

# Comparison of the Wet-Milling Properties of Opaque-2 High-Lysine Corn and Normal Corn<sup>1</sup>

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## ABSTRACT

The physical and chemical properties of a high-lysine corn hybrid, recessive for the opaque-2 gene, were compared with those of two normal hybrid corns. The wet-milling characteristics of the three corns were compared by a conventional procedure. Comparisons of physical and milling properties were somewhat confounded by wide differences in composition: 13.6% protein for opaque-2 and 10.4% and 8.0% for the normal corns; 64.4% starch for opaque-2, 71.4 and 73.6% for the normal corns, respectively. Opaque-2 kernels were about three-fourths as large as normal kernels, 22 vs. 31 g./100 kernels; they were 12 and 15% lower in kernel density and bulk density, respectively. Upon steeping, opaque-2 corn swelled to twice the volume of normal corn and contained 52.3% moisture (42.8 and 44.6% for the normal corns). Aqueous extraction of dry ground aliquots solubilized 6.5% of the dry substance from opaque-2 (4.5% from normal corn). As predicted from analytical data, the wet-milling of opaque-2 corn produced high yields of steep-water, germ, oil, and fiber and low yields of gluten-protein and starch. The amount of lysine in steepwater-protein was about the same for both types of corn, but gluten-protein from opaque-2 was significantly higher in lysine and tryptophan than that from the normal corn. Higher lysine values were accompanied by lower zein contents in the gluteins. Starch recovered from the opaque-2 corn was similar in all properties to normal starch.

The nutritional inadequacy of corn as a protein source for humans and monogastric animals has been shown to be due to inadequate levels of lysine and tryptophan in the endosperm protein (1,2). Attempts to improve the nutritional value of corn by intensive nitrogen fertilization of the crop or by breeding for a greater amount of total protein have been unsuccessful because of large increases in the proportion of zein, a protein essentially devoid of tryptophan and lysine (1,3).

A genetic approach to increasing lysine and tryptophan contents of normal corn inbred lines by recurrent selection methods was not very successful (4). Recently, however, indigenous races of corn from Mexico were shown to vary significantly in lysine content (5). Concurrently, Mertz *et al.* observed alteration of lysine content of corn by the action of single recessive genes, opaque-2 (6) and floury-2 (7). These genes have been recognized for many years by their effect of producing floury or opaque endosperm, but this is the first report on any compositional effects. The higher lysine and tryptophan values of these corn mutants were shown to be due largely to a reduction in the proportion of zein and an increase in glutelin in the endosperm proteins.

The opaque-2 mutant, commonly known as high-lysine corn, as the only source of dietary protein has produced excellent growth of rats (8) and swine (9). Opaque-2 corn is expected to be equally valuable as a protein source in the human diet. The potential value of high-lysine corn seems large.

<sup>1</sup>Contribution from the Exploratory Research Division, Moffett Technical Center, Corn Products Co., Argo, Ill.

Much corn-breeding work is currently under way to incorporate the opaque-2 and floury-2 genes into inbreds suitable for commercial production. It appears that the first opaque-2 hybrid developed by Purdue University (10) is probably rather primitive; yet, when quantities of grain became available it seemed desirable to determine its wet-milling properties. Since about one-third of the corn input into a wet-milling plant becomes feed products, a determination of the distribution of the high-lysine component among wet-milled fractions seems appropriate. Although high-lysine corn will be used primarily as food and feed, it may be useful at this time to elucidate undesirable wet-milling characteristics that might be correctable by breeding.

#### MATERIALS AND METHODS

##### Corn

The opaque-2 hybrid corn is a yellow single-cross hybrid, (B-37  $\times$  540 opaque)  $\times$  W64A<sub>op</sub>, obtained from Purdue University, Lafayette, Indiana<sup>2</sup>. The recessive opaque-2 gene was introduced into B-37 by the back-cross technique. The grain used in this experiment was grown on the Purdue Agronomy Farm in 1965.

The normal hybrid No. 335 is an unidentified commercial hybrid grown on the Purdue Agronomy Farm in 1965. Normal hybrid No. 3 is Funk G-94 hybrid grown at Clinton, Illinois, in 1949. Quality has been preserved by storage in a dry atmosphere at 5°C.

