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DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

EXTRACTION OF CASSAVA (*Manihot esculenta* Cranz) STARCH WITH THE AID OF PECTOLYTIC ENZYMES FROM SACCHAROMYCES CEREVISIAE (ATCC 52712): EFFECTS OF CASSAVA VARIETAL DIFFERENCES ON THE PROCESS.

BY

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Declaration

I, Japheth Kwame Agyepong, hereby declare that this piece of work (thesis) herein now submitted as dissertation for a Masters Degree in this University is the result of my own findings and that no previous submission of this work for a Masters degree has been made elsewhere except those references which have been duly acknowledged in the text.

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ABSTRACT

This work sought to study and compare the effects of various dosages of pectolytic enzymes from *S. cerevisiae* ATCC 52712 on yield and physicochemical parameters of starch fromfive cassava varieties ('Nkabom', 'Afisiafi', 'Dokuduade', 'Bankyehemaa' and 'Esambankye') and to explore feasibility for scale-up production with the variety that gives the best response to enzyme treatment. Generally, application of pectolytic enzymes 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 variety,enzyme dosage and holding time for enzyme action. An enzyme dosage of 0.02% was found to significantly ($P<0.05$) peak starch yield in 'Esambankye' and 'Nkabom'varieties at 0.5 hrs and 1 hour in the 'Afisiafi' variety; 0.025% enzyme dosage at 0.5 hours holding time was the optimum treatment combination for starch yield in both 'Bankyehemaa' and 'Dokuduade' varieties. Enzyme treatment significantly enhanced the release of larger starch granules thus subsequently enhancing water binding capacity, solubility and swelling power of starches from most varieties; starches from the treated samples were also significantly($P<0.05$) lighter in colour;crude fibre, crude protein and ash contents were however not affected by enzyme treatment.Enzyme treatment also significantly ($P<0.05$) reduced the time and temperature required for starch gelatinization without affecting the starch's viscosities. Time required for attainment of peak viscosity was significantly ($P<0.05$) reduced in starches from the 'Afisiafi' and 'Esambankye' butdid not have any significant effect ($P>0.05$) on peak viscosities of starches from the 'Bankyehemaa'. Apart from starches from the 'Afisiafi', all other starches from enzyme treated mashes were significantly less stable at 95^{O}C. Hot paste viscosity of 'Bankyehemaa' starches were also significantly ($P<0.05$) enhanced by enzyme application. The technology did not also affect the stability of starches from the 'Nkabom' and 'Bankyehemaa' varieties when their starches were held at 50^{O}C. 'Esambankye' and 'Nkabom' recorded highest values for setback viscosity due to enzyme treatment. Given the good responses of 'Bankyehemaa' to enzyme treatment, its adoption for feasibility studies at the industrial scale gave an overall enhanced yield and flow rates of 44.45% and 46.15% respectively over the control. The study therefore showed that although application of pectolytic enzymes to starch extraction enhances yield, the technology is affected by varietal difference. It also enhances many physicochemical properties of starches for application to industry. Given the heavy dependence of most of Ghana's industries on starch, the technology if made available would greatly boost the productivity of these sectors at relatively lower cost.

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'*If I have seen (any) further, it is by standing on the shoulders of giants*'

– Isaac Newton (1642 – 1727).

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CHAPTER ONE

INTRODUCTION

The 'Country Review' of Ghana, Atkinson (2009) reported that as at the 2008 economic year, the agricultural sector contributed about 37% of Ghana's Gross Domestic Product (G.D.P) being only second to the services sector (which is the leading provider of Ghana's G.D.P.with an average value of 39%). Agriculture is also known to provide approximately 60% of Ghana's workforce (www.iss.co.za/af/profiles/Ghana/Economy). However, land economists continue to show that globally, arable land is drastically degrading and its availability is also on the decline due to human activities (Mackenzie and Mackenzie, 1995). Here in Ghana, it is noted that only 20% of arable land is available for cultivation (www.iss.co.za/af/profiles/Ghana/Economy). In order to ensure food security without compromising infrastructural growth, effective land management is crucial. This would require maximization of agricultural output to meet both export and domestic demands thus placing fewer constraints on the demand for land. This can be achieved by maximizing the efficiency of produce processing mechanisms. Such a move could enhance or stabilize the performance of this sector on the global market.

Alternatively, diversifying the use of agricultural produce such that crop varieties that show much better potential for the production of a particular agricultural product is concentrated on more in that regard.

Cassava (*Manihot esculenta* Cranz) is one crop known to contribute immensely to Ghana's agriculture. The crop is almost exclusively grown in the arid and semiarid tropics, where it accounts for approximately 10% of the total caloric value of staple crops (de Figueroa *et al*., 2001). According to a Food and Agricultural Organization (FAO) statistics, world ranking of total cassava root exports pegs Ghana at sixth position with a total production of 7,845,440 tons (FAOSTAT, 2001). In Ghana, it is also the main source of carbohydrates to meet dietary needs and a regular source of income for most rural dwellers. Cassava also contributes substantially (22 percent) to the Agricultural Gross Domestic

Product, AGDP (http://www.fao.org/docrep/009/a0154e/A0154E07.HTM). The consumption of cassava in Ghana is categorized as follows: fresh cassava roots (55%), *gari* (23%), *agbelema* (18%), *kokonte* (2%), chips (1%), Industrial (1%). The fresh cassava roots are processed and consumed locally as other traditional foods like *tuo-zaafi*, *fufu*, *akyekye* etc. (IFAD, 2006). However, there are the inedible and less popular "bitter cassava" which owes its name to the high concentrations of the cyanogenic glycosides, linamarin and lautostralin present in the plant's tissues (http://www.hort.purdue.edu/newcrop/CropFactSheets/cassava.html).

Cyanogenic glycosides, like many other plant secondary metabolites, are stored naturally in the cell walls of tissues as a defensive mechanism against predation (McKee and McKee, 1999). These glycosides are naturally converted to hydrogen cyanide and released upon rupture of the cell walls (Anderson *et al*., 2001). Hydrogen cyanide apart from being a respiratory toxin has also been implicated in many other pathological situations like tropical amploipia (a form of blindness common in West Africa) and tropical antaxic neuropathy (TAN) (Umoh *et al*, 1984). It is estimated that while the sweet cultivars can produce as little as 20 mg of HCN per kg of fresh roots, the bitter ones may produce more than 50 times as much (www.hort.purdue.edu/newcrop/CropFactSheets/cassava.html).These cassava varieties also grow wild and (due to their high toxicity) are avoided (Nassar, 2000).

Though the edible varieties also contain these precursors, their concentrations are relatively low and processing reduces their presence drastically. It is noteworthy that not all the edible varieties of the crop are mealy, mainly due to differences **in fiber and starch composition** (**IFAD, 2006**). These differences in fibre/starch content could be responsible for the significant variations in the cooking time for the various cassava varieties making some varieties more preferred for food than others. Starch from such non-toxic and non-mealy varieties could be more appropriately applied to the production of adhesives, gums, wallpaper, particle board, biofuels, alcoholic products, drugs, packaging, stain removers, concrete

stabilizers and moisture sequesters (**http://www.research4development.info/PDF/Outputs/R7418h.pdf**). Other industrial applications can be found in the paper, textile and wood furniture industries.

Cassava starch has many remarkable characteristics including high paste viscosity, high paste clarity and high freeze-thaw stability, which are advantageous to many industries (Sriroth *et. al*, 2002).The Food and Agriculture Organization (FAO) has reported that global demand for cassava starch could increase at an annual rate of 3.1 percent, while regional growth rates are expected to be for Asia 4.2 percent, Latin America 3.4 percent and Africa 2.3 percent (FAO, 2000). The Agriculture and Consumer Department of the Food and Agriculture of the United Nations (FAO, 2006) has also reported that starch extraction has become a major source of income for small-scale farmers producing cassava in many countries worldwide (including Ghana). It also added that the extraction industry provides permanent jobs for natives of such starch-producing countries. 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 (IFAD, 2006). These reports apparently show the crop's immense potential for an economic boom in the near future. However, apart from problems with diseases, a major problem that besets this crop, especially in terms of starch production, is the lack of efficient processing mechanisms (Adadevoh, 2008).

In 2002, the Ghana government, as part of his Presidential Special Initiative (PSI) to promote starch production for the local industries and exports, established the Ayensu Starch Factory. The factory as at the time was projected to operate at 70% of its installed capacity. However due to insufficiency of raw materials it produced only at 20% since 2006. Apart from the above-mentioned technical constraints, there were also reports that farmers contracted under the project could only supply 13,000 tonnes of raw materials- a quantity that was woefully inadequate to meet factory demands (Adadevoh, 2008). Some agricultural economists have suggested that the lack of the appropriate raw materials could have influenced adoption of varieties that are relatively low starch yielding or that such varieties were mealy

making them more available for food than for starch production (Doku, 2009). Thus, even though there are many improved varieties of cassava with high starch production capability, some of these varieties are mealy (making them also suited for consumption in our diets) and other varieties which due to their non-mealy characteristics make them more suited for starch production. They contend that the focus should therefore be more on the non-mealy ones (http://www.ghanabusinessnews.com /2009/02/20 /the-biotechnology-option-of-the-president%E2%80%99s). Thus it makes economic sense to avoid incorporating mealy crops into starch production at the expense of the non-mealy ones. Cassava varieties such as 'Abasafitaa' (clone -*TMS (4)1425*), 'Afisiafi' (clone-*TMS 30572*), 'Tek bankye' (coded *Isu-White* (*Isu-W*), 'Gblemoduade' (clone *TMS 50395*) and 'Doku duade' are among the locally bred and selected varieties and have been endorsed as high starch yielding varieties by Ghana's Ministry of Food and Agriculture (MOFA) and the Crop Research Institute (RTIP Fact sheet, 2002).

The histology of plant cells reveal that starch granules are tightly bound and locked up in an intricate matrix of fibre consisting mainly of cellulose, hemicelluloses and pectin (**http://sites.bio.indiana.edu/~hangarterlab/courses/b373/lecturenotes/cellwall/cellwall**). These compounds constitute the main carbohydrate components of cell wall and the lamella of plant cells (Cooper, 2000). In order to make the starch granules available, it would require that this framework be broken, a process which is achieved mechanically by maceration. Conventionally, starch production requires that the tubers be macerated mechanically after which the starch granules are extruded. However, thorough maceration alone does not optimize starch yield since the residual fibre (after mechanical extraction) will still contain some amount of the starch granules bound **(**Kordylas, 1990). In addition, most conventional chemical-aided extraction procedures have been reported (Tischer, 1990) to present problems of toxicity to humans and the environment. These limitations are gradually popularizing the use of enzymes for many bioextraction procedures in industries (Ranalli and De Mattia, 1997; Kashyap *et. al.*, 2001; Vierhius *et al*, 2001). One of such enzymes is the pectinases.

Pectinases are a group of enzymes that attack and hydrolyze the pectin component of plant fibre breaking it down into simpler molecules like the galactouronic acids. This enzyme has been applied in many extraction procedures in the fruit and vegetables industries (Girdharilal *et. al*., 1998; Kashyap *et. al*., 2001; Kashyap and Tewari, 2003; Dzogbefia *et al*., 2006). Pectinolytic enzymes participate in the natural maceration process during and after fruit ripening. The enzyme is also secreted and applied naturally by organisms belonging to the bacteria and fungi mainly for nutrition and is, in some cases, implicated in fungal pathogenicity (Fawole and Odunfa, 2008; Kavanagh, 2005). Fungal and bacterial cells of the genus *Aspergillus, Rhizopus, Trichoderma, Hypocrea, Neurospora, Mucor, Penicillium, Chrysosporium, Myceliophthora, Fusarium, Sclerotia* and *Bacillus, Erwinia, Pseudomonas, Anthrobacter* respectively are noted for their secretion of pectinases (Rombouts and Pilnik, 1980; Manpreet *et. al.*, 2005**).** Fungi of the yeasts genera *Kluyveromyces, Saccharomyces, Schizosaccharomyces, Trichosporon, Schwanniomyces, Hansenula, Pichia* and others of this category are also noted for this property (Aehle, 2000). Microorganisms used for industrial enzyme production are Generally Regarded As Safe (GRAS) and can be obtained from recognised depositories such as the American Type Culture Collection (ATCC), Centraalbureau voor Schimmelcultures (CBS) [Central Office for Mildew Cultures] or Deutsche Sammlung fur Mikroorganismen und Zellkulturen GmbH (DSMZ) [German Collection of Micro-Organisms and Cell Cultures] or any other depository(Aehle, 2000; www.wipo.int/pctdb/ja/ia).

Pectinase enzymes have also been widely applied to the extraction of starch from root crop varieties such as yam (Daiuto *et. al*., 2005), sweet potatoes (Rahman and Rakshit, 2003) and cassava (Sriroth *et al*., 2000**;** Dzogbefia *et. al*., 2008a**).**

Some work has been carried out on enzymatic extraction of cassava starch from native cassava varieties in many places worldwide (Dzogbefia *et. al*., 2008 a, b; Sriroth *et al*., 2000). However, in Ghana, the focus has been on the Afisiafi (Dzogbefia *et. al*., 2008 a and b) – a variety which is also mealy. Thus the

objective of this research is to take a broader look at the effectiveness of the enzyme technology on other local cassava varieties for starch production. The yield of starch from the various varieties would be influenced by their differences in fibre content locking the starch granules. Thus it is hypothesized that differences in fibre content (with regards to both its quantity and biochemistry) between the varieties would have a significant effect on the yield of starch as well as the holding time required for effective enzyme action. The specific objectives of this research therefore are:

1. To **compare** the effects of enzyme treatment on the yield and extraction rates of starch from five selected high starch yielding varieties of cassava.
2. To determine the effect of enzyme treatment on the physicochemical and pasting properties of the selected cassava varieties
3. To determine the effectiveness of the enzyme technology for large scale starch extraction using the best performing cassava variety.

CHAPTER TWO

LITERATURE REVIEW

2.1 CASSAVA- Biology and Taxanomy

Cassava, manioc or yucca (*Manihot esculenta* Crantz) is a perennial shrub of the New World which currently is the sixth world food crop for more than 500 million people in tropical and sub-tropical Africa, Asia and Latin America (El-Sharkawy, 2004; http://www.hort.purdue.edu/newcrop/CropFactSheets/cassava). Taxonomically, the crop belongs to the family Euphorbiaceae and the genus *Manihot* which is known to have about 100 species among which the *Manihot esculenta* Crantz is the only commercially cultivated species (Alves, 2002). Cassava is uniformly diploid with a 2n = 36 chromosomal configuration (www.bath.ac.uk/bio-sci/cassava-project/taxonomy).

According to The American Heritage Dictionary of the English Language (2000), the word 'Cassava' was ultimately derived from the Taino word '*casavi*' which literally means *flour from manioc*. The mature plant grows to an average height of about 1– 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. The plant grows to a height of 1 to 3 m and several roots may be found on each plant (Alves, 2002; www.bath.ac.uk/bio-sci/cassava-project/taxonomy).

Cassava ranks very high among crops that convert the greatest amount of solar energy into soluble carbohydrates per unit of area. Among the starchy staples, cassava gives a carbohydrate production which is about 40% higher than rice and 25% more than maize, with the result that cassava is the cheapest source of calories for both human nutrition and animal feeding **(**Nyerhovwo, 2004). The crop is also noted to be particularly suited to conditions of low nutrient availability and able to survive drought (Burrell, 2003). Although cassava leaves are sometimes consumed, the major harvested organ is the

tuber, which is actually a swollen root. The plant is propagated mostly from stem cuttings. A major limitation of cassava production is the rapid post harvest deterioration of its roots which usually prevents their storage in the fresh state for more than a few days (Okezie and Kosikowski, 1982). Although a perennial crop, the storage roots can be harvested from 6 to 24 months after planting, depending on cultivar and the growing conditions (El-Sharkawy, 1993). In the humid lowland tropics, the roots can be harvested after 6–7 months. In regions with prolonged periods of drought or cold, the farmers usually harvest after 18–24 months (Cock, 1984). Moreover, the roots can be left in the ground without harvesting for a long period of time, making it a very useful crop as a security against famine (Cardoso and Souza, 1999).

The cassava plant, like many other shrubs, is botanically divided into three main parts; leaves, stem and roots.

The roots are the main food storage organ of the cassava which results from secondary growth of fibrous roots. This drastically compromises the ability of the roots to absorb water hence its ability to withstand low soil moisture (Alves, 2002). Anatomically, the mature cassava storage roots consist of three main parts:

- the bark (periderm) forms about 3% of the total weight. It is a thin layer made of few cells thick and as growth progresses the outermost portions usually slough off;
- peel (or cortex) comprises of sclerenchyma, cortical parenchyma and phloem. It constitutes approximately 11–20% of root weight (Barrios and Bressani, 1967) and
- the parenchyma is the edible portion of the fresh root and comprises approximately 85% of total weight, consisting of xylem vessels radially distributed in a matrix of starch- containing cells (Wheatley and Chuzel, 1993).

Moorthy and Ramanujam (1986) reported that starch granule of all the six cassava varieties they studied increased in size up to the 6th month from the time of tuber formation and thereafter remained almost

constant. The amylose content and reducing values did not vary very much at different stages of growth, but the swelling volume and swelling power of starch obtained showed large variation particularly after the 10th month. However these latter changes were peculiar only to the varieties under study. Associative binding forces of starch molecules, as reported (Moorthy and Ramanujam, 1986), is largely determined the stability characteristics of starch under varied environmental conditions.

2.1.1. Nutritional and Dietary Attributes of the Cassava Root

Cassava is primarily a source of carbohydrates and contains very little fat or protein. In fact, its protein content is said to be the lowest among the root crops. On the other hand, it is relatively rich in calcium and ascorbic acid (Chijindu and Boateng, 2008). It is a high starch producer with levels between 73.7 and 84.9% of its total storage root dry weight (Baguma, 2004). Increasingly, there is a need for diverse novel starches for both food and non-food applications (Baguma, 2004). A typical composition of the cassava root tissue is moisture (70%), starch (24%), fiber (2%), protein (1%) and other substances including minerals (3%) (Nyerhovwo, 2004). However, the composition of cassava starch as reported by Leonel *et al*. (2009) was: 12.2% moisture, 88.43% starch, 0.15% fiber, 0.14% total sugar, 0.26% lipids, 0.07% of protein and 0.1% ash.

According to Safo –Katanka and Owusu-Nipa (1992) there is a positive correlation between dry matter content and cooking quality of cassava. Many farmers attach much importance to dry matter content of their selected cassava variety for crop production (Safo-Kantanka and Baafi, 2008). Thus, the lower the dry matter content the poorer the cooking quality of the cassava and the lower its likelihood for market consumption as food. Safo-Kantanka and Baafi (2008) also noted that many elite local cassava varieties have a dry matter content ranging from 34.24 - 46. 35%. Hence, such low dry matter containing varieties, because of their low acceptability as food are considered non-mealy.

2.1.2 Safety and Possible Dietary Toxicity of Cassava

Like many species of the plant kingdom, species of the *Manihot* possess an innate ability to protect itself against predation. They do this biochemically by the synthesizing cyanogenic glycosides, linamrin (α-hydrosyisobutyronitrile-β-D-glucose) and lautostralin (α-hydroxy-α-methylbutyronitrile-β -D-glucose), which hydrolyze under certain conditions to release hydrogen cyanide- a process known as cyanogenesis (Rickard, 1985).

The cyanide present in cassava may be considered to be of two types. Bound cyanide present as the cyanogenic glycoside and free cyanide which is a gas above 26^{o}C (under alkaline conditions). It is the amount of the free cyanide that is used to indicate the potential of the cyanogenic glycosides in the root tubers or leaves (Enidiok *et al*, 2008). Cyanide is widely distributed in nature and is a normal constituent of blood, usually in low concentration, <12 mol L^{-1} (Solomonson, 1981). However, cassava processing, if done improperly could increase blood CN- concentrations when consumed leading to goiter, cretinism, paralysis and neurological disorder (Delange and Ahluwalia, 1983; Bradbury *et al*., 1991)

Of the over hundred species available, early literature on cassava described the genus with only two edible species, *M. ultissima* Phol or sweet and *M. aipi* Phol, delineating species which have high and low cyanogenic glucoside concentrations respectively, although soil and climatic conditions determine the amount of these compounds found in the roots (www.bath.ac.uk/bio-sci/cassava-project/taxonomy).

Studies on tuber formation pattern, ash and protein contents of four wild cassava varieties (*M. oligantha* Pax emend. Nassar subsp. nestili; *M. tripartita* Muell.; *M. zehntneri* Ule, and *M. anomala* Pohl) sampled in parts of Brazil showed that bitter cultivars differ from sweet ones, not only in the amount of HCN they contain, but also in the proportions of nutrients (Nassar, 1980). Ceballos *et. al*. (2004) and Cardoso *et. al.* (2005) have both reported that cultivars with roots containing less than 50 mg of HCN per kg are considered sweet. However, many reports have stated that crude protein content ranges from 2.2 mg/kg in sweet to 2.7 mg/kg in bitter cultivars and fiber ranges from 3.1 to 10.3% (Nassar, 2000). In addition,

notably high percentages of protein occur in wild species in comparison to cultivated cassava. Some reports have referred to a protein percentage as high as 5 or 7% in some cassava cultivars (Nassar, 2000). It is also noteworthy that this estimation of protein based on total nitrogen could be influenced by the breakdown products of cyanogenic glycosides. Compared to the parenchyma (pulp), the cassava peels normally have higher concentration of cyanogenic glucosides making the fresh peel unsuitable for animal feed (Ubalua , 2007).

Although boiling destroys the endogenous enzyme linamarase and eliminates hydrocynic acid, prolonged eating of high cynide cassava because of the presence of linamarin B, can cause chronic cynide toxicity when cassava is consumed without sufficient protein (Balagopolan, 2002).

2.1.3 SELECTED CASSAVA VARIETIES GROWN LOCALLY

Cassava was first introduced into Ghana by Portuguese slave ships from Brazil in 1750 (Sarfo-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 such names 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 (Sarfo-Kantanka, 2004). These cassava cultivars can be distinguished by 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 glucosides in the tuber and leaves (IITA, 1990).

Crop selection by farmers is mainly targeted at helping to alleviate many of the problems that confront the cassava crop production; most especially those concerned with disease (such as the African Cassava

Mosiac Disease, ACMD), nutrition and yield. Thus the myriads of problems confronting cassava production in Ghana have influenced the production of varieties that show resistance to particular problematic factors. However, decisions on the selection of a particular crop variety for cultivation could also be market driven or be due to socio-cultural influences (Agwu, 2002; Dorp and Rulkens, 1993).

2.1.4. Morphology, Biochemical Properties and Uses of some cassava varieties in Ghana.

Afisiafi

The clone (*TMS 30572*) was introduced from IITA to Ghana in 1988 under the code *GC/88-07*. The morphological characteristics are:

- Light green petiole
- Brownish grey mature stem
- Light brown outer skin of tuber with cream inner skin
- Can be grown in both major and minor seasons
- Highly tolerant to major pests and diseases
- Suitable for the preparation of *gari*, *agbelima* and *kokonte*
- Not suitable for *fufu* and *ampesi*
- Suitable for industrial uses- starch and flour

Yield

Average yield (12 months maturity)

- Fresh tubers: 27-30 ton/ha
- Dry tubers: 9-10 ton/ha

Cyanogenic potential mg HCN/100g (FWB)

- unpeeled fresh tuber: 13.0 - 25.5

- peeled fresh tuber: 15.3
- *kokonte* (dried unfermented chips): 8.2
- *agbelima* (tubers milled into dough): 2.7
- *gari* (grated, fermented, sieved and fried mash): 1.1 - 5.2

(Source: **RTIP Fact sheet, 2002**)

Some information on the uses and yield of other locally grown cassava varieties are as shown in Table 2.1:

TABLE 2.1: Average yield and uses of some local cassava varieties

CASSAVA VARIETY	AVERAGE YIELD (Tons/ Ha)	USES
Nkabom	28.0 - 32.0	*Konkonte*, *Fufu*, *Ampesi*, *Agbelema*, *Gari*, Starch, flour
Esam' bankye	32.8	Flour
Bankyehemaa	27.1	Flour
Doku duade	28.2	Starch (24.6%)

Source: Adjekum, 2006

On studies of the flour quality produced from local cassava varieties, Apea-Bah *et al.* (2007) reported 'Afisiafi' and 'Tek bankye' to both have peak flour yields (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 which it 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. Other physicochemical changes noted within the studied cassava varieties were the apparent granule size of starches from all cultivars studied did not change significantly. However, apparent amylose content of starches changed, decreasing in the older roots; granule size distribution was also affected by age of the root, gradually changing from normal to bimodal distribution when harvested very late during the trial. The integrity and crystalline structure of starch granules also depended on the environmental conditions which could result in the difference in water uptake of starches, and their consequent swelling power and gelatinization; the pasting temperature of all starches increased during the dry period, and was lowered during the wet period; the peak and final viscosity of starch decreased from early to mid-harvest time when environmental conditions became drier, and increased close to or greater than the original value when conditions became wet again. Breakdown and setback also followed a similar trend to viscosity.

It is reported (Scott *et. al.*, 2000) that Sub-Saharan Africa is expected to experience the most rapid growth in food demand in root and tubers averaging 2.6 percent per year through 2020. This growth will account for nearly 122 million metric tons with most of the increase coming from cassava, 80 million metric tons (66% of the total). Cassava demand is estimated to grow at 2.0% annually for food and 1.6% per year for feed in developing countries, while total cassava production is projected to reach 168 million tons by 2020 based on the current production rate. They however added that the amount can be far surpassed in developing countries with the right policies and incentives. With the increasing establishment of starch-utilizing industries in developing countries, the production of starch will have to increase beyond the projected figures.

2.1.5. Processing of Cassava for Starch Production:

2.1.5.1 Comparison of Traditional and Industrial Methods

There are two main methods of cassava processing for starch. There is the traditional approach (preferred mostly in India and some Latin American countries) and the modern approach also known as the "Alfa Laval type" used mainly for large-scale industrial processes in many parts of the world. However, both methods are similar to that used in many other parts of the world.

In the traditional process, fresh roots are washed and de-barked before crushing in a rotary rasper (Balagopalan *et al*. 1988). Starch is separated from the crushed pulp before passing through a series of reciprocating nylon screens of decreasing mesh size (50250 mesh). The resultant starch milk is settled over a period of 4-8 hours using a shallow settling table or a series of inclined channels laid out in a zigzag pattern. Settled starch is sun dried on large cement drying floors for approximately 8 hours. During this period the moisture content reduces from 45-50% down to 10-12%. To achieve efficient drying, sunny conditions are required with ambient temperatures of >30°C and relative humidity of 20-30%. Dried starch is ground to a fine powder and packaged for sale. A flow chart illustrating this is shown in Figure 2.1:

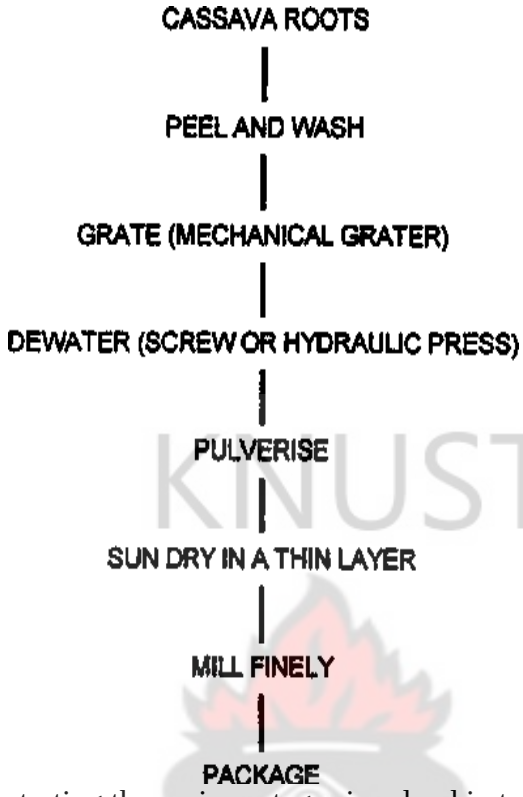

Figure: 2.1: Flow chart illustrating the various stages involved in traditional cassava starch Extraction. Source: Onabalu and Bokanga (1998)

In the modern "Alfa Laval type" process, roots are washed and de-barked, sliced and then crushed in a rotary rasper. Starch pulp is passed through two conical rotary extractors to separate starch granules from fibrous materials, and then fed via a protective safety screen and hydro cyclone unit to a continuous centrifuge for washing, and concentration. The concentrated starch milk is passed through a rotary vacuum filter to reduce water content to 40 - 45% and then flash dried. The flash drying reduces moisture content to 10-12% in a few seconds, so starch granules do not heat up and suffer thermal degradation (http://www.fao.org/docrep/007/y5287e/y5287e07.htm).

