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PROTEIN DISTRIBUTION, AMINO ACID COMPOSITION, AND MICROSTRUCTURE OF MODIFIED OPAQUE-2 CORN ENDOSPERMS

by

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B.S., University of Sante Fe (Argentina), 1966

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1973

Approved by:

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Introduction

Corn is the third largest cereal crop in the world, both in amount produced and acreage planted (1). Corn is the staple food for about 150 million people and in addition is the main feed grain used in temperate countries (2).

Corn protein has low nutritional value for monogastric animals including man, mainly due to a deficiency in the essential amino acids lysine and tryptophan. The nutritional value of corn could be improved by raising the content of those essential amino acids in the protein or by increasing the quantity of that protein.

The homozygous recessive opaque-2 gene causes a substantial increase in lysine and tryptophan content of corn endosperm. Since that discovery was reported the opaque character has been incorporated into many different genetical backgrounds.

Certain undesirable side effects were observed in opaque-2 converted materials: lower yields, lower kernel density, higher susceptibility to diseases, slower rate of maturing, more mechanical damage to kernels during harvest and post-harvest operations, and dull, unattractive appearance of the kernels. In addition, the soft endosperm is less suitable for milling purposes than normal hard endosperm.

There has been evidence that those kernel characteristics can be improved. Certain lines in S_2 generations derived from opaque-2 parents produced two phenotypic classes: completely opaque and modified opaque. The modified opaque kernels show different proportions of translucent endosperm. Those modified phenotype kernels could have a major significance

in high lysine corn breeding since, by selection, one could find translucent kernel phenotypes which are homozygous opaque-2 genotypes.

The translucent appearance of hard endosperm in normal corn has been attributed to the presence of numerous zein bodies in the cytoplasmic protein matrix. If this were true for the hard endosperm of opaque-2 modified kernels, then the protein quality would be questioned, since zein protein, the main constitutent of zein bodies, has a low lysine content. Therefore, even though such kernels carry the opaque-2 gene in a homozygous condition, the protein in their hard endosperm portions may contain a relatively high zein protein fraction, thus being low in lysine and impairing protein quality of the whole endosperm. If that were true, the goal of obtaining opaque-2 hard endosperm phenotypes without a substantial loss of the endosperm protein quality could not be reached.

The purpose of this study was to determine the differences, if any, in the protein composition of hard and soft types of endosperm within the modified opaque-2 kernels.

Literature Review

Mertz et al (3) found that corn homozygous for the mutant gene opaque-2 had an endosperm lysine content about twice that of a normal hybrid corn. The nutritional value of opaque-2 corn on monogastric animals, including man, has been demonstrated in several studies (2).

Lambert et al (4) reported that 17 opaque-2 hybrids tested at 2 locations in Illinois, averaged 8% lower in yield, 5% lower in weight per 1000 kernels, 20% higher in grain moisture, 90% higher in percent

of cracked kernels compared to their normal counterparts. Opaque-2 genotypes were found to be more susceptible to disease damage than normal corn. In addition, milling behavior was adversely affected by the opaque character (5, 6). Lack of horny endosperm means that it was almost impossible to obtain flaking grits or coarse brewer grits, and the larger ratio of germ to endosperm would decrease the amount of starch recovered during wet milling.

Several reports (7, 8, 9) indicate that phenotypical appearance of opaque-2 kernels can be improved by selecting for modifying genes. These genes cause portions of the endosperm to be translucent even in the presence of the opaque-2 gene in a homozygous condition.

Duvick (10) attributes translucence of hard endosperm in normal corn to the presence of zein bodies. According to his hypothesis the proteinaceous material consists of a matrix protein and a granular component (zein bodies). During drying the protein matrix ruptures forming voids, and the opaqueness of soft endosperm is caused by light refraction due to the air in those voids. In translucent endosperm, the granular protein which is missing in soft endosperm fills the voids thus preventing light refraction. This hypothesis has recently been contradicted (11) since normal appearing kernels completely devoid of zein bodies have been found.

Scanning electron micrographs of soft and hard portions of sorghum endosperm showed that the starch granules of the soft endosperm were loosely associated with papery sheets of protein material, while the hard endosperm was tightly packed with a rigid protein matrix. The author (12) attributes hardness to the structure of the starch-protein

matrix, since the floury and horny areas of the endosperm were observed to contain identical quantities of nitrogen.

Paulis et al (13) found that opaque-2 and normal corn glutelins had similar amino acid patterns. Murphy and Dalby (14) harvested normal and opaque-2 corn at varying stages of maturity and fractionated the protein by the Osborne procedure. During ripening, the zein to glutelin ratio decreased in opaque-2 corn, and increased for normal corn.

Musiiko et al (15) studied protein composition of whole kernel and endosperm of normal and mutant corn. Protein from the whole kernel of mutant corn compared to normal corn contained twice the amount of albumin and globulin, less than one half the amount of zein, and increased amounts of glutelins and scleroproteins. Flour from the endosperms of normal corn and mutant corn contained more lysine and less tryptophan than flours from the whole kernel of normal corn. Flours from mutant corn contained increased amounts of methionine.

Salamini et al (16) examined normal and opaque phenotypes of ten segregating F_2 lines for total protein, solubility fractions, and amino acid patterns. Protein content was significantly and positively correlated to the alcohol soluble fraction in normal looking kernels and to the water plus salt soluble fraction in the opaque-2 kernels.

Moureaux and Landry (17) extracted corn proteins using the following sequence of solvents: 0.5M sodium chloride, 55% isopropanol, plus 2-mercaptoethanol (ME) at pH = 10, ME at pH = 10 and sodium lauryl sulfate (SLS) at pH = 10. They reported a nitrogen recovery of 93.5% and (18) 63 lysine residue per 1000 in the salt soluble fraction, one in the alcohol

soluble fraction, three in what the authors call glutelin-1 (fraction soluble in isoPrOH plus ME), 11 in glutelin-2 (Fraction soluble in ME) and 51 in glutelin-3 (fraction soluble in SLS).

Misra et al (19) fractionated the protein of the single mutants opaque-2, opaque-7, floury-2 and brittle-2 and of the double mutant opaque-2 brittle-2 by the Moureaux-Landry procedure. All mutations were incorporated into same genetical background: Oh43. They found that single mutants had higher levels of albumin, globulin and glutelin-3 than their normal counterpart. The double mutant was found to be particularly high in those fractions.

Materials and Methods

Corn Samples

Fourteen corn samples obtained from 3 different sources were used in the study. Three samples, HL1, HL27, and HL46 were furnished by the Kansas State University (KSU) corn breeding program. Those modified opaque-2 seeds were produced by interpollination among a group of opaque-2 hybrids.

Nine samples were furnished by CIMMYT (International Maize and Wheat Improvement Center): Composite K (Comp. K), Veracruz 181xAntigua Group 2 (V181) and CIMMYT O.P. 2 (COP2) each in three phenotypes: normal, opaque, and modified (Fig. 1).