##### Wet-Milling

The corn was steeped 48 hr. at 50°C. by a procedure previously described in detail (11). Steep medium for the first 36 hr. was a solution containing initially 0.05% SO<sub>2</sub> (as potassium metabisulfite) and 1.5% (by weight) lactic acid; and for the last 16 hr., 0.1% SO<sub>2</sub> and 0.5% lactic acid. The pH was held between 3.7 and 4.0 for the entire period. After steeping, the softened corn was wet-milled by a laboratory procedure designed to simulate the commercial process (12). Briefly, the steeped corn was ground to release the germs, which were recovered by flotation on the starch slurry. The starch was released by fine milling, separated from fibrous components by screening on nylon bolting cloth, and separated from gluten by tabling. The gluten was dried by lyophilization. The starch surface was washed gently with a stream of water and the resulting "squeegee" collected. All solid products except gluten were dried in a forced-draft oven at 50°-60°C. Steep-water dry-substance values were corrected for introduced chemicals.

##### Physical Methods

Kernel density was measured in a Beckman air comparison pycnometer and bulk density by weighing a pint of corn. Viability was determined by sprouting 100 kernels in a water-saturated atmosphere at 35°C. for 5 days.

##### Chemical Methods

Moisture content was measured by determination of weight loss at 120°C. under vacuum for 4 hr. Oil was extracted with carbon tetrachloride, and the weight of oil was determined after evaporation of the solvent. Total fat in starch was measured by extraction after acid hydrolysis of the starch.

<sup>2</sup>O. E. Nelson; personal communication.

Protein was determined by the official AACC Kjeldahl method for cereal products (13). Lysine, tryptophan, and methionine were determined by the microbiological procedure of Henderson and Snell (14). Xanthophyll and carotene contents of gluten were analyzed by a column chromatographic procedure (15). Fatty-acid components of oil were determined by gas-liquid chromatography (16).

#### Native Solubles

Corn was freshly ground to pass a 20-mesh screen, and 25 g. was extracted with 200 ml. 0.1% mercuric chloride solution in a Waring Blendor (model FC1) for 2 min. at full speed. The slurry was then centrifuged for 2 min. at 2,000 r.p.m., and the supernatant was filtered through a Hormann D-6 filter pad to give a sparkling-clear filtrate. Total dry substance, determined by evaporation of an aliquot to dryness, was expressed as percent of the original corn dry matter. The mercuric chloride serves to inactivate hydrolytic enzymes which would otherwise alter the composition of the extract.

#### Carbohydrates

Reducing sugars were determined by the Schoorl method (15) and expressed as dextrose. Sucrose was estimated as the increase in reducing sugars upon hydrolysis with 6*N* HCl (13). Water-soluble polysaccharides were determined by extracting the finely ground corn with 10% trichloroacetic acid solution; material precipitated with 75% methanol at 10°C. is weighed as polysaccharide. Starch was determined by the AACC polarimetric procedure (13).

### RESULTS AND DISCUSSION

#### Kernel Properties

The opaque-2 gene produces corn kernels that are completely opaque to transmitted light, in contrast with the brilliant translucency of normal flint and dent corn kernels (Fig. 1). Bulk density, which measures the composite

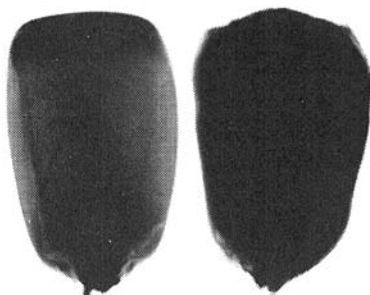


Fig. 1. Normal (left) and opaque-2 (right) corn kernels viewed in transmitted light.

of kernel density and packing volume, is 13% less for opaque-2 than for normal corn (Table I). Actual kernel density, as measured by the air-displacement method, shows that opaque-2 kernels have 11% lower actual density than normal corn No. 3. The latter is closest to average commercial corn

