

## WET-MILLING HIGH-AMYLOSE CORN CONTAINING 66- TO 68-PERCENT-AMYLOSE STARCH<sup>1</sup>

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### ABSTRACT

High-amylose corn lots with starch containing up to 68% amylose, obtained from two corn breeders, were wet-milled on a laboratory and pilot-plant scale, to gather information pertinent to the genetic development of this new corn. Processing characteristics of an inbred sample with starch of 68% amylose content were poorer than those observed in the wet-milling of corn with lower amylose contents. Another corn sample, a hybrid with starch containing 66.7% amylose, exhibited extremely good wet-milling characteristics, contrary to a previously apparent pattern of increasingly poor processing as the amylose content of corn increased. The recovery of 82.7% of the starch present was over 10% greater than the amount recovered from corn containing 57%-amylose starch. The 0.48% protein content of the starch recovered from the 66.7%-amylose corn is comparable to that of ordinary corn starch. Differences in genetic background probably account for the marked improvement in processing characteristics of this new corn.

In a previous article we compared the wet-milling characteristics of two dent hybrid corns having starch with 49 and 57% amylose content with those of ordinary dent corn (3). Two significant differences in processing were increased swelling of the high-amylose

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corn kernel and decreased purity and yield of the high-amylose starch. These differences became more pronounced as the amylose content of the starch increased. Similar investigations were continued on several samples of high-amylose corn containing starch with 66 to 68% amylose, to provide plant breeders with information on the response of the corn samples to wet-milling and to develop optimum conditions for starch recovery. Results of these investigations are compared with those obtained in the previous work.

### Material and Methods

*Materials.* High-amylose corn having starch with amylose contents of 66 to 68% was obtained from two corn breeders. One sample, NRRL 38225, was supplied by M. S. Zuber, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, stationed at the Missouri Agricultural Experiment Station, Columbia, Missouri. This corn is an inbred line in early stage of development, produced from crosses giving an amylose content of about 68%.

R. P. Bear, Bear Hybrid Corn Company, Decatur, Illinois, provided the second sample, NRRL 39990, which is a field-type dent hybrid with starch containing 66.7% amylose. This corn has not yet been developed into a commercial hybrid. The history and composition of these corn samples, and of the ordinary corn used as control, are given in Table I. Although the analyses of the samples received from

TABLE I  
HISTORY AND COMPOSITION OF CORN USED IN WET-MILLING EXPERIMENTS  
(All percentages on a moisture-free basis)

HISTORY AND COMPOSITION	HIGH-AMYLOSE CORN (INBRED), NRRL 38225	HIGH-AMYLOSE CORN (HYBRID), NRRL 39990	ORDINARY DENT CORN (CONTROL), NRRL 18429
History:			
Plant breeder	Zuber	Bear	.....
Crop year	1958	1959	1956
Composition:			
Protein, %	14.5	11.3	10.1
Crude fat, %	4.9	5.2	4.5
Starch, %	57.8	61.4	73.8
Amylose content of the starch, %	68.1	66.7	24
Total solubles, %	7.1	7.1	5.6
Total sugars, %	2.5	3.4	2.3

the two breeders are similar, the appearance of the grains is quite different. In Fig. 1, ears of the high-amylose corns are compared with an ear of the ordinary dent hybrid corn. The ear from the inbred line is considerably smaller and less colored than the hybrid ear. Kernels from the smaller ear are also small and shriveled. The

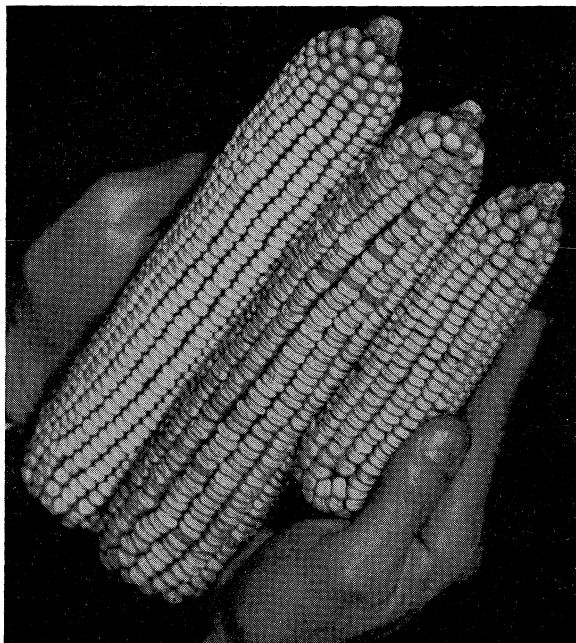


Fig. 1. From top to bottom, ear of an ordinary dent hybrid corn, NRRL 18429; ear of high-amylose dent hybrid corn (66.7% amylose), NRRL 39990; and an ear of high-amylose inbred corn (68% amylose), NRRL 38225.

hybrid ear of high-amylose corn is similar to the ear of ordinary corn.

