THE DIGESTIBILITY OF HIGH-AMYLOSE CORN STARCHES COMPARED TO THAT OF OTHER STARCHES. THE APPARENT EFFECT OF THE *ae* GENE ON SUSCEPTIBILITY TO AMYLASE ACTION[1]

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ABSTRACT

There is much variation between the raw starches in their susceptibility to the action of pancreatic alpha-amylase. In general the resistance to digestion was not directly associated with amylose content. A 40%-amylose starch from corn was the most susceptible, quite similar to waxy corn starch with less than 5% amylose. Wrinkled-pea starch with 65% amylose was intermediate in digestibility, whereas a 61%-amylose corn starch was highly resistant but not as resistant as potato starch with 22% amylose. Among the high-amylose corn starches, those from corn homozygous for either the *du* or su_2 gene, or both, were highly susceptible to digestion whereas those from corn homozygous for the *ae* gene alone or in combination with su_2 or *du* were resistant to digestion. A high-amylose corn having a starch highly resistant to enzyme action proved to be highly resistant to digestion when fed to rats and chicks, confirming the *in vitro* laboratory digestions.

High-amylose corn starch is one of the promising starches on the industrial horizon. At present, corn yielding starch with 60 to 65% amylose is being grown under contract for industrial research purposes, and an intense breeding program is in progress designed to increase the amylose content. The present industrial goal is the production of a hybrid corn with good agronomic characteristics which will yield starch with 80% or more amylose.

In the course of studies on the structure of starch granules, the susceptibility of various raw starches to enzyme action was investigated. It was noted that a high-amylose corn starch (Amylin with an amylose content of 55 to 60%) was much more resistant to the digestive enzymes than were the other cereal starches. This was unexpected, since the raw cereal starches, in comparison to the resistant raw "root" starches, were all thought to be readily susceptible to digestion in the animal digestive tract (3). Such indigestibility could be important, since, if at some time it were produced in excess of industrial requirements, it would normally be diverted into feed channels. Accordingly, a number of high-amylose corn starches varying in amylose content from 35 to 67% and, for feeding purposes, a corn yielding a starch with 63% amylose were obtained for further study.

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Laboratory Methods and Materials

Digestibility of Raw Starch. The method used was a modification of that described by Gates and Sandstedt (5). Essentially it consisted of a 20-hour digestion of 0.4 g. of raw starch in 15 ml. of a solution containing 30 mg. calcium chloride, 30 mg. gelatin, 1 ml. chloroform and pancreatin (to give 25 SKB units of alpha-amylase) at 30°C. and at pH 6.5 with continuous shaking. The undigested residue was filtered, dried, and weighed. The loss in weight expressed as percent of the starch is designated as the digestibility of the starch. However, under these conditions the accumulated end-products of digestion are present during the digestion and, in cases of high digestibility, these end-products may materially inhibit enzyme action. The spread in digestibility accordingly may be wider than that shown.

Determination of Alpha-Amylase. The alpha-amylase content of the enzymes was determined by the SKB method (12), modified by using the glass color-standard as suggested by Redfern (10) and the "beta-amylase for analytical purposes" from the Wallerstein Laboratories.

Digestibility of Starch in Ground Corn. The method was essentially determination of the CO_2 pressure produced by fermentation of sugars obtained from pancreatic digestion of the starch in the ground corn. A 2-g. sample of the corn, ground in a Wiley mill to pass a 1-mm. sieve, was placed in a 50-ml. beaker, mixed with 10 ml. water containing 160 mg. pancreatin (200 SKB units of pancreatic alpha-amylase) and 0.3 g. of baker's compressed yeast. The beaker and contents were placed in a pressuremeter (11) and allowed to ferment for 20 hours at 30°C. The CO_2 pressure (mm. Hg) produced was read at suitable intervals and plotted against time. The curve obtained was corrected for a blank representing fermentable sugars present in the grain, or in some cases possibly representing damaged starch (13). This blank was obtained by extrapolating to zero time a straight line drawn from the points representing the pressures obtained at 2 hours and at 8 hours.

