Pearl Millet Amylases. II. Activity Toward Intact and Heated Starch Granules[1]

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ABSTRACT

The effects of pearl millet amylases on intact starch granules and heated starch suspensions were studied. Amylases in crude millet extracts showed higher amylolytic action on wheat starch than on millet starch, both in the amylograph determinations and in studies on hydrolysis of raw starches. Products formed from raw and gelatinized millet starch and starch fractions by hydrolysis with purified millet α-amylase were studied by paper chromatography. The action pattern of millet α-amylase was similar to that of other cereal α-amylases, with the rate of appearance of hydrolysis products being dependent on the particular starch substrate.

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Pearl millet populations and hybrids contain an active amylase system, with purified α-amylase showing characteristics similar to those reported for amylases from immature cereal grains (Beleia and Varriano-Marston 1981). Although pearl millet starch has been extensively studied (Badi et al 1976, Beleia et al 1980), no information has been published on its susceptibility to amylolytic attack. We report here the effects of pearl millet amylases on millet starch and starch fractions.

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MATERIALS AND METHODS

Materials

A random mating bulk population of pear millet (HMP02), grown at Hays Branch Experiment Station in 1978, was used as the source of crude amylases and of purified α-amylase.

Commercial wheat starch was obtained from Midwest Solvents.

Pearl millet starch was isolated from the grain according to Beleia et al (1980). Starch samples contained less than 0.5% starch damage as determined by the AACC method (1972).

Alpha-Amylase Isolation

α-Amylase was isolated and purified from pearl millet by solvent fractionation and glycogen complex formation. Details of the purification procedure and enzyme properties have been reported (Beleia and Varriano-Marston 1981).

Starch Amylograms

We determined effects of crude enzyme extracts from pearl millet (HMP02) on wheat and millet starch amylograms. Ground millet (3–60 g) was extracted with phosphate-citrate buffer (pH 5.3) for 1 hr and centrifuged at 4,000 rpm for 15 min. Phosphate-citrate buffer was used to extract the amylase from the grain because that is the standard buffer used for determining diastatic activity in flours (AACC 1972). The precipitate was discarded and the supernatant diluted with buffer to 460 ml. The resulting solution then was used to determine the effect of amylases on wheat starch (11% db, w/v) or millet starch (9% db, w/v) amylograms. Total enzyme concentration in the buffer extract was estimated by the Phadebas method (Barnes and Blakeney 1974). Phadebas tablets were calibrated against purified enzyme in enzyme milliunits (mU). One unit of amylase activity was expressed as μmoles of apparent maltose produced per milliliter of digest per minute. The starch suspension was heated from 30 to 95°C in a Brabender Amylograph having a 700-cm-g head and operating at 75 rpm.

Paper Chromatography

The nature and sequence of products formed from various substrates by the action of purified α-amylase were determined by qualitative paper chromatography. The substrates studied were gelatinized and raw millet starches and amylose and amylopectin prepared from millet starch by the method of Montgomery and Senti (1958). All substrates except raw millet starch were used at 0.5% concentration; raw starch was used at a concentration of 50 mg/0.5 ml of solution. For studies on starch fractions and gelatinized starch, we used an enzyme concentration of 430 mU/mg of substrate. Higher enzyme concentrations (2,150 mU) were used for studies on raw millet starch. All samples were incubated at 35° C and sampled as hydrolysis progressed. A few drops of toluene were added to prevent microbial growth. Chromatograms were devloped by the method of Robyt and French (1963). Carbohydrate standards were produced by acid hydrolysis of wheat starch and subsequent separation on a charcoal column.

Starch Hydrolysis

The hydrolysis of raw wheat and millet starch by a dialyzed crude extract of millet was followed by incubating 250 mg of raw starch with 7 ml of millet extract (20 g of meal plus 50 ml of 0.01 *M* acetate buffer, pH 4.8, containing 0.01 *M* $CaCl_2$) at 35° C. After designated times, the suspension was centrifuged (2,500 rpm, 10 min), and total carbohydrate in the supernatant was determined by the phenolsulfuric acid method (Dubois et al 1956).

Scanning Electron Microscopy

Starch granules hydrolyzed by millet amylases were examined by scanning electron microscopy. Granules were separated from the amylase solution by centrifugation, rinsed three times with distilled water, and dehydrated with ethanol. The dried starch was sprinkled on double-sided adhesive tape attached to specimen stubs. Samples were coated with gold-palladium, and micrographs were taken on an Etec U-1 scanning electron microscope at an accelerating voltage of 10 kV.