TABLE I  
PHYSICAL PROPERTIES OF OPAQUE-2 AND NORMAL HYBRID CORN

|                            | OPAQUE-2 | NORMAL<br>No. 3 | NORMAL<br>No. 335 |
|----------------------------|----------|-----------------|-------------------|
| Before steeping            |          |                 |                   |
| Initial kernel moisture, % | 9.08     | 9.5             | 9.22              |
| Kernel density, g./100 cc. | 116      | 131             | 128.5             |
| Bulk density, lb./bu.      | 54.0     | 62.3            | 62.2              |
| Kernel weight, g./100      | 22.1     | 30.8            | 31.2              |
| Viability, %               | 100      | 92              | 99                |
| After steeping             |          |                 |                   |
| Kernel moisture, %         | 52.3     | 42.8            | 44.6              |
| Volume increase, %         | 57       | 29              | 29                |

with respect to protein content and proportion of horny to floury endosperm. This internal kernel difference is, of course, reflected in the kernel weight. The weight of 100 kernels of the opaque-2 hybrid is only about three-fourths as great as the kernel weight of the two normal hybrids. The reasons for these differences are apparently crown collapse (Fig. 2), some internal voids, and smaller endosperm size. Duvick (17) has suggested that the "floury" endosperm region of a normal dent corn kernel refracts light differently because of microscopic voids around each starch granule. He speculated that these voids are produced when the protein matrix ruptures as a result of

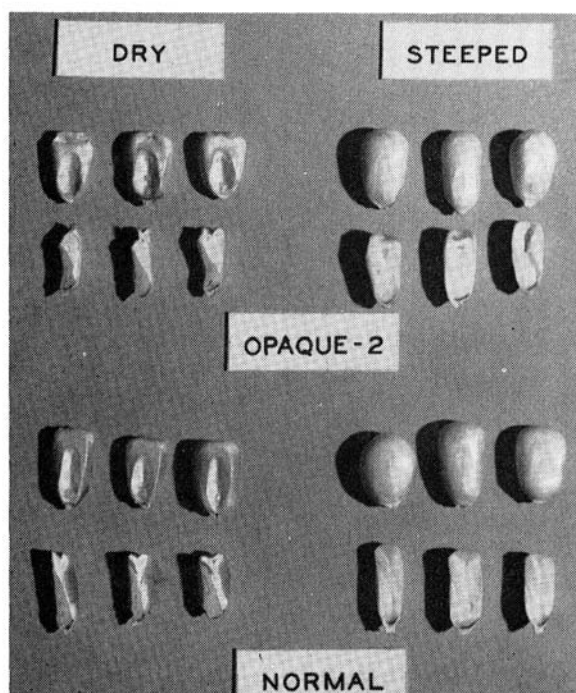


Fig. 2. Dry and steeped kernels of corn: above, opaque-2; below, normal corn.

shrinkage during drying. These small voids probably contain entrapped air which will not only refract both transmitted and incident light but will also result in lower density of the floury endosperm tissue.

The significance of the collapsed crown in wet-milling practice is shown by the much greater swelling volume of the opaque-2 kernels over the normal kernels (Table I). The reason for this may be seen in the swollen appearance and the high moisture content of the steeped opaque-2 kernels (Fig. 1, Table I). Apparently, the pericarp is normally developed. Therefore, water entering the kernel during steeping distends the pericarp and fills the void between the pericarp and the underdeveloped endosperm. Such a large increase in volume during steeping would necessitate a significantly larger steep-house size for a wet-milling plant. It is hoped that further breeding work can improve kernel fill. Nelson (10) believes that differences of 4 to 15% in size of opaque-2 kernels indicate breeding potential for improving this property.

The reduced endosperm size in the opaque-2 kernels was revealed by hand-dissection of steeped kernels (Table II). The opaque-2 endosperm