Comparison of the Traditional and Modern processes of starch production is as shown in Table 2.2:

TABLE 2.2: Comparison of traditional and modern methods of native cassava starch production

Factor	Traditional factory processing 4000 tonnes roots/annum	Modern factory processing 42 000 tonnes roots/annum
Quantity of roots processed in tonnes	4 000 tonnes per annum	42 000 tonnes per annum
Quantity of dry starch produced in tonnes	1 000 tonnes per annum	10 500 tonnes per annum
Processing capacity (tonnes of roots per hour)	4.5 tonnes roots/hour	6.0 tonnes roots/hour
Total processing time (from fresh roots to dry starch)	2 days	1 hour
Capital cost for basic equipment (1)	US$25 000	US$2.5 million
Pay off period (2)	1-2 years	2-5 years
Minimum operating season for economic return (3)	4-6 months/year	10 months/year
Materials used for construction (4)	Mild steel to reduce costs at expense of slight discoloration of product.	Stainless steel to avoid colour problems.
Water consumption without water conservation	7.5 cubic meters per tonne of roots	5.5 cubic meters per tonne of roots
Water consumption with water conservation	3.0 cubic meters per tonne of roots	1.5 cubic meters per tonne of roots
Power consumption	20 kW per tonne of roots	75 kW per tonne of roots
Fuel oil for flash dryer	Not applicable	18 kg per tonne of roots
Sulphur for sulphur dioxide generator	Not applicable	1.1 kg per tonne of roots
Land requirement	Large space is required for settling tanks and drying yards	Small factory is compact and can be contained in one bldg.
Labour requirement	Semi-skilled labour to operate and maintain factory	Skilled labour to operate and maintain factory
Quality of starch	High quality but not as good as product from modern factory. Starch loses some quality Because of long process time.	Highest quality possible but depends on quality of roots used.

Source: Trim and Curran (1993); Alfa Laval Limited (1992) and Nivoba Engineering (1995)

2.1.6. Properties of Cassava Starch for Non-dietary Applications

Apart from the dietary and nutritional roles played by cassava, the starch produced from cassava also plays very important roles in other industries owing to their useful properties. According to Nyerhovwo (2004), starch is a worldwide multibillion dollar business that finds application in several industries. Cassava starch can perform most of the functions where maize, rice and wheat starch are currently used. Some of such industrial applications include:

2.1.6.1 Paper and Board Industries where starch is used in large quantities at three points during the manufacturing process:

- at the end of the wet treatment, when the basic cellulose fibre is beaten to the desired pulp to increase the strength of the finished paper and to impart body and resistance to scuffing and folding;
- at the size press, when the paper sheet or board has been formed and partially dried, starch (generally oxidized or modified) is usually added to one or both sides of the paper sheet or board to improve the finish, appearance, strength, and printing properties;
- in the coating operation, when a pigment coating is required for paper, starch acts as a coating agent and as an adhesive (http://www.cassavabiz.org/index.asp).

Properties of cassava starch that influence their use in this industry are its ability to form strong film, clear paste, its good water holding properties, and stable viscosity (http://www.cassavabiz.org/index.asp). Shewmaker and Stalker (1992) reported that once starch is extracted from plants and is in solution, its amylose forms hydrogen bonds between molecules, resulting in rigid gels. However, depending on the concentration of amylose, degree of polymerization, and temperature, starch may crystallize and shrink (retrogradation) after heating.

2.1.6.2 Textile Industry: Starch is used in three main ways in this industry:

- Sizing: Approximately 80% of the starch employed in this industry is used to shape or form individual fibres of yarns into warp. This is achieved by passing the fibres into sizing solution that

coats the surface of the twisted warp. The coated yarn is then heated to dry the size and a beam of warp is ready for weaving.

- Textile printing requires a carrier for the dyes and pigments and modified starches have found special uses in these applications. Printing pastes are high viscosity media that preferably will not change on ageing and will resist the effect of added acids or alkalis as required by color agents. A sharp image is required and thus a short non stringy paste. Modified starches are frequently mixed with other industrial gums to give the required viscosity and paste characteristics.
- Finishing is an inclusive term, meaning the transformation of grey goods from the loom to a finished product that is attractive to the consuming public. It includes the process of covering blemishes or defects that may be in the yarn.

These attributes are mainly due to starch's flexibility, ability to resist abrasion; ability to form a bond to fibre and to penetrate the fibre bundle to some extent and to have enough water holding capacity so that the fibre itself does not rob the size of its hydration. Starches that provide high film strength due to high cross-linking between the starch (such as that found in corn and potatoes), high amylose varieties and their derivatives, as well as some cationic starches are used in glass fiber sizing of special textile fiber for electrical insulation and circuit boards (I.I.T.A., 2005).

2.1.6.3 Adhesive Industry: Cassava starch adhesives are noted to be more viscous and smoother to work with. They are fluid, stable glues of neutral pH that can be easily prepared and can be combined with many synthetic resin emulsions. Corn and rice starches take a much longer time to prepare and a higher temperature to reach the same level of conversion. It is slightly stronger than a potato starch adhesive while being odorless and tasteless making it ideal for top quality adhesives; certain potato pastes have bitter tasting properties while cereal starches exhibit a cereal flavor.

Cassava starch also has the added advantage of reducing the amount of sodium hydroxide (gelatinisation modifier) and borax (viscosity enhancer) required to prepare a suitable adhesive for paperboard manufacture, thus further reducing costs (dTp Studies Inc., 2000).

Quality adhesives should have low shear resistance or "slip" permitting the paper to be aligned precisely without losing contact with the substrate, good open time (range of tack), and slow setting speed. And for gummed tapes, middle-of-the-range viscosities are employed. Such adhesives should also have rapid absorption of water by the dried film, high cohesive strength (tackiness) when wet, limited tendency to curl, stable re-moistening properties in the dried film, and low application viscosity. To meet these requirements it is important that the starch should have little or no tendency to retrograde in the dried film. Using starches low in amylose can most readily achieve this property and/or by subjecting the starch to retrogradation inhibiting treatments on base starches such as cassava, potato, or waxy cereal starches (dTp Studies Inc., 2000).

Cassava starch also has several other uses such as its application as an additive in cement to improve the setting time, and it is used to improve the viscosity of drilling muds in oil wells.

Owing to the inability of packaging materials made from petroleum products to be degraded by the environment other biofriendly alternatives have been found in cassava starch. Studies by Carr *et. al.* (2006) showed that by altering the fibre contents of cassava starch, foamy characteristics (high and low density foams) could be produced with cassava starch which has proved to be of comparable quality to the conventional petroleum packing materials. It was however noted that (after processes of swelling, gelatinization and network building by heating thermopressing) the source of the fibre was not significant but quantity proved to be significant in the quality of the foamy material produced.

Ghana's industries also employ starch to a large extent in many of their operations with the major sectors being the textiles and the wood industries. This is as according to estimates from a local

Ghanaian market survey shown in Table 2.3. Below this are some physicochemical properties that enhance utilization of some other local cassava varieties (Table 2.4):

Table 2.3: Utilization of cassava starch on the Ghanaian market

Sector	Market share (%)	Metric tonnes per annum (estimated)
Textiles	40	1 680
Pharmaceuticals	20	840
Paper	10	420
Food	3	126
Plywood (glue extenders) and others	27	1 134
Total	**100**	**4 200**

Source: Graffham *et al*. (1997)

Table 2.4: **Physicochemical Properties of Starch produced from three locally selected Cassava varieties**

Cassava variety	pH	Moisture Content (%)	Solubility (%)	Water Binding Capacity (%)	Amylose Content (%)	Swelling Power (%)	Viscosity Requirement (cp) Brookfield dial viscometer Spindle/rpm
Abasafitaa	6.20	40.26	12.00	133.00	24.10	23.10	44,000 LV 3/6
Afisifi	6.40	44.70	7.00	144.00	21.60	22.80	7,750 LV 3/6
Gblemoduade	7.30	35.84	6.20	126.00	23.40	23.90	9,600 LV 3/6

Source: Darkwa *et. al*. (2003)

2.1.7.0. **Constraints in cassava starch production**

A Repository Document of the Food and Agriculture Organization (http://www.fao.org/docrep/007/ y5287e/y5287e07.htm) reported on the bottlenecks in cassava starch production to include:

2.1.7.1 Cassava supply and seasonality of supply: Starch factories require at least 20 - 40 tonnes of high quality fresh roots a day for a period of at least 100 days in a year to be effective. To meet these demands cassava has to be treated as a high value cash crop and grown on a large-scale. Reliability of supply could be a serious issue in an area where cassava had always been perceived as a low value, food security or marginal crop. To be effective starch factories need a long processing season with continuous supplies of roots of consistent quality. In many cases climatic problems reduce the season to between four to six months. Factories relying on sun drying face the additional difficulty of finding that the peak season for root availability often coincides with periods of wet and cloudy weather, which are unsuitable for sun drying.

2.1.7.2 Root maturity: Starch factories have highly critical requirements in terms of root maturity. However, farmers will be tempted to harvest roots early to maximize use of available land, and may occasionally leave roots in the ground for too long in the hope of getting a better price.

Though cassava has its origin in South America, cassava has become an indigenous crop in the tropics having been widely grown and used. It has played vital roles in the diets of many African countries as a major source of low cost carbohydrates (O'Hair, 1990).

2.1.7.3 Water and power supplies: These two factors are most likely to cause problem during the dry season, and limited supplies of either could disrupt processing.

2.1.7.4 The Crop: The high degree of perishability and high water content (70% of fresh weight) of the root makes handling of the crop quite problematic. The high water content of roots introduces the added expense of transporting a large amount of water from field to factory for no useful purpose. This problem might be overcome by producing dry chips, but starch recovery and quality from dry chips is poor.

Local processing of native cassava starch is often seen as an attractive option, because it offers a means of converting a relatively low cost raw material into a high value product, which can readily substitute for imported starch.

2.1.7.5 Capital and running costs: Production of native starch from cassava on an industrial scale requires a high level of capital investment, followed by high running costs. A traditional factory would require a minimum of US$ 150 000 to establish. A proposal for a modern starch factory would typically budget US$ 8 million-US$ l0 million for initial capital investment and significant amounts of additional funding to cover running costs in the first few years of production. In addition such (modern) factories may require five years to pay off the initial investment, and start providing an economic return. The risk involved in investing in starch production is high because of the number of variables involved.

2.1.7.6 Market size, access and price competitiveness: The micro-economic environment in many developing countries including Ghana, Malawi and Benin significantly limits the growth of the national markets for starch. This makes it difficult to imagine a modern starch factory providing an economic return by basing its supply only to the domestic market. The alternative may be to aim primarily at the export markets.

2.2. STARCH BIOCHEMISTRY

As early as 4000 – 3500 B.C, Egyptians obtained the first starch from wheat. According to a Sumerian tablet (dating as far back as approximately 1750 B.C.), the art of beer production using barley, a starchy grain, was documented (McKee and McKee, 1999). At present, starches are made from many different raw materials such as maize, rice, potato, cassava, barley, sweet potato and wheat (Baguma, 2004).

On the basis of chemical composition, starch can be chemically fractionated into two types of glucan polymer: amylose and amylopectin:

2.2.1 Amylose

Amylose consists of predominantly linear chains of α (l→4) linked glucose residues, each 1000 residues long. Amylose is usually branched at a low level (approximately one branch per 1000 residues) by α (l→6) linkages and makes up 30% of starch. This proportion, however, may vary considerably with the plant species (a range of 11 to 35% was found in a survey of 51 species; (Whistler and BeMiller, 2009) and variety (a range of 20 to 36% was found in a survey of 399 maize varieties; (Whistler *et. al*., 1984)) and also with the plant organ, the developmental age of that organ, and, to some extent, the growth conditions of the plant (Shannon and Garwood, 1984).

The molecular weight of amylose may vary from 150,000 to 600,000. Because the linear amylose molecule forms long, tight helices, its compact shape is ideal for its storage function

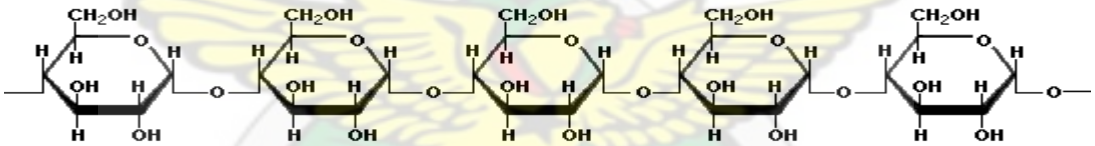

Figure 2.2: Chemical structure of Amylose
(Source: http:/www.scientificpsychic.com/fitness/carbohydrates1.html)

The common iodine test for starch works because molecular iodine inserts itself into these helices. The intense blue colour of a positive test comes from electronic interactions between iodine molecules and the helically arranged glucose residues of amylose (McKee and McKee, 1999).

2.2.2 Amylopectin

Amylopectin, which consists of highly branched glucan chains, makes up 70% of starch. Branching is attributed to the presence of a branching enzyme (similar to the glycogen branching enzyme) found in the chloroplast (Nelson and Cox, 2004). Chains of roughly 20 α (l→4)-linked glucose

residues are joined by α (1⟶6) linkages to other branches. The branches themselves form an organized structure. Some are not substituted on the six positions and are called A chains. These chains are α (1⟶6) linked to inner branches (B chains), which may be branched at one or severa1 points. A single chain per amylopectin molecule has a free reducing end (the C chain). The branches are not randomly arranged but are clustered at 7 to 10-nm intervals. An average amylopectin molecule is 200 to 400 nm long (20 to 40 clusters) and 15 nm wide (Kainuma, 1988; Smith and Martin, 1993).

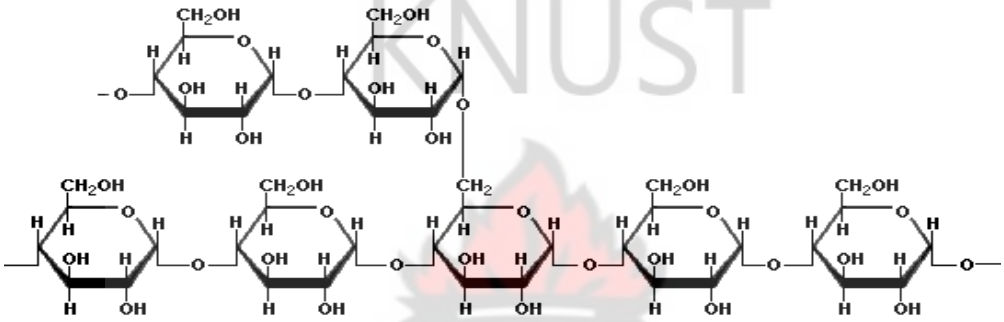

Figure 2.3: Chemical structure of Amylopectin

(Source: www.scientificpsychic.com/fitness/carbohydrates1.html**)**

After extraction, amylopectin has more limited hydrogen bonding than amylose in solution and is more stable, remaining fluid and giving high viscosity and elasticity to pastes and thickeners.

2.3 Pectin Biochemistry as a Plant Cell Wall Material

The cell wall of plants is mainly composed of cellulose, hemicelluloses and pectins. The pectins comprise an average of 30 % of the plant cell wall material (Hong and Tucker, 2000).

Pectin is a group of complex polysaccharides, which contribute firmness and structure to plant tissues as a part of the middle lamella (Daengkanit and Ketsa, 1999) with a molecular weight of 200,000 or more (Uchida and Watebe, 1998). The basic unit of pectic substances is the galacturonan (α- D-galacturonic acid). Thus, they contain a large number of negatively charged galacturonic acid residues. The 6th carbon atom (C6) of the molecule is oxidized to form carboxyl groups, which are sometimes modified by esterification with methyl groups, forming methoxyl groups or by complete neutralization by sodium,

potassium or ammonium salts (Jayani *et al*, 2005). Some of the hydroxyl groups on C2 and C3 may be acetylated (Alkorta *et al*, 1998).

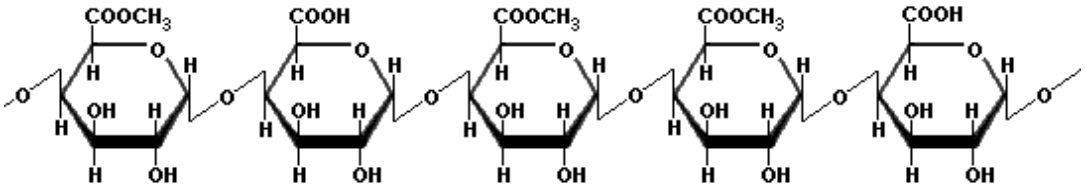

Figure 2.4: Primary structure of the pectin molecule (Source: Alkorta *et. al*. 1998)

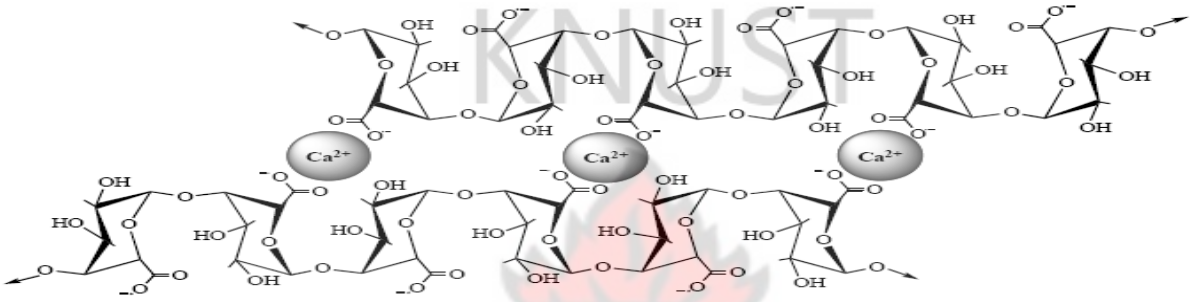

Figure 2.5: Interaction of calcium ions with the carboxylic ends of two homogalacturonan molecules (Source: Pedrolli *et al*., 2009)

Because of the multiple negative charges, pectins bind positively charged ions (such as Ca^{2+}) and trap water to form gels. In the cell wall, the pectins form a gel-like network that is interlocked with the cross linked cellulose microfibrils (Cooper, 2000). Pectic substances are of two parts: homogalacturonan and heterogalacturonan (rhmnogalacturonan I and II) (Gummadi *et al*., 2007; Pedrolli *et al*., 2009). The main polymer chain in the homogalacturonan consists of an α –D-galacturonan units linked by (1- 4) linked α –D-galacuronates and with about 2-4% L-rhamnose units that are β (1-2) and β (1-4) linked to D-galacturonate units (Whitaker, 1991). The side chains of rhamnogalacturonan consist of L-arabinose or D-galacturonic acid units and have a pH of 5 when negatively charged. Other side chains that could be present include arabinan, galactan, arabinogalactan, xylose or fucose which are connected to the main chain through their C 1 and C 2 atoms.

Classification scheme by the American Chemical Society groups the pectic substances as being of four main types:

(I) Protopectin: is the water insoluble pectic substance present in intact tissue. It is a high methyl ester pectin that yields pectin or pectic acids on restricted hydrolysis.

(II) Pectic acid: is the soluble polymer of galacturonans that contains negligible amount of methoxyl groups. Normal or acid salts of pectic acid are called pectates.

(III) Pectinic acids: is the polygalacturonan chain that contains >0 and <75% methylated galacturonate units. Thus it has intermediate methyl ester content. Normal or acid salts of pectinic acid are referred to as pectinates.

(IV) Pectin (Polymethyl galacturonate): is the polymeric material with intermediate methyl ester content; at least, 75% of the carboxyl groups of the galacturonate units are esterified with methanol. It is colloidal and is known to confer rigidity on cell wall when it is bound to cellulose in the cell wall. (Alkorta *et al.* 1998).

Other cell wall materials present are a variety of glycoproteins that are incorporated into the matrix which are thought to provide further structural support.

Madhav and Pushpalatha (2002) have reported that the properties of pectin in cell walls are sometimes modified by low levels of hydroxyl esterification with acetyl groups. The presence of acetyl groups however inhibits gel formation. Distribution of acetyl groups in pectin is unknown but in sugar beet, pear and apricot pectin, acetyl levels are reported to approach four per cent (Ranganna, 1986).

Pectin is present in all plants but the content and composition varies depending on the species, variety, maturity, plant part and growing conditions (Holloway *et al*., 1983).

2.3.1. Industrial Uses and Application of Pectins

Because of its colloidal properties, pectins have been applied as jelling agents in the production of jams and thickening foods. Examples of such foods include yoghurt, cake, ketchups and fruit jelly (Holloway

et al., 1983). Madhav and Pushpalatha (2002) have also reported that pectinases used in pharmaceutical preparation as filler, as an agglutinator in blood therapy and also to glaze candied fruits. In addition, it can be used to increase the foaming power of gases in water.

Pectic substances have also been found to have medicinal benefits. Various effects such as a defecation improving effect (due to its strong activity as dietary fibre), an effect of repressing the level of cholesterol content of blood, and effect of repressing the formation of gallstones, and a hypertensive repression effects have been reported (Uchida and Watebe, 1998). There are also reports that pectins have been used to increase insulin resistance, relieve diarrhea, used as a regulator and protectant of the gastrointestinal tract, as a immune system stimulant and as anticancer and antinephrotic agent (Holloway *et. al*., 1983).

2.4.0. PECTINASES: Biochemistry

Pectinases are a heterogeneous group of enzymes that catalyze the breakdown of pectin- containing substrates (Reda *et al*, 2008). They are one of the most widely distributed enzymes in bacteria, fungi and plants (Jayani *et. al*., 2005). The activity of pectinases in the degradation of pectins is expressed as endo-PG unit (U) and is defined by Gainvors *et al*. (2000) as the quantity of enzyme which caused a reduction in viscosity of 50% in 30 minutes of reaction under standard conditions. Endo-PG activity is expressed as units per gram of dry medium (U/g).

Alkorta *et al*. (1998) classified the pectinases as being of three types: depolymerizing enzymes (pectinases: hydrolases and lyases), de-esterifying enzymes (pectinesterases) and protopectinases. The depolymerases split the ß-(1→4) glycosidic bonds between galacturonic monomers in pectic substances by either hydrolysis (hydrolases) or ß-elimination (lyases). The hydrolases are also divided into two groups: those preferring pectate are called polygalacturonases and those preferentially degrading pectin are called polymethylgalacturonases.

Protopectinases catalyze the solubilization of protopectin. Polygalacturonases hydrolyze the polygalacturonic acid chain by addition of water and are the most abundant among all the pectinolytic enzymes. Lyases catalyze the trans-eliminative cleavage of the galacturonic acid polymer.

Pectin esterases liberate pectins and methanol by de-esterifying the methyl ester linkages of the pectin backbone. Fungal pectin esterases have been reported to be active at pH ranging from 3.0 -5.0 (Pastore, 2001)

Below is a table (Table 2.5) of the nomenclature and biochemical action of the depolymerizing components of the pectic enzymes:

TABLE ON PECTINASE FAMILY OF ENZYMES

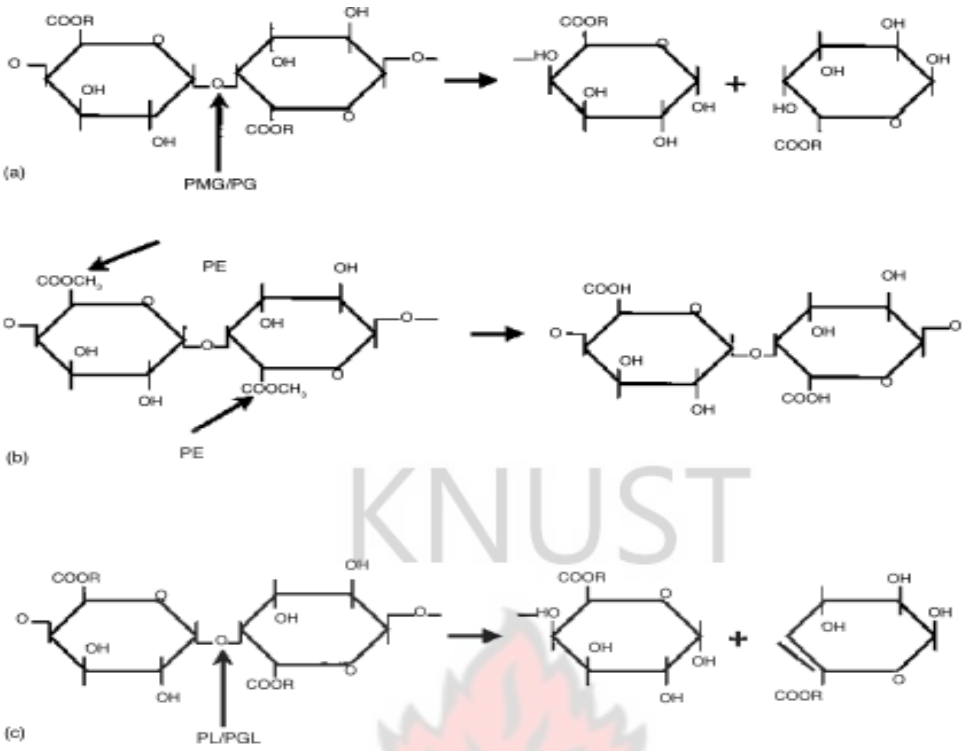

Diagram showing the mode of action of the pectinases

Figure 2.6: Mechanism of action of the pectinases: In the above diagram (a) R=H for PG and CH_3 for PMG (b) PE and (c) R=H for PGL and CH_3 for PL. The arrow indicates the place where the pectinase react with the pectic substrates . Extract from the Journal *Microbial pectinolytic enzymes: A review* by Jayani *et al.* (2005)

2.4.1 Aftermath of Pectinase action on Pectic Substances

The action of pectinase on a solution of pectin results in some physiological changes. These include:

- a rapid lowering of the viscosity of the solution;
- a failure of the solution to form a gel on the addition of ethanol or solutions of calcium salts, only a gelatinous precipitate or a cloudy suspension being formed;
- an increase in the titratable acidity of the solution;
- an increase in the reducing power of the solution, as measured by the iodine-reducing value. (Kaur and Sharma, 2013; Martos *et. al.*, 2013)

These effects are due to the action of the pectin esterase and polygalacturonase (Alkorta *et. al*, 1998). The increase in acidity is due to the demethylation of the pectin by pectin esterase (Martos *et. al.*, 2013). According to Blanco *et al.* (1997), the lowering of viscosity, failure of gelation, and increase in reducing power are due to the degradation of pectin by polygalacturonase which act only very slowly on true pectin but rapidly on demethylated pectin (pectic acid). Changes in viscosity and gelling properties are brought about fairly rapidly even by very weak enzyme preparations, but marked changes in reducing power only appear with active preparations.

