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Comparative studies of starch susceptibilities to α-amylase degradation of different cereal and root crops of Nigeria

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The objective of this work was to determine the susceptibilities of starches from Nigeria's high yielding local varieties of cassava and maize crops to α-amylase. Amylose/amylpectin content of each starch samples was determined. Susceptibilities to α-amylase were studied. The amylose/amylopectin content of the four starch samples varied; amylose content of starch from maize varieties was higher than those of cassava. Large differences in enzymes susceptibilities were observed when studied within 4 h with white maize having the highest value of dextrose equivalent of 42%, followed by yellow maize 37.5%, and cassava varieties, *okoyawo* 27.3% and *odongbo* 24.75%.

Key words: *Odongbo, Okoyawo,* α-amylase, amylase, amylopectin.

INTRODUCTION

The world's attention is gradually shifting from the conventional sources of energy to the renewable. The consequences of climate change, the unstable price of fossil fuel and the recent global recession are key drivers of renewable energy. Thus, an additional emphasis is being placed on the development, production, and use of alternative fuels considered being friendlier to the environment than fossil fuels. Among these fuels or sources of energy is ethanol, which traditionally is being obtained from starch (Ugarte, 2003).

Starch is a carbohydrate consisting of a large number of glucose units joined together by glycosidic bonds. This polysaccharide is the most abundant form of storage polysaccharide in plants as it is produced by all green plants as an energy store. It is the most important carbohydrate in the human diet and is contained in such staple foods as potatoes, wheat, maize (corn), rice and cassava. Starch consists of two molecules: amylose and amylopectin. Amylose is formed from glucose linked by α-1,4 and amylopectin is formed from α-1,4-linked chains of glucose with α-1,6-linked branch points. Starch, by virtue of its various chemical and physical properties, can be processed into products that can have a variety of performance applications. These vary from the use as an energy carrier, an adhesive to a variety of food or feed applications or use as a source of the chemical glucose used as a feed stock for the fermentation industry to produce bio-ethanol (Guzman-Maldonado and Paredes-Lopez, 1995; Giordano et al., 2000).

The hydrolysis of starch to products with low molecular weight, catalyzed by α-amylase is one of the most important commercial enzyme processes. The enzymatic susceptibility of starch granules has been studied by various authors (Leach and Schoch, 1961; Franco and Ciacco, 1987; Franco et al., 1988). The susceptibility and mode of enzyme action depend on the starch source and enzyme system (Franco et al., 1988). Also, the susceptibility of starch to hydrolysis by α-amylase has been

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shown to vary with botanical origin. Extent of digestibility is known to be related to crystalline polymorphic forms, and it is accepted that starch with 'A' type X-ray diffraction is more susceptible to amylolysis than that with a 'B' type pattern (Jane et al., 1997; Planchot et al., 1997; Valetudie et al., 1993). A number of work have been conducted on enzymatic hydrolysis of starch in Nigeria, but none has really been reported, directed at investigating the susceptibility of different native starches to α-amylase hydrolysis.

Nigeria (world leading producer of cassava) has a strong incentive to become a major player in the development of the Nigerian bio-ethanol industry, by investigating which of her abundant starch sources is/are appropriate for economical conversion to ethanol. To that end, the present work was accordingly undertaken to elucidate the apparent differences in the hydrolysis of starches high yielding local cultivars of cassava and maize in Nigeria.

Furthermore, most of the previous work on cassava and maize starches susceptibility to α-amylase was based on studies on a single cultivar. This approach makes the results difficult to interpret, since it is not known, whether the data truly represents the species in general. Thus, a comparative study of the susceptibility of cassava and maize starches belonging to different cultivars towards α-amylase may lead to the identification of the structural factors that limit α-amylolysis. This in turn may help us understand as to which source of starch is most appropriate for bio-ethanol production in Nigeria's bio-ethanol programme. The objective of this study was three fold: (1) to determine the amylose/amylopectin contents of starches from cultivars of cassava and maize. (2) to determine the susceptibility of the above starches towards α-amylase hydrolysis; (3) to relate the differences in the rate and extent of α-amylase hydrolysis to differences in amylose/amylopectin contents.