TABLE II  
HAND-DISSECTED FRACTIONS OF STEEPED KERNELS OF OPAQUE AND NORMAL CORN

| FRACTION <sup>a</sup> | OPAQUE-2 (332)     |              |        |      | NORMAL NO. 3 |              |        |      | NORMAL NO. 335 |              |        |      |
|-----------------------|--------------------|--------------|--------|------|--------------|--------------|--------|------|----------------|--------------|--------|------|
|                       | D. S. <sup>b</sup> | Pro-<br>tein | Starch | Fat  | D. S.        | Pro-<br>tein | Starch | Fat  | D. S.          | Pro-<br>tein | Starch | Fat  |
|                       | %                  | %            | %      | %    | %            | %            | %      | %    | %              | %            | %      | %    |
| PTC                   | 8.5                | 6.0          | 3.2    | 0.35 | 6.7          | 4.4          | 0.7    | 0.68 | 6.6            | 4.4          | 2.2    | 0.50 |
| G                     | 11.6               | 27.2         | 2.0    | 42.4 | 8.2          | 12.6         | 2.1    | 58.3 | 7.4            | 12.6         | 1.8    | 50.8 |
| E                     | 72.3               | 10.1         | 81.2   | 0.58 | 83.5         | 9.3          | 84.3   | 0.6  | 82.2           | 6.2          | 86.1   | 0.54 |
| S                     | 7.2                | 73.9         | ....   | .... | 3.4          | 40.0         | ....   | .... | 4.0            | 63.4         | ....   | .... |
|                       | 99.6               |              |        |      | 101.8        |              |        |      | 100.2          |              |        |      |

<sup>a</sup> PTC, pericarp and tip cap; G, germ; E, endosperm; S, steep-water.

<sup>b</sup> Expressed as percent of the whole-kernel dry substance. All other values are percent of the dry substance in the component parts.

comprised a smaller proportion of the kernel than was the case with the normal kernels; consequently, germ and pericarp made up a larger proportion of the opaque-2 kernel than of the normal kernel. The smaller proportion of endosperm also is reflected in a lower total starch content, 64.4%, and a higher oil content, 5.5%, for whole kernels of opaque-2 corn (Table III). The starch content of dissected steeped opaque-2 endosperm is lower and protein content is higher than in the normal corn endosperm. Composition of dissected germ (Table II) showed that starch was low and variable. Protein was unusually high in the opaque-2 germ, but this was probably due to retention of soluble protein, since the germ obtained in wet-milling (Table IV) was closer to normal germ. The lower oil content of opaque-2 germ compared with normal germ was the result of higher levels of protein and starch.

The extremely high protein content of the raw opaque-2 kernels (Table III) is not easily explained, but high soil nitrogen may have been partly responsible. Mertz *et al.* (6) have indicated that the opaque-2 gene does not affect total protein content of the whole kernels or endosperms, yet the opaque-2 corn is significantly higher in protein content than normal corn

TABLE III  
CHEMICAL COMPOSITION OF OPAQUE-2 AND NORMAL HYBRID CORN

| CONSTITUENT            | OPAQUE-2 | NORMAL No. 3 | NORMAL No. 335 | CONSTITUENT            | OPAQUE-2 | NORMAL No. 3 | NORMAL No. 335 |
|------------------------|----------|--------------|----------------|------------------------|----------|--------------|----------------|
|                        | %        | %            | %              |                        | %        | %            | %              |
| Protein                | 13.6     | 10.4         | 8.0            | Sucrose                | 2.1      | 1.5          | 1.3            |
| Starch                 | 64.4     | 71.4         | 73.6           | Reducing sugars        | 0.66     | 0.35         | 0.30           |
| Oil                    | 5.5      | 5.2          | 4.2            | Soluble polysaccharide | 1.4      | 1.6          | 1.4            |
| Native solubles, total | 6.6      | 4.6          | 4.4            | Xanthophyll, p.p.m.    | 26       | 14           | 22             |
| Soluble protein        | 1.40     | 0.39         | 0.41           | Carotene, p.p.m.       | 4        | 2            | 4              |

No. 3, which was known to have been grown in highly fertile soil. Nothing is known about the soil condition for normal hybrid No. 335, but its very low protein content suggests that the amount of soil nitrogen available during growth was severely limited. Schneider *et al.* (3) reported that a good-quality commercial hybrid can vary from 7 to 13% in kernel protein in response to different levels of soil nitrogen or planting rate, or both.

A significant characteristic of opaque-2 kernels is the large amount of water-soluble material (native solubles, Table III). The soluble nitrogenous substances in the water extract probably include protein, peptides, amino acids, and other simple nitrogenous compounds. The opaque-2 hybrid corn yielded more than three times as much soluble "protein" as the normal hybrids. Soluble polysaccharide content was unaffected by the opaque-2 gene. The sucrose and reducing-sugar contents of opaque-2 corn were higher than normal, but this difference could be entirely due to the larger germ in opaque-2 kernels.

