PROTEIN COMPOSITION OF DENT, WAXY, AND HIGH-AMYLOSE CORNS[1]

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ABSTRACT

Protein content and composition were examined in three types of corn, alike in hereditary factors except for *ae* and *wx* genes which determine the amylose and amylopectin contents of the starches. Globulin, zein, and glutelin proteins, prepared by selective extractions from dent, waxy, and high-amylose corns, were subjected to gel electrophoresis and amino acid analysis to elucidate possible variations in proteins. No differences were observed in the electrophoretic patterns of the proteins from the different genotypes. Although the globulins and zeins from the different strains were similar in amino acid content, the glutelin fractions differed significantly. The starch-gel electrophoretic patterns and amino acid compositions of reduced globulins, zeins, and glutelins from dent corn were also compared. Reduced glutelin electrophoretic components have counterparts in reduced zein and reduced globulins; also, glutelin amino acid composition is intermediate between that of the other two proteins.

Significant quantities of corn from strains having starch rich in amylose, or starch exclusively composed of amylopectin, are being grown for specific uses. Zuber *et al.* (1) have shown that as amylose content of the grain is increased, the endosperm size is diminished as evidenced by increased yield of germ and pericarp. Although in high-amylose (amylomaize) varieties the amount of protein in the germ does not change, the amount in the endosperm increases significantly. Studies of wet-milling by Anderson *et al.* (2,3) established that high-amylose corn mills with greater difficulty than dent varieties and that recoveries of starch and gluten protein are lower. Since the protein of corn influences wet-milling and since the gluten derived is a valuable by-product, it was desirable to establish whether selection of corn variety for starch might significantly change not only the quantity but the kind of protein in the grain. The protein composition is also important, because surplus quantities of high-amylose or waxy-corn varieties may be used directly for feed. Variation in starch biosynthesis results from enzymatic differences that may be reflected in component proteins in different strains of corn.

Carefully controlled corn breeding has produced varieties differing only in the *ae* gene, which regulates amylose production, and in the *wx* gene, responsible for amylopectin synthesis. Studies on protein composition were therefore conducted on normal dent and related waxy and amylomaize hybrid strains to ensure that the variations reflected only changes due to the introduction of starch-modifying genetic factors. The three strains were compared as to protein content, amounts of different types of protein (i.e., globulins, zein, and glutelin), patterns from starch-gel electrophoresis, and amino acid content of the separated protein fractions.

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Materials and Methods

Treatment of Grain. The three samples of corn were the seed generation of dent, waxy, and amylomaize hybrids containing the same basic germ plasm and differing only in the *wx* gene in the waxy and the *ae* gene in the amylomaize. The samples, provided by Bear Hybrid Corn Co., Inc., were from respective lot numbers 19P, 119P, and 117P for the normal dent, waxy, and amylomaize. Their nitrogen and amylose contents are given in Table I.

TABLE I

NITROGEN AND AMYLOSE CONTENTS OF AMYLOMAIZE, WAXY, AND DENT CORNS AND PERCENTAGES OF NITROGEN EXTRACTED WITH VARIOUS SOLVENTS

			Nitrogen Extracted							
					Successive Extractions					
Corn	Nitrogen Content	Amylose Content of Starch	Saline-Phosphate	Saline-Acetate	1 Water	2 Saline	3 Ethanol	4 Alkali	5 Alkali-Copper	Total (Extracts 1-5)
	%	%	%	%	%	%	%	%	%	%
Dent	2.07	25.9	11.8	11.3	10.9	6.6	22.6	26.6	9.5	76.2
Waxy	1.76	2.4	13.5	12.6	14.1	7.1	18.5	26.1	6.6	72.4
Amylomaize	2.45	52.8	11.4	10.7	11.0	6.7	22.1	25.8	7.4	73.0

Portions of each sample were cracked in a burr mill and ground in a hammer mill to pass a 1/16-in. screen, then defatted with pentane-hexane for 1 hr. at 5°C., and air dried.

Extraction of Proteins. The water-soluble proteins were obtained by stirring samples of the defatted corn meals with water in a 5:1 water-to-meal ratio (v./w.) for 1 hr. at 5°C. and centrifuging the suspension. The meal residues were re-extracted with water in a 5:2 water-to-meal ratio (v./w.) for 1 hr. at 5°C., and the resulting suspensions were centrifuged. The combined supernatants were then lyophilized.

Similarly, the saline-solubles were isolated from the water-extracted meal with 0.5*M* NaCl solution as the extractant, then dialyzed and lyophilized.