*Processing Methods.* Experiments were carried out in duplicate on a laboratory scale and on a small pilot-plant scale. In the laboratory, 1,500 g. of corn were steeped in 2,800 ml. of distilled water containing 0.25% sulfur dioxide at 125°F. (52°C.). After 48 hours of steeping, the water was drained, the grain was coarsely ground in a Quaker City<sup>2</sup> drug mill, and the germ was recovered by simple flotation. The fibrous material remaining was finely ground in the drug mill. The resulting slurry was screened first on a 0.039-in. perforated copper sieve to remove coarse fibers and then on a 200-mesh stainless-steel sieve to remove fine fibers. Starch was recovered from the ordinary corn by a single tabling. The starch was recovered from high-amylose corn by tabling the mill starch first to remove the gluten. Then the crude starch was removed from the table, reslurried, and again tabled to recover a purer starch. This procedure for recovering starch from high-amylose corn was described previously (3).

<sup>2</sup>Mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

In the pilot-plant experiments, 2 bu. of grain were steeped in tapwater containing sulfur dioxide. The water was drained after steeping was completed, and the corn was ground in a Bauer mill equipped with jaw-toothed plates to crack the kernel and free the germ. The germ was recovered by flotation. The remaining material was then ground in a Rietz disintegrator equipped with a 1/16-in. round, perforated screen. Coarse fibers were removed from the slurry by passing it over a Rotex gyratory shaker equipped with a 26-mesh screen, and fine fibers were recovered by passing the slurry over the same shaker equipped with a 200-mesh screen. The mill starch was separated into starch and gluten by tabling. The pilot-plant equipment and procedures have been described by Anderson (2).

*Analytical Methods.* The moisture of the corn and of the various recovered fractions was determined by drying a sample for 4 hours at 110°C. under a vacuum of 28 in. of mercury. Protein ( $N \times 6.25$ ) was determined by an improved Kjeldahl method for nitrate-free samples (1). Starch content was determined polarimetrically by the procedure of Earle and Milner (6). Apparent amylose content of the corn was determined by the iodine sorption method of Bates, French, and Rundle (5), essentially as modified by Wilson, Schoch, and Hudson (7). The amylose analysis results in figures that are generally high, as it has been shown that the amount of material which can be isolated as amylose from this type of corn is significantly lower (8). Fat analysis was carried out according to the official AOAC method of analysis (4). Solubles and sugar analyses were conducted according to AACC laboratory methods (1). Sulfur dioxide in the steepwater was determined by titration with iodine solution using starch as the indicator. All recovery and analytical data presented are on a moisture-free basis (m.f.b.).

## Results and Discussion

*Processing Characteristics of High-Amylose Corn (66-68%).* Laboratory-scale wet-milling investigations were carried out on the two samples of high-amylose corn and on a sample of ordinary dent corn. Processing characteristics of the two high-amylose corns were compared with those of ordinary corn to show differences thought to be caused by genetic background.

Steeping characteristics were closely examined throughout the complete steeping cycle by analyzing, at intervals, the steeped corn for moisture and the steepwater for pH and sulfur dioxide content. The steeped-corn moisture-curve from both high-amylose corn samples

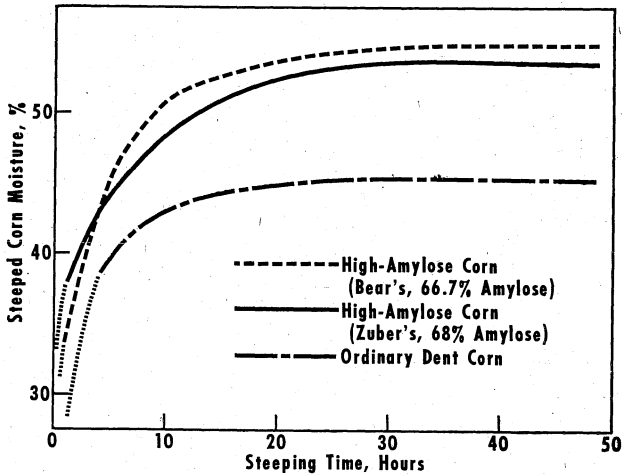


Fig. 2. Effect of steeping time on steeped corn moisture.