Factors for the Conversion of CO_2 Pressures (mm. Hg) to mg. Maltose. A 500-mg. sample of maltose monohydrate was mixed with the substance under study (ground corn, starch, gelatinized starch, etc.) plus all ingredients that were used in the regular fermentation except the enzyme (pancreatin, malt, or other). This mixture was fermented for 20 hours. A blank containing no maltose or enzyme was also fermented. The pressure obtained from the maltose fermentation was corrected for the blank. Then 500 divided by the corrected pressure

= maltose per mm. pressure. This factor may vary according to the yeast nutrients and activators present with the fermenting maltose mixture (2).

Starches. All starches were unmodified. The 38-, 40-, 47-, 54-, 57-, and 61%-amylose samples were prepared in the Nebraska Laboratory from corn furnished by H. H. Kramer of Purdue University and the 63%-amylose starch from a double-cross hybrid corn furnished by American Maize-Products Co. The other starches were furnished by National Starch Products Co. and American Maize Co. The information concerning the amylose content and the genetic composition of the high-amylose starch samples was furnished by the individuals who supplied the samples.

Pancreatin. Fisher Scientific Co. Assayed for alpha-amylase content.

Results and Discussion

The data presented in Fig. 1 compare the susceptibility to digestion by pancreatin of a number of raw starches. The values given are averages of closely agreeing duplicates.

"Root" Starches. It is often stated that the so-called "root" starches (tubers, bulbs, enlarged root stocks, corms, and rhizomes) are exceedingly difficult to digest. The data of Fig. 1 indicate that there was considerable variation in resistance to enzyme action among these starches, canna being only 3% digested and potato 5%, but cassava was ten times higher. To be certain that this was representative of cassava starches, three samples obtained from different sources over widely separated periods of time (about 10 years) were analyzed; digestibilities of 53, 55, and 58% were obtained. Cassava starch has an amylose content of about 18% and potato starch about 22%.

Cereal Starches. The cereal starches were exceedingly variable, the widest variations being in the maize starches. On arranging the maize starches in the order of increasing amylose content, it was noted that digestibility apparently was not directly related to amylose content. The waxy corn starch with less than 5% amylose was highly susceptible, but so were the samples with intermediate amylose content (36, 38, 40, and 47%). The 40%-amylose sample was the most susceptible of the entire series of starches. Yet with greater increases in amylose content (48% and higher amylose), digestibility dropped drastically.

Genetic Composition and Susceptibility to Amylase Action. The genetic composition and interaction of the genes of high-amylose corn have been thoroughly reviewed by Kramer, Pfohler, and Whistler (7). Unfortunately only a few high-amylose samples with known genetic composition were available for this particular study, but it

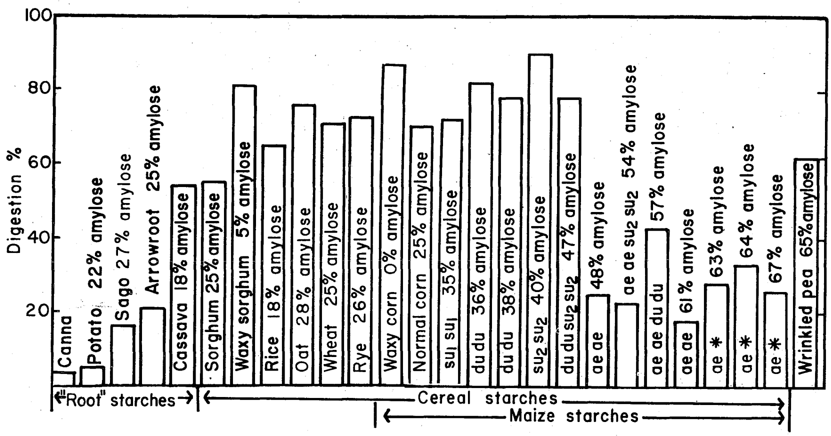

Fig. 1. A comparison of the susceptibility of various starches to pancreatic digestion. Asterisk denotes unknown genetic composition.