Zein, the 70% ethanol-soluble protein fraction, was prepared by a procedure similar to that described previously (4). Meals remaining after the water and saline extractions were washed free of salt with water before extraction with 70% ethanol.

To obtain the glutelin fraction, the meal residues previously extracted with water, saline, and 70% ethanol solutions, and washed free of solvents with water, were stirred with 0.1*N* NaOH in a 5:2 solvent-to-meal ratio (v./w.) for 4 hr. at room temperature. The meal residues were washed twice with fresh 0.1*N* NaOH solutions in a 1:1 ratio (v./w.) and the supernatants were combined after centrifugation. The combined solutions were dialyzed against water until neutral and then lyophilized.

The alkaline copper-sulfite procedure described by Mertz and Bressani (5)

was modified for the final extraction. Meals were mixed with the alkaline copper-sulfite solution and stirred for 3 hr. at room temperature and centrifuged. The supernatants were frozen and thawed, and the insoluble starch residues were removed and discarded. The clarified supernatants were then dialyzed against 0.02*N* HCl and lyophilized.

The meal residues were frozen between extractions and thawed when required for further investigation.

Two independent extractions of albumins and globulins were made from corn meals that had not been previously extracted. In one, a 0.1% phosphate-1*M* NaCl solution, pH 6.7, was stirred with samples of the corn meals in a 4:1 (v./w.) solvent-to-meal ratio for 30 min. at room temperature as described by Pence and Elder (6). The suspensions were centrifuged, dialyzed, and lyophilized. The other extraction was made with an acidic-saline medium (0.1*N* acetic acid-0.5*M* NaCl) in a 5:1 (v./w.) solvent-to-meal ratio for 3 hr. at room temperature. The suspension was centrifuged, and the supernatant was dialyzed against 0.1*N* acetic acid and then lyophilized.

Analytical Methods. Aliquots of the extracts or weighed-dried materials were assayed for nitrogen by a semimicro-Kjeldahl method in which mercuric oxide served as digestion catalyst.

Amylose was determined spectrophotometrically as the iodine-starch complex by the method of Deatherage *et al.* (7).

Reduction of portions of the extracted proteins was carried out in a pH 8.0 phosphate buffer (ionic strength 0.02) containing 8*M* urea by reaction with a twenty-fold molar excess of mercaptoethanol (8) (assuming 1 mole of ½ cystine per 7,500 g. protein) for 3 hr. A tenfold excess of acrylonitrile was added for alkylation (9) of the thiol groups of protein and mercaptoethanol and reacted for 1 hr. at room temperature. The solutions were adjusted to pH 3.0 and dialyzed against either water or 0.01*M* acetic acid before lyophilization.

For amino acid analysis, 50-mg. protein samples were hydrolyzed with 10 ml. constant-boiling HCl in sealed glass tubes at 105°C. for 24 hr. Amino acids were quantitatively determined with a Phoenix automatic amino acid analyzer, Model K-8000, by the Spackman, Stein, and Moore (10) procedure.

Electrophoresis was carried out in starch gels containing 8*M* urea as described by Turner *et al.* (4). However, the length of time of electrophoresis varied and was determined for each protein by its mobility relative to that of a spot of safranin O. This dye, used routinely to follow the progress of electrophoresis, served as a basis for relative mobilities. Globulins have greater mobility than zein and required electrophoresis for only 6 hr. compared to 24 hr. for zein resolution.

Results and Discussion

Extraction Yields. The percentages of the total nitrogen extracted from the three corn genotypes with each of the solvents described are shown in Table I. The three corns differed markedly in protein content (N × 6.25): dent, 12.9%; waxy, 11.0%; and amylomaize, 15.3%. The high protein con-

tent of the amylomaize sample is consistent with other observations that high protein levels are associated with the *ae* gene (1,3). In contrast, the low protein content of the waxy strain is not expected, since MacMasters and Hilbert (11) indicated that these corn varieties had an average higher protein content than normal dents. However, analyses by these workers of several samples of waxy corn from different varieties and crop years showed considerable variation in protein content, and the lower value in our sample is within the range of variance. Despite the differences in protein content, the percentage of total nitrogen extracted from the three corns by successive extraction is approximately the same: 76.2, 72.4, and 73.0%.

