties.html>).

One of the reasons cited for the collapse of the Ayensu Starch Factory, set up in 2008 as part of the Ghana governments Presidential Special Initiative (PSI) on cassava, was the lack of raw materials needed to feed the factory. Attempts at recovering starch using enzyme technology have achieved remarkable results, boosting yields and impacting positively the utility of the resulting starches. However, this feat (in many cases) has only been achieved at the lab scale with single crop varieties. Response of variety to effects of the technology on the physicochemical properties of the ensuing starches is therefore poorly studied. Previous work using crude pectolytic enzyme preparations showed some amylase activity (Agyepong and Barimah, 2017). However, as starch granules from different varieties of the same crop species show some differences in granule morphology and chemistry (including starch and amylose content), it was expected that the technology will impact the resulting starches differently. Effects of the (enzyme) technology on starches from the various cassava varieties will help inform the utility of the ensuing starches as yields are also enhanced.

MATERIALS AND METHODS

Plant materials and chemicals

Root tubers of fresh local cassava (*Manihot esculenta* Cranz) varieties *Afisiafi*, *Esam bankye*, *Bankyehemaa*, *Nkabom* and *Doku duade* harvested at 9 months after planting (MAP) were obtained under a running project at the Department of Agriculture Engineering, K.N.U.S.T. (Kumasi, Ghana). 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.

All chemicals used for the project were (analytical grade) products of SIGMA® and FISON®. These were obtained from the Labs of the Departments of Biochemistry and Biotechnology and Theoretical and Applied Biology, KNUST.

The microbe used for enzyme production was yeast (*Saccharomyces cerevisiae* ATCC 52712). This strain of yeast, purchased from America Type Culture Collection, Maryland, USA, had been maintained on agar slant at the Department of Biochemistry and Biotechnology, KNUST, Kumasi.

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 malt extract 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) 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.

Production and assaying of crude pectolytic enzymes

Four milliliters (4 ml) of M.E.B. culture (cell density 6.32×10^{12} per 100 ml) was subcultured in 100 ml of 1% pectin medium (formulated based on a modification of the method used by Ranganna (1986)) for 8 days at 28°C to induce carbon catabolite repression and to stimulate the production of yeast pectolytic enzymes in the medium. During this period, the concentration of crude protein (enzyme) was monitored (using the Biuret test) 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.

Extracts from the 1% pectin medium were also monitored (during the 8 day incubation period) for their pectolytic activity (Jayani et al., 2005). One unit of polygalacturonase (PGase) activity was as defined by Jayani et al. (2005).

Endogenous amylase enzyme assay

Crude pectolytic enzyme preparations have been reported (Dzogbefia et al., 2008a) to contain some amylase activity. Hence, over the 8-day period of incubation, amylase activity in crude enzyme extract was assayed with laboratory grade starch (BDH). This was based on modification of the method described by Bernfeld (1955).

1% starch solution was prepared by dissolving 1 g starch in 100 ml of slightly warmed sodium acetate buffer (0.1 M, pH 4.7). The extraction buffer was 1 M potassium hydrogen phosphate, pH 6.5. One milliliter (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 5 min. While the test tubes and its content was warm, 1 ml of 40% potassium sodium tartrate solution was added and the content cooled in running tap water. The volume was then made up to 10 ml by the addition of 6 ml water. The absorbance was read at 560 nm. In the case of 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 (10-100 mg) of maltose.

Preparation of cassava mash for starch extraction

Freshly harvested cassava varieties (*Afisiafi*, *Doku duade*, *Esam bankye*, *Nkabom* and *Bankye hema*) all harvested at 9 months after planting, MAP) were each sorted, washed under running tap water and knife peeled. Moisture content of the pulp from the cassava varieties was determined using the AOAC Method (1990). The peeled cassava was washed in distilled water and cut into 2-3 cm³ chunks. 100 g of diced cassava pulp from each variety (in triplicates) were selected and frozen after which they were blended separately in a double screw waring blender (model 32 BL80 (8011), USA) set at low speed (18000 RPM) for 1 min. The chilling treatment was to minimize starch gelatinization when blending and the low rotor speed was to minimize granule shearing as this could influence enzyme activity, granule structure and other starch physicochemical properties. Cassava mash of each variety was transferred to into 600-ml conical flask and labeled accordingly. 100 ml of distilled water was added to each of the samples and shaken to dissolve the ensuing mash.

Stages for the enzyme-based starch extraction are as shown in the flow chart (Figure 1). Starches obtained from optimum treatment combinations (retention time versus crude protein dosage) for yield were sampled and effects of enzyme treatment on their physicochemical parameters were investigated. Optimum starch yield was recorded with the 0.2% crude protein at 0.5 h (retention time) in the *Nkabom* and *Esam Bankye*; *Afisiafi* required similar crude protein dosage but at 1 h retention time. *Doku duade* and *Bankye hema* both recorded yield optima with the 0.25% dosage at 0.5 h retention time (Agyepong and Barimah, 2017).

Microscopic observation of starch granules

Starches from controls and treated samples of all varieties were mounted in distilled water and observed under binocular compound microscope. Observation was done at high power (X 400). For starch granule diameter measurements, a micrometer disk (eyepiece graticule) and a stage micrometer were used. Starch granules from each (control) variety and for those obtained from their corresponding (enzyme) treatments, were categorized as *Small*, *Medium* and *Large* based on gross visual inspection. Thereafter, their corresponding measurements were carried out by selecting randomly three granules from each category and the mean size determined.

Determination of starch pH

The extracted cassava starch (10 g) was weighed and made into slurry using 25 ml of distilled water. The pH of the slurry was determined using the Corning pH meter (model 240). This was done for both control and enzyme treated samples.

Determination of starch moisture content

Two grams (2 g) of samples were weighed into previously dried and weighed glass crucibles. The crucibles with the samples were then placed in thermostatically controlled oven (XOV 880, Gallenkamp, England) at 105°C for 5 h to obtain starch dry mass. At the end of the period, the crucibles were removed and placed in a desiccator to cool, and their weights recorded till a constant weight was obtained. Percentage moisture contents of the starches were calculated (AOAC, 1990).

Determination of ash content of starch

Ash content of the starch samples was determined by the Dry

Ashing Method (AOAC, 1990). Two grams (2 g) of samples was weighed into previously ignited and weighed porcelain crucibles. The crucibles and their contents were then placed in a Muffle furnace (Model AS 260D, Gallenkamp, England) preheated to 600°C and heated for 2 h. The ash content in each was calculated and expressed as a percentage.

