Modified Opaque-2 Corn Endosperms. I. Protein Distribution and Amino Acid Composition[1]

J. L. ROBUTTI[2], R. C. HOSENEY[3], and C. W. DEYOE[3]

ABSTRACT

Normal, *opaque-2* and modified *opaque-2* corn endosperm samples and hand-dissected soft and hard endosperm portions were analyzed for protein content, protein distribution (based on solubility), and amino acid content. In addition, the amino acid content of the extracted protein fractions was determined. Protein distribution was determined by successive extractions with water, ethanol:water (70:30 v./v.), 0.5M sodium chloride, 0.6% 2-mercaptoethanol at pH 10, and 0.5% sodium lauryl sulfate at pH 10. The protein content of *opaque-2* endosperms was less than that of their normal counterpart. Protein contents of the modified *opaque-2* endosperms were equal to or higher than those of the normal ones. Hard and soft portions of modified endosperms had equal protein contents. The *opaque-2* materials had an altered protein distribution; they had higher amounts of water plus salt-soluble protein and lower amounts of alcohol-soluble protein than did normal materials. Modified endosperms had protein distributions that, although closer to opaque than to normal patterns, were significantly different from *opaque-2* endosperms. The hard endosperm portion was responsible for that difference.Compared with normal samples, *opaque-2* materials had increased levels of lysine, arginine, glycine, and aspartic acid and decreased levels of alanine, leucine, and phenylalanine. The values found for those amino acids in modified endosperms were, in general, intermediate between those values for opaque and normal phenotypes. The amino acid composition of modified soft endosperm was similar to that of opaque phenotypes. The amino acid pattern of modified hard endosperm was not similar to normal or opaque samples but, in general, was intermediate between the two types.

Corn protein has a low nutritional value for monogastric animals and man, mainly because it is deficient in the essential amino acid lysine.

Mertz et al. (1) reported that the *opaque-2* mutant gene substantially increases the lysine content of the endosperm protein. The mutant gene has been incorporated into many genetic backgrounds. Lambert et al. (2) reported that the *opaque-2* - converted materials are lower in yield and weight per 1,000 kernels, higher in percentage of cracked kernels, and less resistant to diseases than are their normal counterparts. Also, milling behavior is adversely affected by the opaque, soft endosperm character (3,4). Several reports (5,6,7) indicate that those undesirable side effects can be improved by selecting for modifying genes, which cause portions of the endosperm to be translucent and hard even in the presence of the homozygous recessive *opaque-2* gene. Then it would be possible to find, by selection, translucent kernel phenotypes that are high in lysine.

However, translucence has been associated with the presence of zein bodies in the cytoplasmic protein matrix (8). Because zein is low in lysine, one could assume either that (a) as the amount of translucence increases in the kernel the percentage of lysine decreases, or (b) the amount of zein is not related to translucence. Only if the latter of those two is true would it be possible to produce translucent kernels that are high in lysine. In an attempt to answer that question, we determined the protein composition of hard and soft types of endosperm within modified *opaque-2* kernels.

[1]Contribution No. 818, Department of Grain Science and Industry, Kansas Agricultural Experiment Station, Manhattan 66506.

[2]Graduate Student, Department of Grain Science and Industry. Present address: Estacion Experimental Agropecuaria Pergamino, C.C. 31, Pergamino (B.A.), Argentina.

[3]Respectively: Associate Professor and Professor, Department of Grain Science and Industry.

MATERIALS AND METHODS

Corn Samples

Thirteen corn samples were used in the study. Three samples, HL1, HL27, and HL46, were modified *opaque-2* seeds produced by interpollination among a group of *opaque-2* hybrids; they were provided by the Kansas State University (KSU) corn breeding program.

Nine samples–Composite K (Comp. K), Veracruz 181XAntigua Group 2 (V18l), and CIMMYT O.P. 2 (COP 2) each in three phenotypes: normal, opaque, and modified (Fig. 1)–were furnished by CIMMYT (International Maize and Wheat Improvement Center). Analysis of the normal Comp. K sample raised questions about its validity; thus it was not included in the study.

Two samples, a bulk normal (++) and bulk opaque (o_2o_2), were obtained from the KSU feed mill.