MATERIALS AND METHODS

Yellow and white maize varieties were bought from a local Alamisi Market in Ikirun. Preparation of starch from maize varieties, as well as production and characterization of α-amylase used was shown in our previous works (Adejumo et al., 2009, 2012). Cassava tubers *Okoyawo (Manihot esculenta)* and *Odongbo (Manihot utilisima)* were bought from a local farmer in Ejigbo. Cassava starch was isolated from fresh roots according to the method described by Srichuwong et al. (1999).

Cassava tubers were washed with water, peeled, then, cut into small pieces. The pieces were suspended in water (1:4 w/v) and ground in attrition mill. The milled cassava was filtered through two layers of muslin cloth. The filtrate was filtered through a net with a 125 µm mesh width and allowed to settle. The supernatant was discarded and the precipitate re-suspended in water. The supernatant was discarded together with brown material on the surface of the precipitate and the starch layer was re-suspended in water. The re-suspension steps were repeated five times and the final material sun dried.

Determination of starch concentration in prepared cassava and maize starch flour

Starch flour (1.0 g) was gelatinized in 100 ml distilled water (1.0% starch slurry). The quantities of 0.2, 0.4, 0.6, 0.8 and 1.0 ml aliquots were taken and mixed with 5.0 ml of iodine solution (0.5% KI and 0.15% I_2). The final volume was adjusted to 15.0 ml by adding distilled water. The absorbance was measured at 550 nm against a blank containing 5.0 ml of iodine solution and 10 ml of distilled water. Absorbances were then converted to starch concentration using the calibration curve (Apar and Ozbek, 2004). In preparing the starch caliberation curve, the concentrations of starch were varied between 0.1 mg/ml to 1.0 in seven test tubes. 5.0 ml of iodine solution was added to each tube, and appropriate volume of distilled water was added to bring the volume in each tube to 15.0 ml.

Amylose/amylopectin content determination

Amylose content of the starches was determined by following the method of Williams et al. (1970). A starch sample (20 mg) was taken and 10 ml of 0.5 N KOH was added to it. The suspension was thoroughly mixed. The dispersed sample was transferred to a 100 ml volumetric flask and diluted to the mark with distilled water. An aliquot of this solution (10 ml) was pipetted into a 50 ml volumetric flask and 5 ml of 0.1 N HCl was added followed by 0.5 ml of iodine reagent. The volume was diluted to 50 ml and the absorbance was measured at 625 nm. The measurement of the amylose was determined from a standard curve developed using amylose and amylopectin blends.

Enzymatic hydrolysis of various starches

Enzymatic hydrolyses of cassava and maize starches were performed according to the method of Franco et al. (1987) with some modifications. Two high yielding local varieties of cassava (namely: *Okoyawo and Odongbo*) as well as maize varieties (white and yellow maize) were chosen for the study. Starch samples hydrolysis were conducted at a temperature of 70°C in a 50 dm^3 batch reactor to be gently stirred with a simple paddle agitator and no baffle. 10.0 kg (20%) sample of starch was dispersed in 0.2 M accetate buffer (pH was adjusted to 6.0) in the hydrolyser. The α-amylase concentration of 9.0 KNU/100 g suspension (1 KNU equals 1000 Units) was used. The mixtures were hydrolyzed for 4 h, during the period, and samples were taken at 30 min interval. The samples taken were centrifuged after stopping the enzymatic activities by placing the samples in boiling water for 5 min. The extent of liquefaction was determined by measuring a decrease in residual starch and an increase in reducing sugar content by analysing the supernatant for reducing sugar by DNS method. The percentage of hydrolysis was calculated using the following equation:

$$\text{Percentage of hydrolysis} = \frac{(g\ of\ starch\ before\ hydrolysis\ (dry\ basis) - (g\ of\ starch\ after\ hydrolysis\ (dry\ basis)}{(g\ of\ starch\ before\ hydrolysis\ (dry\ basis))} \times 100$$

Determination of the residual starch concentration

For determination of the residual starch concentration (Astolfi-Filfo, 1986), samples were taken at timed intervals to determine the starch concentration in the reaction solution. 5 mL iodine solution, (0.5% KI and 0.15% I_2) and 3 ml of the samples were mixed. The

Table 1. Total starch concentration.