followed the characteristic water-absorption curves obtained from steeping ordinary corn (Fig. 2). Although the time of maximum water absorption was approximately the same for all samples, about 10 hours, the moisture content of the high-amylose corns was 7 to 9% more than that of the ordinary corn. The pH of the steepwater obtained from these corn samples followed similar curves, but the pH of the steepwater from the ordinary corn was somewhat higher than that for the hybrid sample of high-amylose corn, and was slightly lower than that for the inbred sample, as illustrated in Fig. 3. The sulfur dioxide disappearance from the steepwater was about the same for

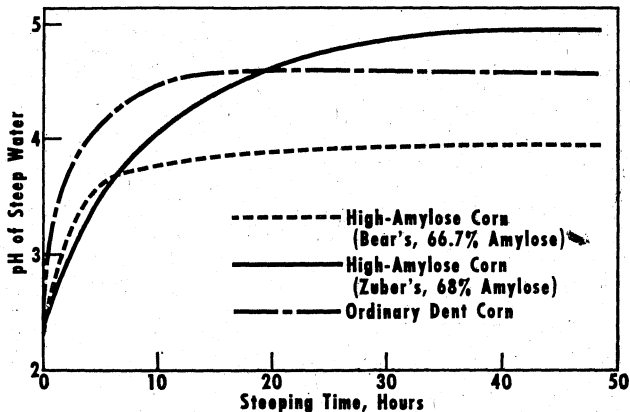


Fig. 3. Effect of steeping time on pH of the steepwater.

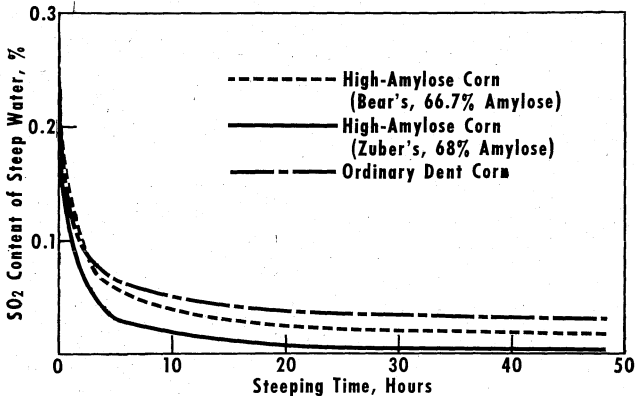


Fig. 4. Effect of steeping time on sulfur dioxide content of the steepwater.

the three samples; only a trace remained after steeping of the high-amylose corn inbred (Fig. 4). The yield of solids in the steepwater and their protein content averaged 4.1 and 30.8%, respectively, and were about the same for all three samples. The comparison of this processing characteristic and others is shown in Table II.

During steeping, the high-amylose corn inbred kernel exhibited an increase of 121% of its original dry volume; whereas the hybrid had an increase of 105%, and the ordinary corn only 63%.

The germ yield from the processing of both samples of high-amylose corn was higher than that from ordinary corn, 5.8 and 7.6%, as compared with 4.7%. The oil content of the germ from the high-amylose corn was 7 to 12% less than the 53% present in the ordinary corn germ. But total oil recovered from high-amylose corn was the same or slightly higher than that from the ordinary corn.

The response of the coarse and fine fibers to wet-milling was about the same for all three corns. However, fiber recovery from the inbred sample was considerably greater than that from the high-amylose hybrid or the ordinary corn. Because it was more difficult to wash starch from the fibers of the inbred corn, a relatively high amount of starch remained in both coarse and fine fibers. The fibers recovered from the high-amylose corn hybrid were washed free of starch quite readily, so that their starch content was less than that generally found in ordinary corn fibers.