Determination of crude fiber content of starch

Crude fiber content of samples was determined by the AOAC (1990) method.

Determination of starch protein content of starch

The Association of Official Analytical Chemists (AOAC, 1990) Kjeldahl procedure was used to determine the crude protein content of starch samples.

Determination of amylose content of starch samples

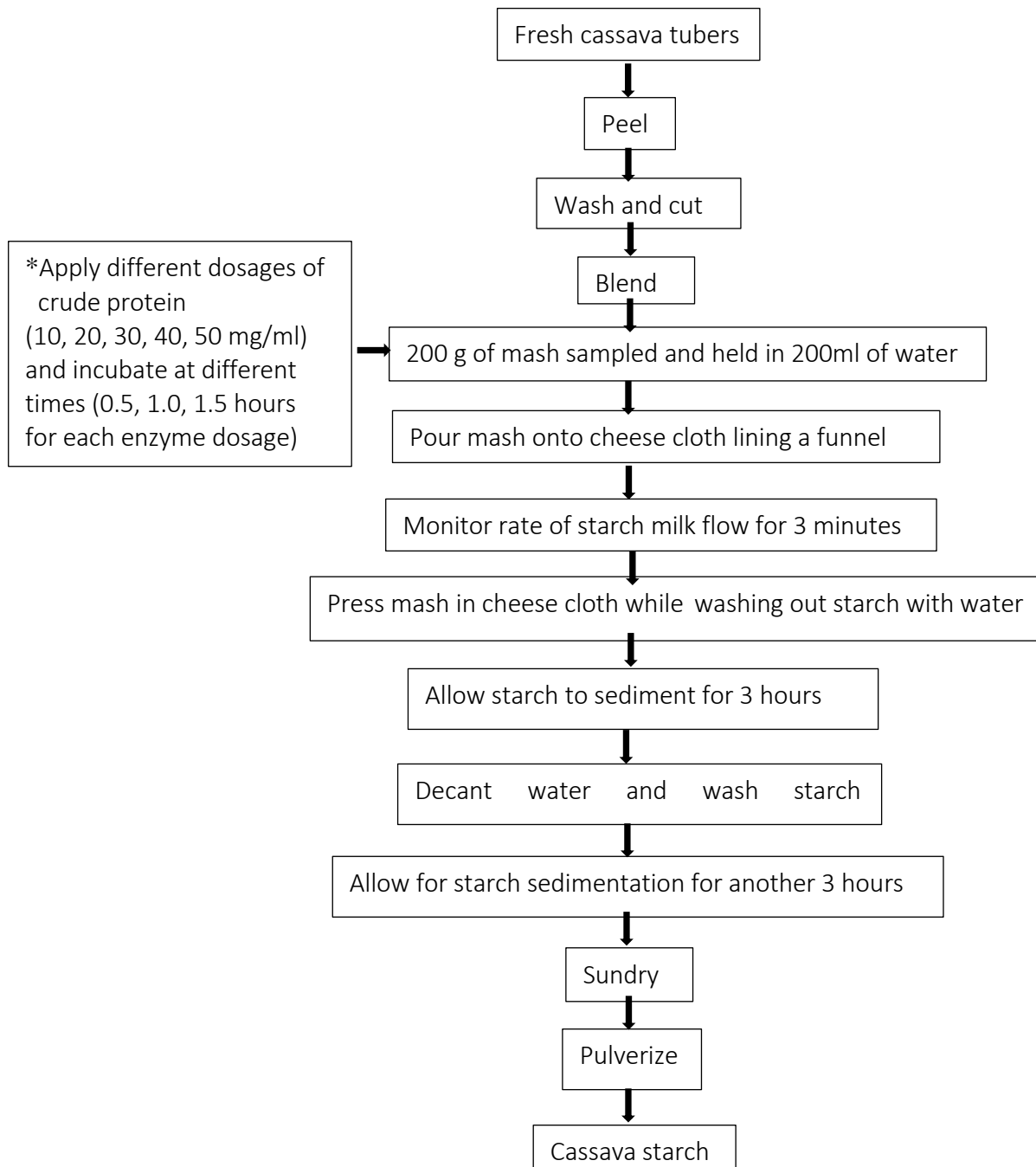
Amylose content of the starch samples was determined based on the iodine colorimetric method described by McCready and Hassid (1943). One hundred milligrams (100 mg) each of cassava starch samples was introduced into 100 ml volumetric flask, wetted with 1 ml ethanol and 10 ml distilled water. The content was dissolved by adding 2 ml of 10% NaOH, and heated in water bath to form a clear solution. The flask with its content was cooled and diluted to the mark. Five milliliters (5 ml) portion (equivalent to 5 mg) of the alkaline starch solution was introduced into a 500-ml volumetric flask; 100 ml of water added and slightly acidified with 3 drops of HCl. The contents were well mixed by shaking the flask and 5 ml iodine was added to the mixture and diluted to 500 ml with distilled water. A blank was set by diluting 5 ml iodine solution to 500 ml with distilled water in place of the standard sample. The absorbance of each starch sample was read against the blank in the spectrophotometer set at 640 nm. The percentage amylose of samples was determined using an equation derived from a standard calibration curve for amylose.

Determination of water binding capacity

Water binding capacity of starch samples was determined following the method of Yamazaki (1953) as modified by Medcalf and Gilles (1965). An aqueous suspension was made by dissolving 2 g of cassava starch in 40 ml distilled water in a previously dried and weighed centrifuge tube. The suspension was agitated for 1 h on a Griffin shaker and centrifuged at 2200 rpm for 10 min. The centrifuge tube was inverted for 10 min to drain off unbound water. The centrifuge together with its content was weighed and bound water was calculated and expressed as a percentage.

Determination of solubility and swelling power

Solubility and swelling power of the starch samples were determined based on the modified method of Leach et al. (1959). One gram (1 g) of cassava starch sample was dissolved in 40 ml distilled water in a previously weighed 50-ml centrifuge tube. The suspension was stirred just sufficiently and uniformly avoiding excessive speed to prevent starch granule fracture. The suspension was heated at 85°C in a thermostatically regulated water bath for 30 min with constant stirring. It was then centrifuged at 2200 rpm for 15 min. The solubility was found from the residue after evaporating the supernatant in a previously dried and weighed glass crucible.



** Control samples did not require this step*

Figure 1. Stages involved in the processing of cassava tubers for enzyme-aided starch extraction. Source: Agyepong and Barimah (2017).