Variety	Yield of pure starch (%)
Odongbo	47.5
Okoyawo	42.97
Yellow maize	39.5
White maize	42.86

final volume was made up to 15 mL by addition of distilled water. The absorbance was measured at 550 nm against a blank containing 5 mL of iodine solution and 10 mL of distilled water. Absorbances were converted to starch concentration using standard curve prepared under the same condition.

RESULTS AND DISCUSSION

Starch isolation

The isolation of starch from cassava roots was difficult due to the presence of insoluble flocculent protein and fine fiber which co-settled with the starch to give a brownish deposit. Similar difficulties were encountered during starch extraction from sour cassava (Rani, 1998). Re-washing with water resulted in removal of most of the remainder of the attached protein. The yields of starch recovered from the cassava roots and maize grains are as shown in Table 1.

Amylose/amylopectin content

From Table 2, the starches have significantly different amylose/amylopectin contents. Results reported are the average values of measurements in triplicate. The starch from the two cassava varieties *okoyawo* and *odongbo* had the lowest amylose contents of 22.11 and 27.96%, respectively. The amylose contents of maize varieties were higher than those of cassava starches, with white maize (40.68%) higher than yellow maize starch (35.77%). Conversely, the amylopectin content was higher for cassava starch than maize starch. They were in the order of 77.89, 72.04, 64.24 and 59.33% for *okoyawo, odongbo*, yellow maize and white maize, respectively. These values are similar to those of waxy maize amylopectin reported by Bello-Perez et al. (1998), Yokoyama et al. (1998) and Klavons et al. (1997). These low values for the corn amylopectin fraction might be caused by molecular degradation during the starch desolution treatments or by a low recovery due to the loss of large amylopectin molecules (You and Lim, 2000).

Traditionally, differences among cassava varieties, and maize varieties were attributed to botanical sources and field growing conditions (Charles et al., 2005). Moorthy and Ramanujam (1986) reported that the amylose contents of cassava starch obtained from six varieties grown in India did not vary significantly with the age of the crop. However, varietal differences in amylose contents were noticed. The authors analyzed cassava starch of six varieties harvested at monthly intervals between 2 and 18 months. In agreement with the amylose contents of *okoyawo*, they reported amylose contents varying between 17 and 24%.

Asaoka et al. (1991) found amylose contents in cassava starch isolated from roots of four cultivars harvested 10 months after planting to vary from 16 to 20%. From their study, the authors concluded that cultivars did not differ significantly in starch amylose contents, although some impact of the season of cultivation was noted.

Progress of hydrolysis of various starches

Starches from two different varieties of cassava and maize were employed in this study to understand their susceptibilities to α-amylase. In this work, the extent of hydrolysis of maize and cassava starches by α-amylase extracted from *Bacillus cereus* is presented in Table 2. From the results, it was apparent that maize starches were better substrates than cassava starches, undergoing 42 and 37.5% hydrolysis in 4 h, respectively. The corresponding values for cassava starches ranged from 24.75% in *odongbo* to 27.3% *okoyawo*. All the starches showed fairly close similarities in hydrolysis rates during the initial 2 h, and only in the later stages were differences in degree of hydrolysis apparent among the starches. Starches of different sources display considerable differences in their susceptibility to enzyme action. Jane et al. (1997) reported that cassava starch with B-type X-ray diffraction pattern is more resistant to amylolysis than cereal starches with A-type pattern. Jane et al. (1997) postulated that the difference in amylolysis among different crystalline types arrived from variation in the location of their amylopectin branch points. The presence of more A-chains (DP 6–12) and branch linkages in the crystalline lamellae of A-type starches produced ‘weak’ points that were more susceptible to enzyme hydrolysis. In B-type starches, more branch points were found in the amorphous region and thereby provide a more superior crystalline structure that is resistant to hydrolysis.