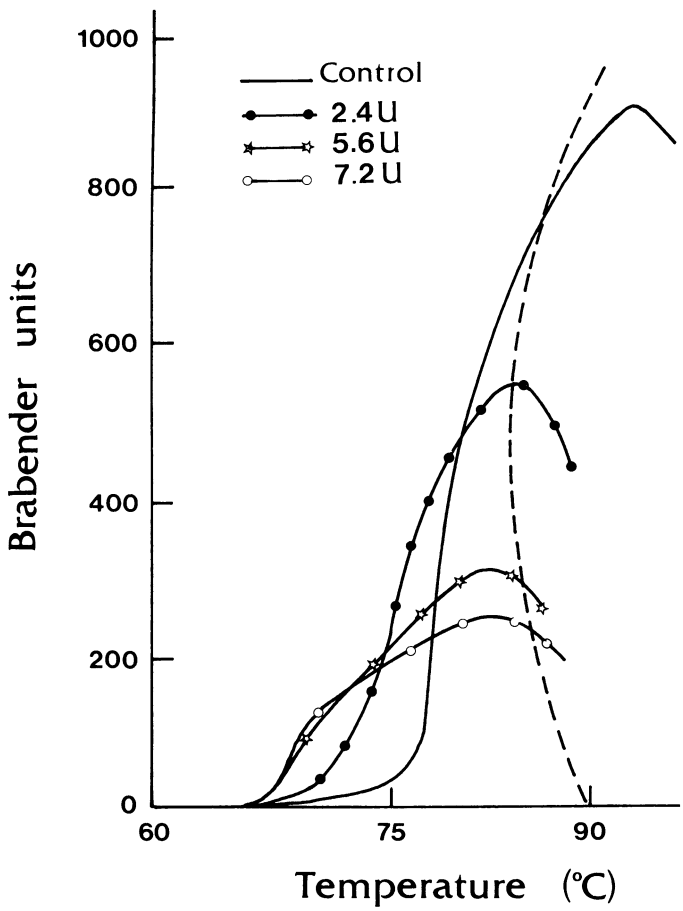

Fig. 1. Amylograms of wheat starch and a buffer extract of millet meal. U = enzyme units.

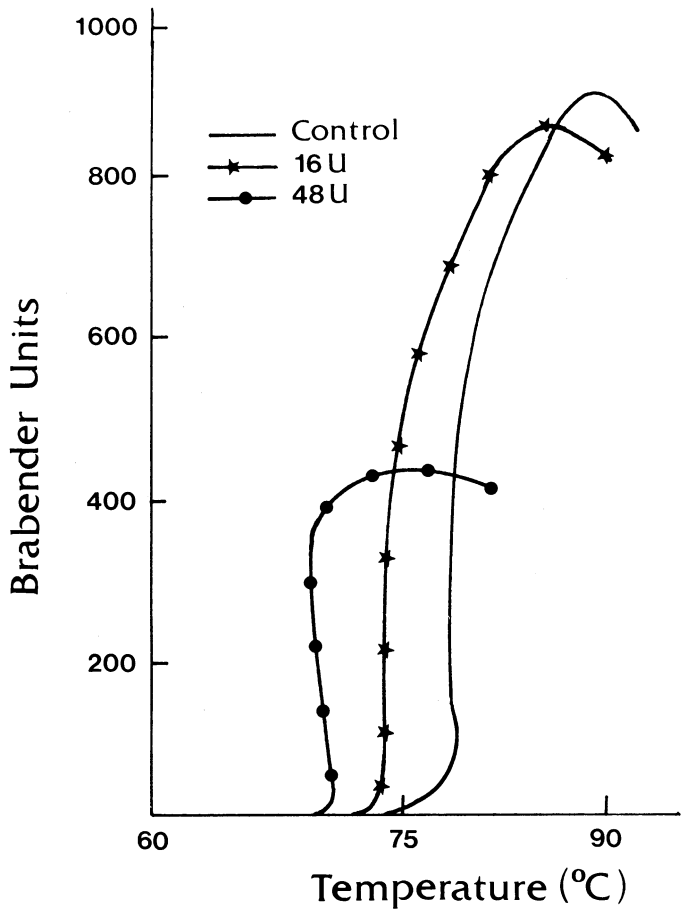

Fig. 2. Amylograms of millet starch and a buffer extract of millet meal. U = enzyme units.