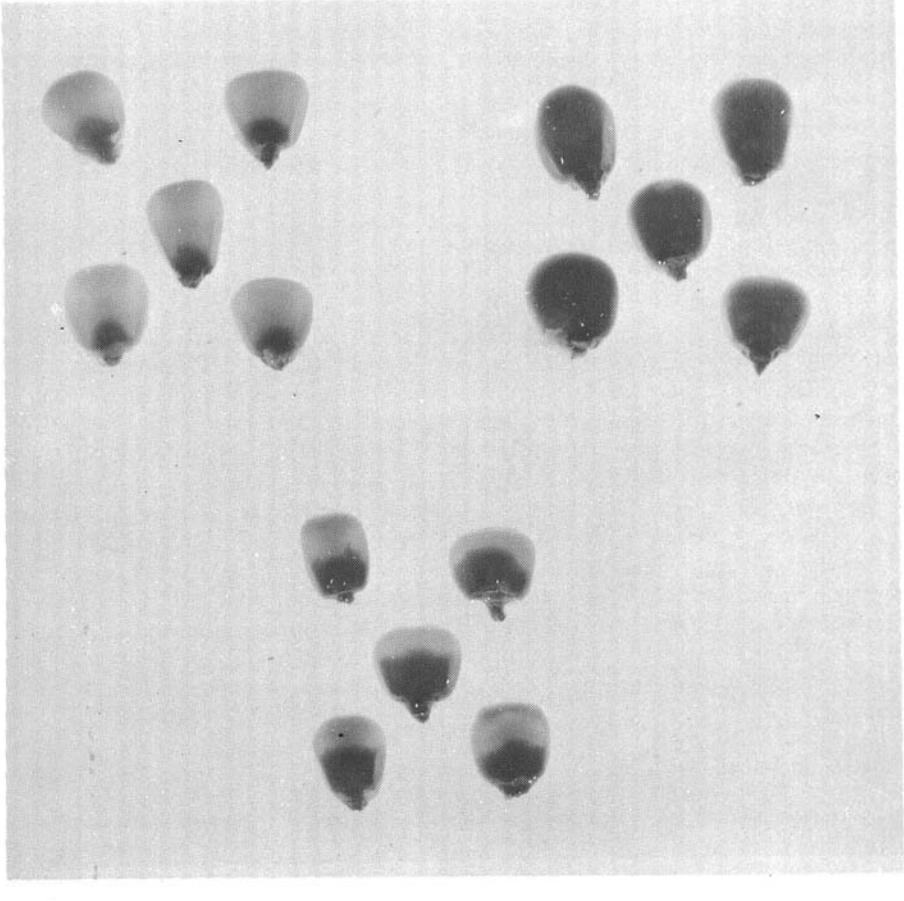

Fig. 1. Normal (top left), *opaque-2* (top right), and modified (bottom) corn kernels.

Hand Dissection of Kernels

Randomly selected kernels from each of the samples were soaked in water for 2 to 3 min. and then peeled and degerminated with the aid of tweezers and scalpel. Four of the modified samples (HL1, HL27, HL46, and Comp. K) were separated into opaque and translucent portions with a scalpel. The hard endosperm chunks could not be completely freed of floury endosperm. On the other hand, the soft endosperm portion was nearly free of hard endosperm (Fig. 2). Each endosperm portion and whole endosperms were ground in a Wiley mill to pass a 40-mesh sieve.

Protein Fractionation

Proteins from endosperms of normal, *opaque-2*, and modified *opaque-2* kernels, as well as the soft and hard endosperm portions from modified kernels, were fractionated on the basis of solubility. Samples (3 g.) were extracted with a magnetic stirrer, at room temperature to a solvent-to-meal ratio of 4:1 (v./w.), using a modified Landry and Moureaux procedure (9,10). The sequence of solvents