In the successive extractions, the relative yields of extracted protein fractions in the waxy samples differed markedly from those of the normal dent and amylomaize. The waxy maize contained a significantly greater amount of water-soluble and saline-soluble proteins and a smaller proportion of alcohol-solubles than the other strains (Table I). The amylomaize and dent corn were similar, except that a higher proportion of dent-corn protein yielded to extraction by alkali-copper treatment.

The saline-phosphate and the saline-acetate solutions were similar in efficiency of extraction. The buffered saline solutions extracted less nitrogen, however, than the combined water and saline extractions.

Only small differences existed between nitrogen contents of extracted solids from the different corns (Table II), except in the water-solubles and

TABLE II

PERCENT NITROGEN IN EXTRACTED SOLIDS

CORN	METHOD OF PROTEIN EXTRACTION				
	Water (Albumin)	Saline (Globulin)	Ethanol (Zein)	Alkali (Glutelin)	Alkali-Copper
Dent	3.7	9.8	14.2	14.7	N.D.[a]
Waxy	3.0	13.4	13.5	15.0	N.D.[a]
Amylomaize	3.4	14.2	14.3	15.6	16.2

[a] N.D. = not determined.

the saline-solubles. The low nitrogen of the water-solubles indicates extensive extraction of nonprotein constituents. Although the relatively easy dispersion of the polysaccharide from waxy corn may explain why its water extract was the lowest in protein, there is no explanation for the unusually low nitrogen analysis of the saline extract of dent corn.

Comparison of Proteins by Starch-Gel Electrophoresis. The starch-gel patterns of the three globulin preparations—saline, phosphate-saline, and acetate-saline solubles—are shown in Figs. 1, 2, and 3, respectively. The three globulin preparations were made because it was expected that differences would be most likely to appear in this class of proteins. The saline (Fig. 1) and phosphate-saline (Fig. 2) preparations are very much alike in the complexity of their patterns and in the mobilities and large numbers of components. The acetate-saline preparation (Fig. 3) differs greatly from the other two; there are fewer components, and resolution is much improved

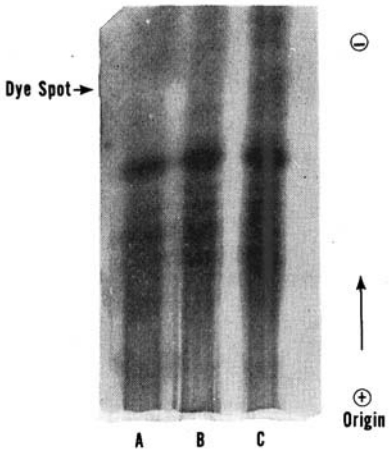

Fig. 1 (left). Starch-gel electrophoretic patterns of saline-soluble proteins of dent (A), waxy (B), and amylomaize (C) corns.

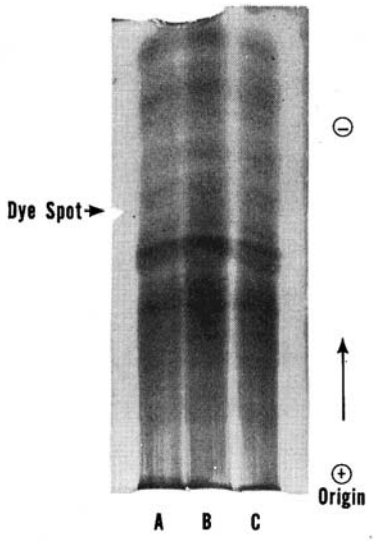

Fig. 2 (right). Starch-gel electrophoretic patterns of phosphate-saline-soluble proteins of dent (A), waxy (B), and amylomaize (C) corns.

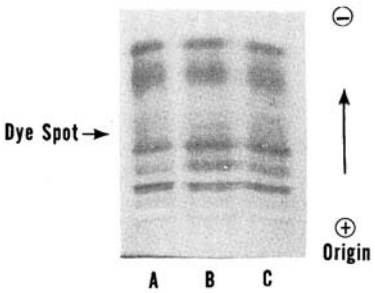

Fig. 3. Starch-gel electrophoretic patterns of acetate-saline-soluble proteins of dent (A), waxy (B), and amylomaize (C) corns.

because of lack of origin material and of streaking. The globulins in each preparation from the three genotypes bear a striking similarity in electrophoretic pattern, with no significant detectable differences. The electrophoretic patterns of water-solubles (not shown) also exhibit no differences.

Two major bands, plus material at the origin, are visible for zeins (70% alcohol-solubles) from the three corns (Fig. 4). Although the electrophoretically mobile components of the native zeins appear identical, the possibility existed of differences in the disulfide-crosslinked immobile material (4). Therefore, the zeins were reduced to disrupt the disulfide bonds, to allow migration of component polypeptides of the immobile material into the gel.