The percent solubility and swelling power were then determined.

Determination of starch colour

The colour of samples of starch from both treated and untreated mashes of each variety were examined using a colour meter

(Minota, chromameter CR-2001). The L_aa_b* (Chroma meter) colour system was used to calibrate a white tile.

Statistical tool(s) and analyses

For all parameters measured, statistical analyses were carried out

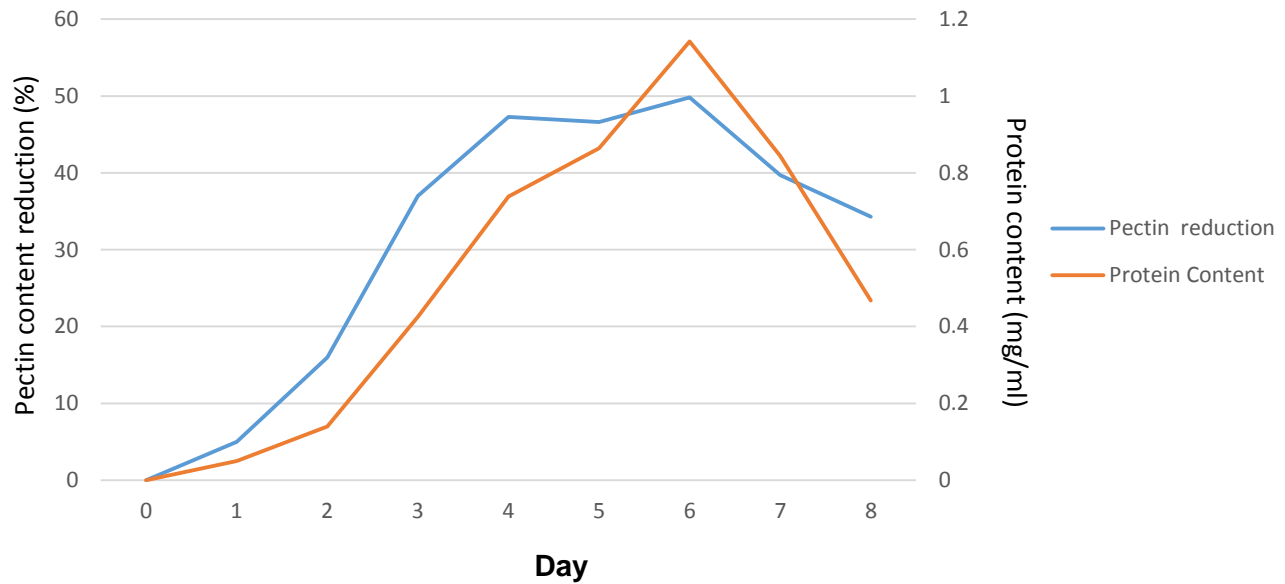


Figure 2. Protein content and pectolytic activity of crude protein extracts during incubation with *S. cerevisiae* (ATCC 52712).

with SigmaPlot for Windows Version 11.0 by Systat Software Inc. © 2008. Graphs were plotted using Microsoft Excel 2007 (from Microsoft Inc.).

The Completely Randomized Design (CRD) was used to organize all data. Data were subjected to a one-way ANOVA (unless otherwise stated) and significant differences were tested using the Duncan's New Multiple Range Test. All results were presented as mean \pm standard values of three replicates ($n=3$) and Least Significant Difference (LSD) were also determined for all parameters. Significant differences between treatment means were accepted at $P<0.05$ (unless otherwise stated).

RESULTS AND DISCUSSION

Pectolytic and amylase activities in crude enzyme extracts

On the 6th day of culturing *S. cerevisiae* in the 1% Pectin medium (Figure 2), the resulting extract recorded its highest protein content and pectolytic activity of 4.91 U (with specific activity 4.21 U/mg) and a 0.293 U/ml (with a specific activity of 0.257 U/mg) of endogenous amylase activity in the extract.

Starch granule size and structure

Enzyme treatment enhanced the sizes of medium to large starch granules extracted from all varieties. However, sizes of small starch granules extracted from most varieties were not improved by the technology (Table 1).

Photomicrographs of starch granules from root (pulp) mashes of untreated and treated cassava varieties are presented in Plates 1 to 5.

Starch granule size affects starch composition and other functional properties such as gelatinization and pasting properties, enzyme susceptibility, crystallinity, swelling and solubility (although several other factors, including amylose/amylopectin ratio and molecular weight and granule fine structure, are also influential) (Lindeboom et al., 2004). An improvement in the recovery of starch granule size (as a result of enzyme treatment) suggests a general enhancement in digestibility and in its performance as a binding agent (Sandhan et al., 2017). Starch granule sizes obtained ranged from 5.33 - 23.34 μm (Table 1). This range of values agrees with the 4 - 35 μm range reported by Adejumo et al. (2011). Dzagbafia et al. (2008b) recorded similar granule sizes for the various categories of starches they extruded from untreated *Afisiayi* (aged 6MAP). This confirms reports (Moorthy and Ramanujam, 1986) that the granule size of starch does not increase significantly after the 6th M.A.P. However, larger starch granules were observed in our treated samples for the same variety. This observation could be due to the differences in enzyme activity on the *Afisiayi* cassava mash. Thus, the crude enzyme used for this work, probably had slightly varied composition of pectolytic enzymes that was more effective at hydrolyzing pectins in the mash thus liberating much bigger starch granules that could have otherwise been trapped within the fiber matrix.

On morphology of all (non-fractured) granules, the photomicrographs (Plates 1 to 5) showed near-spherical starch granules, each with three shallow equidistant surface fissures that radiate from a deep central groove towards the edge of the granule; the grooves diminish as they approach the edge of the granules. Yuan et al. (2007) confirmed the presence of incomplete hemisphere

Table 1. Sizes of starch granules extracted from the treated and untreated cassava root mashes.