TABLE IV  
WET-MILLING FRACTIONS FROM OPAQUE-2 AND NORMAL HYBRID CORNS

| FRACTION                 | OPAQUE-2 | NORMAL No. 3 | NORMAL No. 335 | FRACTION              | OPAQUE-2 | NORMAL No. 3 | NORMAL No. 335 |
|--------------------------|----------|--------------|----------------|-----------------------|----------|--------------|----------------|
|                          | %        | %            | %              |                       | %        | %            | %              |
| GERM                     |          |              |                | SQUEEGEE              |          |              |                |
| Yield, d.s. <sup>a</sup> | 9.2      | 7.1          | 6.7            | Yield, d.s.           | 4.4      | 6.7          | 3.7            |
| Protein                  | 15.2     | 11.6         | 10.8           | Protein               | 3.7      | 13.4         | 3.3            |
| Oil                      | 49.6     | 56.8         | 52.2           | Oil                   | 0.46     | 2.2          | 0.4            |
| Starch                   | 6.9      | 10.8         | 9.5            | Starch                | 93.0     | 81.8         | 93.4           |
| Oil yield                | 4.6      | 4.3          | 3.5            | STARCH                |          |              |                |
| FIBER, COARSE            |          |              |                | Yield, d.s.           | 53.0     | 59.2         | 65.1           |
| Yield, d.s.              | 10.2     | 9.4          | 8.3            | Protein               | 0.36     | 0.24         | 0.21           |
| Protein                  | 8.8      | 12.2         | 8.2            | SOLUBLES, STEEP-WATER |          |              |                |
| Oil                      | 3.0      | 3.1          | 3.4            | Yield, d.s.           | 7.2      | 4.0          | 3.9            |
| Starch                   | 11.5     | 17.0         | 14.1           | Protein               | 73.9     | 58.6         | 63.4           |
| GLUTEN                   |          |              |                | SOLUBLES, MILLING     |          |              |                |
| Yield, d.s.              | 8.5      | 7.9          | 6.9            | Yield, d.s.           | 4.0      | 2.5          | 2.2            |
| Protein                  | 36.5     | 46.0         | 40.8           | Protein               | 51.9     | 44.0         | 36.3           |
| Oil                      | 11.0     | 8.3          | 6.4            |                       |          |              |                |
| Starch                   | 41.0     | 37.4         | 43.2           |                       |          |              |                |

<sup>a</sup>d.s. = dry substance.

## Wet-Milling Characteristics

Yields and compositions of the wet-milling fractions of the three corns are given in Table IV. Each fraction except solubles was analyzed for dry substance (moisture), protein, starch, and fat. Each value is an average of closely agreeing duplicate runs. These data were recalculated to give total recovery figures for each component (Table V). Recovery of all com-

TABLE V  
DISTRIBUTION OF DRY SUBSTANCE, PROTEIN, STARCH, AND FAT  
AMONG WET-MILLING FRACTIONS

| FRACTION         | NORMAL        |       |         | OPAQUE-2 | NORMAL |         |
|------------------|---------------|-------|---------|----------|--------|---------|
|                  | OPAQUE-2      | No. 3 | No. 335 |          | No. 3  | No. 335 |
|                  | %             | %     | %       | %        | %      | %       |
|                  | Dry substance |       |         | Protein  |        |         |
| Germ             | 9.2           | 7.1   | 6.7     | 10.2     | 7.9    | 9.0     |
| Fiber            | 10.2          | 9.4   | 8.5     | 6.6      | 10.8   | 8.7     |
| Gluten           | 8.5           | 7.9   | 6.9     | 22.6     | 34.6   | 34.8    |
| Squeegee         | 4.4           | 6.7   | 3.7     | 1.2      | 8.2    | 1.4     |
| Starch           | 53.0          | 59.2  | 65.1    | 1.4      | 1.4    | 1.7     |
| Steepwater       | 7.2           | 4.0   | 3.9     | 38.9     | 22.2   | 30.7    |
| Milling solubles | 4.0           | 2.6   | 2.2     | 15.5     | 11.1   | 9.9     |
| Sum              | 96.5          | 96.9  | 96.8    | 96.5     | 96.0   | 96.3    |
|                  | Oil           |       |         | Starch   |        |         |
| Germ             | 81.3          | 78.2  | 83.4    | 1.0      | 1.0    | 0.8     |
| Fiber            | 4.6           | 5.6   | 5.4     | 1.9      | 2.2    | 1.7     |
| Gluten           | 17.4          | 12.6  | 11.2    | 5.6      | 4.2    | 4.2     |
| Squeegee         | 0.4           | 2.7   | 0.3     | 6.0      | 7.8    | 4.8     |
| Starch           | 0.9           | 1.6   | 0.9     | 83.0     | 82.7   | 88.1    |
| Steepwater       | ....          | ....  | ....    | ....     | ....   | ....    |
| Milling solubles | ....          | ....  | ....    | ....     | ....   | ....    |
| Sum              | 104.6         | 100.8 | 101.2   | 97.2     | 97.8   | 99.6    |