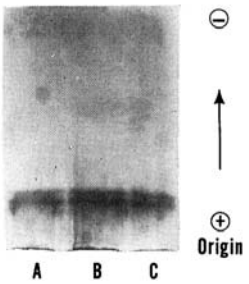

Fig. 4 (left). Starch-gel electrophoretic patterns of alcohol-soluble proteins (zein) of dent (A), waxy (B), and amylomaize (C).

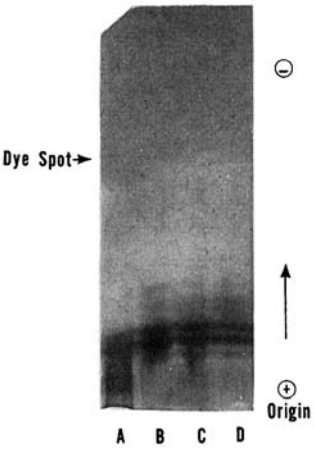

Fig. 5 (right). Starch-gel electrophoretic patterns of alcohol-soluble protein (zein) of dent corn (A); alkylated-reduced zeins of dent (B), waxy (C), and amylomaize (D) corns.

Figure 5 illustrates the starch-gel electrophoretic pattern of the alkylated-reduced zeins from each genotype. A pattern of native zein is also shown in Fig. 5 to demonstrate changes in mobilities that follow reduction and alkylation (4). There are no differences apparent in alkylated-reduced zeins from corns of different genotypes.

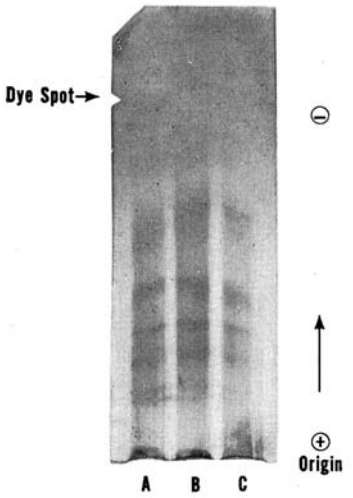

Fig. 6 (left). Starch-gel electrophoretic patterns of alkylated-reduced alkali-soluble proteins (glutelin) from dent (A), waxy (B), and amylomaize (C) corns.

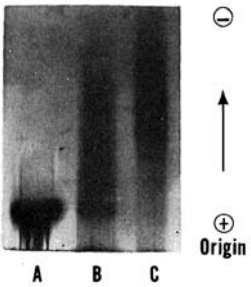

Fig. 7 (right). Starch-gel electrophoretic patterns of reduced alcohol-solubles (A), reduced alkali-solubles (B), and reduced saline-solubles (C) from dent corn.

The electrophoretic patterns of alkylated-reduced glutelins in Fig. 6 show several mobile components plus material at the origin. Nonreduced glutelin does not migrate into the gel (not shown). The presence of immobile material could indicate incomplete reduction. No differences are detected in the mobile components of reduced glutelin from the three genotypes.

Comparison of three proteins—globulin, zein, and glutelin—from normal dent corn by starch-gel electrophoresis is shown in Fig. 7. Each of the proteins was reduced but not alkylated, and electrophoresis was performed in gels containing mercaptoethanol to maintain the proteins in a reduced state. The reduced globulins migrate more rapidly than the reduced zeins. The two slowest-moving bands in the reduced glutelin correspond to the major zein components, and the fastest-moving bands have counterparts in the reduced globulin pattern. Therefore, glutelin is composed, at least partially, of polypeptides resembling those of both zein and globulin proteins.

Amino Acid Composition. Amino acid composition for separate protein classes in each corn genotype was examined to detect possible differences not revealed in the electrophoretic studies.

The marked differences in amino acid composition among the globulins, zeins, and glutelins from dent corn are shown in Table III. The globulins are the richest in the basic amino acids and glycine. The higher content of basic amino acids in globulin proteins than in zein accounts for their greater mobility in starch-gel electrophoresis in acid buffer. Zein contains a much higher content of glutamic acid, proline, alanine, and leucine than do globulins. The amino acid content of the glutelin appears to be midway between that of globulins and zein, except for the higher content of histidine and proline in the glutelin fraction. The possibility that glutelin consists of some globulin-like and zein-like proteins linked by disulfide bonds is consistent with the relative electrophoretic mobilities of the three protein types after reduction (Fig. 7).