Cassava variety		Categories of starch granules and their average diameter (μm)			
		Small	Medium	Large	Avg. size
<i>Nkabom</i>	Control	6.67 \pm 1.9	13.34 \pm 0.0	18.67 \pm 0.0	12.89
	Treated	6.67 \pm 1.9	13.34 \pm 0.0	20.01 \pm 1.9	13.34
<i>Afisiafi</i>	Control	6.67 \pm 1.9	13.34 \pm 0.0	18.67 \pm 0.0	12.89
	Treated	6.67 \pm 1.9	12.94 \pm 0.6	21.34 \pm 0.0	13.65
<i>Bankye hema</i>	Control	9.34 \pm 1.9	13.34 \pm 0.0	21.34 \pm 3.8	14.67
	Treated	9.34 \pm 1.9	14.67 \pm 1.9	23.34 \pm 0.9	15.78
<i>Esam Bankye</i>	Control	5.33 \pm 0.0	14.67 \pm 1.9	20.01 \pm 1.9	13.34
	Treated	7.34 \pm 0.9	18.34 \pm 0.0	22.67 \pm 1.9	16.12
<i>Doku duade</i>	Control	7.33 \pm 0.9	13.34 \pm 0.0	20.01 \pm 1.9	13.56
	Treated	4.67 \pm 0.9	13.34 \pm 0.0	23.34 \pm 1.9	13.12

Values are represented as mean \pm standard deviation (n=3); n = number of replicate mashes.

granules with fissures on tapioca starch. Starch granule size, shape and their surface characteristics are important in characterizing and identifying the botanical source of starch (Robertson et al., 2006).

From the photomicrographs (Plates 1 to 5), it is clear that generally, enzyme treatment did not have much detrimental effect on starch granule structure. However, it is observed that starches from the *Bankyehama* and *Nkabom* (Plates 1 and 4 respectively) recorded fractures in some granules from both the treated and untreated mashes. These fractures result from effects of processing mechanisms such as maceration, freezing of mashes during storage (Sujka and Jamroz, 2007) and solar drying of granules (Huber and BeMiller, 1997). According to Sujka and Jamroz (2007), freezing starches of different water content causes granule surface crushing and destroys granule inner structure (acting in a way similar to high pressure).

Fractures observed, especially in the central grooves of starch granules from the treated *Doku duade* and *Esam bankye* (Plates 2 and 3 respectively) varieties, suggest that enzyme treatment contributed to granule fracture in those regions. There are reports (Sujka and Jamroz, 2007) that the presence of pores, channels and cavities on the surface of starch increases the surfaces potentially available for chemical and enzymatic reactions. Thus, activities of endogenous amylases were probably high in these grooves and micropores (which cannot be resolved by conventional light microscopy) making the granules more vulnerable to fracture in these areas. The effects, however, might have been minimized by the shorter retention times adopted for mash incubation (since not all treated granules showed the detrimental effects) during enzyme treatment and starch extraction.

Thus effects of enzyme treatment on starch granule structure of different cassava varieties varied. The technology rendered some granules more susceptible to fragmentation whereas others were not significantly affected.

Moisture content and pH

Starch samples from all enzyme-treated mashes had higher moisture content than their respective controls (Table 2). As moisture content correlates negatively with amylose content (QinKe-xin et al., 2014), it is possible that reduction in amylose-water interaction (due to amylolysis) might have caused this. Moisture content of starches from both treated and untreated varieties differed significantly but agreed with values reported in literature (Oladunmoye et al., 2014; Belibi et al., 2014). Higher than reported moisture content reduces the shelf life of the starch as it tends to enhance the growth of moulds which subsequently affects other important qualities like its colour, protein and amylose contents.

Moisture content of starches from different cassava varieties can be affected by a number of factors which include intensity and duration of thermal exposure, prevailing ambient humidity (Apea-Bah et al., 2011) as well as aeration: morphological features of the starches' microstructure could also influence water sorption.

Enzyme treatment also reduced the pH of the ensuing starches. Acetate buffer used for enzyme production (for cassava mash incubation) might have influenced this outcome. Starch pH ranged from 5.2 to 7.6 (the lowest pH being recorded in the *Doku duade* and the highest from the *Esam bankye* varieties). However, these do not

Table 2. Physicochemical properties of starches from the various cassava varieties before and after enzyme treatment.

Physicochemical Parameter	Cassava varieties									
	<i>Nkabom</i>		<i>Esam Bankye</i>		<i>Bankyehemaa</i>		<i>Afisiafi</i>		<i>D. duade</i>	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
pH	6.12 ^a (0.01)	6.03 ^b (0.00)	7.86 ^c (0.00)	7.62 ^d (0.02)	7.19 ^e (0.02)	7.04 ^f (0.00)	6.92 ^g (0.00)	6.81 ^h (0.01)	6.29 ⁱ (0.01)	6.22 ^j (0.02)
Moisture (%)	8.21 ^a (0.29)	9.32 ^b (0.25)	11.69 ^c (0.58)	12.39 ^d (0.12)	10.26 ^e (0.43)	10.47 ^f (0.41)	10.72 ^g (0.34)	11.05 ^h (0.17)	12.07 ^c (0.44)	12.10 ^c (0.46)
Total ash (%)	0.98 ^a (0.01)	0.96 ^a (0.01)	0.50 ^b (0.01)	0.49 ^b (0.01)	0.38 ^c (0.01)	0.39 ^c (0.01)	0.49 ^b (0.01)	0.49 ^b (0.01)	0.50 ^b (0.01)	0.50 ^b (0.01)
Crude Protein (%)	0.09 ^a (0.00)	0.09 ^a (0.00)	0.04 ^b (0.00)	0.04 ^b (0.00)	0.03 ^c (0.00)	0.03 ^c (0.00)	0.06 ^d (0.00)	0.06 ^d (0.00)	0.09 ^a (0.00)	0.09 ^a (0.00)
Crude Fiber(%)	0.15 ^a (0.08)	0.14 ^b (0.05)	0.12 ^c (0.05)	0.12 ^c (0.07)	0.12 ^c (0.06)	0.12 ^c (0.04)	0.13 ^d (0.07)	0.12 ^c (0.03)	0.14 ^e (0.04)	0.14 ^e (0.06)
Solubility (%)	4.81 ^a (0.37)	22.66 ^b (0.18)	1.29 ^c (0.01)	4.14 ^a (0.07)	2.00 ^c (0.02)	19.08 ^d (0.08)	7.38 ^e (2.21)	8.00 ^e (0.12)	1.70 ^c (0.03)	22.08 ^b (0.05)
Amylose (%)	23.79 ^a (0.17)	20.57 ^b (0.05)	36.33 ^c (0.20)	29.29 ^d (0.80)	42.47 ^e (0.44)	41.47 ^f (0.41)	27.40 ^g (1.11)	19.71 ^b (0.35)	48.79 ^h (0.68)	41.09 ^f (0.20)
Water Binding Capacity (%)	52.80 ^a (0.51)	62.57 ^b (0.09)	66.56 ^c (0.03)	66.91 ^c (0.27)	71.91 ^d (0.09)	77.93 ^e (1.53)	61.86 ^b (2.32)	80.03 ^f (0.57)	69.88 ^g (0.30)	65.40 ^c (0.53)
Swelling Power (%)	13.31 ^a (0.12)	13.49 ^a (0.11)	8.62 ^b (0.09)	13.29 ^a (0.13)	10.58 ^c (0.02)	17.15 ^d (0.26)	14.47 ^e (0.05)	14.15 ^f (0.15)	6.41 ^g (0.47)	10.70 ^c (0.41)

a) Values in parenthesis () are standard deviations of mean (triplicate) determinations

a) Means in a row followed by the same letter are not significantly different ($P>0.05$)

deviate from starch pH values reported in literature (Garrido et al., 2014).