Two samples were furnished by the KSU feed mill: bulk normal (++) and bulk opaque (o_2o_2).

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

Hand Dissection of Kernels

Modified kernels were hand picked from those completely normal and those completely opaque. The separation was aided by viewing them over a frosted glass illuminated from underneath with a fluorescent light. The materials obtained from CIMMYT had previously been sorted.

Selected kernels were soaked in water for 2-3 minutes and then peeled and degerminated with the aid of tweezers and scalpel. The opaque and translucent portions were separated from each other with a scalpel. The hard endosperm chunks could not be completely freed of floury endosperm. On the other hand, the soft endosperm portion was practically 100% free of hard endosperm (Fig. 2). Each endosperm portion and whole endosperms were ground in a Wiley mill to pass a No. 40 sieve.

Protein Fractionation

Proteins from endosperms of normal, opaque, and modified kernels, as well as soft and hard endosperm portions from modified kernels were fractionated on the basis of solubility. Extractions were performed on 3 g. samples with a magnetic stirrer at room temperature, with a solvent to meal ratio of 4:1 (v:w). Fractionations were carried out in triplicate unless otherwise designated. Sequence of solvents employed and the length of extraction are shown in Table 1 and Figure 3.

After each extraction, the suspension was centrifuged at 3000 rpm (1500 x G) for 15 minutes and protein content of supernatant and residual meals determined. In most cases the water soluble and the salt soluble fractions were combined before proceeding with the analysis.

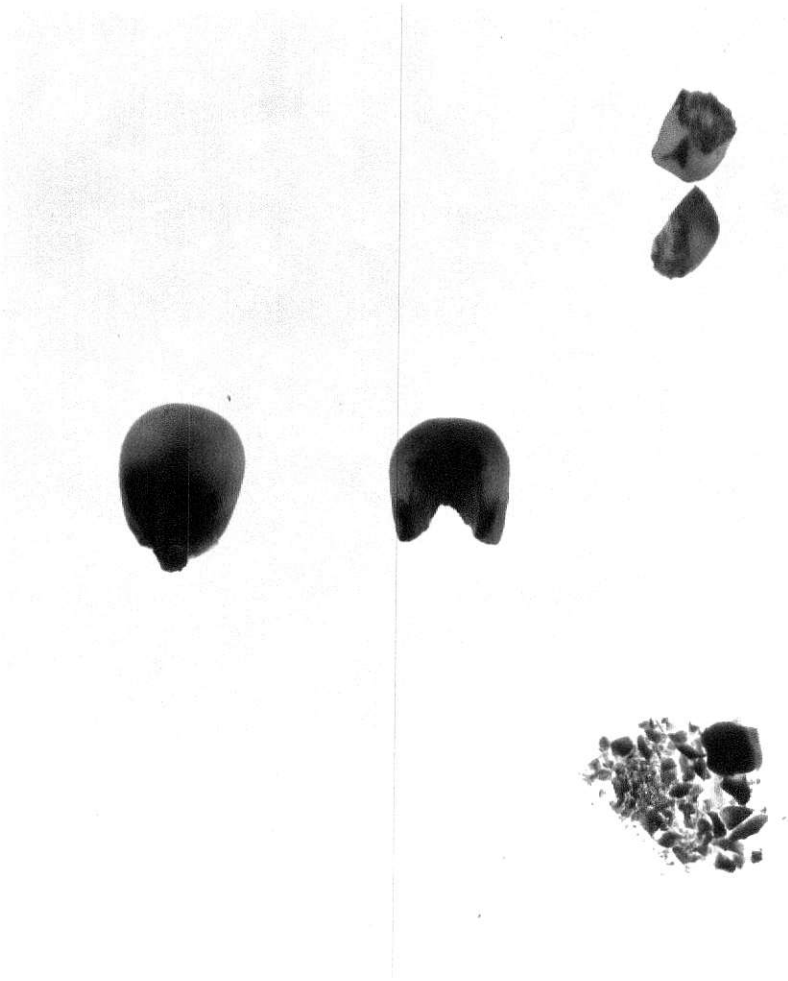

Fig. 2. Modified whole corn kernel (left) endosperm, after peeling and degerming (center), and (right) hard (top) and soft (bottom) portions after hand dissection of endosperm.

Table 1. Solvents, order, and length of extractions used for fractionating corn endosperm proteins.

Solvents and Order of Extraction	Extraction Time (min.)		
	1st	2nd	3rd
Water	60	30	5
Ethanol:Water (70:30, v:v) with 0.5% Sodium Acetate	240	120	5
0.5M Sodium Chloride	60	30	5
0.6% (v:v) 2-Mercaptoethanol in a Sodium Carbonate-Sodium Bicarbonate Buffer (pH 10)	60	30	5
0.5% (w:v) Sodium Lauryl Suflate in a Sodium Carbonate-Sodium Bicarbonate Buffer (pH 10)	60	30	5