The amino acid analyses of the globulins, zeins, and glutelin fractions are compared for the three different types of corn in Table III.

Some variations in the contents of amino acids between the three genotypes were statistically significant at the 0.05 probability level. Estimates of variation (using logarithms) were calculated from the sums of squares of the differences between results for the duplicate assays. For the glutelins and globulins, standard deviations (in log units) were 0.00635 and 0.00522, respectively; for the zeins, 0.01438. The relative precision calculated from the antilogs gave relative errors of 1.2% for globulins, 1.5% for glutelins, and 3.4% for zeins.

Although some significant differences were observed in the amino acid content of the globulins, zeins, and glutelins (Table III) in the three corns, none were sufficiently marked to indicate a major deviation from protein type.

The amino acid composition of glutelin proteins showed the greatest variation between dent, waxy, and amylomaize types (Table III). The dent corn glutelin is the highest of the three in the major amino acids of zein (glutamic acid, proline, alanine, and leucine). The waxy corn glutelin contains the highest amounts of the amino acids associated with the globulins

TABLE III

AMINO ACID COMPOSITION OF GLOBULINS, ZEINS, AND GLUTELINS FROM THREE GENOTYPES[a]
(mmoles/g. N)

	GLOBULINS			ZEINS			GLUTELINS		
AMINO ACID	From Dent	From Waxy	From Amylomaize	From Dent	From Waxy	From Amylomaize	From Dent	From Waxy	From Amylomaize
Lysine	2.35*	2.56*	2.76*	0.05	0.06	0.04	1.08*	1.25*	1.14*
Histidine	1.56	1.42	1.48	0.58	0.60	0.60	1.84	1.88	1.84
Ammonia	5.30*	5.44*	5.19*	11.93	11.99	12.21	8.28	8.37	8.10
Arginine	4.58	4.50	4.46	0.71	0.64	0.68	1.84	2.00	1.84
Aspartic acid	3.76	3.72	3.86	2.94	2.88	2.88	2.58	2.66	2.44
Threonine	2.04*	2.06*	2.16*	1.66*	1.80*	1.68*	2.22	2.18	2.12
Serine	3.52	3.59	3.46	3.24*	4.02*	3.32*	3.34*	2.99*	2.82*
Glutamic acid	6.12	6.14	6.10	11.90	11.64	11.39	9.37*	8.64*	8.51*
Proline	2.84*	2.64*	2.84*	6.77	6.76	6.37	7.56*	7.06*	6.94*
Glycine	5.04	5.08	4.88	1.22	1.19	1.21	3.80*	4.18*	3.90*
Alanine	4.39	4.41	4.38	8.06	7.84	7.69	5.40*	5.22*	4.88*
1/2 Cystine[b]	1.41	1.36	1.49	0.27	0.18	0.18	1.02	0.93	1.01
Valine	3.04	2.98	2.98	2.40	2.29	2.24	2.90*	3.32*	3.18*
Methionine	0.58	0.59	0.59	0.18*	0.42*	0.20*	1.00	1.18	1.20*
Isoleucine	1.67*	1.60*	1.62*	2.60*	2.33*	2.49*	1.58*	1.74*	1.65*
Leucine	2.79	2.80	2.78	11.14	11.00	10.53	6.18*	5.71*	5.48*
Tyrosine	1.24*	1.39*	1.36*	2.08	2.14	1.96	1.96	1.91	1.83
Phenylalanine	1.84*	1.96*	1.76*	3.59	3.72	3.84	1.94*	1.94*	1.76*

[a] Tryptophan not determined.
[b] Minimum value; not included in significant difference evaluation.
*Variation between at least two of the three types is significant.

(lysine, arginine, and glycine). These analyses could indicate a high proportion of zein-like protein in dent glutelin and a high proportion of globulin-like proteins in waxy glutelin.

Conclusions

High-amylose corn contains more total protein than dent hybrid; however, differences between the two corns in composition and relative proportion of each protein class were not observed. The germ content of the amylomaize grains is greater than that in standard dent varieties (1), but the ratio of globulin, in which the germ is rich, to the zein or glutelins, which are found mostly in the endosperm, is not different from that in dent varieties. Therefore, the apparent increase in all proteins is probably explained by a decrease in the amount of starch and reflected in the smaller endosperm, which still contains normal complements of each protein.