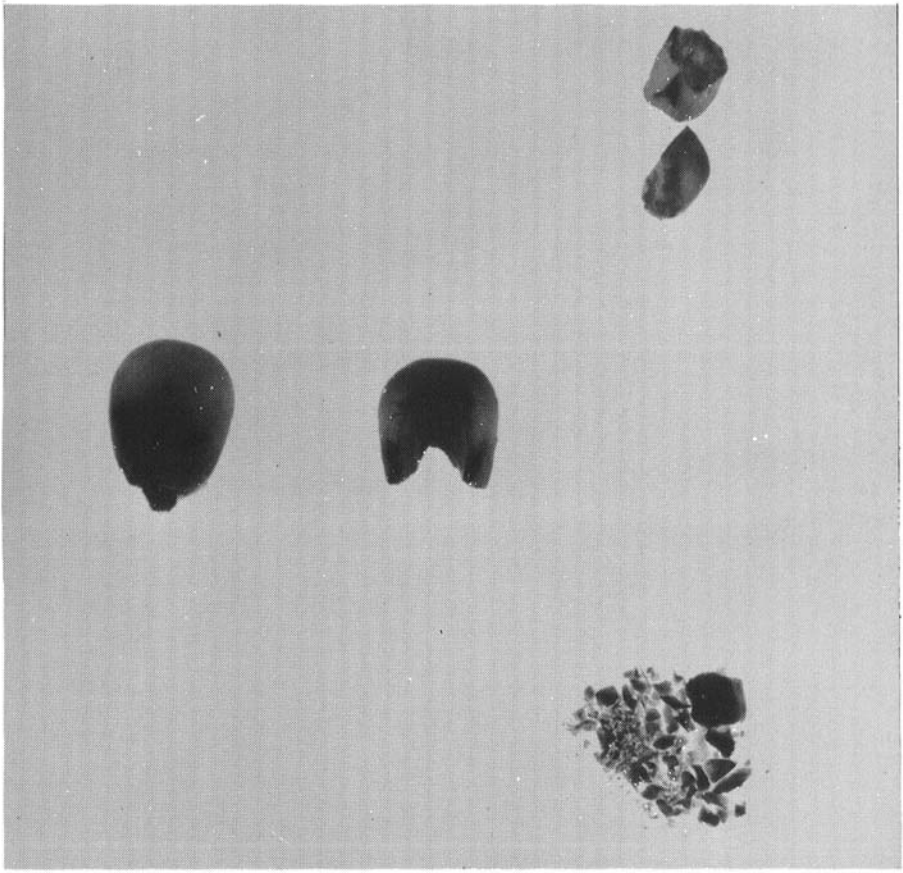

Fig. 2. Modified whole corn kernel (left), endosperm after peeling and degerming (center), and hard (top right) and soft (bottom right) portions of hand-dissected endosperm.

TABLE I. SOLVENTS, ORDER, AND LENGTH OF EXTRACTIONS USED FOR FRACTIONATING CORN ENDOSPERM PROTEINS

Solvents and Order of Extraction	Extraction Time, min.		
	1st	2nd	3rd
Water (W)	60	30	5
Ethanol:water (70:30, v./v.) with 0.5% sodium acetate (alcohol)	240	120	5
0.5M Sodium chloride (S)	60	30	5
0.6% (v./v.) 2-mercaptoethanol in a pH 10 sodium carbonate-sodium bicarbonate buffer (ME)	60	30	5
0.5% (w./v.) sodium lauryl sulfate in a pH 10 sodium carbonate-sodium bicarbonate buffer (SLS)	60	30	5

employed and the length of each extraction are shown in Table I.

After each extraction, the suspension was centrifuged at 3,000 r.p.m. (1,500 X g) for 15 min. and the protein contents of the supernatant and the residue were determined. In most cases the water-soluble and the salt-soluble fractions were combined before they were analyzed.

After protein analysis, the combined supernatants were dialyzed against water, freeze-dried, and analyzed for amino acids. The alcohol-soluble fractions were evaporated under vacuum prior to dialysis.

Analytical Procedures

Protein content (N X 6.25) was determined by a slightly modified micro-Kjeldahl procedure (11). Moisture was determined on 0.5-g. samples heated overnight (to a constant weight) in a forced-draft oven at 50° to 60°C.

Amino acid composition was determined on a 120B Beckman amino acid AutoAnalyzer. Samples were hydrolyzed for 22 hr. with 6N HCl at 110°C. in sealed tubes. No correction was made for possible losses during hydrolysis.