2.4.2 Industrial Application of Pectinsases

Pectinases enjoy enormous industrial applications. It is known that of all the enzymes employed worldwide for industrial purposes, the pectinases account for about 10%. (Stutzenberger, 1992). Among the many industrial uses are:

1. **Fruit Juice Extraction and Wine making:** The fruit juice and wine making industries are by far the ones that enjoy the most industrial application of the pectinases. The industry is estimated to employ approximately 25% of global sale of all food enzymes as pectinases **(**Jayani *et. al.*, 2005). It is reported that the use of pectinases in combination with xylanases and cellulase have been found to reduce filtration time by approximately up to 50% and in such fruits as banana, grape and apple, it has been found to increase the fruit juice volume when the fruits' pulps were treated with the pectinases **(**http://pec.biodbs.info/Applications.html; Kaur *et. al*., 2004**).** Their use in combination with other enzymes such as the cellulases, arabinases and xylanases has also helped increase pressing efficiency of the fruits for juice extraction. Citrus fruits have also been softened and easily peeled when their peels were (vacuum) infused with pectinases (Baker and Wicker, 1996). Infusion of a combination of pectin methylesterases with calcium salts in stone peaches has been found to firm up the fruits by a factor of four (Brummel *et al*., 2004) to enhance extensive preservation during export.

According to Novozyme A/S (2008), grapes contain pectinesterases and polygalacturonases which degrade cell wall pectin to enhance fruit ripening and softening. However, these natural enzymes are produced in insufficient amounts and therefore do not impart the significant physiological effects needed for grape juice extraction for wine making. However, application of a cocktail of enzymes comprising pectinases, cellulases and hemicellulases (under the trade name Vinozym®) was found to reduce maceration time by as much as 20% and the extraction of valuable tannins, anthocyanins and aroma compounds were much more optimized with the resulting effect of much better clarification, a pleasant fruity aroma and an enhanced mouth feel. Application of much concentrated pectinases (under the trade name ultrazym*100 and Novoclair ^{TM} FCE) for clarification of grape juice yields juice products of much better quality and processing is quicker.

2.4.2.2 Oil extraction: Citrus oils such as lemon oil can be extracted with pectinases. They destroy the emulsifying properties of pectin, which interferes with the collection of oils from citrus peel extracts.

2.4.2.3 **Improvement of chromaticity and stability of red wines**: Pectinolytic enzymes added to macerated fruits before the addition of wine yeast in the process of producing red wine resulted in improved visual characteristics (colour and turbidity) as compared to the untreated wines. Enzymatically treated red wines presented chromatic characteristics, which are considered better than the control wines. These wines also showed greater stability as compared to the control.

(Sources: http://pec.biodbs.info/Applications.html; Novozyme A/S 2008)

2.4.3. Microbial Enzyme Production and Enzyme recovery in Culture Media

Generally, enzyme production can be achieved in two main ways:

2.4.3.1 Solid State Fermentation (SSF): where the microorganisms are cultured on solid substrate with low water activity. Thus, the SSF culture process is carried out under conditions devoid of any free water; in other words, the quantity of water does not exceed the capacity of saturation of the solid substrate in which the microorganisms grow (Laukevics *et al*, 1984).

2.4.3.2 Submerged Fermentation (SmF): involves culturing microbes in media with relatively very high water activity.

Although the SSF process is known to produce higher enzyme titres than the SmF process (Viniegra-González *et. al.*, 2003), the availability of much literature and the predictability of the SmF process makes the process more reliable for application in the production of enzymes. The abundance of knowledge on regulatory parameters and the ease of manipulation of culture conditions such as humidity, pH, dissolved oxygen and CO_2 concentrations make the SmF process a method of choice for enzyme production (Cristobal *et. al.*, 2008) - as accurate regulation of parameters (for process optimization) is crucial to profit-making in industry.

Nutrients used by microorganisms for growth must contain an organic carbon and nitrogen source for energy and biomass formation; minerals that serve as cofactors for growth are also to be provided as inorganic salts. The cultured organism (or biocatalyst), in an attempt to hydrolyze and assimilate the nutrient sources, make use of enzymes. These enzymes could be secreted into the fermentation media as extracellular enzymes or sequestered into the cell mass itself (intracellular enzymes). Depending on the desired enzyme, the biotechnologist could induce the production of a particular enzyme by varying the carbon source thus inducing the microbe to mobilize enzymes for the degradation of that substrate (carbon source).

Enzymes produced can be recovered by methods such as freeze thawing (for intracellular enzymes), filtration, chromatography and centrifugation. These methods however produce impure or technical enzymes (comprising different proteins some of which could be undesired enzymes) with relatively lower enzyme activity. Purification would require chemical treatment as in the case of the salting out procedure with ammonium sulphate. This is carried out by subjecting the mixture to the target enzyme's characteristic salting out point. Additionally, ion-exchange chromatography and dialysis could also be used for enzyme purification (McKee and McKee**,** 1999). The general production cost of

intracellular enzymes is much higher due to the extra cost of employing techniques for breakage of cell wall.

2.5.1 The ASCOMYCETES YEAST (*Saccharomyces cerevisiae*)

The yeast, *Saccharomyces cerevisiae*, is among an ancient group of eukaryotic Ascomycetes fungi, well known for its application in the baking industry since the Sumeria and ancients Babylonian times (B.C. 6000-2000) (McKee and McKee, 1999). The cells of this microbial group are described as being vegetative with an ellipsoidal shape (Kavanagh, 2005). According to Kordylas (1992) more than 800 strains of this vegetative microorganism have been classified with its life cycle involving mitotically propagating haploid forms of two distinct mating types, and a diploid form that can either grow vegetatively or be induced into a meiotic developmental pathway through manipulation of the nutrient conditions of the growth medium. *S. cerevisiae* has a clearly defined and experimentally manipulable life cycle. Its genome is divided up onto 16 chromosomes ranging in size between 250 kb and >2500 kb. Thus with its 12.8 Mb, the yeast genome is about 200 times smaller than the human genome but less than four times bigger than that of *E.coli.* It has a generation time of 80 min making biomass or biocatalyst production (depending on application) easy.

2.5.2. Yeast nutrition and metabolism

The yeast, like other fungi, have relatively simple nutritional needs and most species would be able to survive quite well in aerobic conditions if supplied with glucose (carbon source); ammonium salts (NH_4^+), urea or amino acids (as nitrogen sources); inorganic ions (such as Mg^+, Ca^{2+}, SO_4^{2+}, K^+) and a few growth factors or micronutirents (such as Ni^+, Na_2MoO_4, Zn^{2+} salts) needed mainly for fungal cell growth and enzyme activity. These macro and micronutrients are supplied at millimolar and micromollar concentrations respectively (Kavanagh, 2005).

Generally, substrates are metabolized using either of two pathways:

2.5.2.i Anabolic Pathway which is an oxidative pathway that uses the cofactor $NADP^+$ to produce new cellular materials and

2.5.2.ii Catabolic Pathway: in which an oxidative process of removing electrons from substrates or intermediates are used to generate energy. For this process, NAD^+ is assimilated as cofactor.

2.5.2.1. Galacturonic acid metabolism in yeast

D-Galactose produced from pectin degradation is taken up by *S. cerevisiae* with the help of the enzyme galactose permease (encoded by *GAL2*) and further catalyzed by enzymes (galactokinase, galactose-1-phosphate uridyltransferase, and uridine diphosphoglucose-4-epimerase encoded by the structural genes *GAL1*, *GAL7*, and *GAL10*) of the Leloir pathway (Moat *et. al.*, 2002).

The enzyme phosphoglucomutase then converts glucose-1-phosphate to glucose-6-phosphate, which then enters the glycolytic pathway. The galactose pathway enzymes are coordinately controlled by a positive factor required for the expression of structural genes and a negative factor that interacts with the inducer (galactose) to modulate the function of the positive factor. Expression of the structural genes is controlled by carbon catabolite repression (Moat *et. al*, 2002).

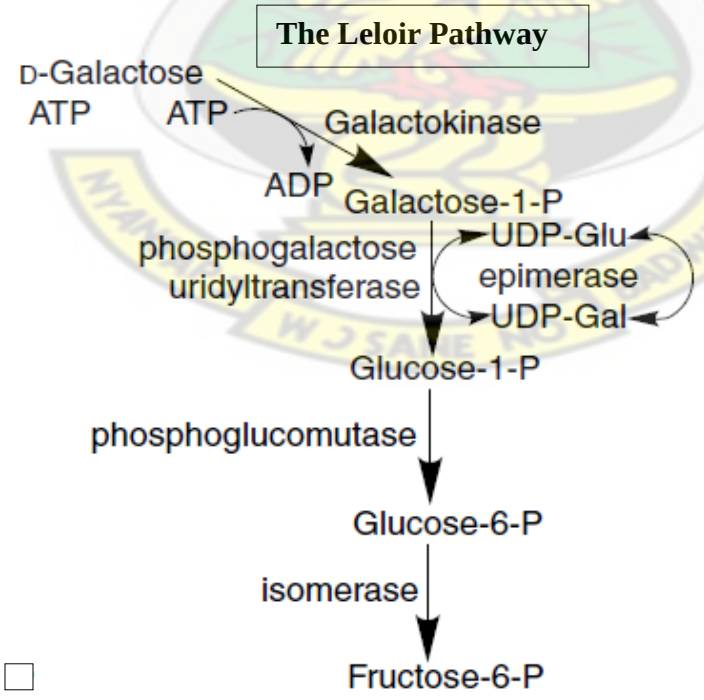

Figure **2.7:** The Leloir Pathway (Source: Moat *et. al.*, 2002)

2.4.4 Yeast for Research and Enzyme Production

The yeast was introduced as an experimental organism in the mid-thirties of the 20th century (Roman, 1981) and has since received increasing attention. It is widely applied for commercial production of enzymes. In fact the the term 'enzyme' was first coined by Kühne in 1878 from the Greek words '*en*' and '*zyme*' literarily meaning "in yeast" to describe the substance within the yeast that was necessary for fermentation (Novozyme A/S, 2008). In more recent times, however, use of the yeast has permeated almost every sector of research and industrial biosciences with its application ranging from the medical and biomedical to food and environmental. A large number of yeast strains carrying auxotrophic markers, drug resistance markers or certain defined mutations are available and maintained at culture collections such at the Yeast Genetic Stock Center (YGSC) and the American Type Culture Collection (ATCC).

Their ease of handling, manipulable nature of their physiology and genetics as well as their GRAS status have endeared the *S. cerevisiae* to the biotechnologist for research and commercial production of many useful products some of which include ethanol, bread making, brewing, single cell protein production, antibiotic and enzyme production (Kavanagh, 2005).

Though the preferred source of carbon for fungal nutrition is glucose, its absence causes catabolite repression when alternative carbon sources are available. This leads to synthesis, mobilization and release of enzymes needed for the degradation of the available carbon source. In the case of plant materials as available substrates, pectinolytic enzymes are among the key enzymes used for such degradation. According to studies by Gainvors and Belarbi (1996), many strains of yeasts are capable of producing one form of pecinolytic enzymes or another, the concentrations of which are naturally low by batch fermentation (Blanco *et al*. 1999)**.** However only one of the local test strains was capable of simultaneously releasing polygalacturanses (PG), pectin lyase (PL) and pectin esterases (PE) and was therefore named *Saccharomyces cerevisiae* Pectinase Plus or SCPP. Their studies also showed that the

production of PG is constitutive and elevated by pectin concentrations, similar to those found in various fruit industries. Molecular studies on this strain showed that the gene *PGL*1 was responsible for encoding *in vivo* active polygalacturonase in both the *S. bayanus* and the *S. cerevisiae* (Gognies *et. al*., 2001). However, current industrial production of pectinases employs most exclusively the *Aspergillus niger* (Gummadi and Panda, 2003).

2.5.4 Enzyme application to Ghana's Industries and its Future

Globally, enzyme production for industries accounts for about 10^9 dollars per annum with the number of patents and research articles related to this field increasing every year (Viniegra-González *et al*., 2003). However, Ghana seems to be left out of this growing industrial scientific trend since very little, if any, documentation on the application of enzymes to indigenous industries is available. Though lots of documented lab-scale research on the feasibility of enzyme application to agro and agro-industrial processing exist (Dzogbefia and Djokoto, 2006; Alemawor *et. al.*, 2010; Tano-Debrah and Ohta, 1996), these have not been adopted and implemented on the industrial scale. Meanwhile, worldwide studies continue to prove enzymes to be much more effective in their rates of biomass conversion and product extraction yields than the conventional chemical extraction methods (Latif *et. al*., 2008; Tröger and Niranjan, 2010). For example, in Ghana, studies conducted by Tano- Debrah and Ohta (1996) on the efficacy of traditional extraction of oils from the *Elias guineensis*, using the roasting and the unroasting kernel method reported that the two processes yielded 20-40% of extractable oils with the roasting method leaving unbleachable dark products which is undesirable. However, using a combination of pectinases with hemicellulase/cellulase and proteases (from the *Aspergillus oryzae*, *A. niger* and the *Trichoderma reesei*) they were able to realize a yield of 62.51 ± 0.4 %. This very significant improvement in yield would not only prove beneficial to commercial production at the small scale level but more especially to large scale industrialist in maximizing the use of biomass. In addition, Dzogbefia *et. al.* (2008a) have reported that treatment dosage of 200g of the 'Afisiafi' cassava variety with 40mg

of technical pectinase enzymes gave much better flow rates of starch milk and a yield of 53% starch without the physicochemical properties of the resulting starch being compromised significantly compared to the control (Dzogbefia *et. al.*, 2008b)

Work by Kordylas (1990) have also shown that during maceration of stem and root tubers for starch production, mechanical means alone still locks up much of the starch grains in the resulting fibre. Thus in the residual fibre much of the useful product (starch grains) is locked up and ends up as agroindustrial waste. A scale up process, involving tons of cassava tubers, could see massive amounts of starch grains ending up as agroindustrial waste leading to gross agricultural and economic losses. There are other reports that application of enzymes for starch production with root tubers such as yam, potato and cassava has produced much better yield than the use of mechanical means alone (Rahman and Rakshit, 2003; Dauito *et al*., 2005; Sriroth *et. al*., 2000).

Statistics globally have shown that the application of enzymes to industries has contributed immensely to industrial productivity and has improved the economy of many countries (Novozyme A/S, 2008). Moreover, these biological agents when applied are far better than their chemical counterparts because of their better precision (due to their specific nature) of converting biomass to the desired product; requirements of milder of operating conditions and the easy availability of the substrates (many of which are from agriculture wastes) (Manpreet *et. al*, 2005). Enzyme application to industries continues to promise an enormous potential of alleviating the myriads of socio-economic problems and environmental hazards plaguing our biosphere. Given the numerous socio-economic and environmental challenges, it is time Ghana finds her place among 'league of nations' applying biotechnology to the latter.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1. MATERIALS

3.1.1. Plant Materials

Fresh local cassava (*Manihot esculenta* Cranz) varieties 'Afisiafi', 'Esam bankye', 'Bankyehemaa', 'Nkabom' and 'Doku duade' harvested at nine months after planting 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 the same environmental conditions.

3.1.2. Chemicals

Malt extract broth, yeast extract broth (both products of DIFCO Laboratories, Michighan, USA) and malt extract agar (product of Park Scientific Limited, Moulton Park, and Northampton) were obtained from the laboratory of the DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY, K.N.U.S.T.

The following chemicals, however, were made available by the DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY. These were all of analytical grade:

Pectin, Bovine Serum Albumin (BSA) and galacturonic acid (SIGMA);

Sodium hydroxide, copper sulphate, sodium carbonate (FISONS);

Hydrochloric acid, concentrated sulphuric acid, sodium acetate, acetic acid, potassium sodium (+) – tartrate, maltose, phenol, 3,5 – dinitrosalcylic acid, carbazole, sodium nitrate, potassium dihydrogen phosphate, magnesium sulphate, ferrous sulphate ammonium sulphate ($FeSO_4.(NH_4)_2SO_4$), sodium chloride, ethanol and starch (BDH).

3.1.3. Microorganism for Enzyme Production

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.

3.1.4. Statistical Tool(s)

For all parameters measured, statistical analysis was done with SigmaPlot for Windows Version 11.0 by Systat Software Inc. © 2008. However, graphs were plotted using Microsoft Excel 2007 (from Microsoft Inc.) and SigmaPlot for Windows Version 11. Experimental determinations were carried out in in three replicates (n=3) (unless otherwise stated).

3.2. METHOD

3.2.1. Media Preparation

Media used to study the growth characteristics and estimation of cell density consisted of nutrient agar and nutrient broth.

3.2.1.a Nutrient Agar Preparation: The agar consisted of 48g of malt extract agar (pH 5.6 ± 0.2 at 25^{O}C) supplemented with 5% yeast extract. These were dissolved together in 1L conical flask of distilled water by heating. After dissolution, 20 ml triplicate portions of the liquid agar were transferred into test tubes. The conical flask and test tubes was then tightly plugged with cotton wool.

3.2.1.b Nutrient Broth Preparation: The broth consisted of 17g of malt extract broth (pH 4.8±0.2 at 25^{O}C) supplemented with 5% yeast extract and were dissolved in 1L of distilled water contained in a 1L conical flask. The nutrient broth was then dispensed as 100 ml triplicates aliquots into 250ml conical flasks. All conical flasks were plugged with cotton wool.

All glasswares containing both broth and agar were wrapped in aluminium foil and sterilized in an autoclave (Portable Autoclave) at 121^{O}C for 15 minutes.

Petri dishes were sterilized in a hot air oven (GENLAB Ltd.) at a temperature of 121^{O}C for 2 hours. After autoclave sterilization of media 10 ml triplicate aliquots of the malt extract agar were dispensed into sterile petri dishes under laminar flow (Bassair Model 52, Bassair Ltd, Southampton). The plates, flasks and tubes were left at room temperature (25-28^{O}C) on the working bench thoroughly sterilized with 70% ethanol and agar left to cool and solidify and left for three days to ascertain the sterility of working environment. Test tubes containing malt extract agar were also slanted and also left to solidify.

3.2.2 Yeast Propagation

After the period, a loopfull of culture (*S. cerevisiae* (ATCC 52712)) maintained at 4^{O}C was obtained and streaked aseptically over the prepared agar slants. Subculturing was done regularly to obtain pure strains which were then kept at 4^{o}C for later use.

3.2.3 Preparation of Experimental Inoculum

An inoculation loop was sterilized over flame and a loopfull of the pure culture was picked and inoculated into each 100 ml nutrient broths. They were immediately plugged with cotton wool and swirled under the laminar flow. The working bench was also sterilized intermittently with 70% ethanol at working intervals. The culture was incubated at 28^{O}C in a Gallenkamp (size two) incubator (Model IH-150, England).

3.2.4 Estimation of Cell Culture Density

Yeast culture density was determined from a standard plot of the log of cell density (Log N in 100ml of medium) against increasing turbidity of culture broth at 540 nm absorbance. Cell counts were done by conventional pour plate method and plate count was done using the Staurt Scientific Colony Counter (United Kingdom). Corresponding turbidity of inoculated culture broth was measured at 540 nm

absorbance at 0, 12, 24, 36, 48 and 60 hours. Four milliliters of freshly inoculated broth served as the blank. A standard graph correlating Log N against absorbance is as shown in Appendix A.1.1

3.2.5 Media Formulation for Enzyme Production

Media for induction of enzyme synthesis by yeast cells comprised (g/l): Pectin (10.0); $NaNO_3$ (2.0); K_2HPO_4 (1.0); $MgSO_4.7H_2O$ (0.5); $FeSO_4$ (0.01), dissolved in 1% NaCl solution. This was referred to as 1% liquid pectin medium.

Pectin, the substrate for pectinase action acts as a carbon source for cell growth. Thus, even though the preferred carbon source (glucose) is absent, the availability of pectins induces the release of pectolytic enzymes into culture media for the hydrolysis of pectin. The subsequent products of pectin hydrolysis are taken up and assimilated by cells. Nitrate salts served as the nitrogen source and NaCl served as an activator of pectolytic enzymes.

After sterilization, the media were allowed to stand for about 2 hours to stabilize and pH measurement adjusted to 4.4. The optimum range of pH for yeast cell growth is between 3.5- 5 (Kavanagh, 2005). The solution was dispensed into 100 ml aliquots into three 250 ml replica conical flasks, plugged with cotton wool and sterilized by autoclaving at $121^{O}C$ for 15 minutes and allowed to cool to room temperature under the aseptic laminar flow work bench. The replica flasks were labeled A, B and C respectively.

At an absorbance measurement of 1.854 (corresponding to a cell density of 6.32×10^{12} per 100 ml), 4 ml of the yeast culture in malt extract broth was aseptically inoculated into each experimental culture medium for enzyme production. The culture replicates were incubated at room temperature ($25\text{-}28^{O}C$) for 8 days without shaking. The culture were monitored during this period to determine the best time for harvesting when maximum cell number, total protein content and enzyme activity would be obtained.

3.2.6 Determination of Culture Density in Pectin Medium

Absorbance reading of freshly inoculated liquid pectin medium was determined during the 8 day period. From the results of the standard graph (Appendix A.1.1), the corresponding cell density was determined for each day.

3.2.7 Preparation of Crude Enzyme Extracts

The crude enzyme extracts were obtained in the supernatant by centrifugation at 3600 g for 10 min in a Centrikon T-42K centrifuge (Kontron Instruments, ALC International Srl, Italy) at 4^{O}C. Total protein concentration and pectolytic enzyme activity in the supernatant were measured for each of the 8 day period and a corresponding graph (Fig. 4.1) plotted.

3.2.8. Determination of Total Protein Concentration in 1% Pectin Culture Medium

Unknown concentrations of protein samples could be measured from standard calibration curves obtained from known concentrations of a pure protein sample and its corresponding absorbance readings at a specific wavelength.

Concentration of total protein produced by *S. cerevisiae* (ATCC 52712) in the 1% Pectin solution was determined using the Biuret Method (Fine, 1935):

This method employs the principle that peptides containing three or more amino acid residues will form a coloured chelate complex with cupric ions in an alkaline environment containing sodium potassium tartrate. A similar colored chelate complex forms with the organic compound biuret (NH_2–CO–NH–CO–NH_2) and the cupric ion. However, single amino acids or dipeptides do not give the biuret reaction, but tripeptides and larger polypeptides or proteins will react to produce the light-blue to violet complex that absorbs light at 540 nm. One cupric ion forms the colored coordinate complex with 4 to 6 nearby peptides bonds. The intensity of the color produced is proportional to the number of peptide bonds participating in the reaction.

3.2.8.1 Biuret Reagent Preparation

Biuret reagent was prepared according to that described by the **Biochemistry and Food Science Lab Manual of the Kwame Nkrumah University of Science and Technology.**

1.5 grams of $CuSO_4.5H_2O$ and 6 g of sodium potassium tartrate ($NaKC_4O_6.4H_2O$) were placed in a 1 litre volumetric flask and 500 ml of glass distilled water added to the flask and stirred with a glass rod. While stirring the contents of the flask vigorously, 300 ml of 10% (w/v) NaOH was added. The stirring rod was removed and the contents topped up with distilled water to 1 liter. The contents were then transferred to a plastic bottle.

3.2.8.2 Protein Standard Preparation

A standard protein solution (stock solution) of 50 mg bovine serum albumin in 50 ml of distilled water was prepared and working standards obtained by diluting 10 ml of the stock solution to 50 ml with distilled water. Various concentrations of 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml were prepared by dilution with distilled water and 1ml of each protein concentration measured into triplicate test tubes. 4 ml of Biuret reagent was added to each test tube and the contents mixed thoroughly by swirling. The samples were then incubated at 37 ^{O}C for 20 minutes after which absorbance reading for each sample was taken at 540 nm. By linear regression analysis plot, an equation was derived (Appendix A.1.2) and was used to determine protein content of samples.

3.2.9. Assay for Pectolytic Enzyme Activity

Pectolytic enzyme activity was determined based on the percentage reduction in pectin content of liquid 1% Pectin medium. The percentage reduction in pectin content was determined by measuring the amount of pectin degraded in 30 min by 0.18 mg (1.8 % w/w of crude protein) of the enzyme protein in 1ml liquid pectin (1%) medium (Ameko, 1998). One milliliter (1 mL) portions of the sterile pectin medium were put into test tubes and 0.18 mg (corresponding to 157.2µL) of the supernatant added to

each tube. The content of the test tubes were shaken immediately to mix well and the tubes incubated at room temperature for 30 min. At the end of the period, the test tubes were immersed in boiling water bath for 5 minutes and then immediately cooled in cold water bath at about $4^{O}C$ to stop enzyme action. The pectin content of the medium was then determined. A control experiment was run concurrently using uninoculated liquid pectin (1%) medium.

3.2.10. Estimation of Pectin Content

Pectin content of medium was determined by a slight modification of the method described by Ranganna (1977) using galacturonic acid as standard. To 120.5 mg of galacturonic acid monohydrate in 1 litre volumetric flask was added 10 ml of 0.05 N NaOH and diluted to the mark with distilled water. After mixing, it was allowed to stand overnight and different concentrations of the standard prepared. To the samples (pectin solution) was added 0.5 ml of 0.05 N NaOH and allowed to stand for 30 min to deesterify. To 1 ml of this solution was added 0.5 ml of 0.1% carbazole reagent. Twelve milliliters of concentrated H_2SO_4 was added with constant stirring and the tubes closed with rubber stoppers and allowed to stand for 10 min to develop colour. A tube with 0.5 ml purified ethyl alcohol in place of carbazole reagent served as a blank. Exactly 15 minutes after the addition of the acid, the absorbance of the standards and the samples was read at 525 nm against the blank and a standard graph plotted. From the graph, the pectin content was estimated.

The pectin content of fresh cassava pulp and that after starch has been extracted was similarly determined. The percentage reduction in pectin content was calculated using the formula in Appendix B.1.

3.2.11. Preparation of cassava mash for starch extraction

Freshly harvested cassava varieties (‘Afisiafi’, ‘Doku duade’, ‘Esam bankye’, ‘Nkabom’ and ‘Bankye hemaa’ all harvested at 9 months after planting, MAP) were each sorted, washed under running tap water and knife peeled. The peeled cassava was washed in distilled water blender and cut into 2-3 cm^3

chunks and 100 g of each (in triplicate) was blended using a warring blender (model 32 BL80 (8011), USA) at low speed for 1 minute. The 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.

3.2.12. Determination of Enzyme dosage and holding time for peak activity

To ensure that results obtained (especially for flow rates) were not biased towards tissues that underwent better maceration, equal amounts of fresh cassava sample (at aged 12 MAP) from the same planting site were prefrozen before blending in a Warring blender. The chilling treatment was necessary to ensure, as much as possible, a reduction in starch gelatinization during blending and to improve maceration as chunks with fluid consistencies have been reported (Meuser *et al*., 1987) to respond poorly to maceration.

To serve as a baseline, a modification of parameters adopted by Dzogbefia *et al*. (2008a) for enzyme-aided starch extraction was adopted. Thus 20 mg (corresponding to 22.84 ml) of crude protein extract was added to 100 g of cassava mash of each of the varieties giving an enzyme dosage of 0.02% ie 10mg/100g. However for optimization, treatment dosage and holding time combinations were carried out on the mashes (then aged 12 MAP) with 0, 5, 10, 15, 20 and 25 mg total protein on 100 g mash at 0.5, 1.0 and 1.5 hours holding times.

The highly perishable nature of freshly harvested cassava roots also necessitated this step to help ensure that enough cassava mash was preserved for the determination of the minimum enzyme dosage and holding times that would maximize starch extraction from the various varieties. Starch samples obtained at the best performing dosages and holding times were then used to carry out other physicochemical tests.