Protein, fiber and ash content

Enzyme treatment did not affect the protein content of the ensuing starch (Table 2) but recorded values were lower than reported (Aldana and Quintero, 2013). High protein content negatively affects starch pasting properties (Iwe et al., 2017) due to the reduction in hydroxyl groups caused by Maillard reactions. Hydroxyl groups in starch are key physicochemical determinants of starches (Silvaa et al., 2017).

Ash content was not significantly affected by enzyme treatment (Table 2) and the values recorded agreed with those in literature (Adjei et al., 2017). Although cultivated in the same soil having similar edaphic features and chemistry, there were significant differences ($P<0.05$) in ash content between varieties suggesting that mineral uptake by cassava root parenchyma differ significantly

between varieties.

Although starches from some of the treated mashes of the *Afisiafi* and *Nkabom* showed significant reduction in crude fibre content, this parameter was significantly ($P<0.05$) not affected by enzyme treatment in the other varieties. Fibre content was generally lower than reported in literature for cassava starch (Dzogbefia et al., 2008a).

Effects of enzyme treatment on amylose content

Amylose content of starches from the *Afisiafi* and *Nkabom* varieties agreed with values for cassava starch cited in some literature (Ojo et al., 2017; Rolland-Sabaté et al., 2012), whereas those from *Bankye hema*, *Esam bankye* and *Doku duade* did not. Amylose content of flour from some cassava varieties in Ghana has been reported to range between 10.9 and 44.3% (Aryee et al., 2006). Despite these disparities our primary objective of investigating the effect of the technology, from (percentage) changes in their physicochemical

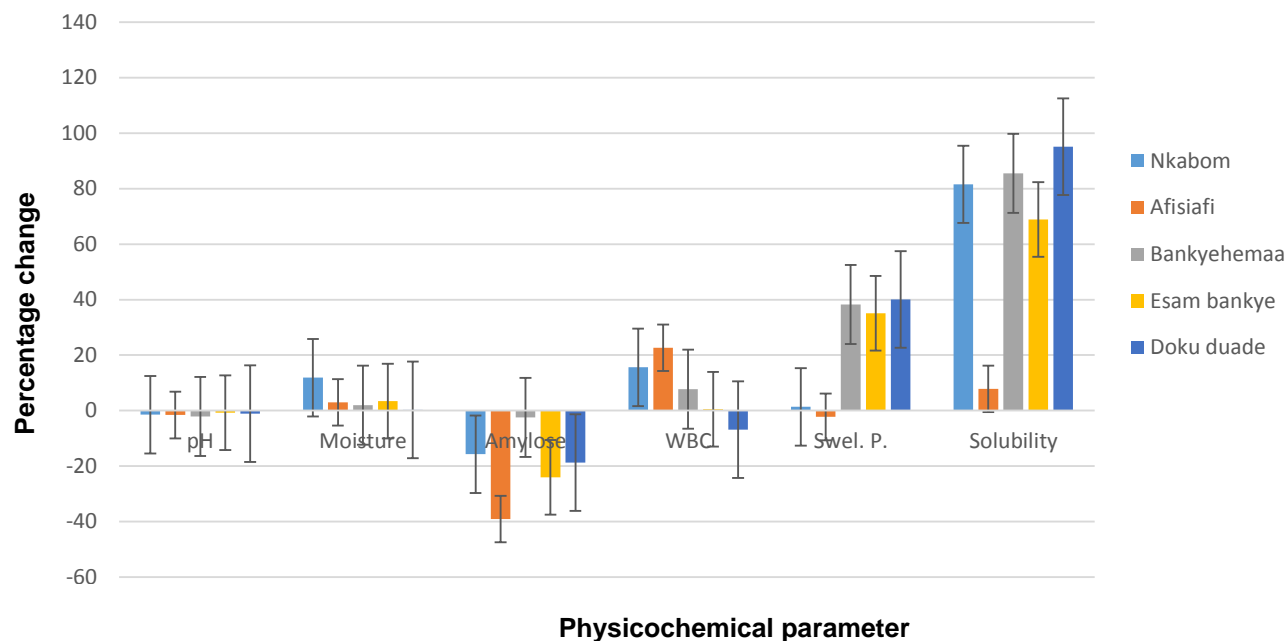


Figure 3. Physicochemical parameters of starches affected by enzyme treatment.

characteristics (Figure 3), was possible since starches from both control and treated mashes were subjected to the same experimental conditions and protocols.

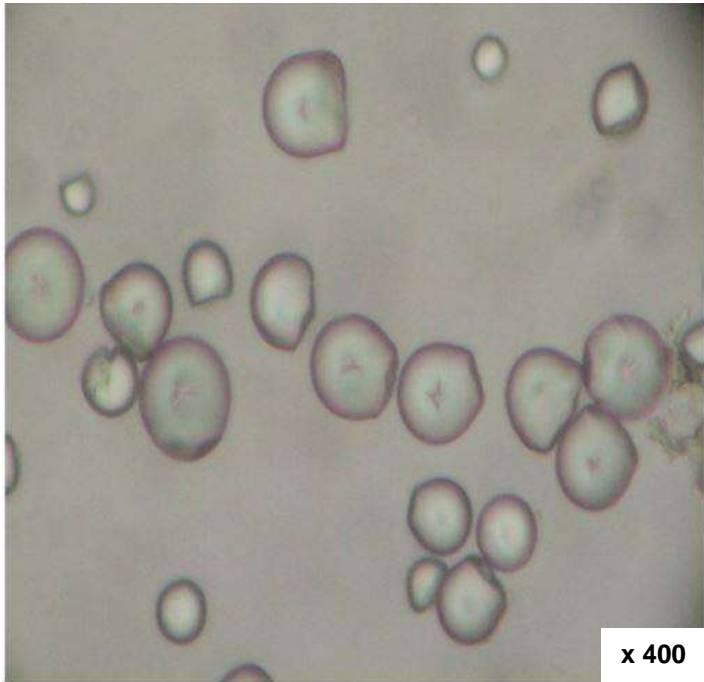
Starches from treated root mashes of all varieties showed significant reduction in amylose (Table 2). High amylose activity recorded in the crude enzyme would explain the generally high amyolytic effects. The very high degradation of amylose in the *Afisiafi* variety could be attributed to the longer period of exposure of its (pulp) mashes to the crude enzymes (1 h incubation time compared with 30 min in the other varieties). Since the enzyme was from a crude source, overall amylose activity would depend on the type and quantity of amylose enzymes present (Bijttebier et al., 2008); type of ions present (which could serve as inhibitors and/or cofactors to amylose and pectinases) in the mash (Bijttebier et al., 2008; Singh et al., 2014) and differences in (amylose) molecular architecture (starch crystallinity), which will determine availability of amylose for amylose action (Polesi et al., 2017). This latter feature (of starch) is variety dependent (Alcázar-Alay and Meireles, 2015).