RESULTS AND DISCUSSION

Protein Content of the Hand-Dissected Endosperms

The protein contents of hand-dissected endosperms from normal, *opaque-2*, and

TABLE II. PROTEIN CONTENT (N X 6.25) OF THE HAND-DISSECTED ENDOSPERMS (% d.b.)

Sample	Endosperm Protein,%		
	Whole	Hard	Soft
Modified HL1	8.1	8.1	8.2
Modified HL27	8.2	8.2	8.4
Modified HL46	9.5	9.3	9.4
Opaque-2 Comp. K	7.3	---	---
Modified Comp. K	10.3	10.2	10.1
Normal V181	9.8	---	---
Opaque-2 V181	8.0	---	---
Modified V181	9.7	---	---
Normal COP 2	9.7	---	---
Opaque-2 COP 2	8.3	---	---
Modified COP 2	11.3	---	---
Bulk opaque-2	8.6	---	---
Bulk normal	10.7	---	---

modified samples are shown in Table II. The *opaque-2*-converted materials were lower in protein than were their normal counterparts. However, the related modified kernels had protein contents essentially equal to those of the normal phenotype. The modified whole endosperms of HL1 and HL27 had similar protein values; HL46 was about 1% higher. The protein content was essentially equal in the hard and soft endosperm portions for the four modified materials analyzed, a finding in agreement with that reported by Palmer (12) for sorghum.

The ratios of soft to predominantly hard endosperm (expressed as w./w.) were found to be 0.96:1, 0.86:1, and 0.90:1 for HL1, HL27, and HL46, respectively.

TABLE III. DISTRIBUTION OF PROTEIN (% OF TOTAL PROTEIN) IN WHOLE, HARD, AND SOFT ENDOSPERMS FROM COMP. K AND IN WHOLE, NORMAL, OPAQUE-2, AND MODIFIED ENDOSPERMS FROM SEVERAL MATERIALS

Sample	W+S-Soluble %	Alcohol-Soluble %	ME-Soluble %	SLS-Soluble %
Bulk				
Normal	5.0	43.5	17.2	28.4
Opaque-2	15.9	15.9	21.8	40.0
V181				
Normal	4.9	40.5	17.4	32.0
Opaque-2	21.0	9.9	21.7	38.6
Modified	12.5	13.4	29.6	35.8
Comp. K				
Opaque-2	18.6	8.2	23.2	41.6
Modified				
Whole endosperm	14.5	15.9	26.4	33.2
Hard endosperm	12.6	17.7	28.1	30.5
Soft endosperm	16.0	8.7	25.2	35.3

Protein Distribution

Protein distributions of a normal and an *opaque-2* corn (bulk samples) are given in Table III. Water-, salt-, and SLS-soluble fractions were much higher and the alcohol-soluble fraction was much lower in the opaque than in the normal corn, which generally agreed with work reported by Misra et al. (13).

The modified V181 (Table III) had a protein distribution intermediate between the values found for V181 normal and V181 opaque kernels, except for the ME-soluble fraction, which was somewhat higher. Apparently there was a shift from alcohol-soluble to ME-soluble protein in the modified material. The V181 normal and *opaque-2* phenotypes had protein distributions similar to those of the bulk normal and *opaque-2* samples, respectively.

Protein distribution for whole modified Comp. K endosperm (Table III) was intermediate between those for the hard and soft portions of the endosperm. The Comp. K *opaque-2* endosperm was higher in water-plus-salt- (W+S) soluble and SLS-soluble fractions but lower in the alcohol-soluble fraction than the modified whole endosperm. Its protein distribution closely resembled that of the modified soft endosperm portion and also that of the bulk *opaque-2* corn. The ME fraction from the modified whole endosperm was higher than that from the opaque

genotype, indicating a shift from alcohol-soluble to ME-soluble in the modified Comp. K (as in the modified V181).