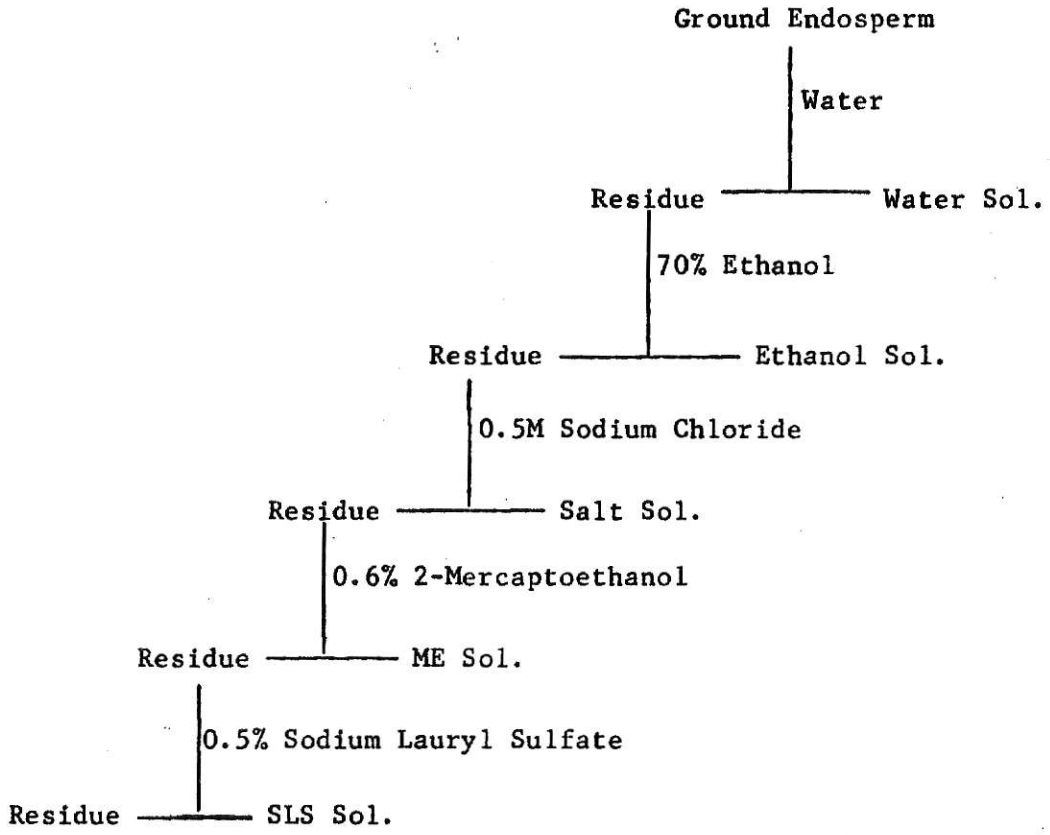

Fig. 3. Fractionation scheme for whole and hand dissected endosperms.

After aliquots for protein analysis were taken, remnants from the three replications were combined and the solutions dialyzed against 2 changes of distilled water at 4°C for about 48 hr. Alcohol soluble fractions were evaporated in a BUCHI Rotavapor-R prior to dialysis. Dialyzed liquid samples were then freeze dried and used for amino acid analysis.

Analytical Procedures

Protein content (N x 6.25) was determined by a slightly modified micro-Kjeldahl procedure (20). Moisture was determined on 0.5 g. samples heated overnight in a forced draft oven at 50-60°C.

Amino acid composition was determined on a 120B Beckman Amino Acid Autoanalyzer. Samples were hydrolyzed for 22 hr. with 6 N hydrochloric acid at 110°C in sealed tubes. The acid was evaporated in a rotary evaporator and sufficient pH 2.2 buffer added to have approximately 1 mg. protein per ml. Basic amino acids were determined in a 7 cm column packed with AA-27 Beckman resin and a sodium citrate buffer at pH = 5.28. The acid and neutral amino acids were determined on a 54 cm column packed with AA-15 Beckman resin and using 2 sodium citrate buffers: pH = 3.28 and pH = 4.25. The buffer switch occurred before the valine peak.

Scanning Electron Micrographs

Certain kernels selected from the samples were cut transversely with a razor blade, mounted on aluminum stubs with Delco No. 93 collodial silver coated with a 150 Å thick gold-palladium layer and viewed and photographed in a ETEC Autoscan scanning electron microscope.

Results and Discussion

Protein Content of the Hand Dissected Endosperms

The protein content of hand disected endosperms from normal, opaque-2, and modified samples are shown in Table 2.

The bulk opaque-2 sample had a lower protein content than the bulk normal. It must be kept in mind that those materials were not genetically related or grown under the same environmental conditions. The opaque-2 converted materials were lower in protein than their normal counterparts. However, the related modified kernels had protein contents essentially equal to the normal phenotype. The modified whole endosperms of HL1 and HL27 had similar protein values while HL46 was about 1% higher. The protein content was essentially equal in the hard and soft endosperm portions for the four modified materials analyzed. This finding is 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.

Protein Distribution

Protein distributions of a normal and an opaque-2 corn (undesignated samples) are given in Table 3. Water, salt, and SLS soluble fractions were much higher in the opaque corn, whereas the alcohol soluble fraction was much lower than in normal corn. This is in general agreement with work reported by Musiiko (15).

The modified V181 (Table 4) has a protein distribution intermediate between the values found for V181 normal and V181 opaque kernels, except

Table 2. Protein content (N x 6.25) of the hand dissected endosperms (% dry basis).

Sample	Genotype	Endosperm Protein		
		Whole	Hard	Soft
Modified HL1	o_2o_2	8.1	8.1	8.2
Modified HL27	o_2o_2	8.2	8.2	8.4
Modified HL46	o_2o_2	9.5	9.3	9.4
Normal Comp. K	++	9.9		
Opaque-2 Comp. K	o_2o_2	7.3		
Modified Comp. K	o_2o_2	10.3	10.2	10.1
Normal V181	++	9.8		
Opaque-2 V181	o_2o_2	8.0		
Modified V181	o_2o_2	9.7		
Normal COP 2	++	9.7		
Opaque-2 COP 2	o_2o_2	8.3		
Modified COP 2	o_2o_2	11.3		
Bulk Opaque-2	o_2o_2	8.6		
Bulk Normal	++	10.7		

Table 3. Distribution of protein (% of total protein) in endosperms from a normal sample and an opaque-2 sample.

Sample	Water Soluble	Alcohol Soluble	Salt Soluble	ME Soluble	SLS Soluble	Residual Meal	Total Recovery
	%	%	%	%	%	%	%
Normal	2.7	43.5	2.3	17.2	28.4	3.5	97.6
Opaque-2	11.3	15.9	4.6	21.8	40.0	6.6	100.2

Table 4. Distribution of protein (% of total protein) in endosperms from 3 phenotypes (normal, opaque, and modified) of V181.

Sample	Water + Salt Soluble	Alcohol Soluble	ME Soluble	SLS Soluble
	%	%	%	%
Normal V181	4.9	40.5	17.4	32.0
Opaque-2 V181	21.0	9.9	21.7	38.6
Modified V181	12.5	13.4	29.6	35.8

that the ME soluble fraction was somewhat higher than in the other two phenotypes. There appears to be a shift of alcohol soluble to ME soluble protein in the modified material. The V181 normal and opaque-2 phenotypes had protein distributions similar to those of bulk normal and opaque-2 samples, respectively.

Protein distribution for whole modified Comp. K endosperm (Table 5) was intermediate between those for the hard and the soft portion of the endosperm. The Comp. K opaque phenotype was higher in water plus salt soluble (W plus S), and SLS soluble fractions, and lower in the alcohol soluble fraction than the modified whole endosperm. Its protein distribution closely resembles that of the modified soft endosperm portion and also that of the unknown opaque-2 corn. Protein distribution for normal Comp. K is similar to those of modified materials rather than normal. We have the strong suspicion that the bag from which the samples were taken had been mislabeled. That suspicion was strengthened by other data discussed later. The ME fraction from the modified whole endosperm was higher than from the opaque genotype indicating that a shift from alcohol soluble to ME soluble had occurred in the modified Comp. K as it did in modified V181.

Protein distributions for the modified samples HL1, HL27, and HL46 are given in Table 6. Results obtained for HL1 and HL27 agree quite well with each other and with those obtained for the modified phenotypes of Comp. K and V181, however, HL46 differs markedly. The water plus salt soluble and the ME soluble fractions were lower for HL46 and the alcohol soluble fractions higher than the corresponding samples for the other two materials. When compared to normal corn (Table 3 or 4)

Table 5. Distribution of protein (% of total protein) in whole, soft, and hard endosperms from modified Comp. K and in whole endosperms of its normal and opaque phenotypes (single determination).