RESULTS AND DISCUSSION

Action of Millet Alpha-Amylase on Heated Starch Pastes

Supplementing wheat flours with millet meal reduces the viscosity of flour pastes (Badi et al 1976). To obtain a better understanding of what levels of α-amylase were required to significantly reduce the viscosity, we ran amylograms containing wheat or millet starch and millet α-amylase. Amylograms of millet starch showed a higher peak viscosity (1,440 BU) than did those of wheat starch (900 BU) at the same concentration, ie, 11% (db), so the concentration of millet starch was reduced to 9% (db) to give a peak viscosity in the same range as that of the wheat starch.

The peak viscosity of the control wheat starch was reduced to 580, 300, and 250 BU by adding 2.4, 5.6, and 7.2 units of pearl millet α-amylase, respectively (Fig. 1). Pasting temperature was lowered and the shape of the curve broadened as amylase concentration increased. Those same alterations in amylograph curves were observed by Anker and Geddes (1944) when takadiastase was added to wheat starch.

Higher levels of α-amylase were needed to reduce the viscosity of millet starch pastes than were needed to reduce the viscosity of wheat starch pastes, indicating that millet starch was less susceptible to amylolytic degradation (Fig. 2). Because millet starch has a higher gelatinization temperature than wheat starch (Beleia et al 1980), α-amylase had less time to degrade the solubilized millet starch before heat inactivation. The peak viscosity of the control millet starch (900 BU) was reduced to 850 BU by the addition of 16 units of millet α-amylase and to only 420 BU by 48 units (Fig. 2). Both pasting temperature and temperature at maximum viscosity were reduced, but shapes of the millet starch amylograms did not broaden as they did with wheat starch.

Hydrolysis of Raw Starch by Millet Alpha-Amylase

Although the amylograph provides information about changes in starch while millet is cooked, it supplies little information on the events that occur at room temperature or at temperatures below 70°C, so we studied rates of hydrolysis of raw pearl milllet and wheat starches by α-amylase at 35°C.

Percentages of raw wheat and millet starch hydrolyzed by amylases in a crude extract during a 32-hr reaction period are shown in Fig. 3. Wheat starch was 30% hydrolyzed at the end of the period, compared to only 19% of millet starch. These data confirm the amylograph results (Figs. 1 and 2) indicating that millet starch is less susceptible than wheat starch to millet amylolytic attack.

Scanning electron micrographs (Fig. 4a–c) clearly showed the erosion of the granule surface by the amylases of the crude extract. Large wheat starch granules (Fig. 4a and b) and spherical millet starch granules (Fig. 4c) were preferentially attacked. Degradation of many of the large wheat starch granules was confined to the interior of the granule, leaving only an outer shell similar in structure to a mushroon cap (Fig. 4b).

Millet starch hydrolyzed by α-amylases in a heat-treated, crude millet extract showed no erosion at the surface of the granules, but pitting of the granules was apparent and similar to millet starch

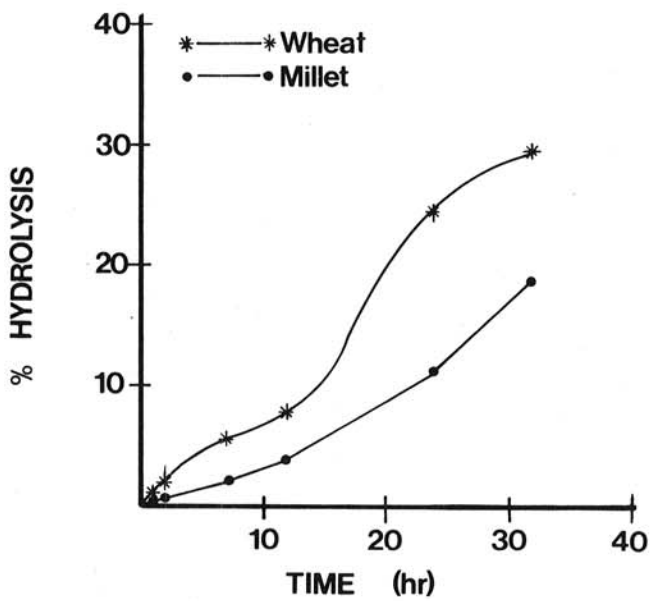

Fig. 3. Percent hydrolysis of wheat and millet starch granules by amylases in a crude extract of millet meal.