Protein distributions for modified samples HL1, HL27, and HL46 are given in Table IV. Results obtained for HL1 and HL27 agreed quite well with each other and with those obtained for the modified phenotypes of Comp. K and V181; however, HL46 differed markedly. The W+S-soluble and the ME-soluble fractions were lower and the alcohol-soluble fractions higher for HL46 than were the corresponding

TABLE IV. DISTRIBUTION OF PROTEIN (% OF TOTAL PROTEIN) IN WHOLE, HARD, AND SOFT ENDOSPERMS FROM MODIFIED OPAQUE-2 HYBRID CORN

Sample	W+S-Soluble %	Alcohol-Soluble %	ME-Soluble %	SLS-Soluble %	Residual Meal %	Recovery %
HL1						
Whole endosperm	15.7	10.1	25.3	40.3	10.0	101.4
	14.9	9.8	25.7	39.0	12.3	101.7
	14.6	9.7	25.4	40.9	10.0	100.6
Hard endosperm	13.0	16.1	28.9	32.5	9.1	99.6
	13.3	15.5	29.1	34.0	8.1	100.0
	13.0	14.9	29.7	33.9	8.5	100.0
Soft endosperm	---	7.8	25.9	40.0	8.6	---
	15.3	7.4	25.9	42.9	8.2	99.6
	16.3	8.0	26.9	43.8	8.0	103.1
HL27						
Whole endosperm	15.2	11.5	27.2	33.1	8.6	95.6
	16.9	12.8	25.7	34.6	10.0	100.0
	14.8	12.0	24.8	36.8	8.6	97.0
Hard endosperm	11.9	17.1	25.8	36.2	8.6	99.6
	11.2	16.1	24.9	35.3	10.6	98.2
	11.2	17.5	25.7	35.5	10.4	100.3
Soft endosperm	14.6	10.4	24.7	38.5	12.1	100.3
	16.4	10.1	23.3	38.3	12.5	100.6
	14.6	9.8	23.4	38.8	11.8	98.4
HL46						
Whole endosperm	5.5	27.9	14.6	42.9	8.6	99.6
	5.6	30.1	14.2	41.2	7.7	99.8
	5.7	28.7	14.2	41.5	7.7	98.3
Hard endosperm	3.3	31.2	14.9	41.5	8.1	99.0
	3.9	33.1	16.2	39.5	7.0	99.7
Soft endosperm[a]	10.0	23.2	17.9	39.7	11.3	102.1
	8.4	22.2	17.0	---	10.0	---
	9.6	23.5	16.4	---	10.7	---

[a]Two analyses gave low values for alcohol-soluble (14%) and correspondingly high values for SLS-soluble (58%), from the whole and soft endosperm value. The value appeared to be in error.

samples for the other two genetic sources. Compared with normal corn (Table III), the HL46 modified whole endosperm had similar values for W+S- and ME-solubles, a lower value for alcohol-soluble, and a higher value for SLS. Thus, the genetic expression of protein distribution in HL46 was only a shift from alcohol- to SLS-soluble, while in the other modified endosperm there was an increase in W+S-solubles, a greater decrease in alcohol-solubles, and an increase in ME-soluble, compared with normal corn. Also noteworthy, the soft endosperm of modified HL46 had a high alcohol-soluble value but low W+S and ME values.

The highest proportion of total protein was recovered in the SLS-soluble fraction for all types of endosperm and all genetic backgrounds. In the three endosperm types (normal, opaque, and modified) the protein distribution for the whole endosperms was intermediate between those for the hard and soft endosperms (as would be expected), and the hard endosperm portion contained more alcohol-soluble protein but less W+S-soluble protein than the soft endosperm portion.

Protein distribution appeared to be different for modified materials than for opaque phenotypes. The W+S- and SLS-soluble fractions were found to be lower and the alcohol-soluble and ME-soluble fractions higher in the modified kernels.

The data suggest that the protein of the hard endosperm in modified kernels was responsible for the change in protein distribution, because the soft endosperm (except for HL46) had essentially the same protein distribution as in *opaque-2* materials. Nevertheless, the modified hard endosperms had higher W+S- and ME-soluble fractions and a lower alcohol-soluble fraction than did normal hard endosperms.