Sample	Water + Salt Soluble	Alcohol Soluble	ME Soluble	SLS Soluble	Residual Meal	Total Recovery
	%	%	%	%	%	%
Normal Comp. K	13.8	15.7	26.4	34.6	9.7	100.2
Opaque-2 Comp. K	18.6	8.2	23.2	41.6	8.2	99.8
Modified Comp. K						
Whole Endosperm	14.5	15.9	26.4	33.2	10.2	100.2
Hard Endosperm	12.6	17.7	28.1	30.5	8.2	97.1
Soft Endosperm	16.0	8.7	25.2	35.3	11.6	96.8

Table 6. Distribution of protein (% of total protein) in whole, hard, and soft endosperms from modified opaque-2 hybrid corn.

Sample	Water + Salt 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
	a/					
Soft Endosperm	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 analysis gave low values for alcohol soluble (14%) and correspondingly high values for SLS (58%), from the whole and soft endosperm value the value appeared to be in error.

the HL46 modified whole endosperm has similar values for water plus salt solubles and ME, but a lower value for alcohol soluble and a higher value for SLS.

The highest proportion of total protein was recovered in the SLS soluble fraction for all types of endosperm and all three genotypes. In all three materials the protein distribution for whole endosperms were intermediate between those for hard and those for soft endosperms as would be expected. The hard endosperm portion contained more alcohol soluble protein and the soft endosperm portion more water plus salt soluble protein in all three genetically different materials.

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

The data presented suggests that the protein of the hard endosperm was responsible for that change in protein distribution, since the soft endosperm-except for HL46-had essentially the same protein distribution as in opaque-2 materials. Nevertheless, the protein distribution of modified hard endosperms was different from that of normal endosperms; higher in W plus S and ME soluble and lower in the alcohol soluble fraction.

Amino Acid Composition of Endosperms and its Fractions

Amino acid composition of whole corn endosperms (peeled and degermed) is given in Table 7. Amino acid distribution for the bulk normal sample and the normal V181 were similar. Also agreeing quite well in amino acid composition were the three opaque-2 genotypes.

Table 7. Amino acid composition (g/100 g. protein) of endosperms from normal, opaque-2 and three phenotypes each of Comp. K and V181 corn.

Amino Acid	Bulk		V181			Comp. K		
	Normal(++)	Opaque-2(o_2o_2)	Normal	Opaque-2	Modified	Normal a/	Opaque-2	Modified
Lysine	1.65	3.75	1.50	3.78	2.96	3.01	3.78	3.27
Histidine	2.58	3.21	2.71	3.66	3.49	3.84	3.47	4.15
Ammonia	2.25	1.83	2.07	2.23	2.38	1.98	1.78	1.81
Arginine	3.44	5.60	2.70	5.16	4.20	4.72	4.81	4.54
Aspartic Acid	5.49	8.77	5.14	8.91	7.81	6.50	9.97	7.65
Threonine	3.15	3.93	3.03	3.91	3.64	3.53	3.84	3.68
Serine	4.64	4.56	4.52	4.31	4.43	4.33	4.26	4.42
Glutamic	20.88	18.31	21.55	17.38	19.51	18.50	20.29	18.68
Proline	8.61	8.97	9.55	9.50	9.62	9.84	8.98	9.75
Glycine	2.93	4.25	2.64	4.34	3.92	3.87	4.24	4.07
Alanine	7.91	6.15	7.94	6.07	6.12	6.07	6.50	6.06
Half Cystine	3.37	3.16	2.73	3.19	4.23	4.88	2.36	4.18
Valine	3.63	4.39	4.53	5.18	5.09	4.45	5.22	5.26
Methionine	2.04	1.10	1.69	1.47	1.13	1.28	1.45	1.48
Isoleucine	3.47	3.30	3.45	3.51	3.31	3.36	3.19	3.33
Leucine	14.84	10.54	15.50	9.58	10.61	11.50	8.48	10.21
Tyrosine	4.04	3.73	3.59	3.62	3.48	3.71	3.61	3.49
Phenylalanine	5.06	4.44	5.17	4.17	4.11	4.61	3.76	3.95

a/ The data reported for the bag labeled normal Comp. K appears to be for modified corn as noted previously.

The opaque materials had higher levels of lysine, histidine, arginine, aspartic acid and glycine and lower levels of glutamic acid, alanine, and leucine. This is in good agreement with data reported previously (3, 16,19). All modified phenotypes had similar amino acid distributions except HL46 which also had an unusual protein distribution.

The amino acid composition of normal comp. K was essentially the same as that of modified Comp. K. This was additional evidence that the normal sample was a mislabeled modified sample.

In general, the modified materials had amino acid compositions intermediate between those values for normal and opaque. The sulfur containing amino acids did not have intermediate values, with methionine being lower and cystine being higher than either the normal or opaque. Of course there is no reason why the modified phenotypes should have intermediate values.

When the whole endosperms were dissected into hard and soft portions, it would be logical to assume that the amino acid distribution of the whole endosperm would be intermediate between the values for the hard and soft portions. In general that was found (Table 8) for most of the amino acids. However, certain amino acids did not follow this general trend. The data for the sulfur containing amino acids were erratic and no general trend could be observed. The data for histidine and glutamic acid showed wide variations. Samples having an abnormally high histidine content also had an unreasonably low glutamic acid content. This would suggest a shift between those amino acids during hydrolysis or while they were being run on the analyzer.

Table 8. Amino acid composition (g./100 g. protein) for whole, hard, and soft endosperm portions of modified corn.