Starch extraction was based on the procedure as described by (Kordylas, 1990). The starch in the cassava mash of each variety with or without enzyme treatment was washed out and filtered through a

clean cheese cloth. The residue was further mixed with distilled water, hand-stirred and strained through the cheese cloth to wash out the remaining starch. This was repeated till a clear solution was expressed (using 100 ml distilled water) that contained little or no starch. The filtrate containing the starch was allowed to stand for 3 hours and the supernatant decanted. The moist starch sediment was further cleaned by resuspending in 100 ml distilled water, allowed to stand for further 3 hours and decanted as before. The wet starch cake that settled was broken and transferred to clean, dried and preweighed shallow aluminium trays for drying in a solar tent drier for 48 hours and weighed. During the extraction process, the rate of extraction and yield of starch were also determined. Flow chart for the processing of cassava for starch production is as shown in **Figure 3.1**. Each determination was carried out in duplicates.

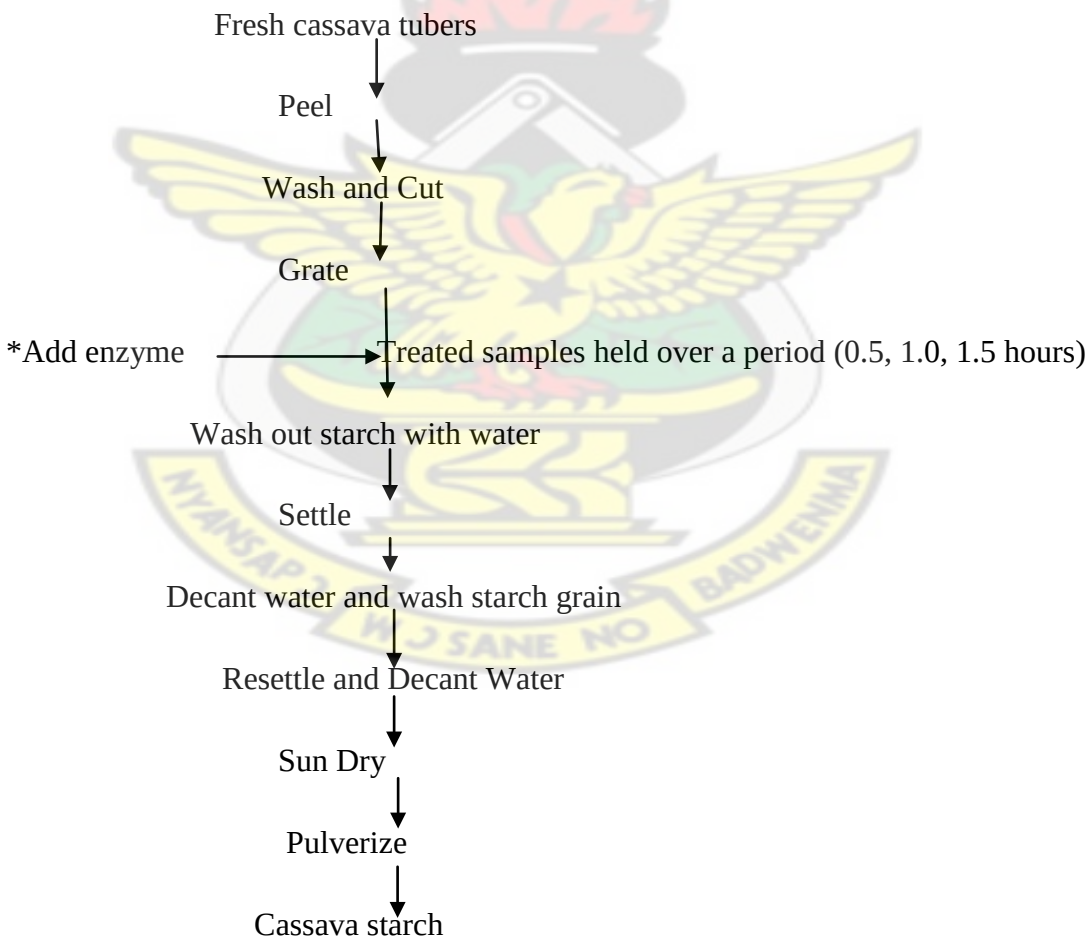

Figure 3.1: Flow Chart showing the stages of enzyme-assisted cassava root processing for starch production

3.2.13. Determination of Rates of Starch Extraction

At the end of the 30 minutes holding time, a mash for one of the varieties was transferred into a funnel (Pyrex Brand 100) lined with clean cheese cloth and the starch milk collected in a graduated cylinder placed beneath. The volume of the starch milk collected was measured at 15 seconds intervals for 3 minutes, and a graph of volume of starch against time plotted. The rate of starch extraction and percentage increase in the rate of extraction of starch for the treated samples compared to the control was also determined. This was repeated in turn for the triplicate of each variety and calculations (Appendix B.2) made.

3.2.14. Determination of Yield of Extracted Starch

The wet starch cake in the flasks was broken and transferred to a clean, dried and pre-weighed shallow aluminium tray for solar drying. This was done for each variety. At the end of the drying period, the starch samples in the trays were weighed and the yield of both enzyme-treated and untreated samples were calculated. The percentage increase in yield due to enzyme treatment was obtained by calculation (Appendix B.4).

3.2.15 Physico-chemical Analyses of Starch Samples

Triplicate samples of the extracted cassava starch (both control and enzyme treated) per variety were used for the various determinations unless otherwise stated:

3.2.15.1 Microscopic Observation of starch granules

Starch from controls and treated samples of all varieties were mounted in distilled water and observed under the binocular compound microscope equipped with camera (Carl Zeiss, West Germany).

Observation was done with high power (x 400 magnification) and by means of the following accessories for length measurement: measuring eyepiece with focusing eyelens, micrometer disk (eyepiece micrometer) and stage micrometer. Focusing and calibration were done for measurement of starch granular diameter.

3.2.15.2 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 ^{O}C for 5 hours. At the end of the period, the crucibles were removed and placed in a dessicator to cool, and their weights recorded till a constant weight was obtained after which their moisture contents were calculated by difference in weight (AOAC, 1990).

3.2.15.3 Determination of starch pH

The extracted cassava starch (10 grams) 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.

3.2.15.4 Determination of Ash Content of starch

The ash content of the starch samples was determined by the Dry Ashing Method (AOAC, 1990). Two grams (2 g) of samples were 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 ^{O}C and heated for 2 hours. The ash content in each was calculated and expressed as a percentage (Appendix B.7).

3.2.15.5 Determination of Crude Fibre Content of starch

The crude fibre content of samples was determined by the AOAC (1990) method. To 2 g of the starch samples in 750 ml Erlenmeyer flask was added 0.5 g of asbestos. To the content was added 200 ml of boiling 1.25 % H_2SO4 and the flask immediately set on a hot plate and connected to a condenser and refluxed for 30 minutes. The sample was then filtered through cheese cloth in a funnel and washed thoroughly with boiling water until it was no longer acidic (litmus test). The charge and ash were washed back into the flask with 200 ml boiling 1.25 % NaOH and the flask connected to the condenser, set on the hot plate and refluxed again for 30 minutes. It was then filtered and washed with boiling water

as before until it was no longer alkaline. This was followed by washing with 15 ml of 95 % ethanol and transferred into porcelain crucible and dried at 100 ^{O}C for 1 hour in an oven.

After drying, the crucibles and contents were cooled in a dessicator and weighed. The crucible with contents was then ignited in a muffle furnace (preheated to 600 ^{O}C) for 30 minutes, cooled in a dessicator and weighed. By calculation (Appendix B.8) the loss in weight was reported as crude fibre.

3.2.15.6 Determination of 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. Two grams (2 g) samples were placed into a Kjeldahl digestion flask. A tablet of selenium was added as a catalyst and 2 ml of concentrated H_2SO_4 added and shaken in a fume chamber. The flask with its content was placed on a digestion burner and heated until digestion was completed (i.e. a clear solution obtained). It was then cooled to room temperature and the content (digest) transferred into a 100 ml volumetric flask and diluted to mark with distilled water. The distillation apparatus was flushed out with steam from boiling distilled water and 25 ml of 2 % boric acid and 2 drops of mixed indicator (methyl red and methylene blue in ethanol) added in a conical flask placed under the condenser with the tip of the condenser completely immersed. Ten milliliters (10 ml) of the digested sample was put in the decomposition chamber and 15 ml of 40 % NaOH solution added and allowed to distil. A colour change of the content in the conical flask from pink to green indicated the presence of trapped ammonia. Distillation was stopped after 5 minutes. The solution in the conical flask was immediately titrated against 0.1 N HCl solution and the endpoint recorded (colour change at the end point was from colourless to pink). A blank was similarly done except that it did not contain the sample. The percentage total nitrogen (treated and untreated/ control) was calculated using the formula in Appendix B.9. Crude protein content was then calculated at 6.25 times this value.

3.2.15.7. Determination of Amylose content

The amylose content of the starch samples was determined based on the iodine colorimetric method described by McCready and Hassid (1943).

3.2.15.7.1 Preparation of Standard Curve for Iodine Colorimetric Method and Calculation of Percentage Amylose

Different concentrations of pure amylose were prepared as follows: 10, 30, 50, 70 and 90 mg of pure amylose were weighed into separate 100 ml volumetric flask and wetted with 1 ml ethanol and 10 ml distilled water. The content of each flask was dissolved by adding 2 ml of 10 % NaOH, cooled and diluted to mark. Five milliliters (5 ml) of each solution was introduced into separate 500 ml volumetric flasks; 100 ml of distilled water was added and slightly acidified with 3 drops of 6 M HCl. The contents were mixed by shaking the flask and 5 ml iodine solution was then added and diluted to the mark in each flask. The absorbance of each standard was read using a spectrophotometer at 640 nm. A blank was set by diluting 5 ml iodine solution to 500 ml with distilled water in place of the standard sample. Absorbance was plotted against percentage amylose. By linear regression analysis an equation was derived and used to determine the percentage amylose in the extracted starch samples (Appendix A.1.4).

3.2.15.7.2 Preparation of samples for Amylose Content determination

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 on 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. The absorbance of each starch sample

was read against the blank in the spectrophotometer set at 640 nm. The percentage amylose of sample was determined using the equation derived from the standard curve.

3.2.15.8 Determination of Water Binding Capacity

The 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 hour on a Griffin shaker and centrifuged at 2200 rpm for 10 min and weighed. The water binding capacity (WBC) was then calculated (Appendix B.11).

3.2.15.9 Determination of Solubility and Swelling Power

The 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 ^{O}C in a thermostatically regulated temperature bath for 30 min with constant stirring. The tube containing the sample was removed, wiped dry on the outside and cooled to room temperature. 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. The percent solubility and swelling power were then determined by calculation (Appendix B.13)

3.2.16. Determination of Pasting Characteristics

The pasting characteristics of starch samples were determined based on the modification of the Shuey and Tipples (1980) method, with a Brabender visco-amylograph (Brabender OHG Duisburg, Kulturstrabe 51 – 55 , D-4100 Duisburg 1) and 700 cmg cartridge. Cassava starch slurry was made by dissolving 30g of starch (moisture free) in 500 ml of distilled water. The slurry was heated at a rate of 1.5 ^{O}C/ min by means of a thermoregulator. When the suspension temperature reach 95 ^{O}C, it was held

constant for 15 min (first holding period), while being stirred continuously. The paste was then cooled to 50 ^{O}C at a rate of 1.5 ^{O}C/min and held at this temperature for another 15 min (second holding period). The following were read from the viscograph amylograph obtained: pasting temperature, peak temperature, peak viscosity, viscosity at 95 ^{O}C, viscosity after 15 min at 95 ^{O}C, viscosity at 50 ^{O}C and viscosity after 15 min at 50 ^{O}C. From these measurements (Appendix A.2.1-5), the paste stability at 95 ^{O}C, paste stability at 50 ^{O}C and retrogradation tendency of starches from both enzyme-treated and untreated cassava mashes were determined (Appendix B.17,18 and Appendix C. 14.10,13,14 respectively).

3.2.17. Determination of Colour

The colour of samples was examined with the naked eyes and was also determined using a colour meter (Minota, chromameter CR-2001). Using the L*a*b* colour system, the chromameter was calibrated with a white tile (**Table 4.10**).

3.2.18 Assay for Amylase Activity

The amylase activity in the crude enzyme preparation was assayed based on a modification of the method described by Bernfeld (1955). A 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 ^{O}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 (10-100mg) of maltose (Appendix A.1.5).

3.2.19 SCALE UP SIMULATION OF CASSAVA STARCH EXTRACTION USING BEST PERFORMING VARIETY

From results obtained from the preceding lab stage studies, the 'Bankyehemaa' cassava variety showed a much better potential for cassava processing with enzyme treatment than all the other varieties and was therefore selected for the scale up starch processing.

A scale up production of pectolytic enzymes was carried out at the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology.

However, traditional scale up starch processing was carried out at the Agric Research Station (at Anwomaso-Domeabra, Kumasi).

Freshly harvested cassava tubers were sorted, knife peeled and hand washed. Fifteen (15kg) of peeled cassava was milled into a fine mash to make it possible to separate the starch granules from the root pulp. The mash was treated as before with 5000 mg of enzyme protein contained in 6584.3 cm^3crude enzyme preparation and held for 30 minutes in plastic containers (as described by Dzogbefia *et al*., 2008). At the end of the holding time, the mash was put into a bucket covered with a piece of clean cloth over a bucket. The starch in the mash was washed out with 30,000 cm^3 water and filtered through the cloth into the plastic bucket. The filtrate was allowed to stand for 8 hours for the starch in it to settle and the supernatant decanted. The wet starch obtained was sun dried for 6 days. During the same period, starch was similarly extracted from cassava mash and dried without enzyme treatment and dried to serve as the control.

The extraction rate for both enzyme-treated and control samples was determined using transparent graduated tall plastic containers. Thus flow rate was record as volume collected at 30 seconds intervals for 6 minutes.

Starch yield from both enzyme-treated and the control mash were determined and percentage increase in the yield obtained by calculation (Appendix B.5). The scale up processing of cassava starch was performed in duplicate.

3.3 STATISTICAL ANALYSES

Using the Completely Randomized Design (CRD), all data (Appendix C) were subjected to ANOVA and significant differences were tested using the Duncan's New Multiple Range Test.

All results (Appendix C) were presented as a mean ± standard values of three replicates (unless otherwise stated) and a one-way analysis of variance (unless otherwise stated) and the Least Significant Difference (LSD) were also carried out for all parameters that were measured.Significant differences between treatment means was accepted at $P<0.05$ (unless otherwise stated).

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CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. DAILY PROTEIN PRODUCTION AND PECTINASE ACTIVITY IN 1% PECTIN MEDIUM

At a peak cell density of 1.092 x 10^{10} cells/100 ml measured on the 6th day of incubation, total protein concentration measured in the 1% pectin medium was 1.142mg/ml. Daily measurements of pectinase activity in the crude medium was also found to be similar to the pattern obtained for biomass and crude protein production having its least activity on day 1 and peaking on day 6 (Figure 4.1).

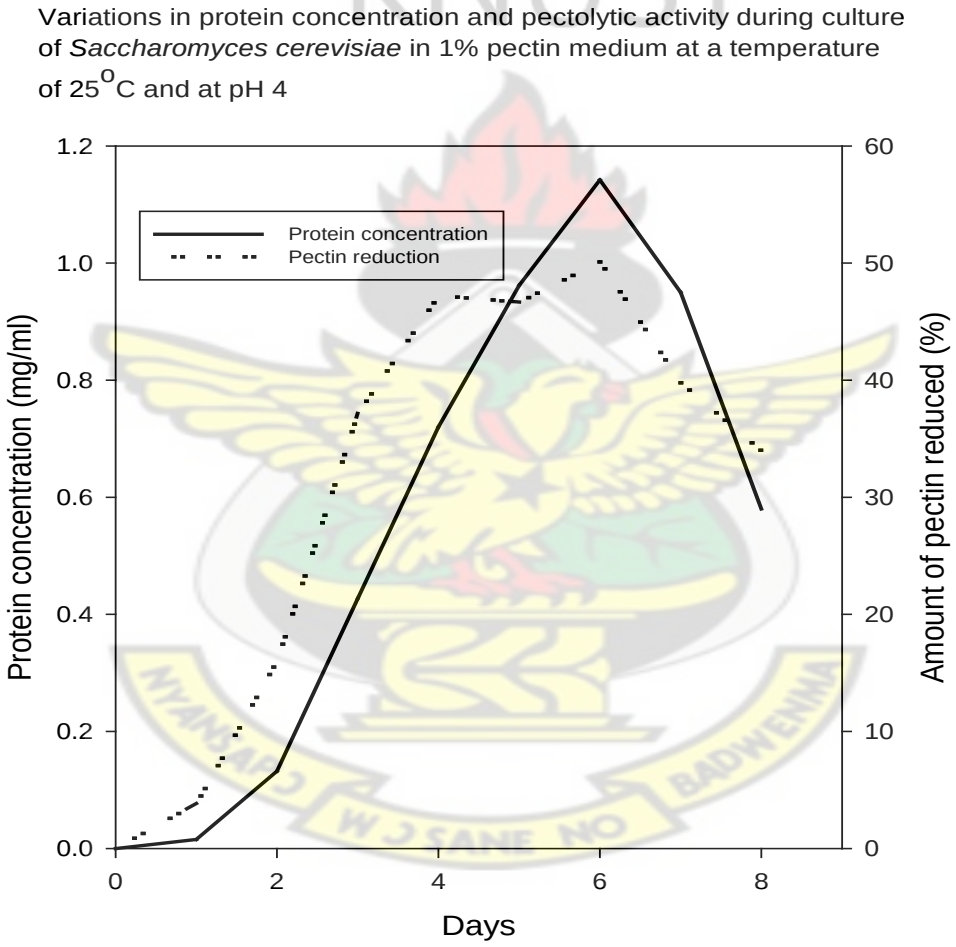


Figure 4.1: Daily variation of protein concentrations and pectolytic pectolytic activity in 1% pectin culture medium.

Enzymes produced on the 6thday were therefore used for extraction procedures. During culturing in the 1% pectin medium, *S. cerevisiae* produced extracellular enzymes (which also includes pectolytic

enzymes) to help hydrolyze pectins for growth (Moat *et. al.*, 2002) hence the observed increase in the amount of protein and enzyme activity in over the period.

Enzyme activity obtained after 30 minutes reaction time on the 1% pectin medium was 4.91µmol/ ml/ min with a specific activity (calculated per minute) of 4.291units/mg protein. Using pure pectin as substrate, Bali (2003) reported a maximum (fungal) pectinase activity of 210.37 nmoles/ml/60min. Polygalacturonase (PGase) activity was calculated as defined by Jayani *et al*. (2005). They defined one unit 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.

Pectic substances are high molecular weight, negatively charged, acidic, complex polysaccharides that form major components of middle lamella of plant cells in the form of calcium pectate and magnesium pectate (Vishal, 1998). Pectic substances account for 0.5–4.0% of the fresh weight of plant material (Kashyap *et. al.*, 2001; Sakai *et al.*, 1993)

4.2. MOISTURE CONTENT OF FRESH CASSAVA ROOT PULP

Results on moisture content of the freshly harvested root pulp of the cassava varieties (Table 4.1) was similar to many literature reports (Nyerhovwo, 2004; Safo-Kantanka and Baafi, 2008).

Table 4.1: Percentage moisture content of freshly harvested cassava varieties at 9 MAP

Cassava Variety	Root Moisture Content (%)
Esam bankye	55.25 ± 0.59
Doku duade	57.39 ± 1.36
Afisiafi	65.86 ± 0.86
Nkabom	61.19 ± 1.82
Bankyehemaa	58.49 ± 0.69

Values are represented as mean ± standard deviation (n=2); n= number of determinations

There are reports that have linked effective maceration to moisture contents of tissues (Meuser *et. al*.,1987; Tombs and Mashingaidze, 1997). For instance Meuser *et. al*. (1987) have reported that a more watery pulp increases hydraulic resistance of the working parts of disintegrators and weakens the impacting effect, thereby limiting starch recovery to about 80%. Maceration is a very important step in enzyme-based extraction procedures as the process influences the amount of surface area available for enzyme action and ultimately the yield of product. Padmanabhan *et al*. (1992) have reported that enzymatic action on macerating chips and disintegrating root cells is dependent on size of chips, presence of peel, temperature, time, agitation and type as well as concentration of enzymes. Thus, yield is optimized by enzymes when the substrate (macerated cassava root pulp) surface area is large enough. It therefore became imperative that differences in moisture content between the various cassava root varieties be noted.

From the above reports (Meuser *et. al*.,1987; Tombs and Mashingaidze, 1997), it is clear that root moisture content has cost implications for industry as the industry would need to expend more energy in macerating root pulp with high moisture content compared with those that have high dry matter content. The adoption of low moisture varieties would therefore most likely enhance profit making in the starch industry.

These results could also very well explain why many traditional production procedures eliminate exogenous water during the initial steps of root tissue maceration.

4.3.0. EFFECTS OF PECTOLYTIC ENZYME TREATMENT ON SOME RHEOLOGICAL PROPERTIES OF ROOT MASHES FROM THE CASSAVA VARIETIES

4.3.1 Effects of enzyme treatment on flow pattern and starch milk recovery

An effect of pectolytic enzyme application is a reduction in viscosity and an enhancement in the flow of milk from root tubers (Rahman and Rakshit, 2003; Dzogbefia *et al.*, 2006; Blanco *et al.* 1997). The flow

pattern of starch milk for the entire 3 minutes and volumes recovered after the period are shown in Table 4.2 and Figures 4.2 – 4.5:

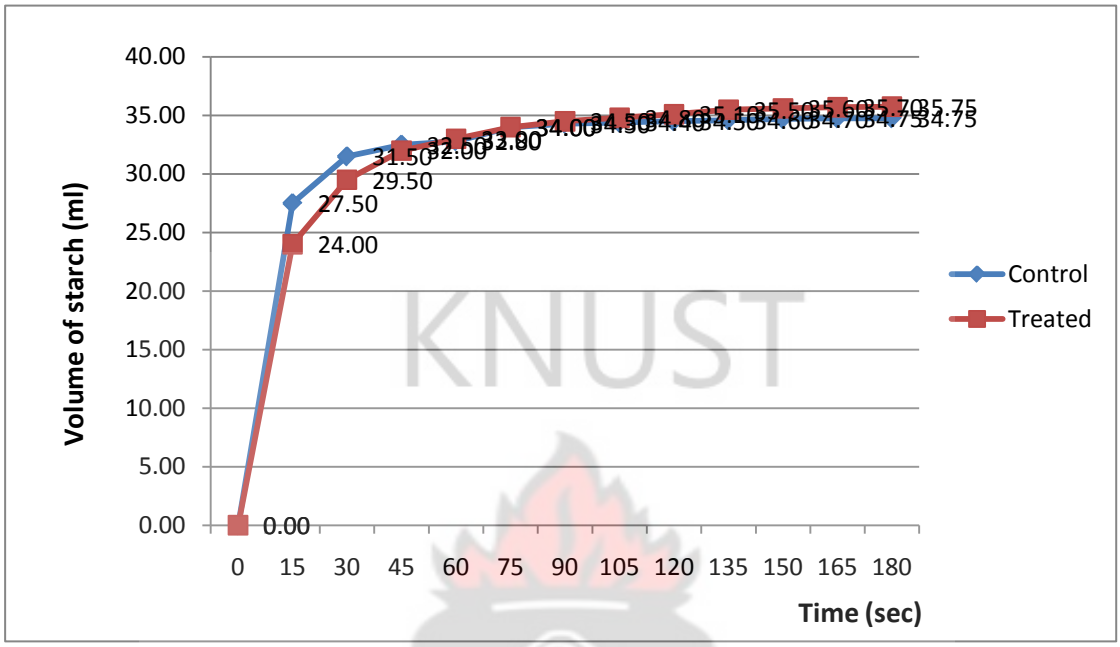


Figure 4.2: Flow pattern of starch milk from 100 g of 'Esam bankye' cassava mash before and after enzyme treatment with 20 mg of crude protein.

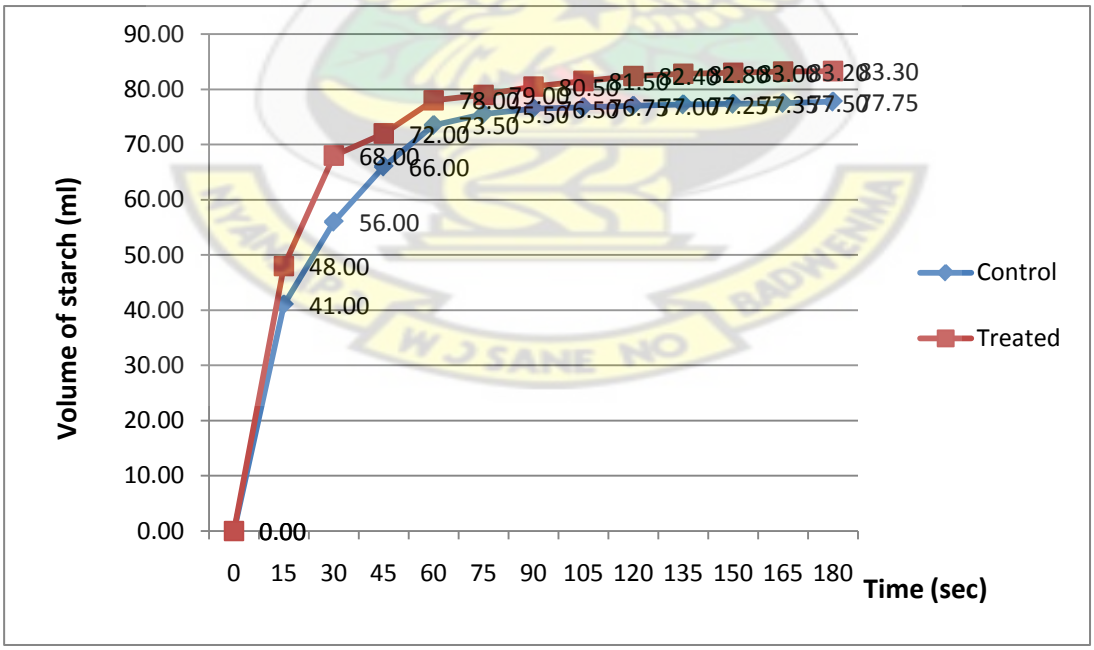


Figure 4.3: Flow pattern of starch milk from 100 g of 'Nkabom' cassava mash before and after enzyme treatment with 20 mg of crude protein.