Amino Acid Composition of Endosperms and Their Fractions

Amino acid composition of whole corn endosperms (peeled and degermed) is given in Table V. Amino acid distribution for the bulk normal sample and the

TABLE V. AMINO ACID COMPOSITION (g./100 g. PROTEIN) OF ENDOSPERMS FROM NORMAL, OPAQUE-2, AND THREE PHENOTYPES EACH OF COMP. K AND V181 CORN

	Bulk		V181			Comp. K	
Amino Acid	Normal	Opaque-2	Normal	Opaque-2	Modified	Opaque-2	Modified
Lysine	1.65	3.75	1.50	3.78	2.96	3.78	3.27
Histidine	2.58	3.21	2.71	3.66	3.49	3.47	4.15
Ammonia	2.25	1.83	2.07	2.23	2.28	1.78	1.81
Arginine	3.44	5.60	2.70	5.16	4.20	4.81	4.54
Aspartic acid	5.49	8.77	5.14	8.91	7.81	9.97	7.65
Threonine	3.15	3.93	3.03	3.91	3.64	3.84	3.68
Serine	4.64	4.56	4.52	4.31	4.43	4.26	4.42
Glutamic acid	20.88	18.31	21.55	17.38	19.51	20.29	18.68
Proline	8.61	8.97	9.55	9.50	9.62	8.98	9.75
Glycine	2.93	4.25	2.64	4.34	3.92	4.24	4.07
Alanine	7.91	6.15	7.94	6.07	6.12	6.50	6.06
Half-cystine	3.37	3.16	2.73	3.19	4.23	2.36	4.18
Valine	3.63	4.39	4.53	5.18	5.09	5.22	5.26
Methionine	2.04	1.10	1.69	1.47	1.13	1.45	1.48
Isoleucine	3.47	3.30	3.45	3.51	3.31	3.19	3.33
Leucine	14.84	10.54	15.50	9.58	10.61	8.48	10.21
Tyrosine	4.04	3.73	3.59	3.62	3.48	3.61	3.49
Phenylalanine	5.06	4.44	5.17	4.17	4.11	3.76	3.95

normal V181 was similar, and the composition of the three *opaque-2* samples agreed quite well.

The opaque materials were higher in lysine, histidine, arginine, aspartic acid, and glycine but lower in glutamic acid, alanine, and leucine than were the normal or modified materials. Those results agreed with data reported previously (1,13). Amino acid distributions were similar in all modified phenotypes (Tables V and VI) except HL46, which also had an unusual protein distribution.

In general, the modified materials had amino acid compositions intermediate between those for normal and opaque. When whole endosperms were dissected into hard and soft portions, it would seem logical to assume that the amino acid distribution of the whole endosperm would be intermediate between the distributions for the hard and soft portions. That generally was so (Table VI), though not for certain amino acids; data for the sulfur-containing amino acids were erratic, and we could observe no general trend.

In general, amino acid distribution was similar in each type of endosperm (whole, hard, soft) for the HL1, HL27, and Comp. K samples. The HL46 sample had higher values for leucine, alanine, and phenylalanine; and lower values for lysine, glycine, and aspartic acid.