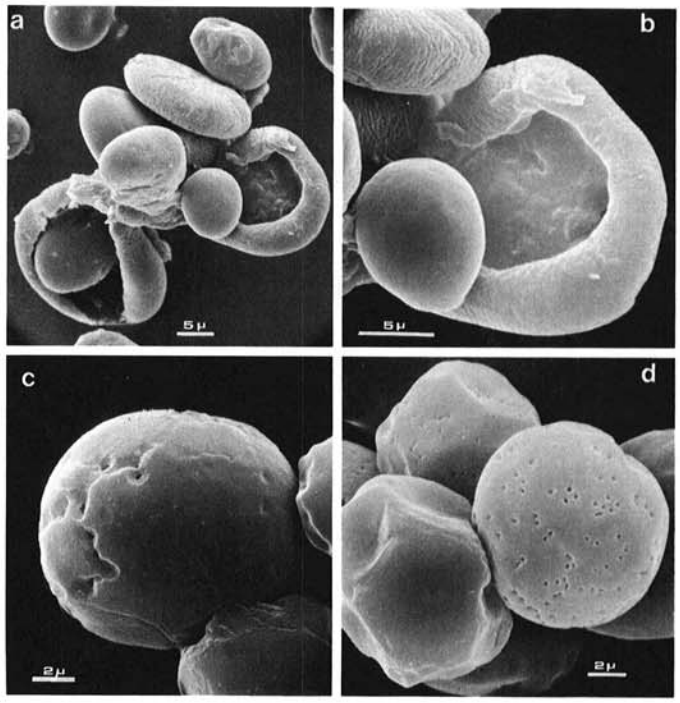

Fig. 4. Scanning electron micrographs of starch granules hydrolyzed by amylases in various extracts of millet meal: **a and b,** wheat starch, crude (unheated) extract; **c,** millet starch, crude (unheated) extract; **d,** millet starch, purified (glycogen complex) α-amylase.

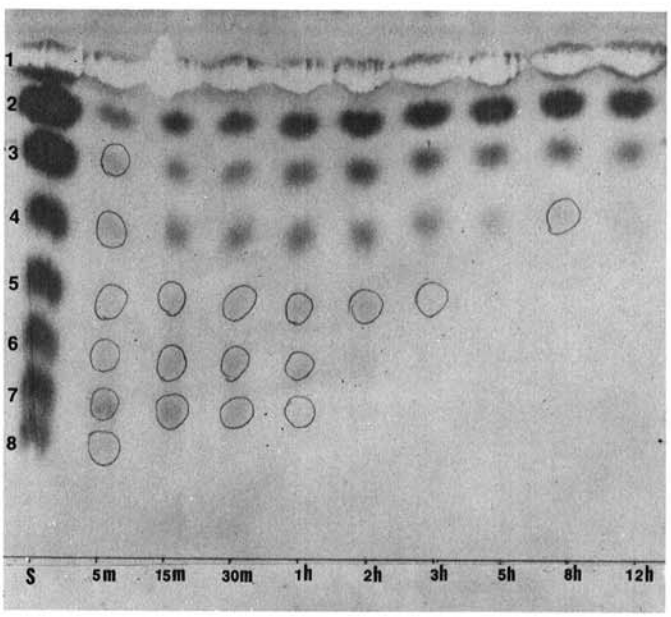

Fig. 5. Chromatograms of products formed by the hydrolysis of millet amylose by α-amylases purified by glycogen complex formation (430 mU/mg of amylose). The standards **(S)** consist of carbohydrate fragments containing 1–8 glucose units.

granules hydrolyzed by purified α-amylase (Fig. 4d). Formation of deep pin holes in the granule surface appears to be a characteristic α-amylase mode of attack. Purified α-amylase attacked a higher proportion of millet polygonal starch granules than did the other α-amylase preparations, probably because of the higher enzyme concentration in that digest.

Hydrolysis Products of Alpha-Amylase Action

Paper chromatography was used to study the products formed by the hydrolysis of millet starch by purified α-amylase. α-Amylase purified from pearl millet produced the same general pattern of action on starch and its fractions as is produced by other cereal α-amylases.

The products of millet amylose and amylopectin hydrolysis were essentially the same (Fig. 5). For both substrates, maltooctaose fragments were present after the first 5 min of hydrolysis but disappeared after 15 min.[3] From 3 to 12 hr, however, oligosaccharides with 4–6 glucose residues disappeared faster from amylose hydrolysates than from amylopectin hydrolysates. This suggests that the initial products of amylose hydrolysis are more susceptible to further degradation by α-amylase than are products from amylopectin hydrolysis. The concentration of maltose in the hydrolysates of both amylose and amylopectin increased throughout the reaction time. Such accumulation of maltose is characteristic of cereal α-amylase action patterns (Greenwood and Milne 1968).