Gallant et al. (1997) proposed that α-amylolysis was affected by the size and arrangement of starch molecules in the amorphous and crystalline lamellae and their interactions with non-starch components. Recently, Zhou et al. (2004) proposed that the formation of crystalline regions from hydrolyzed amylose chains during hydrolysis could also hinder the accessibility of α-amylase to glucosidic bonds. Some researchers proposed that the resistance of cassava starch (B-type) to enzyme hydrolysis may be attributed to its larger blocklets arranged near the surface compared with smaller blocklets in A-type starches (Baldwin et al., 1998;

Table 2. Comparison table of macromolecule components and percentage hydrolysis of starches.

Variety	Amylopectin (%)	Amylose (%)	Percentage of hydrolysis (%)
Odongbo	72.04	27.96	24.75
Okoyawo	77.89	22.11	27.3
Yellow maize	64.24	35.77	37.5
White maize	59.33	40.68	42

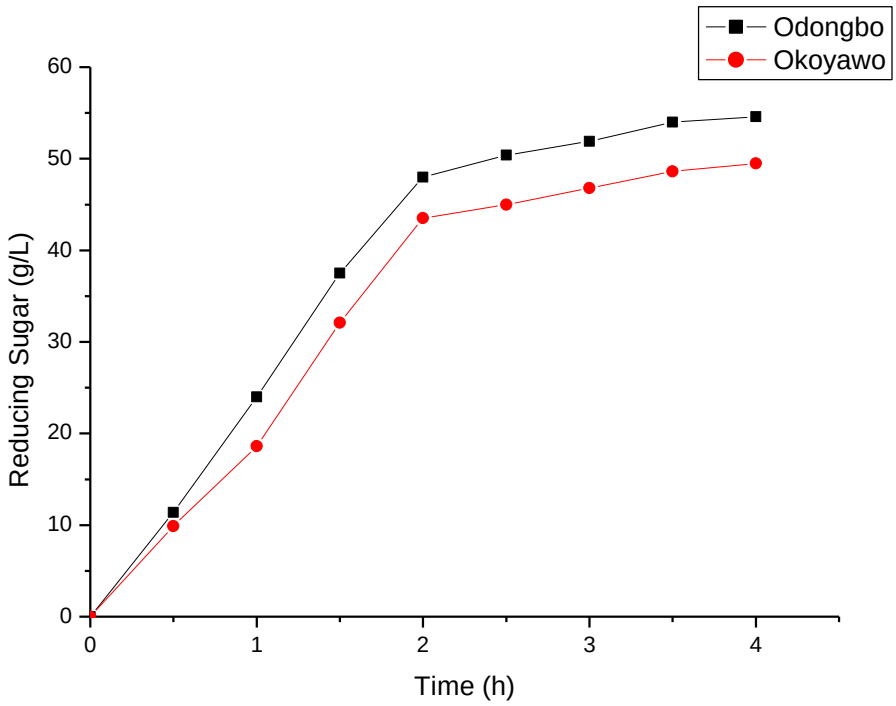

Figure 1. Hydrolysis kinetics of the native cassava starches by *B.cereus* α-amylase in 4 h hydrolysis.

Gallant et al., 1992, 1997; Lin et al., 1998).

These results suggest that α-amylase may preferentially hydrolyze certain granules or that differences in enzyme susceptibility may exist among granules. Moreover, the difference in amylolytic susceptibility between maize and cassava starches could be related to starch molecules in the crystalline region of maize starch being more loosely packed and thus being more accessible to enzymic attack than those of the high-amylose containing starches. Budenhuizen (1959) has postulated that granules which are most susceptible to enzyme have pores or a coarse sponge-like structure with openings of a size sufficient for the entry of enzyme molecules. However, Rosenthal and Nakamura (1972) have shown that jack bean starch, which presents many pores and fissured granules, was much less solubilized than chick pea and lablab beans which were devoid of such fissures and pores.