Effects of amylose on most starches have also been linked with starch granule sizes. Qi and Tester (2016) have reported that small to medium granule starches are more susceptible to enzymatic degradation due to the higher surface area they present. Thus the relative proportions of a particular category of starch granules released (by pectolytic activities) would greatly influence the observed amyolytic effects in the variety: thus interplay of the two groups of enzymes influences the quality of the starches extracted. Varieties that yield more

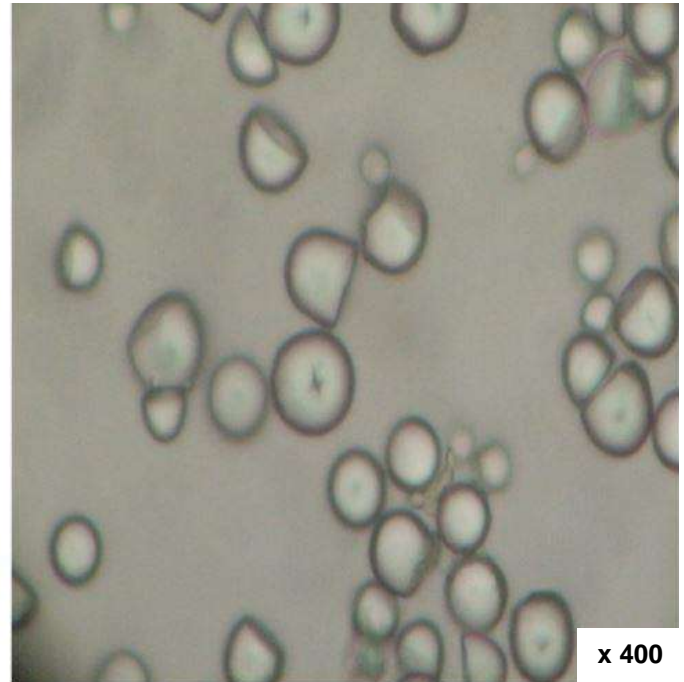
of the larger granules from their mash will be less susceptible to amyolytic effects of the technology. This probably explains why the *Bankyehemaa* variety was more resilient (Figure 3) to amyolysis as the variety recorded the largest granular sizes from both its untreated and treated mashes (Table 1). As high amylose content negatively correlates with digestibility (Fouhse et al., 2015), enzyme application in high amylose/small granule size varieties will yield starches that will be greatly enhanced for application in the food industry. Thus, starches from the *Doku duade* and *Esam bankye*, high amylose varieties (Table 2) which recorded high amyolysis (Figure 3) at short incubation time of 30 min, will be greatly improved for food application if their incubation time with enzymes was extended.

Effects of enzyme treatment on swelling and solubility index

All the starches swelled when heated to a temperature of 80°C. As amylose correlates negatively with swelling power and solubility (Yu et al., 2015), it was expected that the swelling power of high amylose varieties (*Bankyehemaa*, *Doku duade* and *Esam bankye*) would be low (Table 2). Amylose is responsible for maintaining associative forces within granules (Valcárcel-Yamani et al., 2013) hence starches from high amylose varieties would have stronger associative forces that resist swelling upon heating. Reduced swelling also reduces the freeness with which soluble products leach out of



Starch granules from the untreated (Control) mash



Starch granules from the treated mash

Plate 1. Granule structure of starches obtained from untreated (control) and enzyme-treated cassava mash from the *Bankye hema* variety.



Starch granules from the untreated (Control) mash



Starch granules from the treated mash

Plate 2. Granule structure of starches extracted from untreated (control) and enzyme - treated cassava mash from the *Doku duade* cassava variety.



Starch granules from the untreated (Control) mash

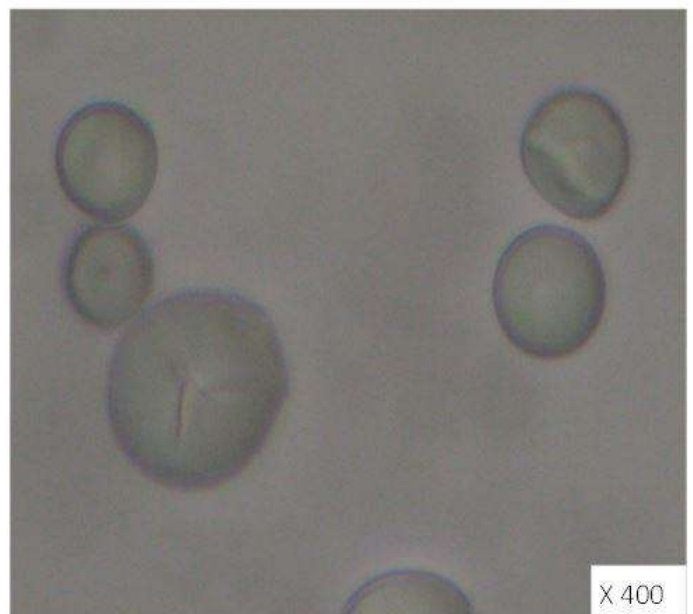


Starch granules from the treated mash

Plate 3. Granule structure of starches extracted from untreated (control) and enzyme - treated cassava mash from the *Esam bankye* variety.