In waxy corn, the lower content of total nitrogen was accompanied by a decrease in the major endosperm proteins, zein and glutelin. The higher globulin content may result from a relatively greater proportion of germ protein. The amount of germ in waxy maize is greater than that in standard dent varieties (1). The waxy corn glutelin contains the amino acids associated with globulins in a greater amount than the dent and amylomaize glutelins.

It must be stressed that these data were obtained on three corn types of identical genetic background except for the respective *ae* and *wx* starch-modifying genes present in high-amylose and waxy varieties. In assessing the contribution of single genes to plant protein characteristics, it is essential that the genetic background of the material be identical except for the genes under investigation. In this study, no significant differences were observed between starch-gel electrophoretic patterns of the different protein classes of the three corns. If enzymatic differences did result from the single gene variation, these were not visibly reflected in the protein patterns. In a survey of protein extracts from markedly different corn varieties, differences in the electrophoretic patterns of some of the protein classes were observed; they will be reported in a future publication.

The results of analysis of the high-amylose corn indicate that the *ae* gene is effective only in changing the starch composition and yield, and that it exerts little detectable effect upon protein composition and distribution. In higher-amylose (68–80%) varieties of more recent development, the endosperm size and starch content have been increased and the relative protein content and germ size reduced (3). These changes during selective breeding probably resulted from the introduction of gene-modifying or other genetic factors.

The nutritional and milling characteristics of high-amylose and waxy grains may be partly influenced by their protein makeup. Waxy corn contains less total protein, but the protein is of a higher quality, being richer in lysine. The high protein-to-starch ratio of the high-amylose corn probably contributes to its more difficult wet-milling.

Significantly, corn glutelin is a high-molecular-weight complex at least

partially composed of disulfide-linked polypeptide subunits. Some components of reduced glutelin migrate electrophoretically like reduced zein proteins; others have mobilities resembling reduced globulins. Amino acid analysis of the glutelins indicates a composition generally intermediate between zein and globulin. The harsh conditions generally employed for extracting glutelins (0.1*N* NaOH) probably disrupt some disulfide bonds, and the extracted material may be partially degraded. Investigations are under way to find more satisfactory solvents for studying these proteins further. The glutelins are important in corn, not only because of their large amounts and possible function as structural protein in the grain, but also because of their potential nutritional value. Mertz *et al.* (12) have found that Opaque-2 high-lysine corn contains a greater proportion of glutelin protein than do standard hybrids.

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Literature Cited

1. Zuber, M. S., Grogan, C. O., Fergason, V. L., Deatherage, W. L., and MacMasters, Majel M. Some observed secondary effects of high-amylose genes in maize. Cereal Chem. **37:** 212–217 (1960).
2. 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).
3. Anderson, R. A., Vojnovich, C., and Griffin, E. L., Jr. Wet-milling high-amylose corn containing 66- to 68-percent-amylose starch. Cereal Chem. **38:** 84-93 (1961).
4. Turner, J. E., Boundy, Joyce A., and Dimler, R. J. Zein: a heterogeneous protein containing disulfide-linked aggregates. Cereal Chem. **42:** 452–461 (1965).
5. Mertz, E. T., and Bressani, R. Studies on corn proteins. I. A new method of extraction. Cereal Chem. **34:** 63–69 (1957).
6. Pence, J. W., and Elder, Angeline H. The albumin and globulin proteins of wheat. Cereal Chem. **30:** 275–287 (1953).
7. Deatherage, W. L., Melvin, E. H., and Dimler, R. J. Spectrophotometric determination of amylose in high-amylose corn. Abstr., AACC 47th annual meeting, St. Louis, Missouri, May 1962.
8. Sela, M., White, F. H., Jr., and Anfinsen, C. B. The reductive cleavage of of disulfide bonds and its application to problems of protein structure. Biochim. Biophys. Acta **31:** 417–426 (1959).
9. Weil, L., and Seibles, T. S. Reaction of reduced disulfide bonds in α-lactalbumin and β-lactalbumin with acrylonitrile. Arch. Biochem. Biophys. **95:** 470–473 (1961).
10. Spackman, D. H., Stein, W. H., and Moore, S. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. **30:** 1190–1206 (1958).
11. MacMasters, Majel M., and Hilbert, G. E. Glutinous corn and sorghum starches. Ind. Eng. Chem. **36:** 958–963 (1944).
12. Mertz, E. T., Bates, L. S., and Nelson, O. E. Mutant gene that changes protein composition and increases lysine content of maize endosperm. Science **145:** 279–280 (1964).