Also, Franco et al. (1987) reported that the hydrolysis products obtained from cassava starch were more efficient in inhibiting enzyme action. This might as well be another reason why hydrolysis percentage of maize starch was higher than that of cassava.

Progress of hydrolysis of cassava starches

The enzymatic action (α-amylase/amyloglucosidase) on the cassava starch granules occurs mainly on the granular surface and is characterized by an erosion and solubilization of the granular surface. The corrosion channels are mainly formed in the larger diameter granules with the formation of a porous surface (Franco et al., 1992).

In this study, percent hydrolysis among the two cassava starches varied significantly with *odongbo* having the higher (27.3%) and that of *okoyawo* having the lower value (24.5%) after 4 h of hydrolysis (Figure 1). At the first 2 h, the two starches were rapidly hydrolysed and then the rate of hydrolysis decreased significantly.

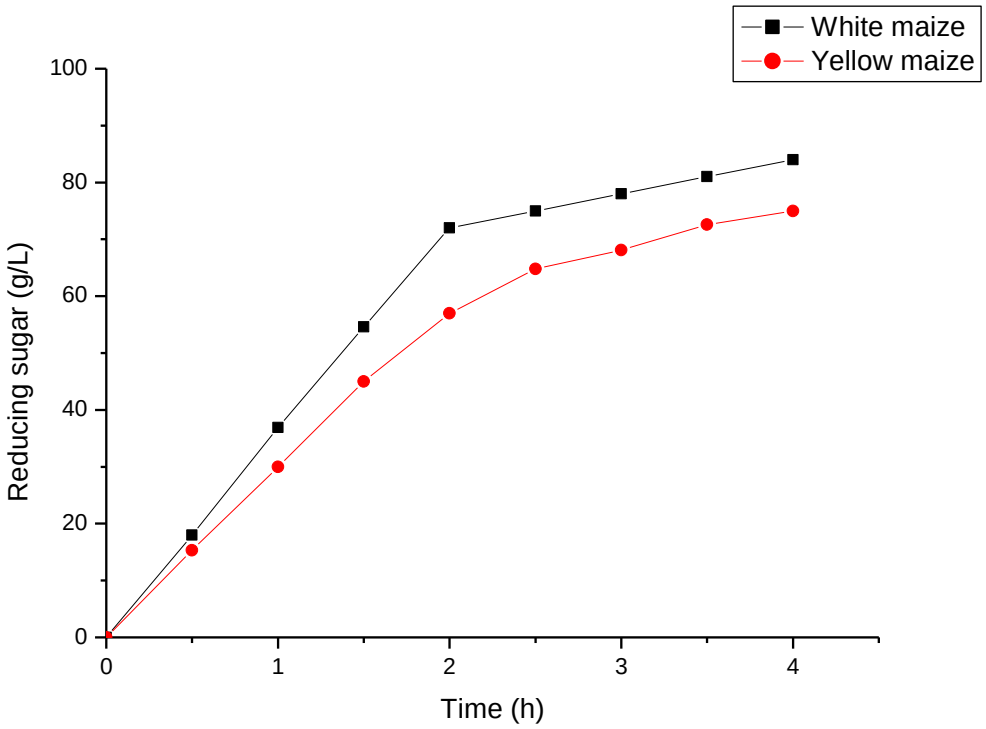

Figure 2. Hydrolysis kinetics of the native maize starches by *B.cereus* α-amylase in 4 h hydrolysis.

The hydrolysis percentages and the amylose content demonstrated that enzymatic hydrolysis of cassava starches followed two distinct steps: in the first one, characterized by a higher rate of hydrolysis, a quick degradation of the amorphous areas of the starch granules occurred in the first 2 h as shown in Figure 1; the second step was characterized by a lower rate of hydrolysis, due to a high resistance to hydrolysis of the granule crystalline regions. Raw starch digestibility is greatly influenced by plant type and depends on physicochemical characteristics of the starch and plant microstructure and composition, and is influenced by processing and storage conditions (Ring et al., 1988). Size of starch granules may affect digestibility, as the relationship between surface area and starch volume, and thus contact between substrate and enzyme, decreases as the size of granule increases (Svihus et al., 2005). Digestibility of starches was negatively correlated to molecular weights (Mw) of amylopectin and amylose. A similar inverse relationship between Mws of amylose and amylopectin and digestibility has been previously reported in chickpea and finger millet starches (Madhusudan and Tharanathan, 1996).

