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Relationship between α-amylase degradation and amylose/amylopectin content of maize starches

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ABSTRACT

Many unique functional properties of starch have been utilized for industrial applications. These properties are influenced by the granular and molecular structures of starch.The amylose/amylopectin content in two maize starch samples of differing cultivars were measured. The starches were then subjected to enzymatic digestibility by α-amylase. The degree of hydrolysis of each starch sample was compared with amylose/amylopectin content. 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 amylopectin content of the two starches are 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. The maize starch with the higher amylose content has the higher value of dextrose equivalent.

INTRODUCTION

Maize (*Zea mays*) is a plant belonging to the cereal family, an important economic crop that can adapt successfully to a wide range of habitats, including marginal regions. Artificial selection of maize, as well as the occurrence of natural hybrids and mutations, has resulted in the existence of a very large number of cultivars. These varieties differ in many of their properties, ranging from the physical appearance and texture of the grains to structure-function properties of the starch. Among a variety of starch cultivars, maize starch has been of particular scientific interest since this kind of starch with different amylose/amylopectin ratios can be directly provided by the nature/agriculture (Liu *et al*., 2011).

Normal starch consists of two types of polysaccharide: amylose and amylopectin. Amylose is fundamentally a linear molecule of α-1,4-linked glucan and occupies approximately 15–30% of starch, while amylopectin, the major component (70–85%), is a larger molecule with highly α-1,6 branched chains. Interest in hydrolysis of starch to products with low molecular weight, catalyzed by an α-amylase has increased in recent years because starch hydrolysis is one of the most important commercial enzyme processes. The hydrolyzed products are widely applied in food, paper, textile and fermentation industries (Nigam and Singh, 1995; Marshal *et al*., 1999; Crabb and Mitchinson, 1999; Pandey and Nigam, 2000).

The enzymatic susceptibility of starch granules has been studied by various authors (Leach and Schoch, 1961; Evers and McDermott, 1970; Franco and Ciacco, 1987; Franco *et al*. 1988; Zhang and Oates, 1999; Srichuwong *et al.*, 2005). These studies have shown that starches vary in their resistance to the action of α-amylase. Starch susceptibility to enzyme attack is influenced by several factors, such as amylose and amylopectin content (Dreher *et al*., 1984; Hoover and Sosulski, 1985; Holm and Bjorck, 1988; Ring *et al*., 1988), crystalline structure, particle size, surface porosity (Huber and BeMiller, 1997; Kong *et al.*, 2003), extent of molecular association between starch components (Dreher *et al.*, 1984), and the presence of enzyme inhibitors.

The susceptibility of maize starches towards α-amylase in terms of enzyme adsorption, action pattern, extent of hydrolysis, hydrolysis products, and structure and properties of the enzyme resistant residues has been reported. However, most of the previous work on maize starch susceptibility to α-amylase was based on studies on a single cultivar. This approach makes the results difficult to interprete, since it is not known, whether the data truly represents the species in general. Thus, a comparative study of the susceptibility of maize starches belonging to different cultivars towards α-amylase may lead to the identification of the structural factors that limit α-amylolysis. To more fully understand difference in susceptibility of the starches to the extracted α-amylase, amylopectin and amylose content of the starches were determined.

The objective of this study was three fold: (1) to determine the amylose/amylopectin content of starches from cultivars of yellow and white maize; (2) to determine the susceptibility of the above starches towards α-amylase hydrolysis; (3) to determine the effect of enzyme concentration on the starch; and (4) to relate differences in the rate and extent of α-amylase hydrolysis to differences in maize starch amylose/amylopectin content.

MATERIALS AND METHODS

2.1. Materials

Yellow and White maize varieties were bought from a local Alamisi Market in Ikirun. Preparation of starch from maize varieties was shown in Adejumo *et al.* (2009). Production and characterization of α-amylase used was shown in our previous work (Adejumo *et al.* in press).

2.2. Methods

2.2.1. 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.

2.2.2. Effect of α-amylase concentration on the hydrolysis of starch in white maize flour

Maize flour (20.0 %, w/w) in suspension in water (pH adjusted to 7.0) was hydrolyzed for 3.0 h by different concentrations of α-amylase (from 1.0 to 12.0 KNU/100g suspension) at 70 °C. 1 KNU equals 1000 Units. Reducing sugar and recovery of starch were 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.

2.2.3. Enzymatic hydrolysis of various starches

Enzymatic hydrolyses of corn starches were performed according to the method of Franco *et al.* (1987) with some modifications. Two high yielding local varieties of white and yellow maize were used for the study.