ponents ranged from 96 to 104% with an average of about 98%. Dry-substance recovery data (Table IV or V) show the higher germ and gluten yields and lower starch yield obtained from the opaque-2 hybrid that were predictable from the data for whole-kernel analysis (Table III) and hand-dissection (Table II).

The higher yield of steep-water and milling solubles from the opaque-2 corn and the higher protein content of the solubles fractions (Table IV) are the outstanding differences in the wet-milling behavior of opaque-2 and normal corn. This difference was at least partially predictable from the higher values for native solubles obtained on the raw grain. Protein content of combined solubles is calculable to be 66.0, 54, and 56% for opaque-2, No. 3, and No. 335, respectively. This accounts for 54.4% of the whole-kernel nitrogen for opaque-2 corn and only 33.3 and 40.6% for normal corns No. 3 and No. 335, respectively.

Starch recovery performance of the opaque-2 corn appears to be quite normal. This is indicated by the acceptable protein content of 0.36% in the starch, the normal recovery of 83% of the original starch by tabling, and the normal yield of squeegee and gluten. Squeegee yield is a fairly sensitive in-

indicator of starch-gluten separation performance, since poor starch separability produces high squeegee yield.

#### Distribution of Essential Amino Acids in Wet-Milled Fractions

Three of the most important essential amino acids for animal nutrition are lysine, tryptophan, and methionine. All fractions were analyzed for lysine, all but fiber was analyzed for methionine; tryptophan was determined only in the whole grain and the gluten. Results in Table VI confirm the expected

TABLE VI  
AMINO ACID ANALYSES OF WET-MILLING FRACTIONS

| FRACTION<br>AND<br>COMPONENTS | OPAQUE-2 HYBRID |            | NORMAL HYBRIDS |            |             |            |
|-------------------------------|-----------------|------------|----------------|------------|-------------|------------|
|                               | In Fraction     | In Protein | No. 3          |            | No. 335     |            |
|                               |                 |            | In Fraction    | In Protein | In Fraction | In Protein |
| Whole grain                   | %               | %          | %              | %          | %           | %          |
| Lysine                        | 0.49            | 3.6        | 0.25           | 2.4        | 0.24        | 3.0        |
| Methionine                    | 0.26            | 1.9        | 0.20           | 1.9        | 0.18        | 2.2        |
| Tryptophan                    | 0.10            | 0.74       | 0.12           | 0.6        | 0.047       | 0.59       |
| Germ                          |                 |            |                |            |             |            |
| Lysine                        | 0.68            | 4.7        | 0.43           | 3.8        | 0.49        | 4.6        |
| Methionine                    | 0.24            | 1.7        | 0.17           | 1.4        | 0.18        | 1.7        |
| Fiber                         |                 |            |                |            |             |            |
| Lysine                        | 0.29            | 3.5        | 0.24           | 2.0        | 0.16        | 1.8        |
| Gluten                        |                 |            |                |            |             |            |
| Lysine                        | 1.27            | 3.5        | 0.60           | 1.3        | 0.92        | 2.1        |
| Methionine                    | 0.81            | 2.2        | 1.15           | 2.5        | 1.31        | 3.0        |
| Tryptophan                    | 0.36            | 1.0        | 0.12           | 0.3        | 0.20        | 0.46       |
| Solubles <sup>a</sup>         |                 |            |                |            |             |            |
| Lysine                        | 2.5             | 3.9        | 2.2            | 4.1        | 1.79        | 3.7        |
| Methionine                    | 1.1             | 1.7        | 1.2            | 2.2        | 1.30        | 2.6        |