Amino Acid	HL1 Endosperm			HL27 Endosperm			HL46 Endosperm			Modified Comp. K Endosperm		
	Whole	Hard	Soft	Whole	Hard	Soft	Whole	Hard	Soft	Whole	Hard	Soft
Lysine	2.89	2.40	3.53	2.73	2.38	3.04	2.00	2.02	2.90	3.27	2.95	4.35
Histidine	3.76	3.64	6.64	3.56	3.69	3.49	2.86	4.89	5.29	4.15	6.14	6.51
Ammonia	1.78	2.21	2.58	1.17	2.21	1.97	2.20	3.42	3.09	1.81	2.76	2.29
Arginine	4.30	3.91	5.05	4.11	3.88	4.33	3.44	3.47	4.37	4.54	4.65	6.01
Aspartic Acid	7.20	6.08	8.31	7.07	6.54	7.56	5.89	5.26	5.85	7.65	7.27	8.90
Threonine	3.66	3.40	4.21	3.69	3.46	3.66	3.17	3.27	3.37	3.68	3.32	4.07
Serine	4.33	4.30	4.40	4.47	4.46	4.53	4.67	4.73	4.63	4.42	4.25	4.56
Glutamic Acid	18.03	19.07	14.93	19.79	19.74	19.19	20.82	17.72	16.22	18.68	16.72	14.83
Proline	9.74	10.20	10.13	9.90	9.98	9.54	10.17	10.28	9.78	9.75	10.63	10.46
Glycine	4.27	3.84	4.00	4.02	3.67	3.99	3.40	2.99	3.68	4.07	3.55	4.67
Alanine	6.23	6.60	5.88	6.38	6.81	6.48	7.20	7.85	7.44	6.06	6.26	6.10
Half Cystine	4.75	3.98	4.17	4.26	3.18	3.97	3.14	1.69	3.22	4.18	3.63	4.06
Valine	5.33	4.72	4.99	5.11	5.07	5.03	4.68	4.31	4.86	5.26	5.10	5.65
Methionine	1.61	1.78	1.18	1.28	1.34	1.43	1.70	2.46	1.88	1.48	1.05	1.35
Isoleucine	3.24	3.22	2.99	3.18	3.31	3.26	3.19	3.14	3.12	3.33	3.26	3.41
Leucine	11.57	12.34	9.38	10.89	12.32	10.87	12.67	14.00	12.13	10.21	11.22	9.41
Tyrosine	3.43	3.96	3.47	3.76	3.61	3.59	4.21	3.70	3.73	3.49	3.10	3.47
Phenylalanine	3.87	4.26	4.14	4.02	4.36	4.05	4.61	4.80	4.43	3.95	4.16	4.38

In general amino acid distribution for each type of endosperm (whole, hard, and soft) for the genotypes HL1, HL27, and Comp. K are in good agreement, if the values for histidine, glutamic acid, cystine and methionine are ignored. The genotype HL46 had higher values for leucine, alanine, and phenylalanine and lower values for lysine, glycine, and aspartic acid.

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

Amino acid compositions of protein fractions extracted from the whole, hard, and soft endosperms of HL1, HL27, and HL46 are shown in Tables 9, 10, and 11. Amino acid distribution for the protein extracted with each extraction medium is remarkably constant. There appears to be little or no variation due to the type of endosperm or the genotype extracted. An average amino acid distribution for each extraction system is given in Table 12.

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

Table 9. Amino acid composition (g./100 g. protein) of the protein fractions extracted from whole, hard, and soft endosperms of HL1 corn.

	Whole Endosperm				Hard Endosperm				Soft Endosperm			
Amino Acid	Water Plus Salt	Alcohol	ME	SLS	Water Plus Salt	Alcohol	ME	SLS	Water Plus Salt	Alcohol	ME	SLS
Lysine	3.04	0.42	0.75	4.61		0.32	0.40	4.03		0.34	0.67	5.08
Histidine	2.34	2.58	7.41	2.27		2.61	7.31	2.30		1.83	7.99	2.68
Ammonia	1.25	4.12	2.21	1.10		3.89	2.70	1.51		3.15	2.17	1.52
Arginine	7.62	2.53	3.44	5.47		2.61	3.97	4.49		2.01	2.93	6.01
Aspartic Acid	9.01	4.86	1.56	9.00		4.65	1.59	8.22		4.87	1.90	8.50
Threonine	3.98	3.24	3.93	4.29		2.85	4.11	4.07		2.64	4.51	4.04
Serine	4.62	5.01	3.82	5.24		4.83	4.23	5.14		5.06	4.32	5.13
Glutamic Acid	11.50	18.63	23.02	15.21		18.18	23.87	17.25		23.02	22.05	15.34
Proline	5.11	10.18	18.07	6.61		10.53	18.26	8.78		9.21	19.55	5.64
Glycine	6.19	2.45	4.55	4.66		2.28	4.92	4.13		1.50	5.00	4.54
Alanine	6.89	8.90	4.62	7.41		8.51	4.95	7.24		9.08	4.77	7.34
Half Cystine	6.10	2.30	1.81	0.77		3.17	0.00	0.00		1.42	0.00	1.57
Valine	12.61	3.74	6.09	5.63		3.99	5.76	4.88		2.93	6.67	5.80
Methionine	3.27	1.18	1.27	2.65		1.23	1.06	3.22		0.59	0.47	2.07
Isoleucine	5.12	2.67	1.81	3.95		3.23	2.21	4.08		3.46	2.41	4.13
Leucine	4.98	17.00	9.74	11.19		16.55	11.17	11.52		17.40	11.05	11.44
Tyrosine	3.26	4.40	2.75	4.69		4.37	1.00	4.44		4.58	0.51	4.14
Phenylalanine	3.08	5.78	2.60	5.32		5.58	2.48	4.60		6.91	3.06	5.04

Table 10. Amino acid composition (g./100 g. protein) of the protein fractions extracted from whole, hard, and soft endosperms of HL27 corn.

	Whole Endosperm				Hard Endosperm				Soft Endosperm			
Amino Acid	Water Plus Salt	Alcohol	ME	SLS	Water Plus Salt	Alcohol	ME	SLS	Water Plus Salt	Alcohol	ME	SLS
Lysine	4.70	0.73			4.13	0.27		4.67	5.44	0.30	0.46	5.54
Histidine	2.49	2.96			2.77	2.74		2.52	4.20	1.05	6.39	2.84
Ammonia	2.61	4.67			1.70	3.37		1.21	1.90	2.03	1.74	1.60
Arginine	7.21	3.10			7.39	2.19		5.91	10.20	1.86	3.10	6.97
Aspartic Acid	9.42	5.02			10.84	4.71		8.44	10.22	5.23	1.83	6.97
Threonine	4.15	3.10			4.90	2.86		4.22	4.34	2.78	4.02	4.29
Serine	4.62	5.02			4.29	5.07		5.18	4.90	5.28	4.28	5.35
Glutamic Acid	14.40	18.25			18.76	19.23		16.31	11.52	19.87	24.64	13.56
Proline	5.57	9.10			7.69	10.41		6.81	5.90	9.42	19.56	6.88
Glycine	6.41	2.24			6.59	1.77		4.28	6.50	1.68	4.86	4.40
Alanine	6.83	8.66			6.79	9.04		7.34	7.15	9.29	4.84	7.31
Half Cystine	6.63	2.57			1.22	2.18		0.52	5.90	2.02	0.00	0.23
Valine	4.95	2.94			4.87	3.55		4.88	4.90	3.12	6.79	5.82
Methionine	1.64	0.79			1.18	0.78		2.43	0.94	0.81	0.82	2.22
Isoleucine	3.64	3.22			3.42	3.41		3.97	3.31	3.60	2.11	4.12
Leucine	6.77	16.49			6.64	17.50		11.67	6.17	20.46	10.50	11.68
Tyrosine	4.01	4.61			3.48	4.70		4.58	3.19	4.73	1.30	4.70
Phenylalanine	3.95	6.53			3.31	6.21		5.06	3.29	6.47	2.76	5.50

Table 11. Amino acid composition (g./100 g. protein) of the protein fractions extracted from whole, hard, and soft endosperms of HL46 corn.