appears from the data obtained (Fig. 1) that the gene *ae* may be associated with both high amylose and high resistance to enzyme action. On the other hand, the genes *du* and su_2, though associated with an intermediate amylose, seem also to be associated with high susceptibility to digestion. The samples homozygous for the *du* and su_2 genes (*du du* and su_2su_2) and their combination, *du du* su_2su_2, were highly susceptible, the su_2su_2 sample being the most susceptible. The sample with 61% amylose, homozygous for the *ae* gene, was the most resistant of the corn samples. The samples homozygous for *ae* and *du* (*ae ae du du*) or *ae* and su_2 (*ae ae* su_2 su_2) were also low in digestibility. If the resistance to digestion is controlled by the *ae* gene and susceptibility by the *du* or su_2 genes, the *ae* gene would appear to exert an epistatic effect on the *du* or su_2 genes, thereby giving not only high amylose but also high resistance to digestion. The only information available concerning the genetic composition of the samples with 63, 64, and 67% amylose is that they contained the *ae* gene.

Susceptibility vs. Amylose Content. Comparisons of other data of Fig. 1 including the noncereal starches give further evidence that amylose content, in itself, is not the factor that determines digestibility. Potato and normal corn starches have approximately the same amylose content but were widely different in digestibility, whereas wrinkled-pea, with an amylose content of about 65% (6), was relatively susceptible if compared to normal corn but highly susceptible if compared to potato. The reasons for differences in susceptibility to enzyme action are still obscure; the answer probably lies in the structure of the starch granules, i.e., in differences in the bonding between the starch molecules; and possibly, also in anomalous linkages within the molecules.

Digestibility of Starches in Ground Whole Corn. Since starch is not fed to animals as purified starch but in the grain, it is of interest to show whether the differences in digestibility of starches will also be shown in digestions of ground corn or whether the differences would be masked by the difficulty of digesting larger pieces of endosperm.

Since the method used for the study of the purified starches could not be used for the digestion of starches mixed with other insoluble materials, a fermentation method for determining the sugars produced by digestion appeared to be the best method available (9,11). This method has an advantage in that the sugars produced are fermented and thus do not inhibit amylase action. Also, pressure readings may be taken at intervals enabling curves to be drawn showing the change in rate of digestion with time. It has the disadvantage, when pancreatin with its pH optimum of about 6.5 is used, of a decreasing pH with time; however, the pH change did not seem to cause a decrease in rate of digestion. Maltose ferments rapidly in the presence of ground corn, indicating that the corn furnishes an adequate supply of yeast nutrients and activators (2).

Figure 2 shows data obtained from a fermentation study of the digestibility of the starch in normal ground corn compared to that in high-amylose (63% -amylose) corn.

The steep slope in the curve for the high-amylose corn during the first 2 hours probably indicates the presence of a small amount of fermentable sugar. This fermentable sugar is to be expected since both the *du* and su_2 genes induce the formation of a "sugary" endosperm (7,8). A straight line running through the 2-hour and 8-hour pressure points extrapolated back to zero-time gives a correction of 90 mm. pressure for this fermentable material. The dashed curve is the curve corrected for this blank. There is no correction for the normal corn; in this case the extrapolated line runs through zero-time at the origin and there is no break in the curve at approximately 2 hours.