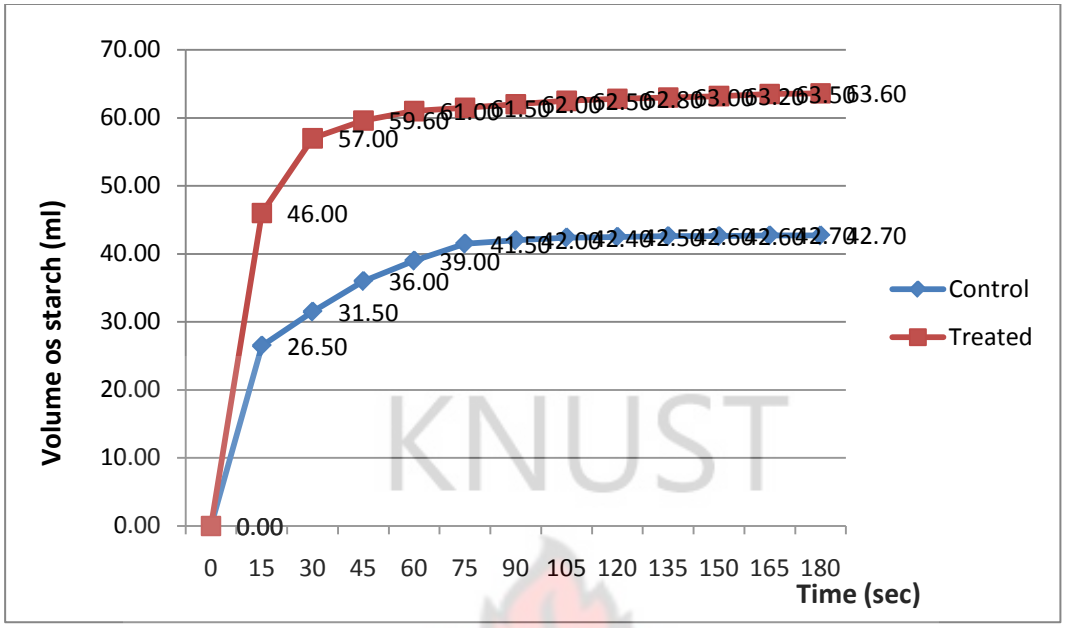


Figure 4.4: Flow pattern of starch milk from 100 g of 'Doku duade' cassava mash before and after enzyme treatment with 20 mg of crude protein.

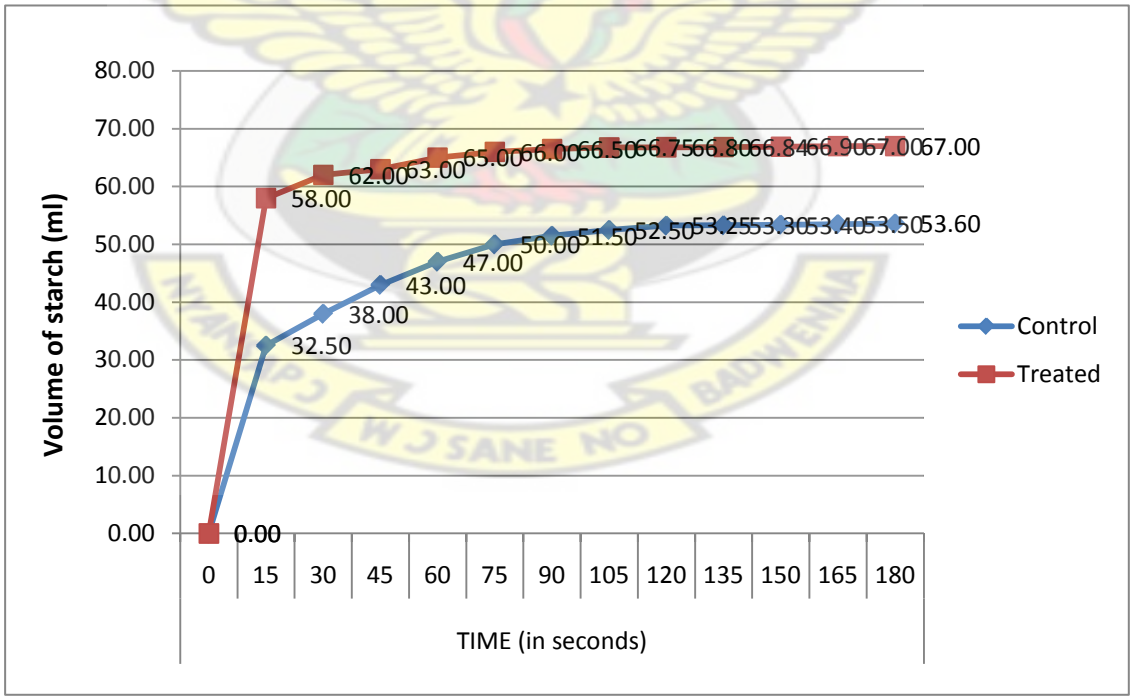


Figure 4.5: Flow pattern of starch milk from 100 g of 'Bankyehemaa' cassava mash before and after enzyme treatment with 20 mg of crude protein.

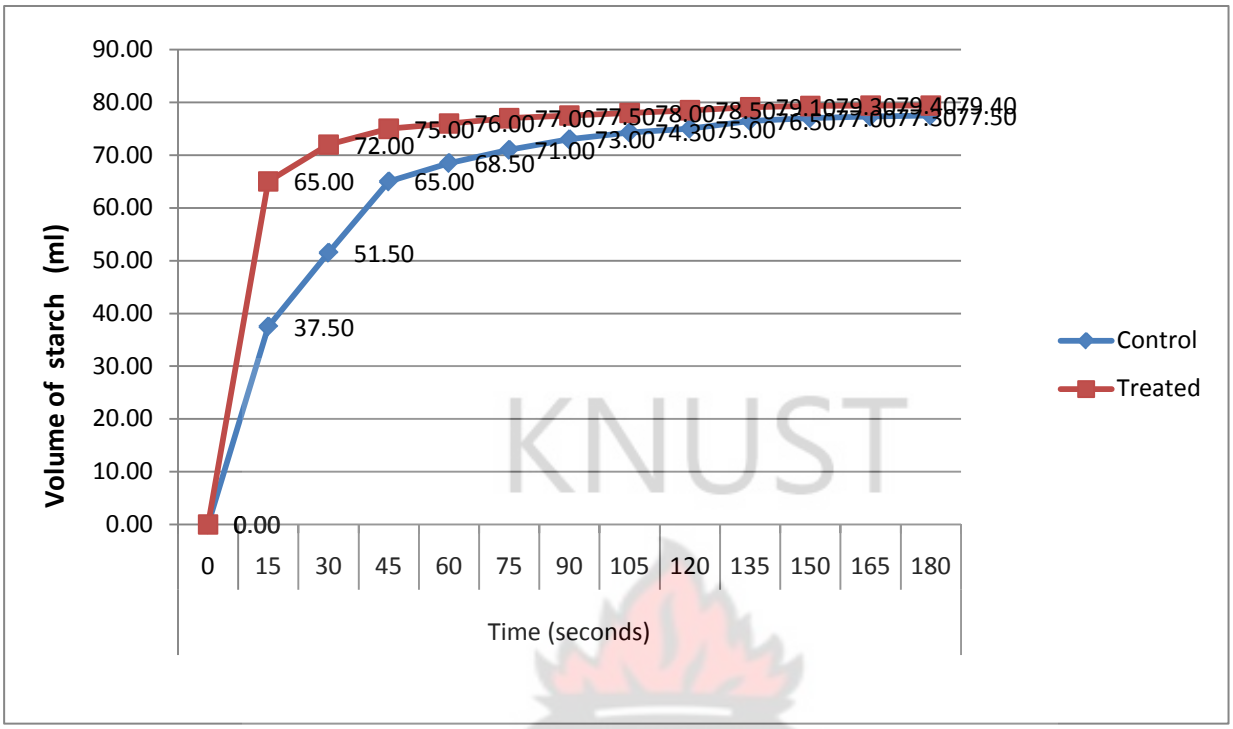


Figure 4.6: Flow pattern of starch milk from 100 g of 'Afisiafi' cassava mash before and after enzyme treatment with 20 mg of crude protein.

Clearly, volumes of starch milk from most treated samples were significantly higher than those from their corresponding untreated ones (Table 4.2). Significant differences were observed in the 'Nkabom' (7.1%), 'Bankyehemaa' (25.1%) and 'Doku duade' (48.9%). Despite the low starch volume increment in the treated 'Afisiafi' (2.4%), this was found to be statistically significant ($P<0.01$) (**Appendix C**. Values from Table 4.2 suggests that 'Esam bankye' recorded a higher percentage starch volume recovery than the 'Afisiafi'; however the observed increment in the 'Esam bankye' variety was not found to be significant ($P>0.05$) probably be due the inconsistent pattern of the flow (as indicated by the high error margin in Table 4.2). The 'Esam bankye'variety's inconsistent response to enzyme treatment and milk flow might also suggest a possible difficulty in estimating milk recovery time should the variety be adopted for enzyme-based industrial starch extraction.

Table 4.2: Average starch milk volume recovered from enzyme-treated and untreated mashes

Cassava variety	Average volume of starch milk (ml)		% Increase in volume over control samples
	CONTROL	TREATED	
Afisiafi	77.5 ± 0.424	79.4 ± 1.273	2.4 ± 1.081
Nkabom	77.8 ± 0.212	83.3 ± 0.707	7.1 ± 1.202
Bankyehemaa	53.6 ± 0.282	67.1 ± 0.141	25.2 ± 0.397
Doku duade	42.7 ± 0.282	63.6 ± 0.566	48.9 ± 2.311
Esam bankye	34.7± 0.791	35.8 ± 0.212	2.9 ± 2.956

Values are represented as mean ± standard deviation (n=2); n = number of replicates

Starch milk volume increments were also significantly different ($P<0.05$) between varieties except for differences between 'Nkabom', 'Afisiafi' and 'Esam bankye' varieties. 'Doku duade' and 'Bankye hemaa' (respectively) were the most positively affected by enzyme treatment as these recorded the highest increase in starch volume (Table 4.2).

During the first 15 sec of flow, the untreated 'Afisiafi' sample gave a volume of 37.5 ml while the treated gave 65.0 ml an increment of about 73.3 %. However, volume of the control sample nearly caught up with that of the treated (Figure 4.6) yielding approximately equal volumes of starch milk at the end of the flow. This was because the treated sample plateaued much earlier especially after the first 45 seconds. The implication of this for industry is that, all conditions being equal, the needed starch volume could be collected within 15 seconds beyond which volumes collected would probably be insignificant.

The very high increase in starch volume observed in the 'Doku duade' and 'Bankyehemaa' varieties (Table 4.2) suggests that fiber in these varieties contained quantities of polymethylgalacturonans (substrates for the enzyme) that were enough to optimize the activity of the enzyme. Thus, the much steady degradation of their fibre led to a corresponding reduction in mash viscosity and a steady release of starch in both mashes. However, the poor result obtained for the 'Esam bankye' variety could be due to the fact that its dry mass was probably relatively higher in starch content than in fiber or probably its

fibre was low in the substrate (polymethylgalacturonans) for which the enzyme targets hence a low activity. Hydroxyl groups on the surfaces of starch granules (in water) have been reported to interact with water molecules giving such starches a viscous consistency (Reis *et. al.,* 2002). Thus a suspension of high quantities of insoluble particles (such as the starch in water) is likely to be viscous. The additional effect of starch being hydrophilic, especially when in high quantities, could have bound more water and further reduced flow. Such high viscosity will impede or limit the flow of moisture also accounting for the relatively very low volumes of milk expelled from both treated and untreated samples compared with the other varieties.

4.3.2. Effects of enzyme treatment on flow rates

Generally, enzyme treated root mashes for all cassava varieties had relatively higher flow rates than their controls (Figure 4.2 – 4.6). This could be due to possible reduction in viscosity of the starch milk upon enzyme treatment. Pectin enzymes are known to cause reduction in viscosity of pectin-containing solutions (Gainvors *et. al.*, 2000; Jayani *et. al.*, 2005; Kaur and Sharma, 2013).

Reduction in viscosity, due to enzyme treatment, could be expressed as a function of volume collected (expressed as percentage increase in product) within the first few seconds of flow. This serves as a qualitative measure of differences in enzyme activity between varieties.

Flow rate was estimated within the first 15 seconds when reaction showed a linear rate (Figure 4.2 – 4.6). Table 4.3 shows the average rates of starch recovery within the first 15 seconds of milk flow:

Table 4.3: Effects of enzyme treatment on starch milk flow rate from different cassava varieties within 15 sec.

Cassava variety	Average rate of starch milk release (ml/ sec)		% Increase in extraction rates over control samples
	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
Bankyehemaa	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

Values are represented as mean ± standard deviation (n=2), n = number of replicates

The results (Table 4.3) showed that enzyme treatment in all cases (except for the Esam bankye variety) resulted in a significantly ($P<0.05$) improved flow rates.

The anomaly recorded in 'Esam bankye' (a negative increase in flow rate) suggests some difficulty in the use of the variety for enzyme-based industrial starch extraction. Thus its inconsistency would discourage its use for any large-scale starch extraction.

From Table 4.1, it is obvious that the 'Esam bankye' variety has the highest dry matter content (suggesting also that it has the least starch/fibre content). Given its low ability to absorb water, we could infer that the variety is relatively low in fiber since fiber (which contains cellulose) has been reported (Ang, 1991; Umaru *et al.*, 2010) to be better at binding water than starch (Lewicki *et al.*, 1978).

The observed low fiber content (hence low content of the target substrate) would again suggest a reduction in enzyme activity due to substrate limitation in the reaction. However, for each variety, viscosities decreased after enzyme treatment due to degradation and solubilization of pectic substances leading to improved flow rates (Rahman and Rakshit, 2003; Blanco *et al*., 1997).

4.3.3. Effects of enzyme treatment on starch yield

Result from starch yield due to treatment (Table 4.4) indicates that the enzyme technology is not limited by variety. Table 4.4 clearly shows that in all the varieties, enzyme treatment was able to achieve a significant ($P<0.05$) increase in starch yield over the controls.

Table 4.4: Yield of starch from cassava varieties with 20mg enzyme dosage at 0.5 hrs holding time.

	% Yield of starch		
Cassava variety	Control (%)	Treated (%)	% Increase in yield
Doku duade	14.9 ± 0.276	18.4 ± 0.311	23.9 ± 4.385
Nkabom	4.0 ± 0.247	5.4 ± 0.354	34.8 ± 17.033
Afisiafi	8.6 ± 0.262	11.6 ± 0.226	34.5 ± 6.688
Bankyehemaa	9.6 ± 0.269	12.5 ± 0.410	29.9 ± 0.634
Esam bankye	12.3 ± 0.453	15.5 ± 0.396	25.6 ± 7.815

Values are represented as mean ± standard deviation (n=2); n = number of replicates

The highest increments in yield due to enzyme treatment were observed in 'Nkabom' and 'Afisiafi' varieties. Ironically, these varieties recorded the least yields within the individual control and treated samples (Table 4.4 and Figure 4.7). The next higher increment in yield was found in the 'Bankyehemaa' with the least being observed in the 'Doku duade'. The varieties ('Doku duade', 'Esam bankye' and 'Bankyehemaa') however, gave the highest yields of starch (Table 4.4) and lowest flow rates (Figure 4.2 - 4.6) again confirming their high-starch-low-fibre status.

This pattern of low rates of starch flow (Table 4.3) coupled with enhanced starch yield (Table 4.4) probably confirms the fact that for varieties with high starch contents their viscosity increases as particle – particle interactions with water increase to increase viscosity thus impeding flow of mositure from starch (http://www.chemeurope.com/en/whitepapers/61207/making-the-connection-particle-size-size-distribution-and-rheology.html.).

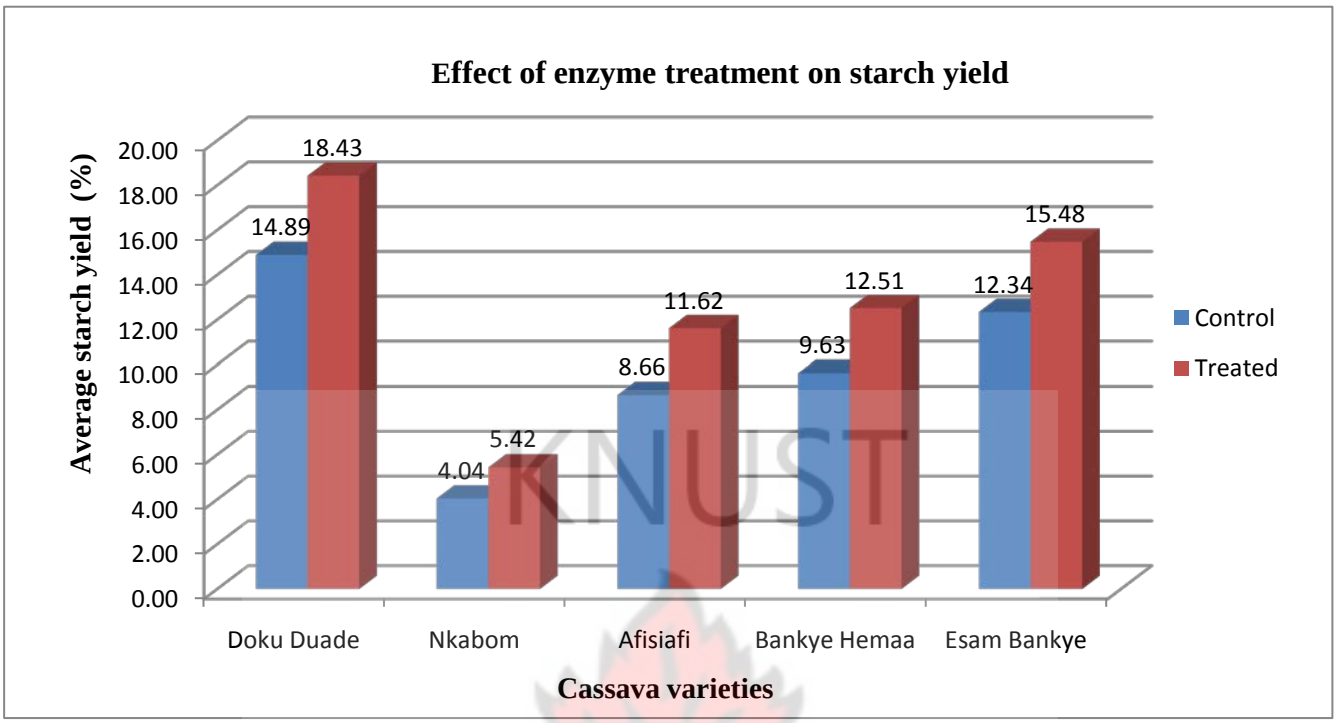


Figure 4.7: Average starch yield from cassava varieties before and after enzyme treatment with 20mg total protein/100g mash.

4.4.0 OPTIMUM HOLDING TIME vrs. ENZYME DOSAGE FOR MAXIMUM STARCH YIELD

There appeared to be a strong interaction between variety, enzyme dosage and holding time (P=<0.001) (Appendix C.17). This indicates that the effect of one factor is not consistent at all combinations of the two other factors; and, therefore, an unambiguous interpretation of the main effects is not possible. In other words, neither enzyme dosage, variety nor holding time alone influenced both flow rates and yields; rather the observation was a result of a strong interplay of all three independent factors.

4.4.1. Effects of enzyme dosage and reaction time on starch yield

Enzyme treatment generally enhanced yield at all dosages (Figure 4.8 – 4.12). Thus, at very low enzyme dosages (between 5-10 mg/100g of mash) improvement in yield was not significantly different from that of the control (P>0.001). Significant differences were however recorded at higher dosages (between 15-25 mg/100g of mash) at increasing holding times in all varieties.

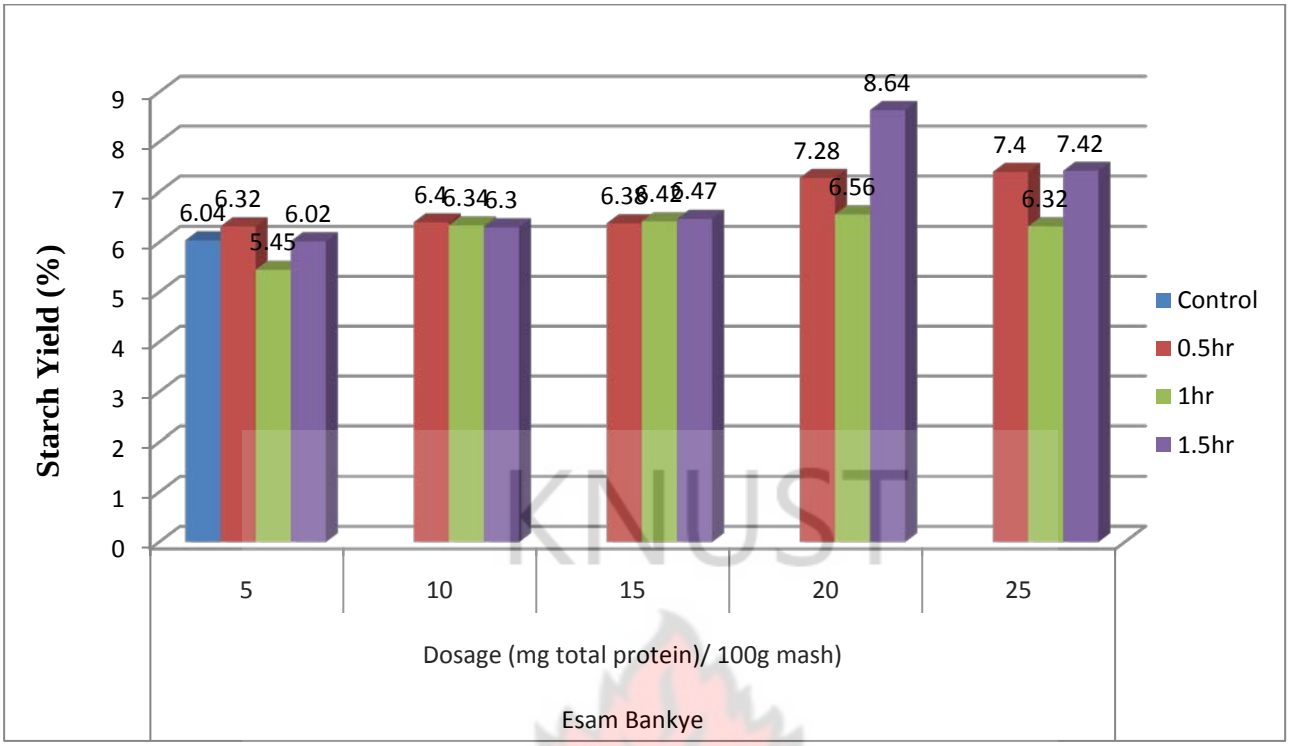


Figure 4.8: Effects of varying enzyme dosage (per 100g) on starch yield in the 'Esam bankye' variety

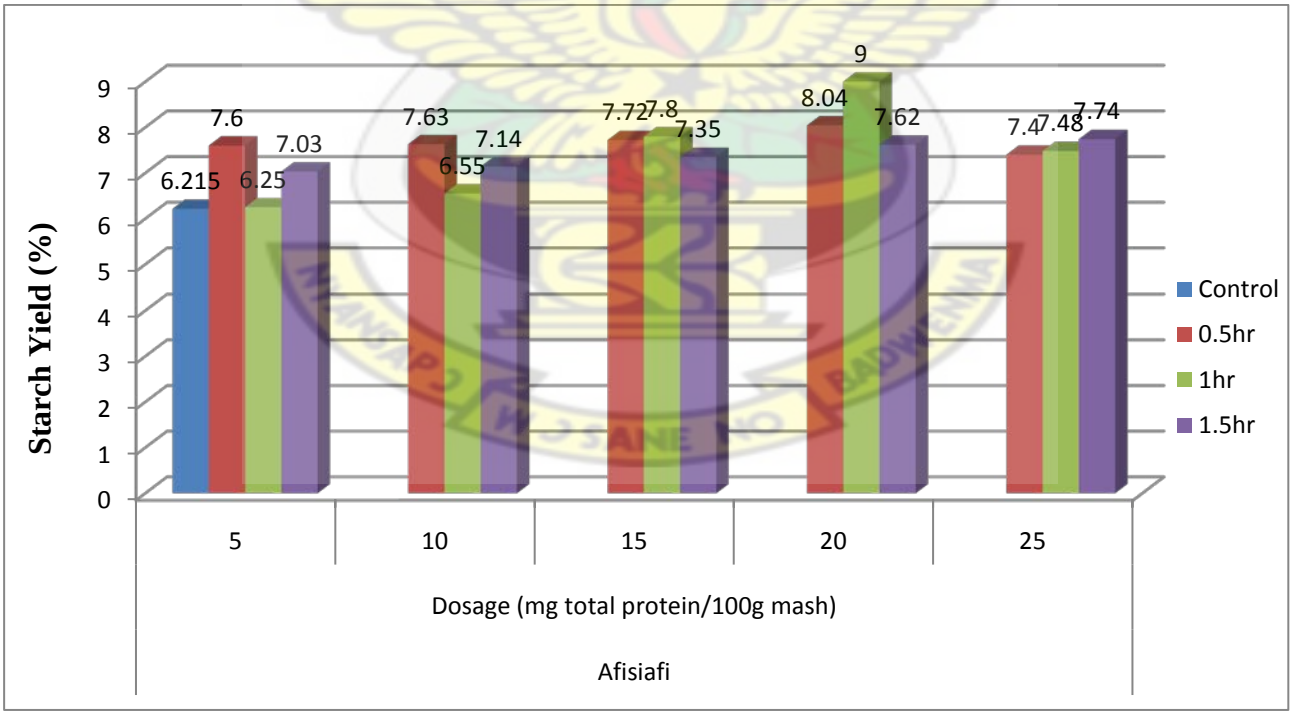


Figure 4.9: Effects of varying enzyme dosage (per 100 g mash) and holding times on starch yield in the 'Afisiafi' variety

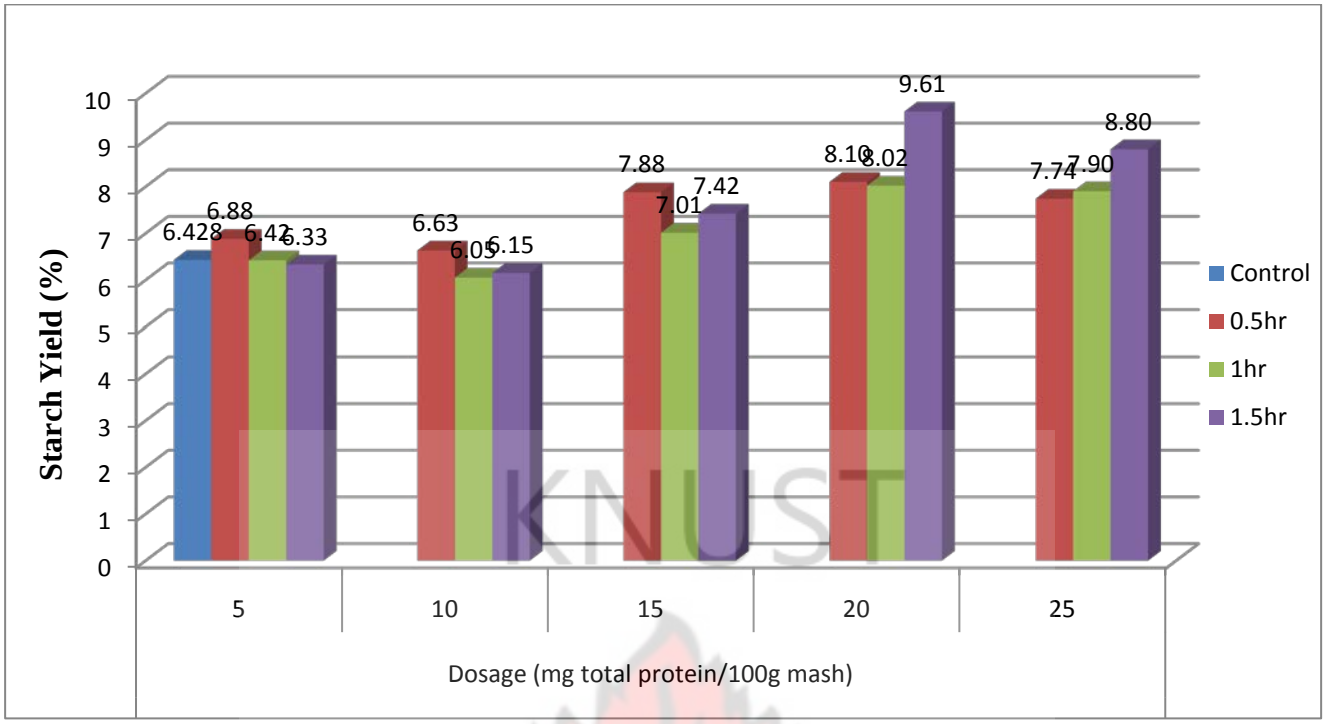


Figure 4.10: Effects of varying enzyme treatment dosage (per 100g mash) and holding times on starch yield in the 'Nkabom' variety

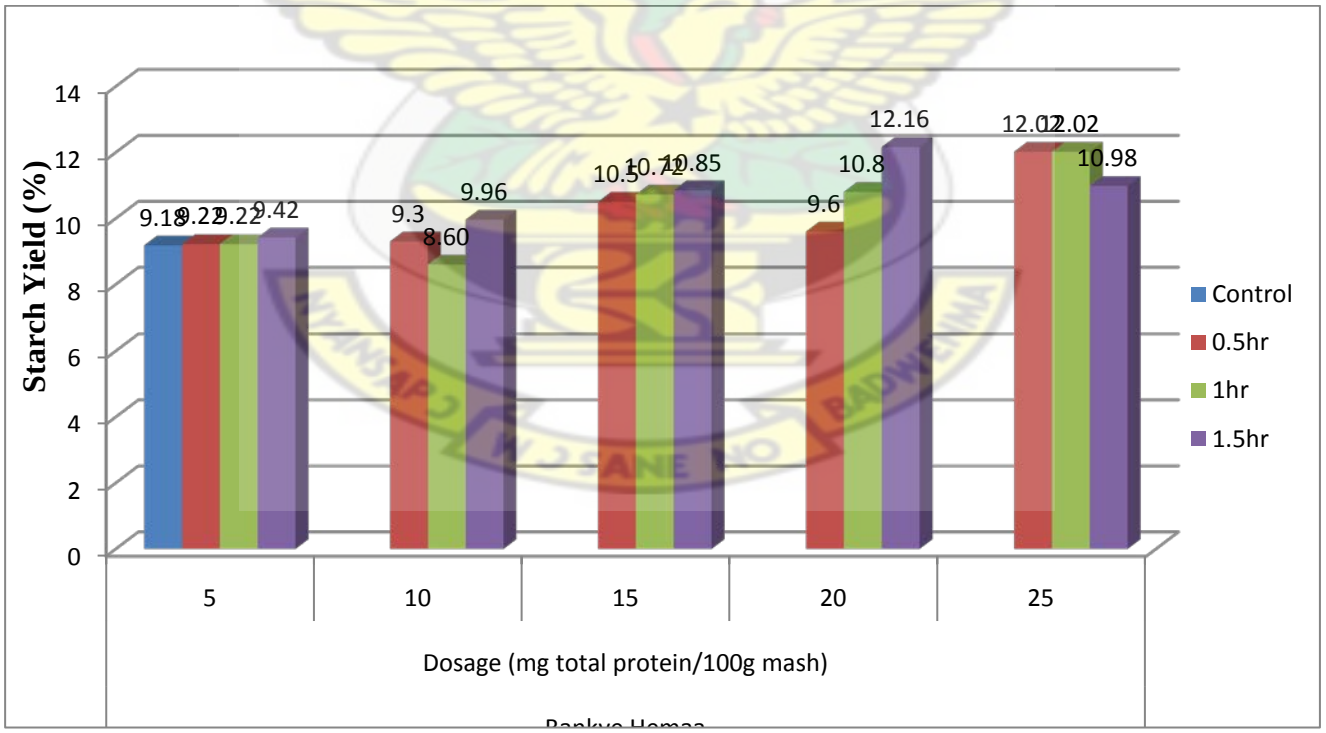


Figure 4.11: Effects of varying enzyme treatment dosage (per 100g of mash) and holding times on starch yield in the 'Bankyehemaa' variety.