TABLE VI. AMINO ACID COMPOSITIONS (g./100 g. PROTEIN) FOR WHOLE, HARD, AND SOFT ENDOSPERM PORTIONS OF MODIFIED CORN

	HL1 Endosperm			HL27 Endosperm			HL46 Endosperm			Modified Comp. K Endosperm		
Amino Acid	Whole	Hard	Soft	Whole	Hard	Soft	Whole	Hard	Soft	Whole	Hard	Soft
Lysine	2.89	2.40	3.24	2.73	2.38	3.04	2.00	1.90	2.95	3.27	2.90	4.22
Histidine	3.76	3.64	3.77	3.56	3.69	3.49	2.86	2.22	3.26	4.15	3.40	3.89
Ammonia	1.78	2.21	1.45	1.17	2.21	1.97	2.20	1.99	2.04	1.81	1.96	1.63
Arginine	4.30	3.91	4.50	4.11	3.88	4.33	3.44	3.08	4.18	4.54	3.72	6.19
Aspartic acid	7.20	6.08	7.79	7.07	6.54	7.56	5.89	5.71	6.67	7.65	7.81	8.65
Threonine	3.66	3.40	3.62	3.69	3.46	3.66	3.17	3.38	3.80	3.68	3.59	3.87
Serine	4.33	4.30	4.30	4.47	4.46	4.53	4.67	4.94	5.05	4.42	4.49	4.66
Glutamic acid	18.03	19.07	17.89	19.79	19.74	19.19	20.82	20.96	20.03	18.68	19.40	16.94
Proline	9.74	10.20	10.14	9.90	9.98	9.54	10.17	9.46	9.34	9.75	9.78	8.64
Glycine	4.27	3.84	4.51	4.02	3.67	3.99	3.40	3.48	3.91	4.07	3.66	4.60
Alanine	6.23	6.60	6.20	6.38	6.81	6.48	7.20	7.82	7.68	6.06	6.31	6.08
Half-cystine	4.75	3.98	4.44	4.26	3.18	3.97	3.14	2.69	2.79	4.18	3.56	3.91
Valine	5.33	4.72	5.80	5.11	5.07	5.03	4.68	4.01	2.61	5.26	5.24	4.97
Methionine	1.61	1.78	1.42	1.28	1.34	1.43	1.70	2.48	1.75	1.48	0.98	1.28
Isoleucine	3.24	3.22	3.28	3.18	3.31	3.26	3.19	3.36	3.09	3.33	3.42	3.34
Leucine	11.57	12.34	10.24	10.89	12.32	10.87	12.67	14.18	12.15	10.21	12.33	9.47
Tyrosine	3.43	3.96	3.35	3.76	3.61	3.59	4.21	3.78	3.99	3.49	3.11	3.52
Phenylalanine	3.87	4.26	4.07	4.02	4.36	4.05	4.61	4.94	4.76	3.95	4.35	4.14

Amino acid distribution in the soft endosperm portions of HL1, HL27, and modified Comp. K was similar to that of opaque materials. However, the hard endosperms of those three genetic sources had distributions that were not similar to normal materials: lysine, aspartic acid, and glycine contents were higher and alanine, leucine, and phenylalanine contents lower. Those amino acids that were similar for normal and opaque materials also were similar for hard and soft portions of the same endosperm.

TABLE VII. AVERAGE AMINO ACID COMPOSITION (g./100 g. PROTEIN) FOR PROTEIN EXTRACTED WITH CERTAIN SOLVENTS

	W+S	Alcohol	ME	SLS
Lysine	4.18	0.46	0.57	4.38
Histidine	2.38	1.28	6.77	2.52
Ammonia	2.36	2.72	2.23	1.68
Arginine	7.35	2.16	3.46	4.49
Aspartic acid	10.06	5.12	1.73	7.90
Threonine	4.60	2.93	3.86	4.04
Serine	5.23	5.11	4.03	5.15
Glutamic acid	14.70	22.18	23.61	16.70
Proline	5.06	9.84	17.83	6.95
Glycine	6.69	2.02	4.72	4.12
Alanine	7.10	9.01	4.92	7.49
Half-cystine	3.73	2.27	0.87	0.64
Valine	5.28	3.43	6.07	5.27
Methionine	1.72	0.94	1.63	2.86
Isoleucine	4.25	3.53	2.23	3.97
Leucine	6.50	17.49	10.28	12.09
Tyrosine	3.25	4.54	2.52	4.72
Phenylalanine	3.57	6.11	2.56	5.31

Amino acid distribution for the protein extracted with each extraction solvent from the whole, hard, and soft endosperms of HL1, HL27, HL46, and Comp. K were remarkably constant. There appeared to be little or no consistent variation because of type of endosperm or genetic source extracted. Average amino acid distribution for the protein extracted with each extraction solvent (all samples extracted with each solvent were used to calculate the average) is given in Table VII.

Those results showed clearly which fractions were rich, poor, or intermediate in a given amino acid. For example, the highest values of lysine were found in the W+S- and SLS-soluble fractions, while the alcohol and the ME-soluble fractions were low in that amino acid. Thus, knowledge of protein distribution would be useful in estimating the amino acid distribution of a given sample.

The amino acid results confirmed the protein-distribution data. For example, the high-lysine opaque materials were high in W+S- and SLS-soluble fractions; alcohol-soluble fractions were high in leucine, and the high-leucine normal phenotypes were high in alcohol-soluble proteins; and the three endosperm types of HL46 were lower in W+S and higher in alcohol-soluble fractions–and therefore lower in lysine, glycine, and aspartic acid and higher in leucine, alanine, and phenylalanine–than were the corresponding endosperms of the other modified materials. Thus, the amino acid data confirmed that the protein distribution of HL46 differed from that of the other modified materials.