Starch samples from the untreated (Control) mash



Starch samples from the treated mash

Plate 4. Granule structure of starches extracted from untreated (control) and enzyme – treated cassava mash from the *Nkabom* variety.

their granules. Starches from the treated samples swell better than those from their respective control samples (Table 2). Reduction in amylose content of starches from the treated mashes, due to activity of endogenous

amylase, reduced amylose-amylose interaction (via hydrogen bonding) in the granules. This also meant that enzyme treatment reduced crystallinity of the starch granules allowing the granules to leach out more amylose

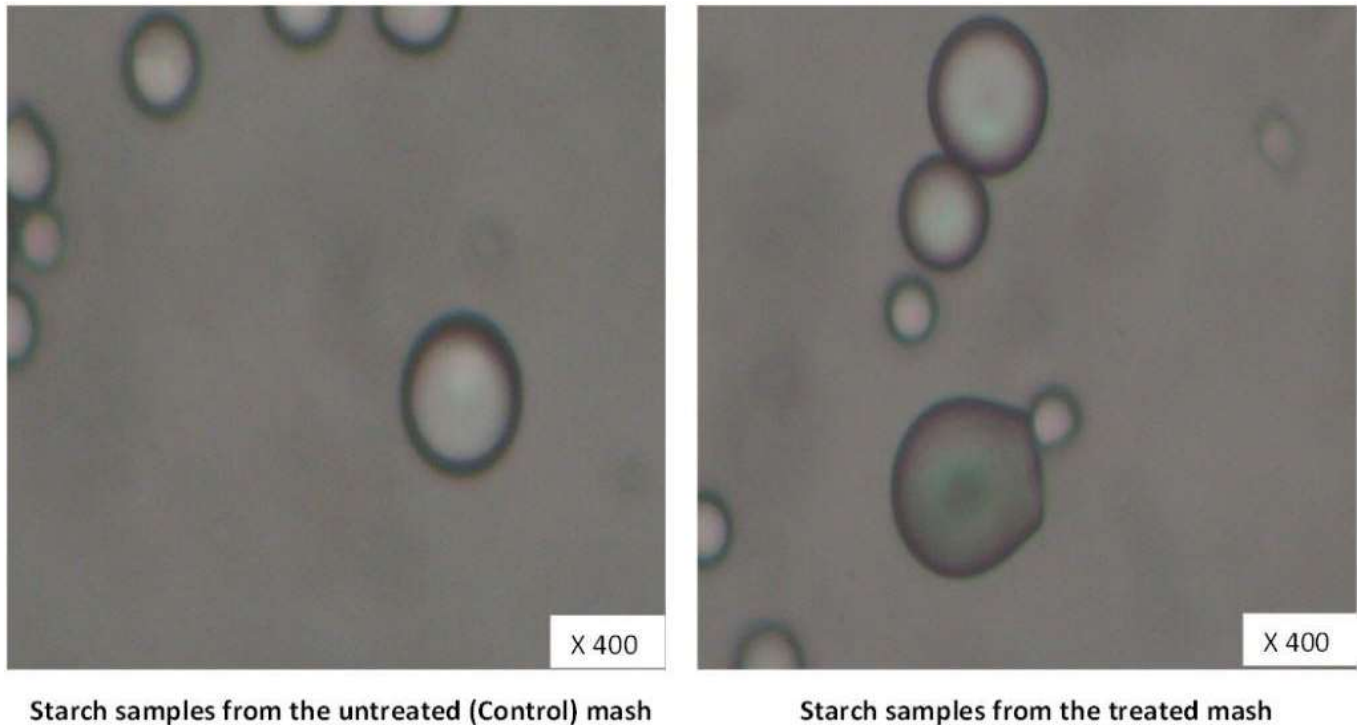


Plate 5. Granule structure of starches extracted from untreated (control) and enzyme - treated cassava mash from the *Afisiafi* variety.

to associate with water. Apparently, starches from varieties whose amylose content were least affected by enzyme treatment could swell better than those from their respective non-treated mashes (Figure 3). Also, swelling association with water. Starches inherently low amylose swell better; however when their amylose content was further compromised, their ability to swell further reduced. This explains the failure of starches from treated *Afisiafi* variety to swell more than its control. The extended incubation time required to peak starch recovery from the variety had detrimental effects on its amylose content. The lowered solubility products recorded in starches from the same variety could be due to its inability to retain (soluble) products as these were probably lost to the supernatant during stages of starch washing and decanting (Figure 1). Starches from all controls samples, especially those from high amylose (cassava) varieties, recorded low solubility values (Table 2) due to their granular thermostability (Omojola et al., 2010).

The relative abundance of the category of starch granules (*Small, Medium or Large*) released might also have influenced the solubility pattern observed. It is obvious that the release of small sized granules (Table 1) by enzyme treatment enhanced solubility values (Table 2). Qi and Tester (2016) have reported that small size starch granules tend to leak out more amylose out of their intact granule than do larger ones at 55°C and higher. Thus, smaller sized granules have higher solubility at temperatures above 55°C. The additive effects of smaller

power of starches from the treated *Nkabom* variety was not significantly ($P>0.05$) affected probably as a result of its ability to resist amylolysis. extensive loss of amylose from low amylose starches drastically reduced amylose granule sizes in providing larger surface area for amylase action (Qi and Tester, 2016) could greatly increase the solubility of such starches. Thus, although high amylose containing varieties (*Doku duade, Bankyehemaa* and *Esam bankye*) provided enough substrate for amylolysis and subsequent solubilization; the same varieties (especially the *Doku duade* and *Esam bankye*) also recorded the least of the granule sizes (Table 1) further providing a larger surface area for enzyme action and rendering them more susceptible to degradation.

The high susceptibility of *Doku duade* starch granules to fragmentation (Plate 2) also explains its exceptionally high swelling power and solubility (Figure 3). The adhesive industry would find amylolytic effects of endogenous amylase undesirable as amylose degradation affects amylose-amylopectin ratio which is an important factor in determining the adhesive strength of starches (Gadhav et al., 2017). Hence, enzyme application aimed at enhancing starch yield for the adhesive industry would require the incorporation of amylase inhibitors, such as maltodextrins and acarbose (Robyt, 2005), to mitigate amylase degradative effects and help to maintain starch granule integrity. However, if the starch is to be applied to food, then such inhibitors could be eliminated as amylases enhance starch

digestibility (Cruz et al., 2015).

Effects of enzyme treatment on water binding capacity (WBC)

Enzyme treatment had varying effects on water binding capacity of the starches produced suggesting some varietal-sensitivity. Enzyme treatment enhanced WBC in the *Bankyehemaa* and *Nkabom* while the starches from the *Esam bankye* were not affected (Table 2). However, WBC for the *Doku duade* and *Afisiafi* recorded significant ($P < 0.05$) reduction.