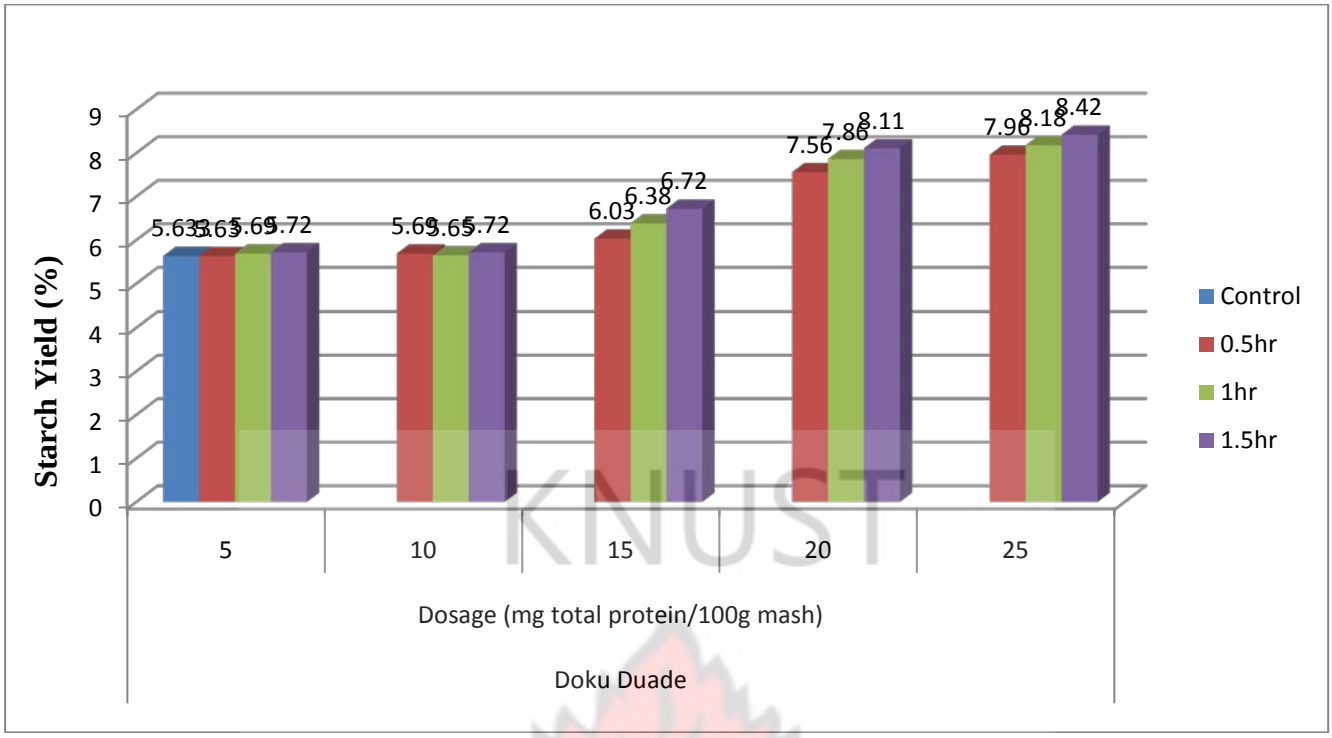


Figure 4.12: Effects of varying enzyme dosage (per 100 g of mash) and holding times on starch yield in the 'Doku duade' variety

With the exception of 'Doku duade' and 'Bankyehemaa' which recorded peak yields with the 25mg enzyme/100 g (0.025%) dosage, all the other varieties ('Afisiafi', 'Nkabom' and 'Esam bankye') gave their highest starch yield with the 20mg/100g (0.02%) dosage. Given the negative implications that longer holding times could have for industry with regards to production rates, shorter holding times that did not significantly affect peak yields were aimed at. Additionally, crude pectinase preparation has been shown to contain some amylase activity (Dzogbefia *et. al*, 2008). Prolonged exposure of starch to amylase and amylase-containing preparations results in breakdown of the starch polymer to glucose (Riley *et al*., 2006) and sometimes maltose (Karkalas, 1985). Thus longer holding times could produce undesirable effects on the functional properties of the resulting starch with regards to granule structure and amylose contents (which ultimately determine the swelling, solubility and pasting properties of starch, Moorthy *et al*., 1993).

Although the 'Esam bankye' recorded the highest starch yield (about 43 %) with the 0.02 % enzyme dosage vrs. 1.5hr holding time combination (Table 4.5 and Fig. 4.8), the negative effects of longer

holding time necessitated the adoption of the 0.02 % vrs 0.5 hr combination resulting in an enhanced starch yield of about 20.53 % over the control. An optimal treatment combination (0.02 % vrs 0.5 hr) in the 'Nkabom' gave an enhanced yield of 26.15 % while in the 'Afisiafi' variety, an optimal holding time of 1hr 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 hr holding time (Fig. 4.9 and Table 4.5). 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 hour holding time with this dosage. These differences could be attributed to differences in activity of the enzyme (neccessitating 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 'Bankyehemaa', however, both required an optimal treatment combination of 0.025% dosage vrs. 0.5 hr holding time to produce optimum yields of 41.46% and 30.91% respectively.

Thus enzyme-aided starch extraction technology is generally applicable although some slight varietal differences are observed. These varietal differences are largely due to the amounts and/ or type of fiber materials (including pectic substances) present (Rose and Vasanthakaalam, 2011) as well as the effectiveness of tissue maceration. Quantitative measurements of polymethyl galacturonase (PMGase) activity on samples using 0.5 grams of mash from each variety gave an activity of 0.121 units/ml on the 'Bankyehemaa' variety; 0.137 units/ml on the 'Doku duade' variety; 0.181 units/ml on the 'Afisiafi' variety 0.067 units/ml on the 'Esam bankye' variety and 0.094 units/ml on the 'Nkabom' variety. This pattern of activities correlated greatly with the starch yield obtained due to the enzyme treatment (Table 4.5). Apparently, pectins from 'Nkabom' and the 'Esam bankye' varieties were lower in their polymethyl galacturonan (PMG) contents. This probably explains why these varities required relatively lower dosages (0.02%) and shorter holding times for maximum activity.

Table 4.5 Summary of optimum enzyme dosage/ holding time combination on percentage increase in yield of starch.

Cassava Variety	Optimum treatment combination (dosage/holding time)	% Increase in yield
Nkabom	0.020% vrs. 0.5hours	26.15 ± 7.367
Afisiafi	0.020% vrs. 1hour	45.10 ± 11.877
Doku duade	0.025% vrs. 0.5hours	41.46 ± 5.165
Bankyehemaa	0.025% vrs. 0.5hours	30.91 ± 4.648
Esam bankye	0.020% vrs. 0.5hours	21.14 ± 11.409

Values are represented as mean ± standard deviation (n=2); where n = number of replicates

4.4.2. Effects of enzyme dosage on flow pattern and starch milk recovery

The flow patterns of starch at the optimum dosage-holding time combinations for the various cassava varieties are shown in Figures 4.13 –17:

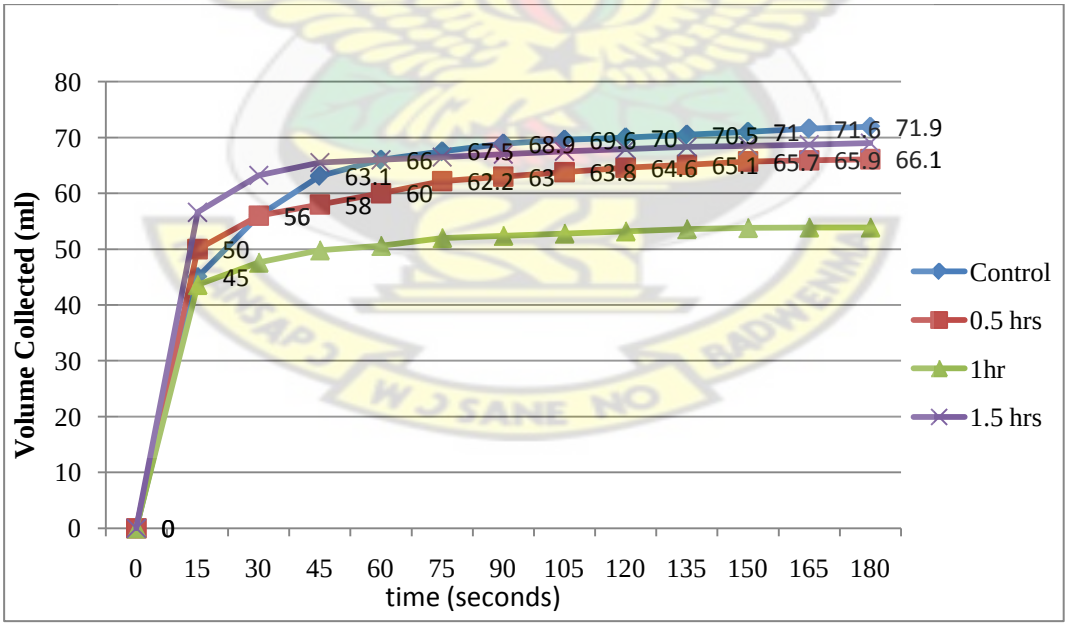


Figure 4.13: Flow characteristics of starch milk from the 'Esam bankye' root mash with 0.020% enzyme dosage at different holding times.

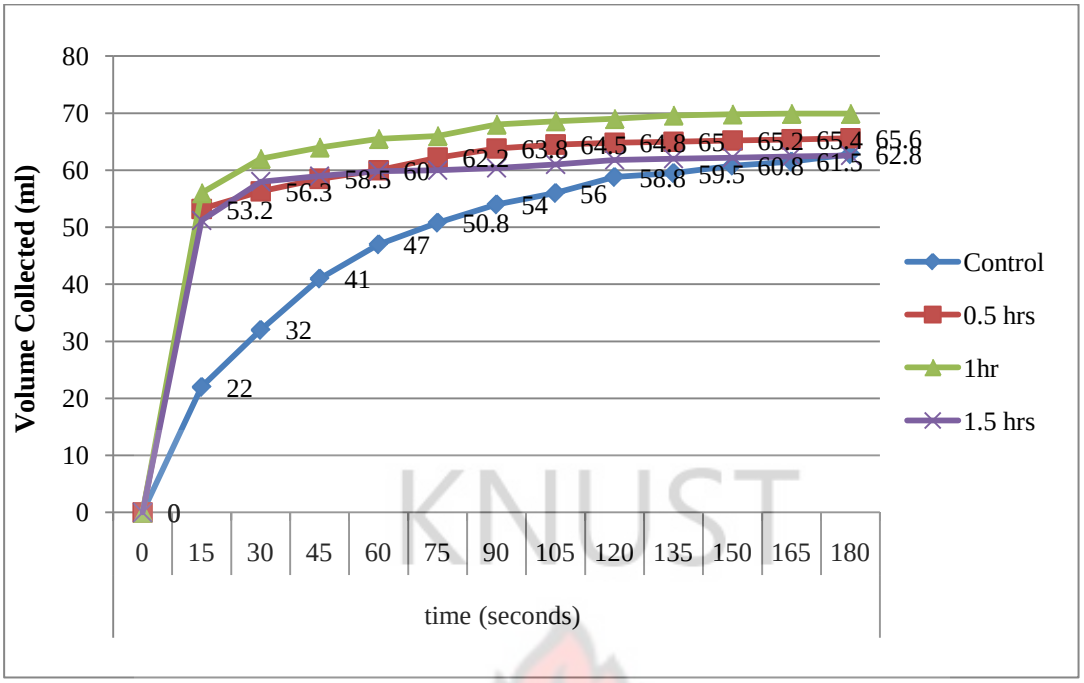


Figure 4.14: Flow characteristics of starch milk from the 'Afisiafi' root mash with 0.020% enzyme dosage at different holding times.

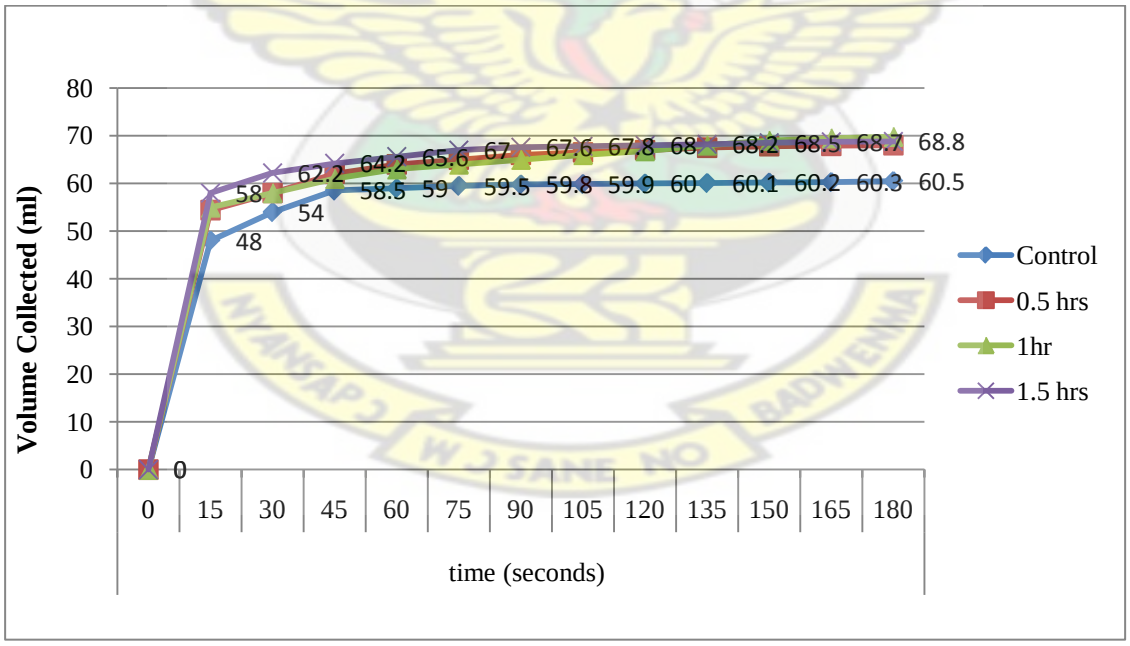


Figure 4.15: Flow characteristics of starch milk from the 'Nkabom' root mash with 0.020% enzyme dosage at different holding times.

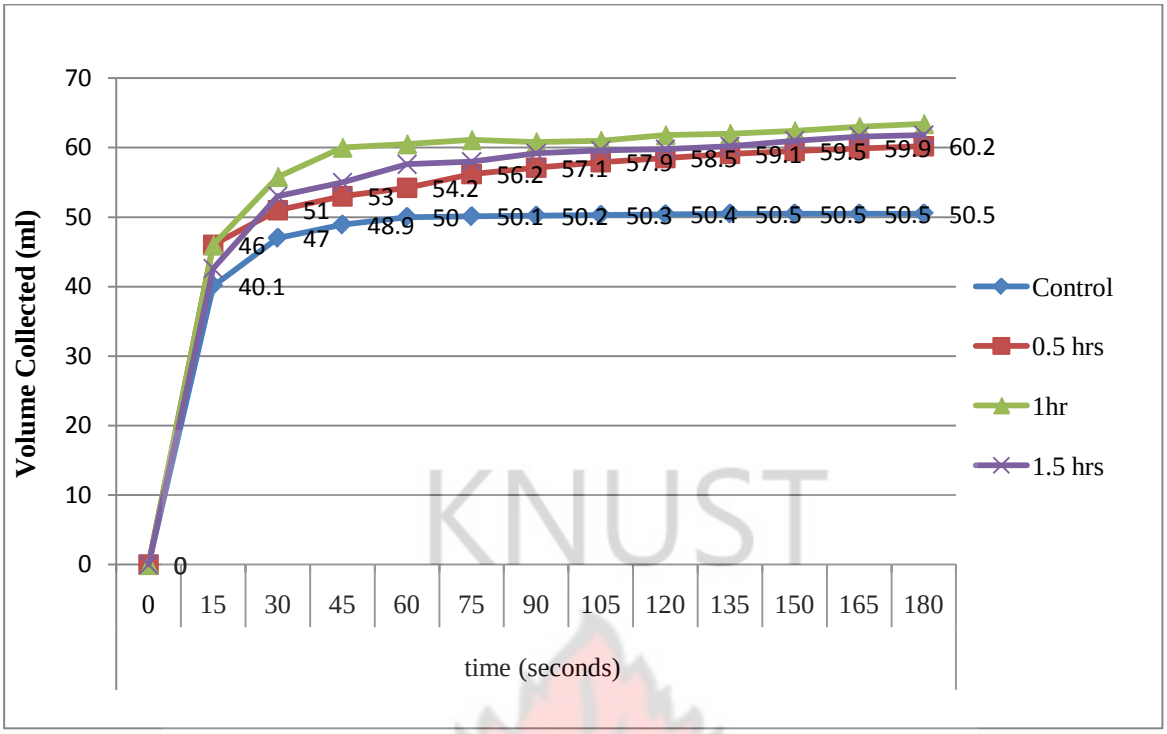


Figure 4.16: Flow characteristics of starch milk from the 'Bankyehemaa' root mash with 0.025% enzyme dosage at different holding times.

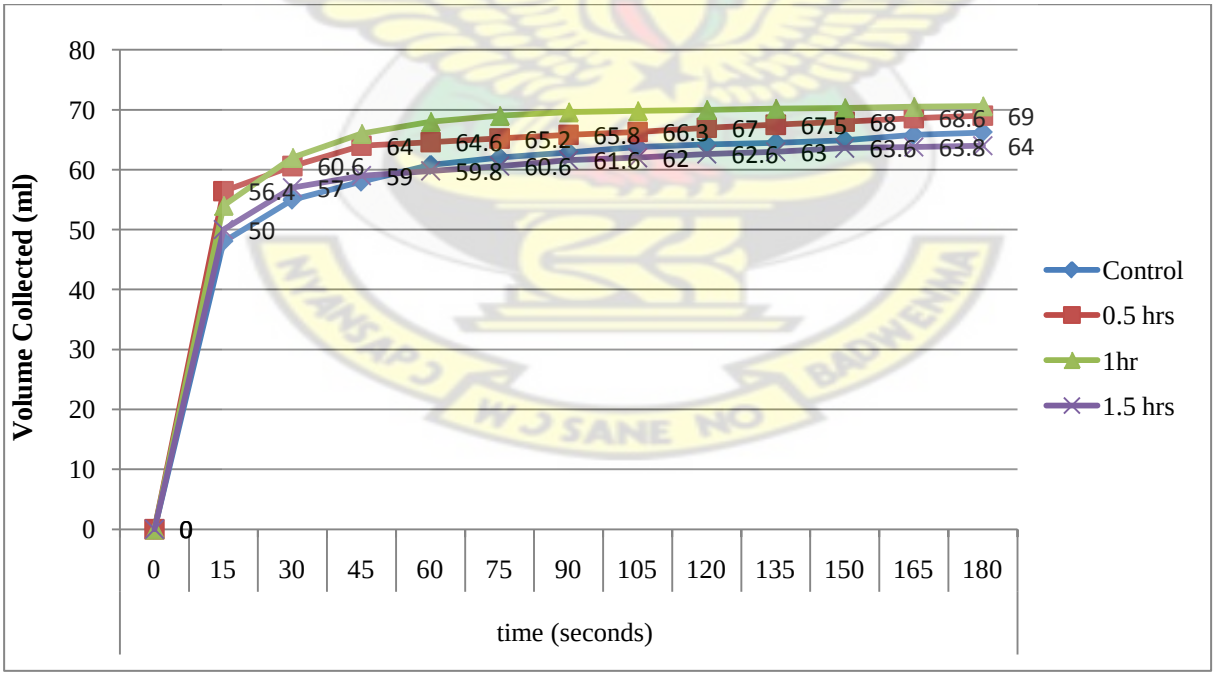


Figure 4.17. Flow characteristics of starch milk from the 'Doku duade' root mash with 0.025% enzyme dosage at different holding times.

Generally all the varieties displayed similar flow pattern of starch milk recovery after enzyme treatment giving higher volumes of starch milk compared with their controls (Figs. 4.11 – 4.17). The only exception to this general trend was the 'Esam bankye' variety where milk recovery for the control compared to enzyme treated samples was higher. This is confirmed by earlier observation where this variety showed a reduction in flow rate over the controls (Table 4.3). Additionally, it was also observed that irrespective of enzyme dosage or reaction time, the flow rates reached an optimum by 15 seconds beyond which no significant increase in flow was observed for all samples (Fig. 4.13-4.17).

This is important in the application of the technology, in that after enzyme treatment of cassava mash, starch recovery will take a relatively short period to reach its optimum.

Knowledge of varietal influence on enzyme technology is vital for the selection of particular varieties for the starch extraction industry. Given the 'Bankyehemaa' variety's higher starch content (comparing starch yield from individual treated and untreated mash and overall enhancement in yield due to enzyme treatment (Figure 4.11 and Table 4.5) to those of the other varieties (Figure 4.(8 – 10, 12)), it is clear that 'Bankyehemaa' would prove to be the best candidate for starch production. Also noteworthy is the fact that almost all the varieties seemed to have had a reduction in starch yield of their controls at 12 M.A.P (compare Figure 4.7 and Figures 4. (8 – 12)) , however, 'Bankyehemaa' variety's yield seemed unaffected within the period as yield at both 9 MAP and 12 MAP were similar.

Despite improvement in yield for all the varieties after enzyme treatment, starch content or yield alone should not be the only selection criterion for industry as poor quality starch would be useless despite its quantity. Thus, higher yield due to enzyme treatment would need to be supported qualitatively by their functional properties ensuring that such treatment does not negatively affect, to a large extent, the starch's original properties.

4.5.0. EFFECTS OF ENZYME TREATMENT ON PHYSICOCHEMICAL PROPERTIES OF STARCH SAMPLES

Measurements on all physical – chemical properties of the starches extracted from both treated and untreated mashes are summarized in Tables 4.6 – 4.10:

4.5.1 Starch granule size and structure

It is obvious (Table 4.6) that enzyme treatment had significant effect ($P<0.05$) (Appendix C. 5) of enhancing the size of extractable starch granules from all treated varieties. Although enzyme treatment did not significantly ($P>0.001$) affect sizes of extractable small starch granules in most of the varieties, significant differences ($P<0.001$) were observed in the extractable medium to large starch granules. This probably suggests that yield might largely have been dependent on the relative amounts of these medium to large size granules especially so, as the process employs sedimentation which is largely dependent on granule size and gravity. A comparision of results on yield (Figures 4.8 - 4.12) with granule size (Table 4.6) confirms this.