The slopes of the curves indicate the relative rates of digestion. The ratios of the pressure at any given time give the relative digestibility of the starches. From 2 to 10 hours the digestion on the high-amylose corn was 40% of that of the normal corn; after this time the digestion on the normal corn was slowing down slightly more than that on the high-amylose corn; accordingly, at 20 hours the high-amylose corn was 44% of the normal corn. According to the data of

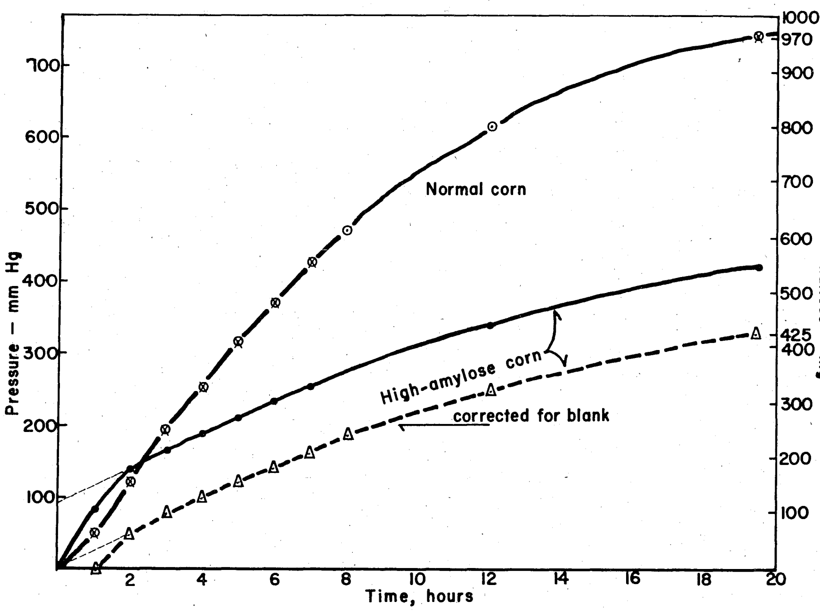

Fig. 2. A comparison of the digestibility of a normal ground corn with that of a 63%-amylose corn.

Fig. 1, the 63%-amylose starch was 37% as digestible as the normal. Considering the wide differences in the mechanical condition of the starches in these two materials (starch vs. ground corn), this may be considered excellent agreement.

In Vivo Studies

The *in vitro* digestibility of the starch contained in the 63%-amylose corn compared to that of normal corn has been substantiated by a study of the comparative utilization of these starches in the ground grains by growing rats and chicks.

Rat-Feeding Trials. Under the direction of R. L. Borchers (4), growing rats were fed equivalent diets containing 75% ground corn; normal corn versus 63%-amylose corn (4). The feed consumption was held at the same level for all rats.

The most dramatic evidence of the relative digestibilities of the starches in the two diets is presented in Fig. 3. This picture compares the 24-hour accumulation of feces from two rats, A on normal corn, B on high-amylose corn. No starch was notable on microscopic examination of the feces from normal corn; this would be expected, since the fragments of granules remaining after 95

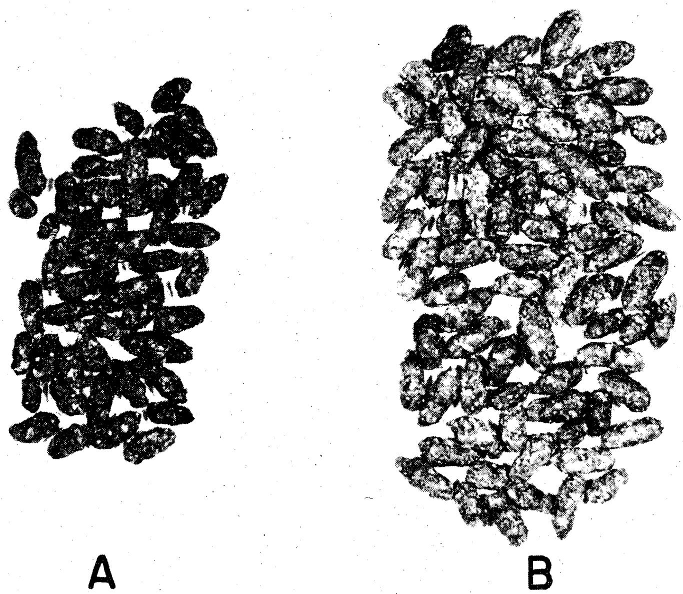

Fig. 3. Visual evidence of the resistance to digestion by rats of a 63%-amylose corn compared to that of normal corn. The 24-hour feces from: A, a rat on a diet containing 75% of normal corn; B, a rat on a diet containing 75% of 63%-amylose corn.