Water binding capacity is a function of granule size and amylose/amylopectin ratio. The treated *Doku duade*, releasing the smallest size starch granules (Table 1) with high amylose content (Table 2), suggests that a unit mass of starch from treated *Doku duade* would present a much larger surface area to volume ratio for its amylose to adsorb water. Its high amylose content (Table 2) and fractured granule morphology (Plate 2) also suggest greater susceptibility to amyolytic attack which subsequently reduces the associative hydrogen and covalent forces in the starch (Polesi et al., 2017). This might also have affected its bulk density (resulting from reduction in the amylose per granule).

Enzyme treatment best enhanced water binding capacity in starches from the treated *Afisiafi*. The long (enzyme) incubation time (1 h) required for peak starch yield in the variety clearly contributed to an enhancement in WBC as amyolysis enhances granule association with water. However the relative abundance of the type/category of granule could also have contributed to this observation. Such improvements in WBC (due to application of the enzyme technology) suggest that the technology, if adopted, would enhance the quality of the starches as drug binders and disintegrants in pharmaceuticals (Adjei et al., 2017). In the food industry, it could also be applied to ketchups to further enhance the stability and prevent separation of water in the food product.

Effects of enzyme treatment on starch colour

A three-way analysis of variance (with interaction) carried out on $L \times a \times b$ parameters of the starches' colour indicated differences ($P < 0.5$) and strong interaction ($P < 0.01$) between variety and effects of enzyme treatment on starch lightness (L values), red to greenness (a values) and blue to yellowness (b values) (Table 3). The sense (positive or negative) of the ($L \times a \times b$) values was also not affected for all the varieties, showing negative values (redness) for the a and positive values for the L and b (whiteness and blueness respectively) (Table 3).

Significant difference in starch lightness between the

varieties could be due to the differences in the type and amounts of proteins and the associated chromogenic compounds (including mineral ions) present in the starch. Studies by Rojas et al. (2007) on mineral absorption pattern of the cassava root revealed that the roots absorb high amounts of K, P, Zn, Br, Cl, S, B and Rb ions. Other well represented ions were from elements of Ca, Mg, Ba, Sr, Cu, Au and Ni and those under represented were those from Na, Fe, Si, Sn, Mo, Cd and the heavy metals like Pb and Hg. Highly soluble salts of these elements, could remain strongly bound to starch granules (at low concentrations after treatment and processing of the tuber) influencing ash content, solubility and colour. Individual or combined interactions of these elements and pigments with white light could have produced the characteristic yellow or blue tints observed in the starches.

All treated samples, for each variety, were significantly lighter compared with their controls (Table 3). Pectinases have been reported to improve the lightness of many processed plant products including starch (Hebeish et al., 2010). At low pH, mineral sorption to cassava starch tends to decrease due to hydrogen ions strongly competing with (mineral) adsorbates (Shah et al., 2015). Hence enzyme treatment might have reduced the amounts of mineral ions in the starches extruded; however this did not seem to have significantly affected ash content (Table 2).

Differences in starch colour observed are also likely to be influenced by the presence of soluble chromogens in the pulp of the cassava parenchyma (Carvalho et al., 2004). Dominant among such compounds are members of the carotenoid group which are known to impart yellow and red colour to the tissues.

White starches are better patronized than dull coloured ones (Lestari et al., 2012) probably because of its contribution to the aesthetics of the final product. Therefore, use of enzyme technology apart from enhancing starch usability, also enhances starch (as well starch-base products) appeal for patronage by the consumer.

Conclusion

Activities of crude pectinases greatly enhanced the extraction of large starch granules from their respective mashes. However, surface morphology of starch granules from some varieties seem to compromise structural integrity (upon treatment) as these granules were more susceptible to fragmentation by endogenous amylases in the crude enzyme preparation. Presence of endogenous amylase in the crude extract generally decreased amylose content; the extent of decrease was however also dependent on starch granule size and amylose content of starches from the varieties. Other physicochemical parameters of the starches such as pH,

Table 3. Comparison of starch sample colour parameters upon enzyme treatment. (L×a×b values for tile).

		L×a×b values for tile		
		L	a	b
		98.00 ± 0.006	-0.16 ± 0.000	1.69 ± 0.050
		L×a×b values for starches from control and enzyme treated mashes of the various cassava varieties		
Cassava variety		Colour co-ordinates		
		L	a	b
Nkabom	Control	75.29 ± 0.00 ^a	-0.69 ± 0.015 ^a	2.77 ± 0.027 ^a
	Treated	77.95±0.006 ^b	-0.71± 0.012 ^b	2.36 ± 0.006 ^b
Doku duade	Control	76.62 ± 0.006 ^c	-0.94±0.000 ^c	3.03 ±0.010 ^c
	Treated	76.47±0.015 ^d	-0.51± 0.006 ^d	2.71±0.012 ^d
Afisiafi	Control	75.72 ± 0.006 ^e	-0.52 ± 0.015 ^e	2.53 ± 0.015 ^e
	Treated	83.47 ± 0.036 ^f	-0.24 ± 0.015 ^f	2.35 ± 0.067 ^b
Esam bankye	Control	78.38 ± 0.015 ^g	-0.66 ±0.012 ^a	3.23 ± 0.012 ^f
	Treated	85.34± 0.012 ^h	0.02 ± 0.021 ^g	0.02±0.017 ^g
Bankyehemaa	Control	67.89 ± 0.017 ⁱ	-0.08 ± 0.012 ^h	3.18 ± 0.015 ^h
	Treated	78.25± 0.069 ^j	0.04 ± 0.015 ^g	2.71± 0.010 ^d

Values are represented as mean ± standard deviation (n=3); n = number of replicates mashes. Values with same superscript within a column are not significantly (P>0.05) different.

ash, fibre and moisture contents were however not compromised. Enzyme treatment also enhanced starch solubility, swelling power, water binding capacities. The technology also rendered the starches lighter in colour. However, extent of interaction of the crude enzymes with mashes (and resulting starches) from the various cassava varieties varied. Thus, the technology impacted the physicochemical parameters (especially regarding swelling power, solubility and amylose content) of starches from some varieties better than those from other varieties. This could influence choice of variety for application of enzyme technology and also greatly impact the utility of the starches extruded. Varietal differences in some physicochemical parameters, with respect to application of enzyme technology, were therefore noted.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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