Table 4.6: Average starch granules sizes extracted from the treated and untreated cassava mashes

CASSAVA VARIETY		AVERAGE GRANULE DIAMETER (µm)			
		Small	Medium	Large	Avg. size
Nkabom	Control	6.67±1.9	13.34±0.0	18.67±0.0	12.89
	Treated	**6.67±1.9**	**13.34±0.0**	**20.01±1.9**	**13.34**
Afisiafi	Control	6.67±1.9	13.34±0.0	18.67±0.0	12.89
	Treated	**6.67±1.9**	**12.94±0.6**	**21.34±0.0**	**13.65**
Doku duade	Control	7.34±0.9	13.34±0.0	20.01±1.9	13.56
	Treated	**4.67±0.9**	**13.34±0.0**	**21.34±0.0**	**13.12**
Bankye Hemaa	Control	9.34±1.9	13.34±0.0	21.34±3.8	14.67
	Treated	**9.34±1.9**	**14.67±1.9**	**23.34±0.9**	**15.78**
Esam Bankye	Control	5.33±0.0	14.67±1.9	20.01±1.9	13.34
	Treated	**7.34±0.9**	**18.34±0.0**	**22.67±1.9**	**16.12**

Values are represented as mean ± standard deviation (n=3); n = number of replicates

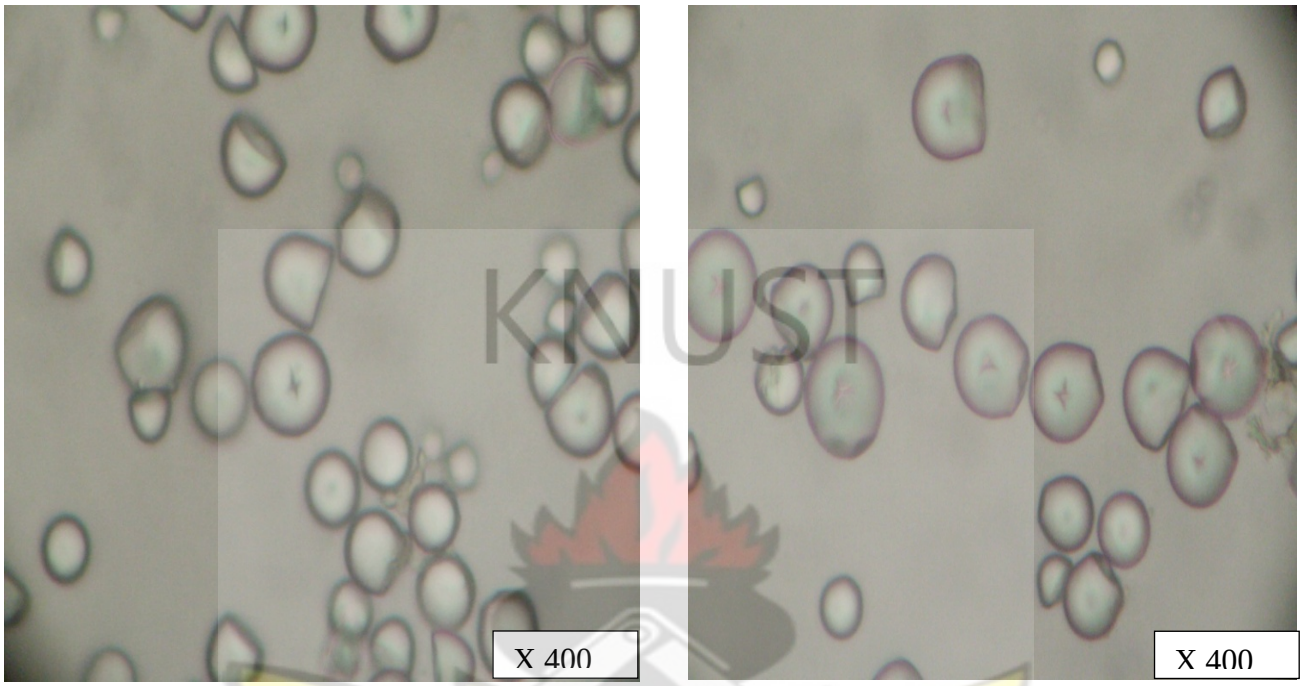
As starch granule size affects starch composition and other functional properties such as gelatinization and pasting properties, enzyme susceptibility, crystallinity, swelling and solubility (although several other factors, including amylose/amylopectin ratio and molecular weight and granule fine structure, are also influential) (Lindeboom *et al*, 2004), an improvement in granule size (as a result of enzyme treatment) suggests a general enhancement in the performance of starch as a binding agent.

Comparing the above results **(Table 4.6**) with that reported by Dzogbefia *et al*. (2008b), it was found that the size of the starch granules for the Afisiafi control sample were very similar. This confirms reports of 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 the treated samples. This variation could be due to the differences in enzyme activity. Thus, under the given working conditions, the enzyme used for this work probably attained its optimum and was therefore 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 the morphology of all intact starch granules, the samples' micrographs showed near spherical structures each with three shallow surface fissures that were equidistant from each other on every starch granule. The fissures were deeper at the center; however, the ridges forming them diminish as they get towards the edge of the granule (**Plate 4.2**). Starch granule size, shape and their surface characteristics are important in characterizing and identifying the botanical source of starch (Robertson *et al*., 2006).

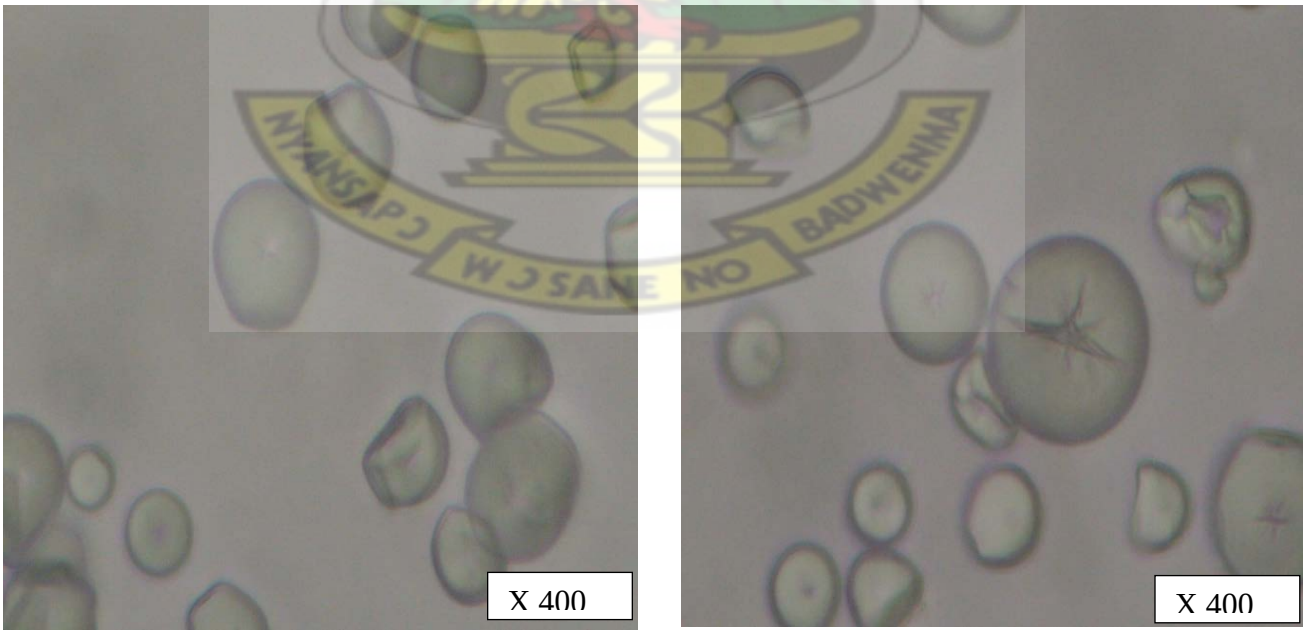
Plate 4.2 Micrographs of starch granules from treated and untreated root mashes of the cassava varieties

1. ***BANKYEHEMAA***



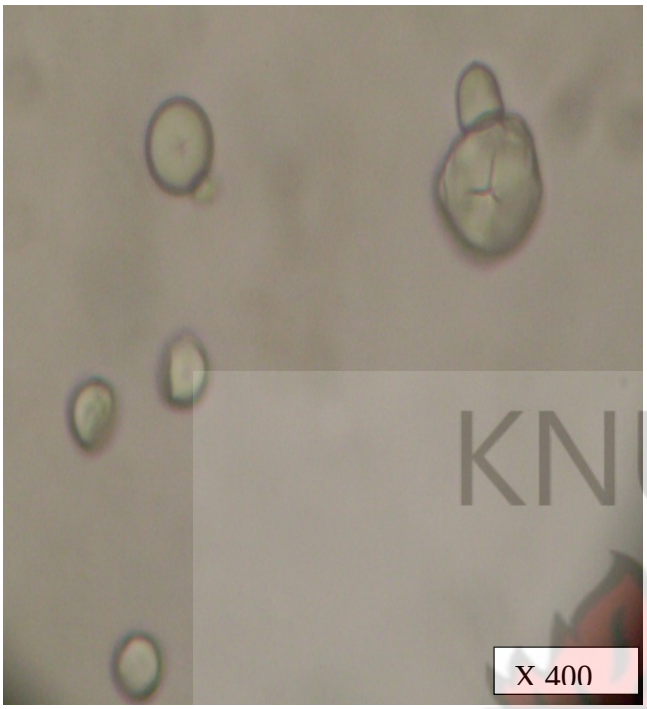
Control Treated

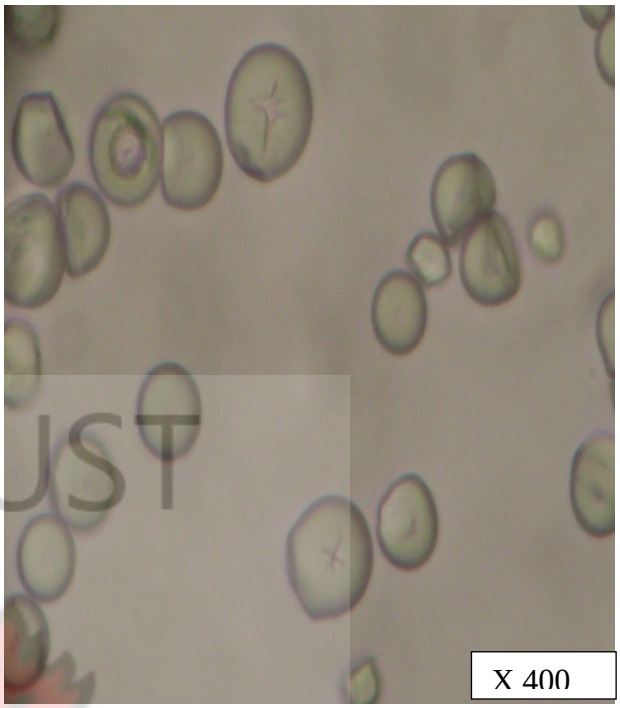
2. *DOKU DUADE*



Control Treated

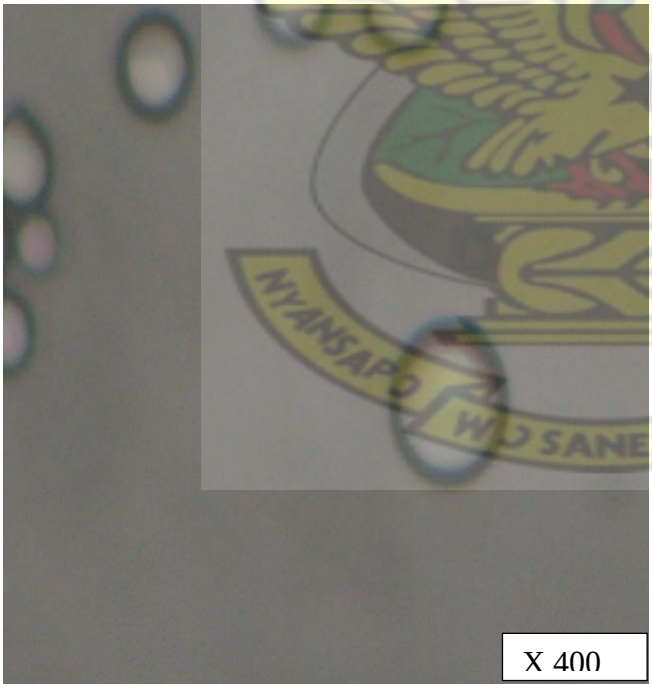
3. *ESAM BANKYE*

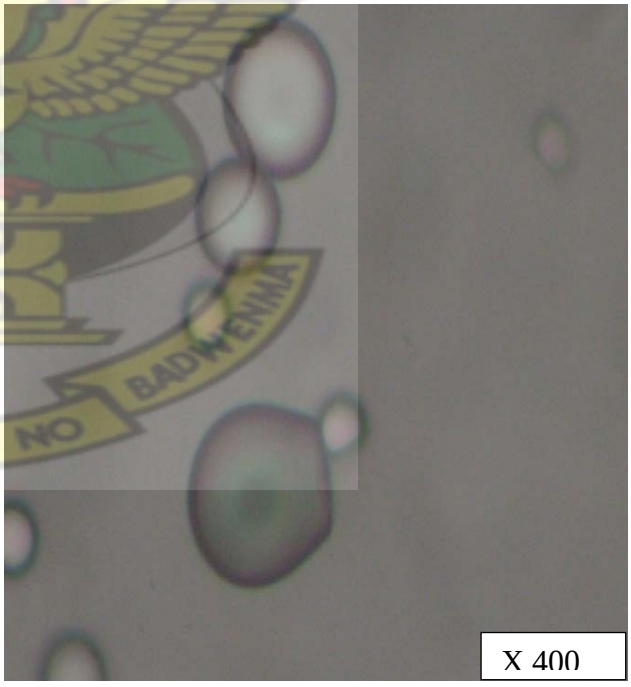




Control Treated

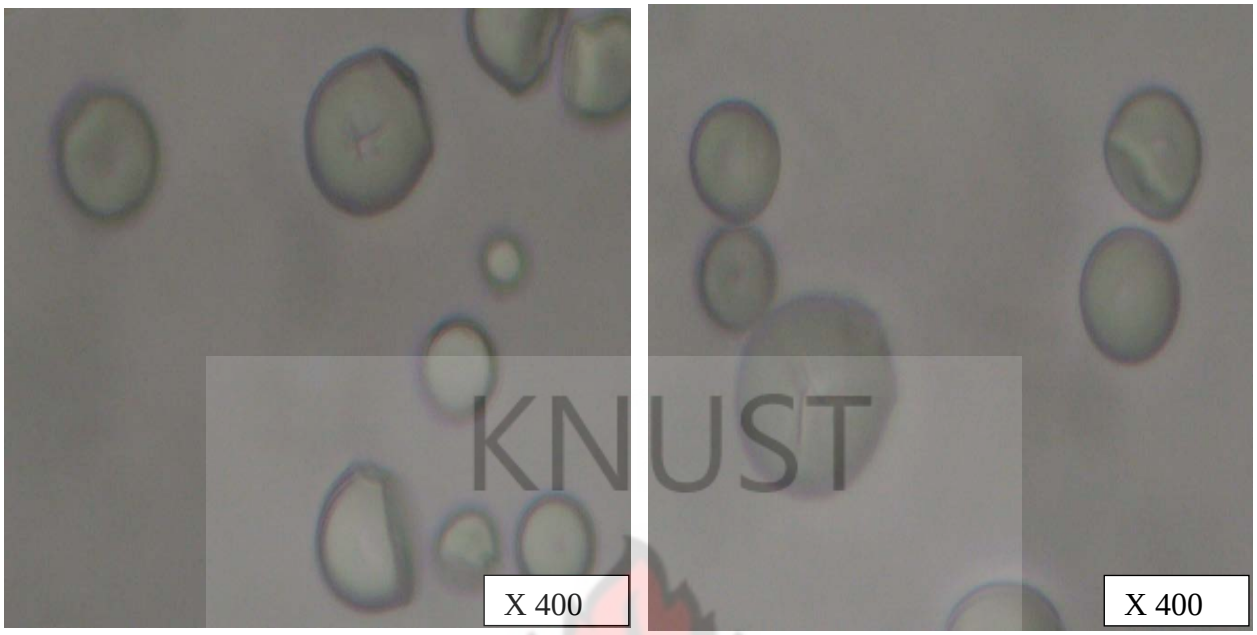
4. *AFISIAFI*





Control Treated

5. NKABOM



Control Treated

Starch granule sizes ranged from 5.33-23.34µm (**Table 4.6)**. This range falls within the 4-35µm range reported by Adejumo *et. al.* (2011**).** Although the minimum, 5.33µm, falls slightly short of the 6 - 31µm range reported by Yuan *et al*. (2006), they affirmed the presence of incomplete hemisphere granules with fissures on tapioca starch.

From the micrographs (Plates 4.2.(1-5)) above, it is clear that generally, enzyme treatment did not have any detrimental effect on starch granule structure. From Figures 4.2.1 and 4.2.5 respectively, it is observed that starches from the 'Bankyehamaa' and 'Nkabom' recorded fractures in some granules from both the treated and untreated mashes alike. These fractures could not be attributed to enzyme treatment but rather to effects of processing mechanisms such as maceration, freezing of mashes (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 in especially the grooves of starch granules from the treated 'Doku duade' (Plate 4.2.2) and 'Esam bankye' (Plate 4.2.3) varieties seem to suggest that enzyme treatment probably contributed to granule fracture in these varieties. Thus, activities of endogenous amylases were probably high in these grooves causing granule degradation in these areas and rending the granules more fragile. Sujka and Jamroz (2007) reported that the presence of pores, channels and cavities on the surface of starch increases the surfaces potentially available for chemical and enzymatic reactions. The effects, however, might have been minimized by the shorter holding times adopted (as not all treated granules showed the detrimental effects).

Thus, it could be inferred from the above results that effects of enzyme treatment on starch granule structure of different cassava varieties also varies. The technology renders some granules more succeptible to fragmentation whereas others are not significantly affected.

TABLE 4.7

TABLE ON PHYSICOCHEMICAL PROPERTIES OF STARCH

4.5.2 STARCH MOISTURE CONTENT AND pH

In general, starches from enzyme – treated samples of all varieties had significantly ($P<0.05$) higher miosture content than their controls (Table 4.7). As moisture content correlates negatively with amylose content (Nuwamanya *et al.*, 2010), it is possible that amylolysis might have decreased the amounts of amylose and therefore reduced the amounts of amylose associative forces within the granules and exposing more of the amylose to bind water. Generally, moisture contents of starches from both treated and untreated varieties, although were found to differ significantly ($P< 0.05$), all agreed with the ranges reported in literature (Leonel *et al*., 2009; Nyerhovwo, 2004; SON, 1988). Thus the enzyme treatment did not have any detrimental effect on this parameter. 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.

Comparison of moisture in the starches from different cassava varieties could be affected by myriads of factors with the obvious ones being the amounts and duration of exposure to radiant energy and humidity (Apea-Bah *et. al*., 2011) as well as aeration. However, the nature of the starches' microstructures also influence water binding and liberation as Nuwamanya *et al*. (2009) have found starch and amylose content to correlate positively but found these to correlate negatively with moisture content. Other possible factors that might also have influenced the amounts of moisture left in starch samples could be relative yield of starch (**Figures 4.8 – 4.12**) as well as the starch granule sizes (**Table 4.6**). This therefore suggests that larger quantities of starch left to dry would likely retain more moisture. Such hydrophylic and crystalline properties are also correlative with their physical properties such as gelatinization temperature and viscosity in viscogram (http://rms2.agsearch.agropedia.affrc.go.jp/ contents/JASI/pdf/acade- m y/25-3140.pdf). Generally the accepted range of moisture in cassava starch as reported by Graffham and Dziedzoave (1998) and Leonel *et. al.* (2009) is between 10 -12.2%.

Enzyme treatment significantly (P< 0.05) rendered starches from the treated samples more acidic (Table 4.7). The low pH of the acetate buffer used for enzyme production (and subsequent starch extraction) might have influenced this result. Starch pH ranged from 5.2 – 7.6 (the lowest pH being recorded in the 'Doku duade' and the highest from the 'Esam bankye' varieties). García-Alonso *et. al.* (1999) have reported that cooking cassava starches below pH 3.5 or above 10.5 significantly reduces the gelatinization temperature and causes starch hydrolysis. Hence the observed pH range (5.2 – 7.6) would therefore not influence the starches' functional properties significantly.

4.5.3 Protein Content

Enzyme treatment did not significantly (P>0.001) affect the amount of protein in the ensuing starch. Crude protein content for all samples was also generally very low (ranging from 0.03% in the 'Bankye hemaa' to 0.09% in the 'Doku duade' and 'Nkabom' varieties) (**Table 4.7**). The observed values are far below the 0.28 - 0.52% reported by Nuwamanya *et al*. (2009) for starches from the varieties he studied. According to Moorthy (2002), protein effects on starch properties depend on its content with high protein content negatively affecting their pasting properties. High protein content would imply a loss or reduction of hydroxyl groups in the starch to Maillard (condensation) reactions especially during starch drying; hydroxyl groups in starch are key physicochemical determinants of starches (Sanni *et. al.*, 2005). Thus, the low protein content recorded implies all the starches are of high quality as their low protein contents would impact positively on their pasting characteristics.

4.5.4. Fibre and Ash Content

Ash content for both enzyme-treated and untreated samples ranged from 0.38 - 0.97% with the 'Nkabom' variety recording the highest and the 'Bankyehemaa' variety recording the least ash contents (Table 4.7). Although cultivated on the same soil having similar chemistry (Appendix A.3), there were significant mean differences (P<0.05) in ash content between varieties suggesting that mineral uptake by cassava root parenchyma differ significantly between varieties. The results obtained were however not

very different from the 0.02 - 0.93% range reported by Nuwamanya *et. al*. (2009) for cassava starch fibre of both parental and progeny lines. Additionally, the ash content between treated and control samples among the varieties also did not differ significantly (P>0.05) suggesting that enzyme treatment did not have any effect on ash content. In all, ash content of samples complied with the regulatory standard of not more than 1.5% ash for starch (SON, 1988).

Fibre was generally lower than reported in most literature for cassava starch (Dzogbefia *et. al*., 2008a; Moorthy *et. al*., 1993). Enzyme treatment however did not significantly (P>0.05) affect fibre contents in most of the varieties, although starches from some of the treated varieties ('Afisiafi' and 'Nkabom') showed significant reduction (P<0.05) in fibre content. The variety that produced starches with the highest fibre content was the 'Nkabom' (0.15% for the control) with those from 'Bankyehemaa' and 'Afisiafi' each recording the least fibre content (0.12%) for their control samples.

4.5.5. Amylose Content

Starches from treated root mashes of all varieties showed a significant reduction in amylose with the 'Afisiafi' variety recording the highest amylose degradation and 'Bankyehemaa' the least degradation (Table 4.7). There are reports **(**Blanco *et al.* 1999; Dzogbefia *et al.*, 2008a**)** that crude pectolytic enzyme preparations from *S. cerevisiae* have some level of amylase activity; thus necessitating assay for the presence of this enzyme. After the assay, the crude enzyme extract was found to have an amylase activity of 0.293 units/ ml (with a specific activity of 0.257 units/ mg) which was higher than the 0.16 unit/ ml and 0.09 units/ mg (for amylase activity and specific activity respectively) reported by Dzogbefia *et al*. (2008a). This relatively higher activity also explains the significant effects the endogenous amylase had on starches from all the varieties. The higher degradation of amylose in the Afisiafi variety could be attributed to the longer period of exposure of the root mashes to the crude enzymes (1 hour compared to 30 minutes for optimum reaction) which allowed extensive degradation of amylose over the period. However, such extensive degradation would lead to the production of higher

amounts of hydrolytic products that would serve as inhibitors to the enzyme (Robyt, 2005) and thereby help mitigate this (degradative) effect.

Variable effects of amylase on most starches have also been linked with variable granule sizes of the starches. For instance, Vasanthan and Bhatty (1996) have reported that small to medium granule starches are more susceptible to enzymatic degradation due to the higher surface areas they present. However, Evers *et al.* (1974) and Myllärinen *et. al.* (1998) have also reported the same amylose content in both small and large starch granules. This similarity in amylose content, as reported by Takeda *et al.* (1999), could probably be due to the large granules containing smaller, less branched amylose polymers. Although both the 'Doku duade' and 'Bankyehemaa' varieties contained relatively higher amounts of amylose, the smaller granule sizes obtained from the treated 'Doku duade' variety (**Table 4.6**) explains the higher degree of amylolysis found; granule sizes from the treated 'Bankyehemaa' was the largest (Table 4.6) and therefore its low susceptibility to amylose degradation.

Amylose degradation in the 'Afisiafi', 'Esam bankye' and the 'Nkabom' varieties are probably more related to their amylose contents than to their granule sizes as they recorded similar granule sizes for their untreated samples.

4.5.6 Swelling and Solubility Index

All the starches swelled when heated to a temperature of 80^{o}C. The swelling power of high amylose varieties ('Bankyehemaa', 'Doku duade' and 'Esam bankye') was however significantly ($P<0.05$) enhanced by enzyme treatment. As amylose is responsible for maintaining associative forces within granules (Ikegwu *et. al*, 2009; Zheng and Sosulski, 1997; Sanni *et al*, 2005), the high amylose content of the 'Doku duade', 'Bankyehemaa' and 'Esam bankye' probably reduced the freeness with which their granules swell in their respective control varieties accounting for their relatively low swelling power (Table 4.7). However high amylolysis observed from their treated samples led to a significant ($P<0.05$) improvement in their swelling power as more and more amylose molecules were free to

assocate with water. Swelling power of starches from the treated 'Nkabom' variety was not significantly (P>0.05) affected probably as a result of its relatively low amylose content. The low amylose content (coupled with the amylolytic effects) explains why the starches (from both the treated and untreated mashes) of the 'Nkabom' variety recorded the highest swelling powers (Table 4.7).

Enzyme treatment also significantly enhanced the solubility of all the starches (**Table 4.7**). From **Table 4.7**, it is observed (for all controls samples) that high amylose containing starches had low solubilities. Reports of high amylose starches having stronger interactive (intramolecular) forces (Omojola *et al.*, 2010***;*** Moorthy and Ramanujam, 1986) suggests that such starches were prevented from opening up freely to interract with water hence their low solubility. On the other hand, starches from treated samples that were most succeptible to the degradative effects of the endogenous amylase released more soluble products and therefore had higher solublities (Table 4.7). Thus products of amylolysis (maltodextrins) might also have contributed to the enhancement of soluble solids of starches obtained from treated samples.

In addition, the release of smaller sized granules (**Table 4.6**) by the treated samples also probably contributed to the enhanced solubility values (**Table 4.7**). Zheng and Sosulski (1997) have reported that small granule starch sizes tend to leak out more amylose out of their intact granule than do larger ones at 55^{o}C and higher. Thus, smaller sized granules have higher solubility at temperatures above 55^{o}C. The additive effects of smaller granule sizes in providing larger surface area for amylase action (MacGregor and Ballance, 1980; Lindeboom *et. al.*, 2004) 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 further providing a larger surface area for enzyme action thus rendering them more succeptible to degradation.

Due to the undesirable effects of endogenous amylase, enzyme application aimed at enhancing starch yield for the adhesive industry would require the incorporation of amylase inhibitors, probably maltodextrins and acarbose (Robyt, 2005), to help mitigate amylase degradative effects. However, if the starch is to be applied to food, then such an inhibitor could be eliminated as amylases enhance starch digestibility (Moorthy, 2002).

4.5.7 Water Binding Capacity (WBC)

Enzyme treatment had varying effects on water binding capacity of the starches produced. Starches from the treated 'Bankyehemaa' and 'Nkabom' all recorded significant ($P<0.05$) improvement in their ability to bind more water than those from their respective control samples. Water binding capacity of starches from the treated 'Esam bankye' variety was however not affected (Table 4.7) but the WBC for the 'Doku duade' and 'Afisiafi' recorded a significant ($P<0.05$) reduction.

As both small and large starch granules have the same composition of amylose and amylopectin (Evers *et. al*., 1974; Myllärinen *et. al.,* 1998), the treated 'Doku duade' releasing the smallest size starch granules (Table 4.6) would imply that a unit volume of starch from treated 'Doku duade' would probably contain a relatively greater amount of amylose (compare with Table 4.7). Thus, the variety would present a much larger surface area to volume ratio for amylose to bind with water. However, its relatively high amylose content (Table 4.7) and fractured granule morphology (Plate 4.2.2), also probably rendered the starches more susceptible to enzyme attack subsequently reducing amylose content (Table 4.7) and the associative hydrogen and covalent forces in the starch (Rincon *et al.,* 1999) significantly ($P<0.05$). This might also have affected its bulk density (as a result of the reduction in the amylose components per granule).

The extensive degradation of amylose (Table 4.7) by the endogenous amylase had the greatest detrimental effect on water binding capacity in starches from the 'Afisiafi'. Thus the high reduction in

the ability of the resultant starches from the treated 'Afisiafi' to bind water (Table 4.7) is due to the longer time allowed (1 hour holding period) for enzyme action.