Progress of hydrolysis of maize starches

Maize starch granules have channels connecting the internal cavity with the external environment (Huber and BeMiller, 1997) therefore the hydrolytic enzymes had access to the interior of the granules via channels (Hood and Liboff, 1983), which results in its high digestibility. Dhital et al. (2010) suggested it is likely that the pores, channels and cavities, characteristic of maize starch cause maize starch to have a much high effective surface area. Qualitative support for this is provided by electron micrographs of partially digested granules (Hood and Liboff, 1983).

The time course of α-amylase hydrolysis of white and yellow maize starches in this study is presented in Figure 2. Hydrolysis occurred in the following phases: rapid hydrolysis (0 to 2 h) and slow hydrolysis (2 to 4 h) leading to maximal hydrolysis. Starches from the two varieties of maize showed variable susceptibilities to *B. cereus* α-amylase attack. The degrees of hydrolysis are 37.5% (yellow maize) and 42.0% (white maize). The slow hydrolysis of yellow maize starch compared to white maize starch could also be ascribed to the presence of pigments (tannin and polyphenol) which may inhibit the enzymatic action.

The amylopectin content of the two starches were 59.33% (white maize) and 64.24% (yellow maize). This suggests that the amylopectin content was inversely related to susceptibility by *B. cereus* α-amylase attack. This result agrees with the result obtained by French (1984). However, the result was at variance with those

found by Franco et al. (1992), where it was reported that susceptibility to the hydrolysis was higher for granules with low levels of amylose, indicating that the enzymatic hydrolysis occured in the branched starch fraction.

Macromolecular components and hydrolysis of starches

The effects of different mutant genotypes on the proportion of amylose and amylopectin are well-documented (Shannon and Garwood, 1984). Therefore, the variations in amylopectin and amylose proportions, structures, and contents would result in starch granules with different chemical and physical properties (Charles et al., 2005). One chemical property of interest is the susceptibility of starches to α-amylase. It is generally accepted that higher amylose content is the major factor contributing to lower peak viscosity and higher setback (Charles et al., 2005). Conventionally, cassava starch (Bahnassey and Breene, 1994), due to the low levels of amylose to reinforce the molecular network within the granules, demonstrated high peak viscosities than maize starch. Based on this findings, it is suggested that there will be more resistance to the mass transfer of enzyme in the more viscous gelatinized cassava starch.

The amylopectin contents of the four starch samples (Table 2) were ranked in the order of *okoyawo*>*odongbo*>yellow maize>white maize, conversely, the order of hydrolysis was odongbo<okoyawo<yellow maize<white maize. This suggests that the amylopectin content was inversely related to susceptibility by α-amylase attack. This result is in accordance with the cluster and double helix structure model proposed by French (1984), in which the lightly bonded double helices offer resistance to enzyme or chemical attack. The amorphous regions, which can easily be degraded by enzyme, are present throughout the granule. Amylose, which has higher enzyme susceptibility, is present in the amorphous regions in the form of single helical structures. Branch points of amylopectin are thought to be located in the amorphous region, but poorly hydrolysed by α-amylase, due to the $(1 \rightarrow 6)$ configuration of the glucosidic bond. In this study, a higher proportion of amylopectin results in greater resistance to enzymatic degradation.

Conclusion

It was inferred from this investigation that starches separated from root and cereal crops, mainly of Nigeria, showed obvious differences in amylopectin and amylose ratio and depended on their varieties. The susceptibilities to α-amylase in cassava and maize starches were primarily influenced by the granule structure, such as amylopectin and amylose ratio. High amylopectin content in the starches showed less susceptibility to α-amylase attack.

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