to 98% digestion (3,4) would be difficult to recognize among the other residues. On the other hand, the feces from the high-amylose corn diet were loaded with intact starch granules. The average daily weight (dry basis) of feces from normal corn was 0.76 g., whereas from high-amylose corn it was 1.97 g., or 2.6 times as much. Assuming that the extra weight of the feces from the high-amylose diet was due to undigested starch and that undigested starch was practically absent from feces from the normal corn, there would be 61% starch in the high-amylose feces.

Borchers' data (4) indicate incomplete digestion of the high-amylose corn starch by rats, but a much more complete digestion than under the conditions for *in vitro* digestions in the laboratory.

Chick Feeding. Evidence similar to that obtained from rat feeding also was obtained by C. W. Ackerson (1) from chick feeding. In this feeding trial, the first week's accumulation of feces from a lot of 15 chicks on normal corn weighed 621 g. (air-dry) as compared to 1,231 g. (air dry) from the lot of 15 chicks on the high-amylose diet, or 1.9 times as much. The visual comparison (Fig. 4) is not as dramatic as the comparison on the rats, since chickens excrete large quantities of uric acid which is white; this obscures the difference due to the white

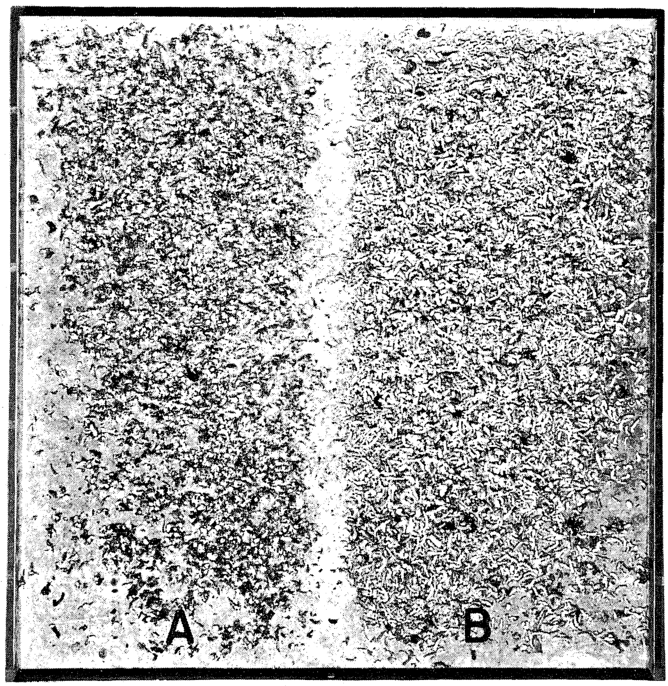

Fig. 4. The digestion by chicks of normal vs. 63%-amylose corn. One week's accumulated feces from a lot of 15 chicks on a diet containing: A, 60% normal corn; B, 60% of 63% amylose corn.

undigested starch. Microscopic examination showed no starch in the sample from the normal corn but much in the sample from high-amylose corn. Again assuming the entire extra weight of the feces from high-amylose corn to be due to undigested starch, the high-amylose sample was 58% starch.

Conclusions

The digestibility of raw corn starch is not directly associated with amylose content; however, the genes involved in producing high-amylose starch in corn are apparently also associated with the digestibility of the starch. According to the data at hand, the su_2 and *du* genes produce susceptibility, whereas the *ae* gene, which seems necessary for the production of high amylose in combination with desirable agronomic characteristics, produces starch highly resistant to enzyme action. Accordingly, high-amylose corn, which is desirable for industrial purposes, will probably contain the *ae* gene and therefore may be resistant to digestion.

Acknowledgments

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