Double-tabling the mill starch from each of the two high-amylose corns produced drastically different results. Based on starch in the inbred corn sample, the recovery of starch was only 69.7%; the starch contained 0.73% protein. The amount of starch obtained from proc-

TABLE II  
COMPARISON OF THE PROCESSING CHARACTERISTICS OF THREE DIFFERENT  
SAMPLES OF CORN  
(Average of duplicate runs)

ITEM	HIGH-AMYLOSE CORN (INBRED), NRRL 38225	HIGH-AMYLOSE CORN (HYBRID), NRRL 39990	ORDINARY DENT CORN (CONTROL), NRRL 18429
Steepwater			
Yield <sup>a</sup> of solids, % m.f.b. <sup>b</sup>	3.9	4.2	4.2
Protein content, % m.f.b.	32	29	31.2
Steeped corn			
Average final moisture, %	53	55	46
Volume increase, %	121	105	63
Germ			
Yield, % m.f.b.	5.8	7.6	4.7
Crude oil content, % m.f.b.	46.2	41.2	53.0
Recovery of total oil, %	55.2	59.7	55.5
Coarse fiber			
Yield, % m.f.b.	17.9	6.7	12.8
Protein content, % m.f.b.	13.3	10.4	14.9
Starch content, % m.f.b.	20.6	9.8	19.8
Fine fiber			
Yield, % m.f.b.	7.7	7.1	6.2
Protein content, % m.f.b.	27.7	18.1	23.8
Starch content, % m.f.b.	36.2	18.9	30.6
Starch			
Yield, % m.f.b.	40.3	50.8	64.4
Recovery, % of total starch	69.7	82.7	87.3
Protein content, % m.f.b.	0.73	0.48	0.51
Gluten			
Yield, % m.f.b.	11.2	11.2	7.8
Protein content, % m.f.b.	35.5	34.6	44.2
Starch content, % m.f.b.	52.3	44.2	...
Squeegee starch <sup>c</sup>			
Yield, % m.f.b.	5.3	4.1	...
Protein content, % m.f.b.	14.0	9.2	...
Starch content, % m.f.b.	54.0	81.9	...
Process waters			
Yield of solids, % m.f.b.	5.7	5.4	2.5
Protein content, % m.f.b.	42.0	36.4	40.0

<sup>a</sup> Yield based on original weight of dry corn.

<sup>b</sup> Moisture-free basis.

<sup>c</sup> Squeegee starch is result of second tabling.

essing the hybrid sample was 82.7%; the starch contained 0.48% of protein. In neither case was a good, hard starch cake formed. As expected from analyses, the high-amylose corns yielded less starch than did the ordinary corn. Although lower yields are primarily due to the smaller amount of starch present in high-amylose corn, the presence of some small and irregularly shaped starch granules also contributes to them.

The yield of the gluten recovered from each of the high-amylose corns was 11.2%, and their protein contents averaged about 35%. However, the gluten from the high-amylose hybrid contained only 44.2% starch, as compared to a starch content of 52.3% in the high-

amylose inbred. Generally, a 7.8% yield of gluten containing 44.2% protein was obtained from the ordinary corn, which is comparable to that obtained from a commercial tabling operation. The "squeegee starch," which was the overflow or "gluten" from the second tabling of high-amylose starch, contained primarily starch.

The solids present in the process water from wet-milling the high-amylose corn amounted to 5.7 and 5.4%, or about twice the amount generally obtained from processing ordinary corn. However, these samples all had approximately the same protein content, about 40%. The larger yield of solids from the high-amylose corns can be attributed to their higher soluble content and also to the presence of very small starch granules which were not recovered in tabling operations.

Experiments carried out in the pilot plant confirmed the data, results, and observations noted in the laboratory processing of the high-amylose corns. Starch recovered in the pilot plant from the high-amylose inbred contained 0.7% protein; starch from the hybrid sample averaged 0.55% protein.

The differences in the results obtained in the processing of these two samples of high-amylose corn indicated that some changes had occurred in the raw material, since processing conditions were held constant. The wet-milling of the high-amylose inbred gave results which followed the general pattern established in the processing of corn with 49- and 57%-amylose starch; that is, as amylose content of the corn increased, starch recovery dropped and protein content of the starch increased. However, when the high-amylose hybrid was processed, an unusually good recovery of starch having low protein content was obtained. Other processing characteristics were also much improved. This hybrid sample, then, is the first high-amylose corn that has given good wet-milling characteristics.

*Comparison of High-Amylose Corn Hybrid (66.7%) with Other High-Amylose Corn Hybrids Containing 49% and 57% Amylose Starch.* Certain significant wet-milling characteristics of the high-amylose corn hybrid (66.7%) were compared with those of two high-amylose corn hybrids with starch containing 49 and 57% amylose, which were also produced by the Bear Hybrid Corn Co. (Table III). The new corn showed improvement in processing characteristics in contrast to past experiences with high-amylose corn.