SUMMARY

Modified *opaque-2* corn had different endosperm protein distribution from that in completely opaque, soft corn endosperm. There was a loss in protein quality in modified *opaque-2*, compared with the opaque phenotype *opaque-2* because modified endosperm protein was lower in lysine than was opaque endosperm protein. Alcohol-soluble protein was lower (14%) in modified phenotypes than in the normal (40%) and was only slightly higher than in the opaque (10%). However, the modified phenotypes were higher in ME-soluble protein (29%) than were either the normal (17%) or the opaque (21%). Thus, apparently there was a shift from

alcohol-soluble to ME-soluble protein upon conversion to the modified phenotype.

In hand-dissected endosperm portions of modified kernels, the protein distribution in the soft endosperm was similar to that of the opaque phenotype endosperm; however, the hard endosperm portion was lower in W+S-soluble and higher in alcohol-soluble fractions (and thus lower in lysine) than was the distribution in the opaque phenotype. The hard portion of the modified endosperm was, therefore, responsible for the loss in protein quality. However, the hard portion of the modified endosperm was lower in alcohol-soluble and higher in W+S-soluble fractions (and thus higher in lysine) than was the normal corn endosperm.

The HL46 kernels did not follow the general protein-distribution pattern. The W+S-soluble fraction was not so high as in the other modified materials; it was similar to that of normal samples. The alcohol-soluble fraction was significantly lower than for normal kernels but higher than for the rest of the modified samples. The ME-soluble fraction did not show values higher than those of normal endosperms. Apparently the modifier genes influenced protein distribution in modified materials.

Amino acid composition of the protein extracted by the various solvents was essentially constant for each solvent and not affected by the genetics of the material extracted. Thus, protein distribution should give useful information on amino acid composition of samples.

Our data suggest that translucent phenotypes of *opaque-2* corn have a substantially better protein quality than do those of normal corn. However, the protein quality of those phenotypes would not be so high as that of opaque types. Because modified endosperms were shown to contain approximately 2% more protein than opaque endosperms, lysine per unit weight of endosperm should be nearly the same in both phenotypes.

Literature Cited

1. 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 (1964).
2. LAMBERT, R. J., ALEXANDER, D. E., and DUDLEY, J. W. Relative performance of normal and modified protein (opaque-2) maize hybrids. Crop Sci. 9: 242 (1969).
3. WATSON, S. A., and YAHL, K. R. Comparison of wet-milling properties of opaque-2 high-lysine corn and normal corn. Cereal Chem. 44: 488 (1967).
4. WICHSER, W. R. Comparison of the dry milling properties of opaque-2 and normal dent corn. In: Proceedings of the High Lysine Corn Conference, Washington, D.C. (1966).
5. CIMMYT (International Wheat and Maize Improvement Center) Annual Report (1968–69).
6. CIMMYT Annual Report (1969–70).
7. PAEZ, A. V., HEHN, J. L., and ZUBER, M. S. Lysine content of opaque-2 maize kernels having different phenotypes. Crop Sci. 9: 251 (1969).
8. DUVICK, D. N. Protein granules of maize endosperm cells. Cereal Chem. 38: 374 (1961).
9. MOUREAUX, T., and LANDRY, J. Selective extraction of protein and particularly of the glutelin fraction from kernels of corn. C. R. Acad. Sci. Ser. D, p. 266 (1968).
10. LANDRY, J., and MOUREAUX, T. Heterogeneity of corn glutelins: Selective extraction and amino acid composition of three isolated fractions. Bull. Soc. Chim. Biol. 52: 1021 (1970).
11. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. Methods of analysis (9th ed.). The Association: Washington, D.C. (1960).
12. PALMER, G. H. Morphology of starch granules in cereal grains and malts. J. Inst. Brew. 78: 326 (1972).
13. MISRA, P. S., JAMBUNATHAN, R., and MERTZ, E. T. Endosperm protein synthesis in maize mutants with increased lysine content. Science 176: 1425 (1972).

[Received May 3, 1973. Accepted July 6, 1973]