Enhancement in WBC due to application of the enzyme technology suggests that the technology would enhance the quality of the starches as drug binders and disintegrants in pharmaceuticals (Zaku *et. al*, 2009). 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.

In general, aside from amylose content which decreased significantly due to enzyme treatment, other physicochemical parameters of the starch such as pH, ash, fibre and moisture contents were not compromised. However, the technology rather significantly ($P<0.05$) improved the solubility, swelling power and water binding capacities of the resulting starches. Varietal differences were however noted.

4.5.8 Pasting Properties

Effects of enzyme treatment on starch stability during processing are key to determining the utilization of the starch for industry. Its effects on the dynamics of pasting parameters such as its viscosity at gelatinization, time and temperature required for maximum viscosity, pasting temperature and peak viscosity are also essential. According to Mayiza-Dixon *et. al*. (2005), gelatinization and pasting are the most important properties which influence the quality and aesthetic considerations of starch in the food industry, since they affect texture and digestibility as well as the end use of starchy foods. These parameters must therefore not be compromised by the technology.

Results on these parameters are presented in Table 4.8:

TABLE ON PASTING PROPERTIES

4.5.8.1 Gelatinization viscosity, Pasting Time and Temperature

Although enzyme treatment did not significantly affect ($P<0.05$) the viscosity at which all the starches gelatinized (Table 4.8), the technology significantly ($P>0.05$) decreased the time and temperature required for gelatinization (Table 4.8). Starch samples from the treated 'Bankyehemaa' variety required the least time and temperature (10.8 min and 65.8 ^{O}C respectively) while starches from the untreated 'Nkabom' variety needed the highest time and temperature (12.8 min and 68.1 ^{O}C respectively) for gelatinization (Table 4.8). Brandam *et al.* (2003) reported that starches with larger granule sizes require lower gelatinization temperatures due to the less degree of (amylose) association. Hence amylose molecules from these starches easily break lose from their amylose-amylose associations to interact with water leading to quicker gelation. This could have positive implications for industry as time and energy costs, factors critical to profit making, for industrial operations are both reduced. Thus enzyme treatment has the effect of reducing gelatinization time and temperature without affecting gelatinization viscosity.

4.5.8.2 Peak Viscosity, Peak Time and Temperature

Peak viscosities recorded for all starches were generally higher than reported in most literature (Ikegwu *et al*., 2009; Nuwamanya *et al*, 2009). The high viscosities might have been influenced by their very low protein content (Table 4.7) as high protein content have been reported to negatively affect pasting properties (Moorthy, 2002). Starches from enzyme treated varieties had significantly ($P<0.05$) higher peak viscosities than their corresponding controls. Peak viscosity of starches from the 'Afisiafi' were however not significantly affected ($P>0.05$) by enzyme treatment despite the long holding time. This could be probably linked to the release larger starch granules from the enzyme-treated 'Afisiafi' mashes (Table 4.6). As large starch granules tend to leach out amylose readily at higher temperatures (Lindeboom *et al.*, 2004), it is possible that the longer holding time allowed the release of more large sized granules whose amylose interacted better with water to improve viscosity.

For enzyme treated samples, starches from the 'Bankyehemaa' had the highest peak viscosity while those from the treated 'Nkabom' recorded the least (Table 4.8). High viscosity is positively correlated with the starch's inherent amylose-amylopectin ratio and granule size (Kigozi *et al*., 2013; Lindeboom *et. al.*, 2004). 'Bankyehemaa' starches had high amylose content (Table 4.7) and were more resistant to enzyme breakdown (Table 4.7); its starch granule sizes were also larger compared with those in the 'Nkabom' (Table 4.6).

Peak viscosity measures the point at which gelatinized starches reaches its maximum viscosity before their physical breakdown (Sanni *et. al.*, 2004: Tsakama *et. al*., 2010). A desirable property of starch, especially for the food industry, is its ability to attain its maximum viscosity by swelling and subsequently gelatinizing upon cooking. This property is often correlated with final product quality (good texture of paste with moderately high gel strength) (Olanrewaju and Moriyike, 2013). Hence application of the enzyme technology to food would enhance the consistency of marmalades and jams and provide better viscosity profiles to confectionaries that apply glucose, fructose and maltose syrups as well as gum Arabic, some hard candies and pizza sauce (http://www.starch.dk/isi/applic/index.asp). The technology would also benefit the textile industry in enhancing the quality of fabrics since the industry uses starch for sizing, finishing and printing of fabrics. The adhesive industry would also see an improvement in the quality of glue and spray starches used in binding surfaces.

In terms of temperature and time requirements, the technology significantly ($P<0.05$) lowered the time and temperature required for attainment of peak viscosity in the 'Afisiafi' and 'Esam bankye' varieties; these parameters were significantly ($P<0.05$) higher in the 'Bankyehemaa' but were not significantly affected ($P>0.05$) in 'Nkabom' variety. The reduction in temperature and time for peak viscosity in 'Afisiafi' and 'Esam bankye' varieties can be attributed to extensive amylolysis (or amylose degradation) (Table 4.7) as this phenomenon has been associated with a reduction in associative forces which exposes amylose to interact with water and subsequently cause its early solubilization. 'Nkabom' and 'Bankyehemaa' on the other hand, recorded the least amylolysis (Table 4.7). Although enzyme

treatment lowered peak temperature in the 'Esam bankye' (Table 4.8), the variety still required the highest temperature and time compared with those from the other treated varieties (Table 4.8). 'Esam bankye' also had the highest diversity of starch granule size with most of their large to medium granules being larger than sizes recorded in the other varieties (Table 4.6).

In terms of the economics, enzyme treatment best favours starches from the 'Afisiafi' and 'Bankye hemaa' varieties as these recorded the highest peak viscosities (810.5 and 838.5 BU respectively) at peak temperatures similar to those in the other varieties (Table 4.8). Thus, the technology signigicantly improved the peak viscosity of starches from especially the 'Afisiafi' and 'Bankyehemaa' at no significant extra energy costs. In addition, the 'Afisiafi' and 'Bankyehemaa' root mashes also recorded the highest starch contents (Figures 4.9 and 4.11) and produced high starch yields due to enzyme treatment (Table 4.5).

4.5.8.3 Paste Stability at 95^{o}C and Breakdown Viscosity

Individual viscosities of starch samples generally decreased when they were further heated to 95^{o}C. However, enzyme treatment rendered starches from the 'Nkabom' and 'Bankyehemaa' varieties significantly ($P<0.05$) more viscous than their respective controls (Table 4.8). The effect of pectolytic enzymes in releasing large starch granules (Dzogbefia *et. al.*, 2008b) might have contributed to this. Viscosity of starches (especially at high temperatures) correlates greatly with starch granule size. At higher temperatures, large-size starch granules disperse and solubilize much easier in water than smaller granules and therefore have a better tendency to associate with water molecules. In addition, the high resistance of these varieties to the effects of amylolytic enzymes (Table 4.7) might also have contributed to the observed high viscosity. Lindeboom *et al.* (2004) have reported that both high amylose content and granule size correlate positively with viscosity.

Despite pectolytic enzymes enhancing the release of large-size starch granules (Table 4.6) in the 'Esam bankye' and 'Afisiafi' varieties, their relative succeptibility to amylolysis contributed greatly to the

marked reduction in viscosity at 95^{o}C. In addition, their relatively larger granule sizes (Table 4.6) rendered the residual amylose more succeptible to futher shearing in the shear field of the Brabender (Abera and Rakshit, 2003)

After the holding period at 95^{o}C, viscosity of all starches fell (Table 4.8). A gradual decrease of the paste viscosity during the hold period indicates thermal breakdown of starch and, thus, may be considered a measure of paste stability (Maziya-Dixon *et. al.*, 2005). Paste stability explains the resistance of a starch paste to viscosity breakdown as shear is applied at a particular high temperature. Thus, differences recorded during the holding time were probably due to further granule disintegration and shearing of amylose (subsequently leading to reduction of amylose-water interactions) by the brabender. Except for the starches from the treated 'Afisiafi' variety, starches from all other enzyme treated mashes recorded significantly ($P<0.05$) higher values (Table 4.8).

Higher values for stability at a particular holding temperature suggests further granule disintegration and loss of starch crystallinity; it also indicates higher susceptibility to shearing (of amylose) in the shear field of the brabender (Maziya-Dixon *et. al.*, 2005). Hence the technology significantly reduced resistance of the starches to disintegration at higher temperatures. Starch samples from the treated 'Bankyehemaa' recorded the highest paste stability value (279 BU) at 95^{o}C. The technology's effect of enhancing the size of extractable granules (Table 4.6) could be linked to this observation as large granules have been reported to easily rupture when cooked to higher temperatures (Maziya-Dixon *et. al.*, 2005).

Generally, starches from all the varieties recorded high breakdown values suggesting after attainment of maximum viscosity, their amylose underwent high degree of fragmentation (Klucinec and Thompson, 2002; Adebowale *et. al.* 2005). Breakdown viscosity measures swelling and subsequent disintegration of starches after attainment of peak viscosity; less stable starches are usually accompanied by high values of breakdown (Shimmelis *et. al.*. 2006). Thus, the shear stabilities of the starches were generally low. From the initially high peak viscosities observed in the various starches (Table 4.8), a correspondingly

high break down value was expected. Tsakama *et al.* (2010) reported a positive correlation between peak viscosity and granule breakdown and also a negative correlation between breakdown viscosity and stability ratio (the ratio between the hot paste viscosity and peak viscosity).

Apart from the 'Nkabom' variety that was unaffected ($P>0.05$), enzyme treatment significantly ($P<0.05$) increased the breakdown value of extracted starches from all cassava varieties rendering them less viscous. This effect of the enzyme suggests that it would find very good application in the food industry. For instance, the technology could help reduce the viscosity of starches to be used in the preparation and production of custards, gravies and other starch food products that require moderately viscous consistencies (http://www.fao.org/docrep/x5032e/x5032 e06.htm). The techology could also be applied to reduce viscosity of starches to be used for low viscosity confectionaries such as gums, jellies and liquorice.

4.5.8.4 Cold Paste Viscosity (Viscosity at 50^{o}C) and Paste Stability at 50^{o}C

Upon cooling to 50^{o}C (after holding starch samples at 95^{o}C), there was a general increase in viscosity in all starch samples. This was due to the residual amylose undergoing re-association to stabilize the paste during the period. Enzyme treatment, however, significantly ($P<0.05$) reduced the viscosity of starch samples from the 'Afisiafi' and 'Esam bankye' varieties (Table 4.8) but recorded a significant ($P<0.05$) increment in viscosity in starches from the treated 'Nkabom' (Table 4.8); 'Bankyehemaa' starches' viscosity was however not significantly affected ($P>0.05$) by enzyme treatment (Table 4.8). The recorded values for viscosity at 50^{o}C give an indication of the starches' tendency to retrograde (Safo-Kantanka and Acquistucci, 1996; Tipples, 1982). Hence, enzyme treatment reduces the retrogradation tendency of starches from the 'Afisiafi' and 'Esam bankye'; enhances retrogradation in the 'Nkabom' but not sensitive to the tendency of 'Bankyehemaa' starches to retrograde. It could be inferred from the trend that initial response of starches to the technology (in terms of starches' viscosity at 50^{o}C) was mainly dependent on variety. At 50^{o}C, the 'Esam bankye' variety recorded the highest paste quality (578.0 BU) for all starches from treated varieties; this was followed by the 'Bankyehemaa' (543.5 BU).

On stability of the paste at 50^{o}C, there were no significant (P>0.05) differences between the stability of starches from the treated and untreated mashes (Table 4.8). However, starches from the untreated 'Esam bankye' and treated 'Afisiafi' varieties recorded negative values (-4.5 and -21.5 BU respectively) suggesting that these starches experienced a further increase in viscosity when starches were held at that temperature (a phenonomenon which is quite uncharacteristic of most cassava starch samples undergoing such treatment). This suggests that upon holding the starches at 50^{o}C, the amylose molecules of starches from the treated 'Afisiafi' and untreated 'Esam bankye' mashes quickly re-established strong hydrogen bonding leading to further viscosity increment of the cold pastes; the observation also suggests that the established bonds were strong enough to resist the shearing forces of the brabender. However, apart from the 'Esam bankye' and 'Afisiafi' varieties, starch samples from the treated 'Nkabom' and 'Bankyehemaa' were more stable as they recorded marginal reduction in viscosity (7.5 and 9.0 BU respectively) (Table 4.8).

Regarding cold paste viscosity (CPV) or starch viscosity at 50^{o}C, response of cassava varieties to enzyme treatment varied. CPV value was significantly (P<0.05) increased in starches from treated 'Nkabom' but decreased significantly (P<0.05) in starches from the 'Afisiafi' and 'Esam bankye' varieties (Table 4.8); enzyme treatment however did not affect (P>0.05) CPV values for 'Bankye hemaa'. For all treated samples, starches from the 'Bankyehemaa', 'Afisiafi' and 'Esam bankye' recorded the highest values for viscosity of the cold paste (CPV) of all treated samples. Cold paste viscosity is a measure of the viscosity of gelatinized starch after holding it at 50^{o}C. CPV is important in especially in foods that require cold thickening capacity like instant soups, creams or sauces. High CPV starches are used for instance in the manufacture of noodles (Akindwande *et al*., 2007). They are also known to affect the textural and sensory properties of food and considered in some food processing operations such as canning (Beta *et al.*, 2001).

4.5.8.5 Setback Viscosities

Setback viscosity of starches from most untreated varieties recorded significantly ($P<0.05$) higher values than those from their corresponding treated samples (the only anomaly occurred in the 'Nkabom' variety). Setback viscosity is a stage where retrogradation or re-ordering of starch molecules occurs after the gelatinized starch has been cooled to 50^{o}C. Thus it measures synaeresis of starch upon cooling of the cooked starch pastes (Sandhu and Singh, 2007). The lower the value, the higher the starch's tendency to retrograde (Agunbiade *et al*., 2011) and form weaker gels. This suggests that the technology had reduced the ability of the starch molecules to reassociate upon cooling. The observation could be as a result of effects of endogenous amylase in the crude pectinase on the starches. Comparing amylose contents (Table 4.7) with the setback values (Table 4.8), it is clear that starches that underwent extensive amylolysis had very low setback viscosities (Table 4.8). Although there was significant ($P<0.05$) reduction in setback viscosity (due to enzyme treatment) in the 'Bankyehemaa' starch samples, the reduction was marginal (Table 4.8) compared with the 'Afisiafi' (the next most viscose starch) which recorded a rather high reduction in setback viscosity (Table 4.8). 'Afisiafi' starches are not as high in amylose as the 'Bankyehemaa' variety (Table 4.7) and since amylose reassociation (at setback) depends on molecular size (Maziya-Dixon *et. al*., 2005), it could be inferred that the extensive loss of amylose in the treated samples of the 'Afisiafi' variety contributed to reduction in associative forces at setback.

From all the pasting parameters measured, it is clear that enzyme application generally improves the pasting quality of most starches. Although hot paste stability of starch is reduced by the technology, starch (viscosity) quality and other processing parameters (such as temperature and time) both at gelatinization and peak viscosity are significantly improved. These parameters are critical to business operations as they reduce energy costs and processing time.

Effects of enzyme application on starch usability at cold temperatures is however variety dependent and given the right choice of variety, starch usability at cold temperatures would not be negatively affected for business operations.

4.5.9 Starch Colour

A three-way analysis of variance (with interaction) was carried out on L*a*b parameters for colour. and there was found significant 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 4.9**). The sense (positive or negative) of the (L*a*b) values were also the same for all the varieties showing negative values (redness) for the *a* and positive values for the *L* and *b* (whiteness and blueness respectively) (**Table 4.9**).

Table 4.9: Comparison of starch sample colour parameters before and after enzmye treatment

*L*a*b* values for tile

L	*a*	*b*
98.00 (0.01)	-0.16 (0.00)	1.69 (0.05)

*L*a**b values for starches from control and enzyme treated mashes of the various cassava varieties

	CONTROL			TREATED		
	L	*a*	*b*	*L*	*a*	*b*
NKABOM	75.29 ± 0.00	-0.69 ± 0.02	2.77 ± 0.03	77.95±0.01	-0.71± 0.01	2.36 ± 0.01
AFISIAFI	75.72 ± 0.01	-0.52 ± 0.02	2.53 ± 0.02	83.47 ± 0.04	-0.24 ± 0.02	2.35 ± 0.07
ESAM BANKYE	78.38 ± 0.02	-0.66 ±0.01	3.23 ± 0.01	85.34± 0.01	0.02 ± 0.02	2.25 ± 0.02
DOKU DUADE	76.62 ± 0.01	-0.94 ± 0.00	3.03 ± 0.01	76.47± 0.02	-0.51 ± 0.01	2.71 ± 0.01
BANKYE HEMAA	67.89 ± 0.02	-0.08 ± 0.01	3.18 ± 0.02	78.25± 0.07	0.04 ± 0.02	2.71± 0.01

Values are represented as mean ± standard deviation (n=3); n = number of replicates

The significant difference in mean lightness between the varieties could be due to the differences in the type and amounts of proteins and the associated chromogenic compounds present in the starch. For all control samples, starches from the untreated 'Esam bankye' variety recorded the highest average value

for lightness (L=78.38) followed by the 'Doku duade' variety (L=76.62) which also recorded the highest value for redness to greenness (a= -0.94). The 'Bankyehemaa' recorded the least value (L=67.89).

All treated samples, for each variety, were also significantly whiter compared with their controls (**Table 4.9**). The slight reduction in starch fiber in starches from all treated samples (Table 4.7), although insignificant, might have contributed to the improved whiteness observed (Table 4.9) as pectinase action has been reported to improve whiteness in many processed plant products including starch (Hebeish *et al*., 2010).

Carvalho *et al*. (2002) have reported that colour differences of the parenchyma (or pulp) of clones of cassava root tubers are mainly due to varying concentrations of certain colour- producing soluble proteins produced by the tissue chromoplasts. Dominant among such compounds are members of the carotenoid group which are known to impart yellow and red colour to the tissues. In addition to proteins and carotenoids, Mégnanou *et. al*. (2009) have reported on the presence of vitamins A and C in the roots of improved selected cassava varieties both of which are possible chromogens in the starch. These as well as other mineral groups could affect the colour of starch extracted from these tissues.

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

This probably could have been influenced by the low pH of the crude enzyme medium used for extraction as adsorbed proteins might have been denatured or rendered insoluble (depending on the pI). This could also account for the significant reduction in the *a* and *b* values and increment in the L values

(as there was less colour interference from such chromogens). *L* values obtained for all controls generally seemed to increase with their corresponding ash contents (Singhal *et. al*, 1997)

There are reports (Chen *et. al*., 2002; Ikegwu *et. al*., 2010; Lestari *et. al*., 2012) that white starches are better patronized than dull coloured ones probably because of its (starch whiteness) contribution to the aesthetics of the final product. Thus, use of enzyme technology apart from enhancing starch usability, also puts starch (as well as its products) at a better position to market itself to a consumer.

4.6 Selection of Cassava variety for Industrial (Scale up) starch Production

Most starches from the varieties examined so far were faced with issues of stability at high temperatures (as is especially the case for those from enzyme treated mashes). Comparing Table 4.4 and Table 4.5, it is also seen that yields of control samples also dipped in most cases between 9 MAP and 12 MAP (respectively). However, the 'Bankyehemaa' variety continued to show good starch yield within the period and gave a better response to enzyme treatment as enzyme treatment did not greatly affect most physicochemical and functional properties of the starches (with some properties rather being enhanced in some cases). Its low root moisture content was also found to be just adequate for enhanced maceration within the 9 and 12 MAP period during which harvesting was done. In addition to its higher yield, its enhanced starch production due to enzyme treatment also seemed unaffected over the 9 to 12 MAP period (Table 4.4 and Table 4.5 respectively) implying a consistency in starch yield. Thus storage of the variety, if well carried out within this period, would not affect its yield of starch. In addition, the variety also showed good resistance to enzymatic degradation; both granules morphology and amylose content where not affected hence enabling the variety to retain (and in some cases even improve upon) most of its physico-chemical properties. The 'Bankyehemaa' variety was therefore selected and its optimum enzyme dosage-time combination (0.025% for 0.5 hours) as established at the lab stage was adopted for scale-up starch extraction.

Starch extraction at the industrial (scale–up) stage also showed impressive results. Flow rate was significantly enhanced by enzyme treatment as 3.9 L of starch milk was collected from the treated mash compared to 2.6 L from the control within the first 30 seconds (**Fig. 4.18**). The loss in the parabolic nature of the curve (as established at the lab stage) was probably due to a better grating of the samples by macerators at the Agric Research Station (Anwomaso-Domeabra, Kumasi). After the first 30 seconds of flow, both graphs were generally linear (**Figure 4.18**). However, the flow pattern of the treated sample was consistently higher than the control. The enhanced flow was due to reduction in viscosity due to enzyme treatment. The untreated mash, however, continued to give a much linear flow rates after the 30 sec. period (**Figure 4.18**) suggesting a longer waiting time for the required starch volume to be realized.

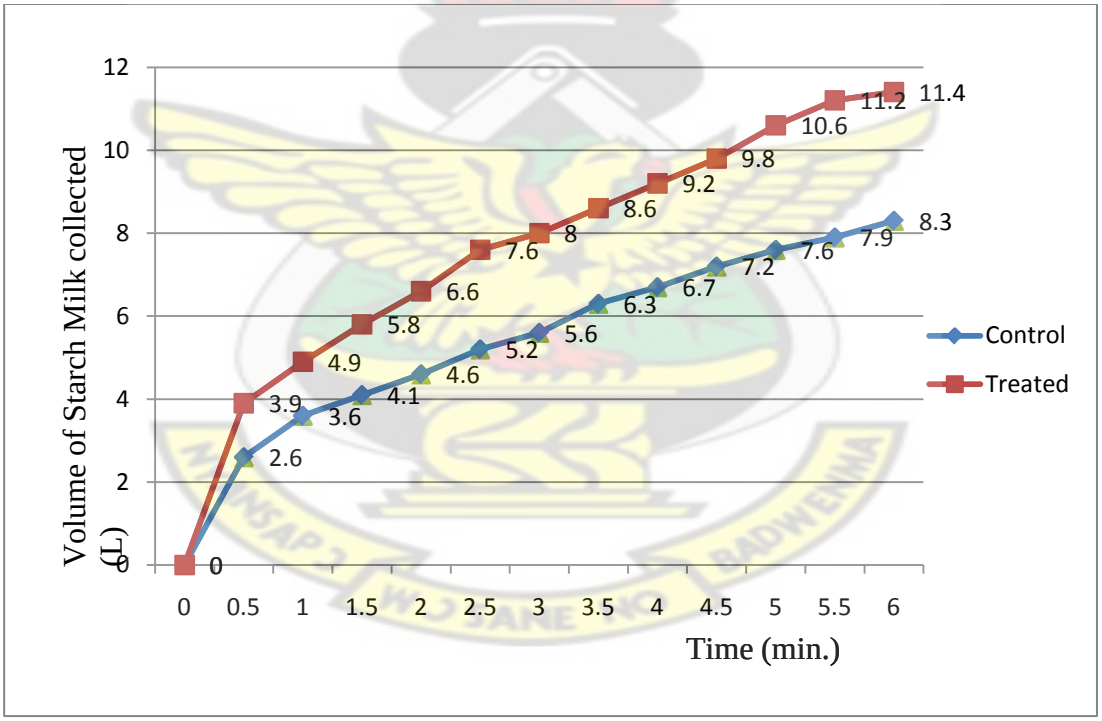


Figure 4.18: Flow Characteristics of starch milk from enzyme treated and untreated 'Bankyehemaa' root mash during scale-up extraction.

Starch yield was also improved as the control which recorded a 21.6% yield was further enhanced to 34.1% in the treated (a 44.5% increase in yield over the control). Such a marked improvement suggests

that given the cassava variety with good physical-chemical qualities and good maceration, the potential of enzyme-assisted extraction for the starch industry is limitless.

Differences in yield and flow rates observed at the industrial scale (Fig. 4.19) compared to its lab scale (Fig. 4.11 and Fig. 4.16) are probably due to differences in processing conditions. For example, large sized funnels had to be improvised in order to contain such large quantities of cassava mash at the industrial scale. Diameter of the funnel used had an outlet whose diameter was larger and would therefore allow higher milk outflow volume. In addition, differences in efficiency of raspers used at both stages would affect the final yield of starch from both untreated and treated mashes (Dzogbefia, 2008 a and b; Kordylas, 1990).

Laboratory scale work on the various cassava varieties has shown that enzyme application is not limited by variety (although responses varied) and that despite its effects of yield enhancements, physicochemical properties of the resulting starches are not affected to any large extent; in some cases, however, some of their functional properties are rather enhanced.

CHAPTER FIVE

5.1 Conclusion

Optimization of starch extraction from cassava using crude pectolytic enzymes from *S. cerevisiae* was found to be dependent on variety as well as the treatment combinations adopted. Generally enzyme dosages of 0.02% (for the 'Nkabom', 'Afisiafi' and 'Esam bankye') and 0.025% (for the 'Doku duade' and 'Bankye hemaa') were required to optimize the yield and time for starch recovery. This was related to the varying biochemical and fibre content of 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 and recovery (due to enzyme treatment) being recorded in the 'Afisiafi' (45.10 ± 11.877 and 154.5% respectively). The longer holding time required for optimization in the 'Afisiafi' variety, however, negatively affected some key physicochemical and pasting parameters such as amylose content and viscosity of the paste at 95^{o}C (as some amylase activity was recorded in the crude preparations) necessitating the adoption of 'Bankyehemaa', which is inherently higher starch yielding and also had most of its physicochemical and pasting parameters most positively affected by enzyme treatment, for scale up at the industrial level. The technology also proved effective on the selected variety ('Bankyehemaa') at the industrial stage as yield and recovery rates of 44.5% and 50.0% were recorded.

Most physicochemical parameters such as size of extractable starch granules, starch colour, water binding capacity, solubility and swelling power were positively affected by enzyme treatment; others such as crude protein, crude fibre and ash were unaffected by enzyme technology.

Thus enzyme technology, though affected, is not inhibited by varietal differences and that given the right treatment combination, the properties of the ensuing starch would not be affected; in some cases, however, such properties are rather enhanced. The work also confirmed that enzyme application is feasible at the industrial level and could therefore be adopted by the starch industry.

5.2. Recommendations:

Given the positive effects of the technology at both the lab scale and industrial level of starch production, there is no doubt that the technology would impact positively on the general operations and productivity of industries that incorporate starch in their activities. However, the observed varietal differences suggest that selection of the right variety for especially enzyme-based starch production is key. The *Bankyehemaa* variety which showed remarkable performance with the technology would be most recommended for application at the industrial scale.

However, the performance of the technology could be improved further by combining the pectinases with other enzyme treatment combinations (such as with cellulases and hemicellulases) which target and hydrolyze other cell wall components.

The presence endogenous amylases in the crude enzyme also affected some parameters of ensuing starch. However, depending on the application, the effects of its presence could either be positive or negative. For instance the food industry would find its presence beneficial as this would enhance digestibility and also provide the desirable viscosity for food application. However, the adhesive industry would find the presence of amylases in the crude extracts rather undesirable due to its effects of reducing starch viscosity. Amylase-inhibiting compounds such maltodextrins or acarbose (which competes with amylose for the active sites of amylase enzymes) (Robyt, 2005) could therefore be added to the crude preparations to help mitigate its activity prior to application in this industry.

It is also recommended that temperature used for incubation of the enzymes with cassava mash be varied to help determine what temperature would further optimize enzyme activity and starch yield.

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