<sup>a</sup> Steep-water and milling solubles combined.

higher level of lysine and tryptophan in the whole opaque-2 corn and the gluten isolated from it. Lysine content of the opaque-2 fiber fraction is also higher than for normal corn, because most of the protein in the fiber fraction is from the endosperm. Germ of opaque-2 corn has lysine and methionine contents equal to those of normal corn No. 335. Mertz *et al.* (6) also found no effect of the opaque-2 gene on lysine content of germ. The lower lysine content of the germ from normal corn No. 3 cannot be explained. The lysine contents of the solubles fractions are remarkably similar for all three corns. This is rather surprising, because previous data have indicated that about one-half of the soluble protein comes from the endosperm through solubilizing action of sulfur dioxide (unpublished data).

#### Gluten Properties

While tabling is the only satisfactory method of separating starch and gluten in the laboratory, all modern wet-milling plants use continuous centrifugal machines which produce gluten of up to 70% protein in order to maximize starch yield. Therefore, gluten and starch yields were recalculated to the 70% protein gluten basis, assuming that dry substance recovered is all starch. These results (Table VII) reveal a subnormal gluten yield from



TABLE VII  
CALCULATION OF GLUTEN AND STARCH YIELD TO THE 70% PROTEIN GLUTEN BASIS

|                                  | OPAQUE-2 | NORMAL CORN |         |
|----------------------------------|----------|-------------|---------|
|                                  |          | No. 3       | No. 335 |
|                                  | %        | %           | %       |
| <b>Starch</b>                    |          |             |         |
| Yield, d.s.                      | 61.3     | 67.4        | 71.5    |
| Recovery                         | 95.1     | 94.4        | 97.1    |
| <b>Gluten</b>                    |          |             |         |
| Yield, d.s.                      | 4.6      | 6.4         | 4.1     |
| Recovery of protein <sup>a</sup> | 34.1     | 62.2        | 51.8    |
| Oil                              | 20.3     | 12.4        | 11.3    |
| Protein                          | 70       | 70          | 70      |
| Lysine                           | 2.5      | 0.9         | 1.5     |
| Methionine                       | 1.5      | 1.8         | 2.1     |
| Tryptophan                       | 0.7      | 0.2         | 0.3     |
| Xanthophyll (p.p.m.)             | 244      | 132         | 120     |
| Carotene (p.p.m.)                | 29       | 12          | 18      |

<sup>a</sup>Based on initial whole-kernel analysis.

opaque-2 corn because of the smaller endosperm size and greater protein solubility during steeping. The normal corn No. 335 is subnormal in gluten yield because of low endosperm protein content. Thus, recovery of kernel protein in gluten is 34.1, 62.2, and 51.8 for opaque-2, normal No. 3, and normal No. 335, respectively.

Lysine and tryptophan contents of gluten from normal corn are expected to decrease with increasing gluten yields, reflecting probable differences in zein contents. Higher levels of total endosperm protein have been shown to reflect increased zein concentration (3,18) and to produce higher gluten yield on wet-milling.

Expected differences in zein contents of the three glutes were confirmed by extraction of the three glutes by the classic Osborn-Mendel (19) series of protein solvents: salt solution, 70% ethanol, and alkali. The opaque-2 gluten was lowest in zein, highest in glutelin and insoluble protein contents (Table VIII). This confirms observations made by Mertz *et al.* (6) by means of an alkaline-copper sulfate fractionation technique. Although no direct evidence can be cited, it appears that steeping has enriched the lysine and tryptophan content of opaque-2 gluten through dissolution of more zein than glutelin.