Starch samples hydrolysis were conducted at a temperature of 70 ^{0}C in a 50 dm^3 batch reactor to be gently stirred with a simple paddle agitator and no baffle. (20 %) of samples of starch were dispersed in 0.2 M accetate buffer in the hydrolyser. The mixtures were hydrolyzed for 4 h, during which period, 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 (Srichuwong *et al.*, 2005).
The percentage of hydrolysis was calculated using the following equation:

Percentage of hydrolysis =

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

2.2.4. Determination of the residual starch concentration

The determination of the residual starch concentration was carried out according to Astolfi-Filfo (1986). Samples were taken at timed intervals to determine the starch concentration in the reaction solution. Iodine solution, 5 mL (0.5% KI and 0.15% I_2) and 3 ml of the samples were mixed. The 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. Absorbancies were converted to starch concentration using standard curve prepared.

2.2.5. Reducing sugar analysis

The extent of hydrolysis was measured by the 3,5-dinitrosalicylic acid (DNS) assay (Miller 1959) which measured total reducing sugar. Samples were centrifuged for 10 min at 4,000 rpm to remove any remaining suspended matter; distilled water was added to dilute the samples within the concentration range of the calibration standard. DNS reagent consisting of an aqueous solution of 1% 3,5 dinitrosalicylic acid, 0.05% sodium sulphite, 20% sodium-potassium tartrate and 1% NaOH solution was added in the ratio 3:1 to the samples in glass tubes, shaken and incubated in a boiling water bath for 8 min. The absorbance of the reacted samples was measured using spectrophotometer at absorbance of 540 nm.

RESULTS AND DISCUSSION

3.1. Amylose/Amylopectin content

Table 1 shows the amylose and amylopectin content of granular ('raw') of white and yellow maize starch. Amylose is a long, mostly linear glucose polymer with a typical molecular weight of about 105–106 corresponding to degree of polymerization of 500–5000 (Takeda *et al.*, 1987). Normal maize starch is composed of 30% primarily linear amylose and 70% highly-branched amylopectin, which are organized in granules with a semi-crystalline structure of double helices (Jiang *et al*., 2010). From Table 1, the starches have significantly different amylose/amylopectin contents which are higher than the values obtained for normal maize. However, the amount of amylose fall within the range of previously reported values of high amylose maize starches by Htoon *et al*., 2009.

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 maize varieties were attributed to botanical sources and field growing conditions (Charles *et al.*, 2005).

Table 1: The amylose/amylopectin content distribution of the various starches

Starches	% Amylose	% Amylopectin
Yellow maize	35.77	64.24
White maize	40.68	59.33

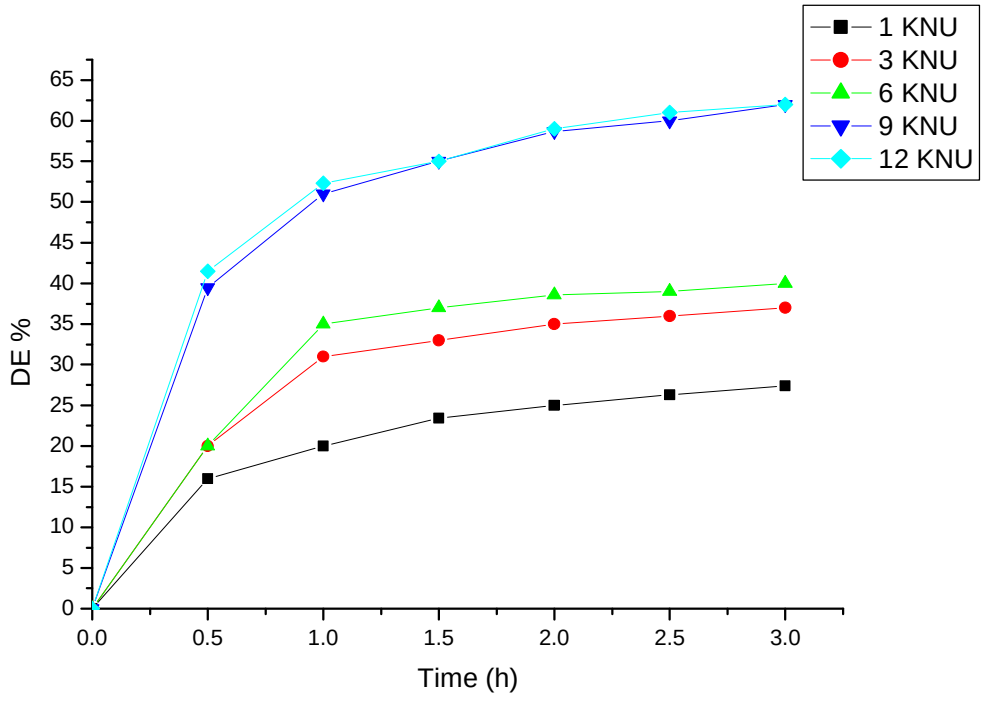

Fig. 1: Effect of different concentrations of α-amylase activity on the Hydrolysis of starch in maize flour as a function of time at pH 7.0 and 70 °C.