[3]The white streak having the same Rf as glucose is an artifact produced during chromatogram development.

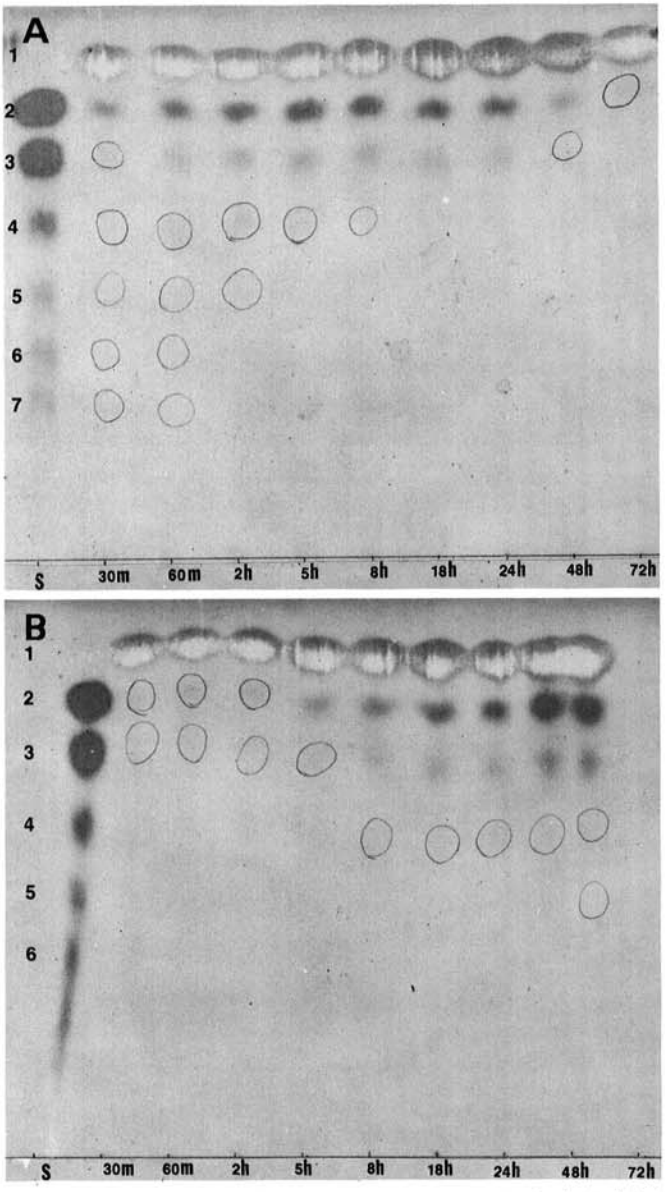

Fig. 6. Chromatograms of products formed by the hydrolysis of gelatinized millet starch **(a)** and raw millet starch **(b)** by α-amylase purified by glycogen complex formation. Enzyme concentrations of 430 mU/mg of gelatinized starch and 2,150 mU/mg of raw starch were used. The standards **(S)** consist of carbohydrate fragments containing 1–7 glucose residues.

Hydrolysis products of gelatinized millet starch after 30 min of digestion ranged from glucose to maltoheptaose (Fig. 6A). As the reaction progressed, higher molecular weight maltodextrins disappeared, and after 72 hr only glucose and maltose remained. Maeda et al (1978), using α-amylase purified from malted barley to hydrolyze soluble starch, found products ranging from glucose to maltoheptaose after 1 min of reaction, but after 30 min, only carbohydrate fragments containing 1–3 glucose units remained. Their much faster hydrolysis of soluble starch, compared with ours, probably stemmed from a higher enzyme concentration rather than to differences in enzyme source; their hydrolysis products were essentially the same as those observed for pearl millet amylase action.

Hydrolysis of raw millet starch by α-amylase (Fig. 6B) produced glucose, maltose, and maltotriose during the first 5 hr of reaction. After 8 hr, maltotetrose could be detected, and at 72 hr traces of maltopentaose were present. Nordin and Kim (1959) found only low molecular weight sugars (glucose to maltotriose) in the hydrolysates of raw sorghum starch by sorghum α-amylase after a three-day digestion period at 30° C. On the other hand, Maeda et al (1978) found maltohexose, maltoheptaose, and higher molecular weight maltodextrins in 24-hr hydrolysates of raw barley starch by malted barley α-amylase.

ACKNOWLEDGMENT

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