	Whole Endosperm				Hard Endosperm				Soft Endosperm			
Amino Acid	Water Plus Salt	Alcohol	ME	SLS	Water Plus Salt	Alcohol	ME	SLS	Water Plus Salt	Alcohol	ME	SLS
Lysine	4.38	0.32	0.60	3.78	0.16	0.72	3.12	3.33	0.48	0.77	4.21	
Histidine	2.77	1.06	7.19	2.88	1.39	5.60	2.17	1.91	1.97	6.71	2.52	
Ammonia	3.23	2.67	2.26	2.13	3.16	2.34	2.35	2.80	3.40	2.11	2.04	
Arginine	6.33	1.55	3.20	5.54	1.82	3.54	3.47	5.41	2.78	3.50	5.11	
Aspartic Acid	9.76	4.85	1.42	7.49	5.45	2.23	7.03	11.85	5.73	1.71	7.58	
Threonine	5.00	2.84	3.72	3.89	3.11	3.40	3.66	5.51	3.12	3.84	3.85	
Serine	5.93	5.03	3.74	5.29	5.56	4.17	5.00	6.07	5.61	3.93	4.83	
Glutamic Acid	15.23	22.78	23.13	19.55	25.51	24.81	18.43	17.57	17.15	22.19	17.98	
Proline	9.15	10.11	18.12	7.69	10.87	13.92	7.52	3.67	8.35	19.06	5.69	
Glycine	6.45	1.85	4.46	4.05	1.70	4.66	3.19	7.89	2.41	4.84	3.69	
Alanine	6.75	9.29	4.14	8.26	10.53	6.08	7.63	7.90	9.57	4.86	7.38	
Half Cystine	0.00	2.36	1.05	0.00	2.21	0.72	1.15	0.00	3.03	1.64	0.90	
Valine	5.40	3.22	6.21	5.11	4.18	5.09	4.58	5.86	3.38	6.48	5.43	
Methionine	2.75	1.11	1.26	4.19	1.36	3.75	3.47	0.00	0.72	1.61	2.64	
Isoleucine	3.97	3.31	3.32	3.92	3.63	1.88	3.67	5.19	3.47	2.04	3.94	
Leucine	6.41	17.59	9.90	13.53	17.34	10.46	13.39	8.17	16.82	9.91	12.26	
Tyrosine	3.10	4.28	3.35	5.85	5.12	4.11	4.28	2.72	5.34	2.59	4.68	
Phenylalanine	3.38	5.79	2.84	5.79	7.04	2.49	5.89	4.13	6.58	2.21	5.27	

Table 12. Average amino acid composition (g./100 g. protein) for proteins extracted with certain solvents.

Amino Acid	Water Plus Salt	Alcohol	ME	SLS
Lysine	4.17	0.37	0.63	4.38
Histidine	2.74	2.02	6.97	2.52
Ammonia	2.20	3.38	2.20	1.68
Arginine	7.32	2.27	3.37	5.37
Aspartic Acid	10.18	5.04	1.75	7.90
Threonine	4.65	3.03	3.95	4.04
Serine	5.07	5.16	4.08	5.14
Glutamic Acid	14.91	20.29	23.35	16.70
Proline	5.93	9.80	18.12	6.45
Glycine	6.67	1.95	4.77	4.12
Alanine	7.04	9.21	4.89	7.49
Half Cystine	3.31	2.36	0.76	0.50
Valine	6.63	3.45	6.17	5.27
Methionine	1.63	0.96	1.44	2.86
Isoleucine	4.11	3.22	2.26	3.97
Leucine	6.50	17.46	10.41	12.02
Tyrosine	3.30	4.68	2.18	4.66
Phenylalanine	3.53	6.32	2.64	5.32

Those results confirm the protein distribution data. For instance, the high lysine opaque materials were high in W plus S and SLS soluble fractions. Alcohol soluble fractions were high in leucine and the high leucine normal phenotypes were high in alcohol soluble proteins. The three endosperms types of HL46 were lower in W plus S and higher in alcohol soluble fractions and therefore lower in lysine, glycine and aspartic acid and higher in leucine, alanine and phenylalanine than the corresponding endosperms of the other modified materials. Thus, the amino acid data confirm that the protein distribution of HL46 is different from that of the other modified materials.

Protein Distribution, Amino Acid Composition and Protein Quality

The protein distribution and amino acid composition indicate that there was a loss in protein quality with the conversion of opaque phenotype to modified phenotype. In modified kernels, the lysine rich fractions were lower, and therefore the lysine poor fractions higher. The loss in protein quality can be attributed to the hard endosperm since the soft endosperm, except for HL46, had essentially the same protein composition as opaque-2 materials. However, the modified hard endosperm protein distribution was different than the normal and higher in lysine.

Data presented would suggest that opaque-2 genotypes with translucent endosperm phenotypes could be bred with a substantially better protein quality than normal corn. However, the protein quality would not be as high as that of nonmodified opaque-2 corn.

Scanning Electron Micrographs

The cellular structure of corn endosperm is shown in Fig. 4. Several cells can be distinctly seen. Certain of the cells were cut open by the razor blade, thus exposing the starch granules and protein matrix. Other cells have their walls intact and can be observed in their entirety. The picture is of opaque corn, however, similar structures have been noted in normal corn.

Starch granules in hard normal endosperm are polygonal in shape (Fig. 5 top). They are tightly packed with no air spaces. A thin continuous layer of protein appears to enclose the granules. Embedded in that protein matrix are small spheroidal bodies which have been shown to be zein protein (10, 22, 23). The picture shows the actual bodies (zb) and the indentations (zbi) left in the starch surface by the bodies which were ripped off by the razor blade.

A photomicrograph of opaque-2 endosperm is shown in Fig. 5 bottom. In contrast to the micrograph at the top of the figure the starch granules are nearly round. The protein matrix is again continuous (except where it has been torn by the cutting) and appears to enclose the starch granules. The starch granules are not tightly packed and as a result there are many intergranular air spaces.

The normal hard endosperm of V181 (Fig. 6 top) is similar to the undesignated normal corn endosperm shown in Fig. 5 top. The zein bodies (zb) and the indentations (zbi) left by them can be clearly seen. Starch granules that have been damaged (dsg) as a result of the cutting are evident in both figures.

The opaque phenotype of V181 (Fig. 7 top) is similar to the opaque-2 endosperm (Fig. 5 bottom).

THIS BOOK CONTAINS NUMEROUS PICTURES THAT ARE ATTACHED TO DOCUMENTS CROOKED.