TABLE VIII  
PROTEIN FRACTIONATION OF OPAQUE-2 AND NORMAL GLUTENS

| PROTEIN FRACTION          | OPAQUE-2 GLUTEN | NORMAL GLUTEN No. 3 | NORMAL GLUTEN No. 335 | COMMERCIAL GLUTEN (WASHED) |
|---------------------------|-----------------|---------------------|-----------------------|----------------------------|
|                           | %               | %                   | %                     | %                          |
| Salt-soluble (globulins)  | 2.8             | 1.5                 | 1.6                   | 1.2                        |
| Alcohol-soluble (zein)    | 32.8            | 65.0                | 53.5                  | 67.7                       |
| Alkali-soluble (glutelin) | 51.0            | 30.4                | 31.8                  | 27.6                       |
| Insoluble                 | 12.0            | 2.8                 | 9.6                   | 3.0                        |
| Sum                       | 98.6            | 99.7                | 96.5                  | 99.5                       |

Xanthophyll and carotene contents of opaque-2 gluten are high in comparison with the glutes from the normal corn. This may be due to a concentrating effect resulting from removal of more soluble protein from opaque-2 endosperm than from normal endosperm. However, some pigment loss must have been encountered, because of the poor recovery of xanthophyll in No. 335 gluten.

#### Starch Properties

In spite of the importance of the gluten fraction from opaque-2 corn, starch must still be the primary product from wet-milling of this grain. Therefore, starch properties from opaque-2 and normal corns were compared (Table IX). No significant differences were found. The amylose contents as measured by iodine affinity were essentially the same, as was Brabender viscosity. The Scott viscosity of the opaque-2 starch was appreciably above normal, but this result cannot be explained in view of the Brabender results.

TABLE IX  
PROPERTIES OF STARCH FROM OPAQUE-2 AND NORMAL CORN

|   | OPAQUE-2<br>HYBRID | NORMAL HYBRIDS |         | COMMER-<br>CIAL<br>CORN<br>STARCH |
|---|--------------------|----------------|---------|-----------------------------------|
|   |                    | No. 3          | No. 335 |                                   |
| Protein, %                              | 0.36               | 0.24           | 0.21    | 0.33                              |
| Total fat, %                            | 0.68               | 0.50           | 0.50    | 0.6                               |
| Oil, %                                  | 0.09               | 0.15           | 0.06    | 0.05                              |
| Gelatinization temp., °C.               |                    |                |         |                                   |
| 2%                                      | 59                 | 57             | 55      | 62                                |
| 50%                                     | 66                 | 64             | 62      | 66                                |
| 98%                                     | 71                 | 70             | 68      | 70                                |
| Swelling power, 85°C.                   | 11.5               | 16             | 13      | 12                                |
| Solubles, %, 85°C.                      | 6.0                | 7.6            | 8.0     | 7.2                               |
| Viscosity, Scott,<br>12 g./50 ml., sec. | 135                | 98             | 79      | 84                                |
| Viscosity, Brabender                    |                    |                |         |                                   |
| Peak                                    | 555                | 560            | 530     | 475                               |
| 95°C., 60 min.                          | 400                | 390            | 360     | 400                               |
| 50°C., 30 min.                          | 870                | 900            | 870     | 845                               |
| Initial temp., °C.                      | 77                 | 74             | 71      | 76                                |
| Iodine affinity                         | 4.74               | 4.62           | 4.63    | 4.90                              |

TABLE X  
FATTY ACID COMPOSITION OF GERM OIL  
(Expressed as percentage of the total oil)

| FATTY<br>ACID | OPAQUE-2<br>HYBRID | NORMAL HYBRIDS |         | COMMER-<br>CIAL<br>CORN<br>OIL |
|---------------|--------------------|----------------|---------|--------------------------------|
|               |                    | No. 3          | No. 335 |                                |
|               | %                  | %              | %       | %                              |
| C-16:0        | 10.4               | 12.0           | 12.4    | 10.9                           |
| C-18:0        | 1.7                | 2.1            | 2.4     | 2.1                            |
| C-18:1        | 23.7               | 27.4           | 26.7    | 25.0                           |
| C-18:2        | 58.5               | 51.6           | 53.0    | 56.0                           |
| C-18:3        | 0.9                | 1.0            | 0.6     | 0.8                            |
| C-20:0        | 0.2                | 0.2            | 0.5     | 0.2                            |

Gelatinization temperature was above normal, as measured by microscopic observation and by the temperature at which viscosity increase began in the Brabender test.

#### Oil Composition

Oil from the isolated germ fraction was extracted and analyzed for fatty-acid components by gas-liquid chromatography. Results are given in Table X. Differences observed in fatty-acid composition are probably unrelated to effects of the opaque-2 gene, because differences of this magnitude are commonly found among different hybrids.

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