3.2. Effect of α-amylase concentration on the hydrolysis of starch in maize flour

Fig. 1 shows the percentage of hydrolysis during the period that the maize starch was incubated with several concentrations of α-amylase. Different α-amylase concentrations used for hydrolysis of 100.0 g maize flour suspension (20 %, w/w) were from 1.0 to 12.0 KNU (ie. 1.0, 3.0, 6.0, 9.0 and 12.0 KNU). 1 KNU equals 1000 Units/ml. Regardless of the concentration of α-amylase the percentage of hydrolysis increased up to a certain incubation time (3 h), with a tendency to stabilize after this time. An increase in the concentration of α-amylase caused increases in the percentage of hydrolysis. Hydrolysis by 9.0 and 12.0 KNU/100 g suspension gave similar results (Fig. 1). At 3.0 h, 62.0 and 62.0 % of the added starch was recovered as sugar/dextrins when 9.0 and 12.0 KNU/100 g suspension were used and hence α-amylase activity of 9.0 KNU/100 g suspension of maize flour (20 %, w/w) was selected.

3.3. Progress of hydrolysis of maize starches

Maize starch granules had 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, 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.

In this study, the time course of α-amylase hydrolysis of white and yellow maize starches is presented in Fig. 2. Hydrolysis occurred in the following phases: rapid hydrolysis (0-2 h), slow hydrolysis (2-4 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 amylopectin content of the two starches are 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 as indicated in table 2 which shows relationship between amylose/amylopectin content and and the degree of hydrolysis. This result agreed with the result obtained in 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.

The slow hydrolysis of yellow maize starch comparing to white maize starch could also be ascribed to the presence of pigments (tannin and polyphenol) which may inhibit the enzymatic action.

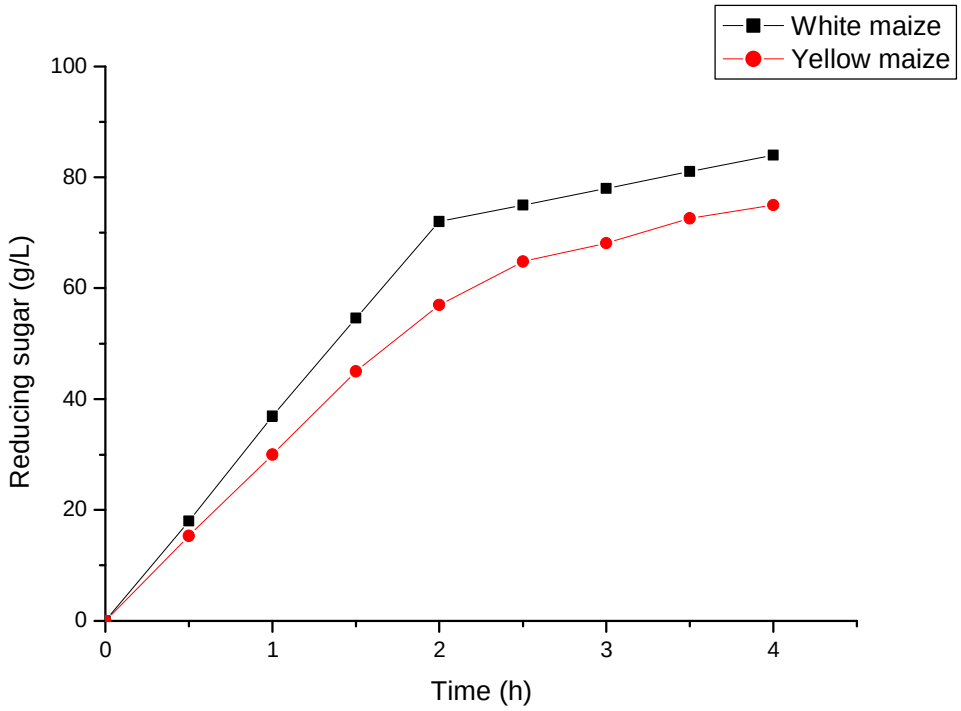

Fig. 2: Hydrolysis kinetics of the native maize starches by *B.cereus* α-amylase in 4 h hydrolysis.

Table 2: Comparison table of macromolecule components and hydrolysis of starches

Variety	Amylopectin (%)	Amylose (%)	Degree of hydrolysis (%)
yellow maize	64.24	35.77	37.5
white mpaize	59.33	40.68	42

CONCLUSION

The hydrolysis percentages and the amylose content demonstrated that enzymatic hydrolysis of both yellow and white maize 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; the second step was characterized by a lower rate of hydrolysis, due to a high resistance to hydrolysis of the granule crystalline regions.

It was shown that white maize starch with higher amylose content had higher degree of hydrolysis than yellow maize. The result indicated that maize starch with higher level of amylose content is more susceptible to α-amylase amylolysis.

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