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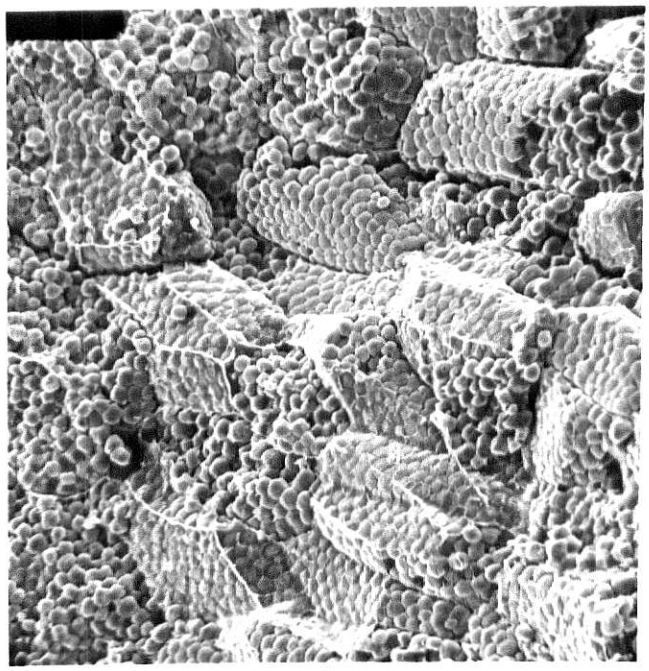

Fig. 4. Scanning electron photomicrograph (140X) of corn endosperm showing the cellular structure.

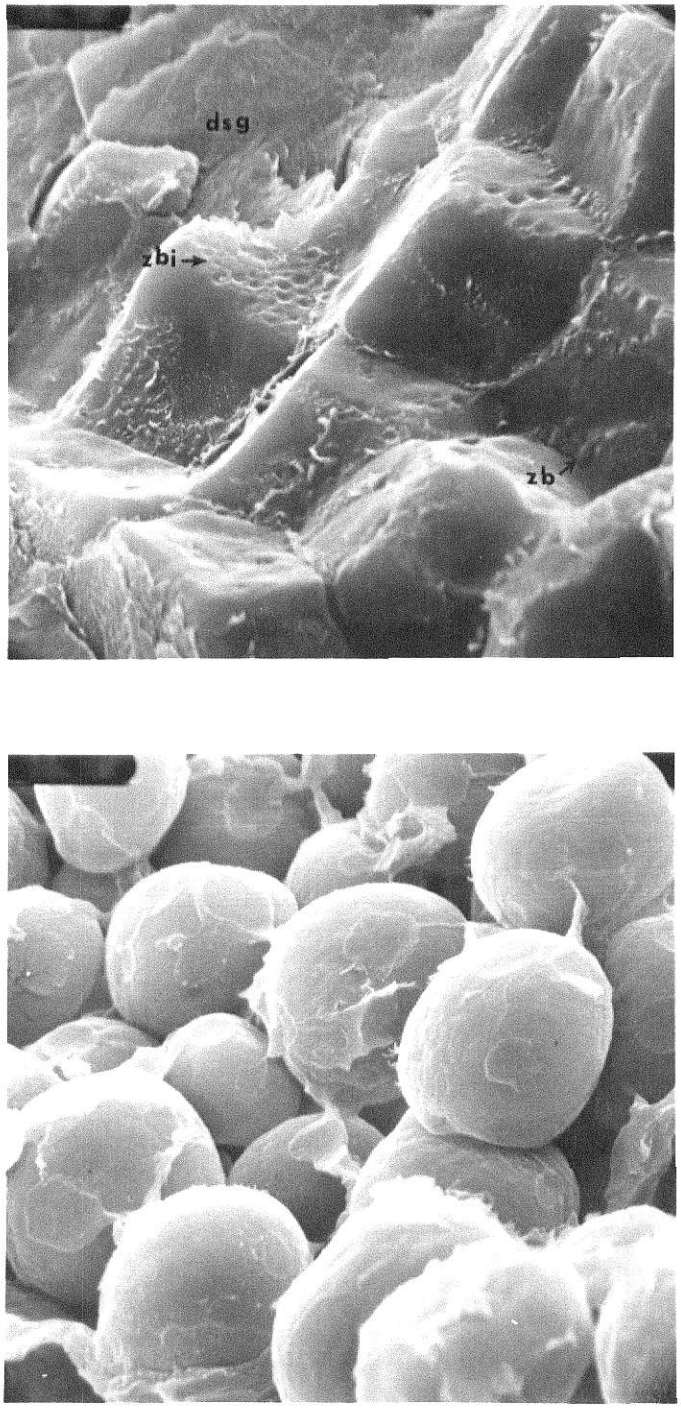

Fig. 5. Scanning electron photomicrographs of (top, 2800X). Normal hard endosperm showing damaged starch granules (dsg), zein bodies (zb) and indentations made by zein bodies (zbi); and bottom, 2900X: opaque-2 endosperm.