The moisture content of the three steeped high-amylose corn samples ranged from 6 to 9% higher than that for steeped ordinary corn. From the pattern observed in the milling of the 49 and 57% hybrid amylose corns, the inbred corns of higher amylose content, and the



TABLE III  
COMPARISON OF SIGNIFICANT WET-MILLING CHARACTERISTICS OF THREE HIGH-AMYLOSE CORN HYBRIDS AND ORDINARY CORN

	AMYLOSE CONTENT, PER CENT			
	24 <sup>a</sup>	49	57	66.7
Steep corn moisture, %	46	54	52	55
Kernel volume increase, %	63	100	128	105
Recovery of total oil, %	55.5	51.4	51.0	59.7
Starch recovery, % of total starch in corn	87.3	80.0	71.4	82.7
Protein in starch, % m.f.b.	0.51	0.53	0.70	0.48

<sup>a</sup> Ordinary corn.

ordinary corn, it would be expected that the volume of the steeped kernels from the hybrid 66.7% amylose corn would increase, or at least remain at about 128%. However, the increase in the volume of the steeped corn kernels of high-amylose corn (66.7%) was 23% less than that of kernels from 57%-amylose corn. This smaller increase in kernel volume will benefit corn processors by permitting a larger quantity of high-amylose corn (66.7%) to be steeped in their present equipment.

The recovery of total oil was significantly greater from the 66.7%-amylose corn than from any of the other corns, including the ordinary dent sample. Almost 60% of the oil was recovered in the germ fraction, as compared to 51% from the other high-amylose corns and 55% from ordinary corn. It is recognized that these recoveries are somewhat lower than those generally obtained by commercial processing, but the comparisons show an improved germ oil recovery from the corn containing 66.7%-amylose starch.

In previous studies the starch-gluten separation always became more difficult as the amylose content of the corn increased. Processing of high-amylose corns (49 and 57%) showed a recovery of starch of 80.0 and 71.4%, respectively. The protein content of the starch from the 49%-amylose corn was 0.53%, and from the 57% material, 0.7%. The 82.7% recovery of starch from 66.7%-amylose corn is over 10% higher than experienced in the processing of 57%-amylose corn, and comes within 4.5% of our optimum starch recovery obtained from the milling of ordinary corn. Also, the protein content of the starch recovered from high-amylose corn (66.7%) was about the same as in ordinary corn starch.

While the over-all processing results obtained with 66.7%-amylose corn were not quite as good as those obtained with ordinary dent corn, processing quality was reasonably good and improvements shown over the milling of 49%-, 57%-, and other high-amylose corns were very significant. A survey of the genetic background of

the 66.7%-amylose corn revealed that its "pedigree" had been altered to introduce more favorable characteristics to the corn<sup>3</sup>. This genetic control of milling quality of high-amylose corn, concurrent with increased amylose content, shows promise for future development of high-amylose corns with excellent processing characteristics, similar to those of ordinary corn.

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#### Literature Cited

1. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minnesota (1957).
2. ANDERSON, R. A. A pilot plant for wet-milling. *Cereal Sci. Today* **2**: 78-80 (1957).
3. ANDERSON, R. A., VOJNOVICH, C., and GRIFFIN, E. L., JR. Wet-milling high-amylose corn containing 49- and 57-percent-amylose starch. *Cereal Chem.* **37**: 334-342 (1960).
4. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Official methods of analysis (8th ed.). The Association: Washington, D.C. (1955).
5. BATES, F. L., FRENCH, D., and RUNDLE, R. E. Amylose and amylopectin content of starches determined by their iodine complex formation. *J. Am. Chem. Soc.* **65**: 142-148 (1943).
6. EARLE, F. R., and MILNER, R. T. Improvements in the determination of starch in corn and wheat. *Cereal Chem.* **21**: 567-575 (1944).
7. WILSON, E. J., JR., SCHOCH, T. J., and HUBSON, C. S. The action of *Macerans* amylase on the fractions of starch. *J. Am. Chem. Soc.* **65**: 1380-1383 (1943).
8. WOLFF, I. A., HOFREITER, B. T., WATSON, P. R., DEATHERAGE, W. L., and MAGMASTERS, MAJEL M. The structure of a new starch of high amylose content. *J. Am. Chem. Soc.* **77**: 1654-1659 (1955).

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<sup>3</sup>Communication with M. L. Vineyard, Bear Hybrid Corn Co.