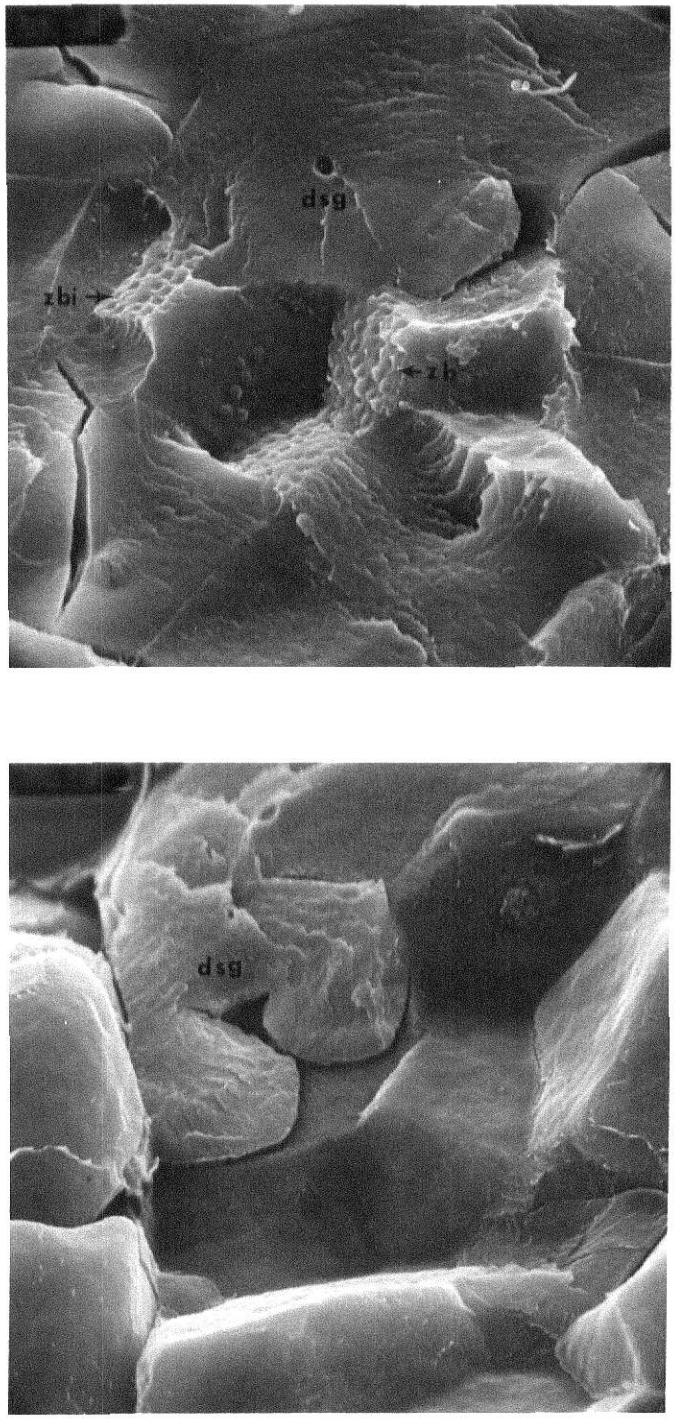

Fig. 6. Scanning electron photomicrographs of (top, 2300X) normal V181 endosperm, showing damaged starch granules (dsg), zein bodies (zb) and indentations made by zein bodies (zbi); and (bottom, 2900X) hard endosperm of modified V181 showing damaged starch granules (dsg) and absence of zein bodies.

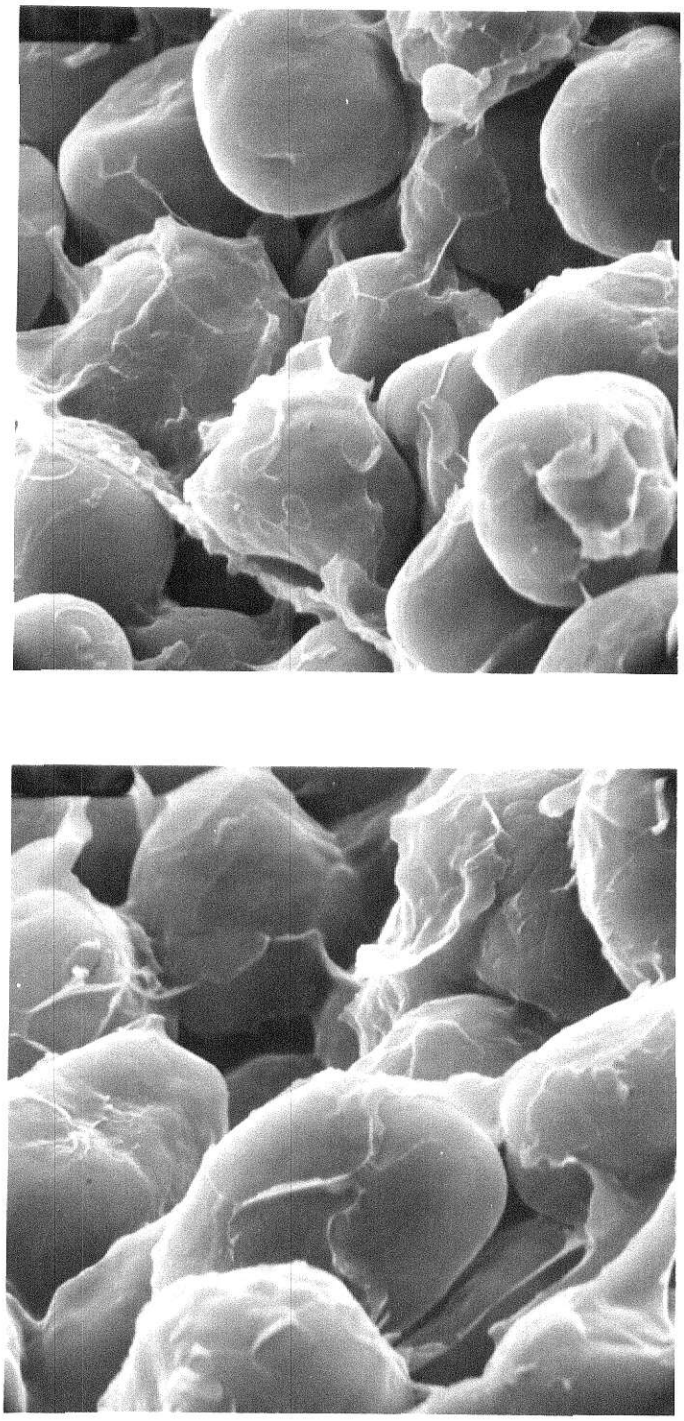

Fig. 7. Scanning electron photomicrographs of (top, 2300X) opaque-2 V181 endosperm and (bottom, 2900X) modified V181 soft endosperm.

A photomicrograph of the hard endosperm of modified V181 (Fig. 6 bottom) shows that the starch granules are angular and tightly packed and the appearance is similar to that of a normal hard endosperm (Figs. 5 top and 6 top). However, there is one distinctive difference; the modified protein matrix does not have zein bodies. The photomicrograph of the soft endosperm of modified V181 (Fig. 7 bottom) is similar to those of opaque corn endosperms (Figs. 5 bottom and 7 top). Scanning electron micrographs of the hard endosperm labeled normal Comp. K showed no zein bodies. The absence of zein bodies was another factor in our conclusion that this material was a modified and not a normal genotype.

Based on the scanning electron photomicrographs and the chemical data several conclusions on the structure of corn seem warranted.

Physical Properties. The loose structure of the soft endosperm with high proportion of intergranular air spaces would explain the lower density reported for opaque materials. Those air spaces would cause the opaqueness, by refracting light as Duvick (10) pointed out. The air spaces, loosely packed starch granules and fine protein matrix would allow the endosperm to give under stress and thus be responsible for its soft character.

Duvick (10) had hypothesized that the hard endosperm was translucent because the intergranular spaces were filled with zein bodies. Based on the scanning electron micrographs we found very little intergranular spaces in the hard endosperm. The intergranular spaces present were filled with the protein matrix, thus leaving no intergranular air space and therefore giving the translucent appearance. We suggest that tight packing of starch granules (number of granules per unit volume) is responsible for the

translucent appearance rather than the amount or type of the protein in the matrix. This is in agreement with finding modified hard endosperms which are translucent and contain little or no zein bodies. The absence of zein bodies confirms the findings of other authors (11). However even though the protein bodies were found only in normal hard endosperms, the proportion of zein in the other types of endosperms was not negligible. Christianson et al (23) suggested that zein was only deposited in zein bodies, if true, the zein bodies in soft endosperms or modified hard endosperms are so small they are not visible under the scanning electron microscope. Transmission electron micrographs showed the presence of zein bodies in opaque-2 endosperms. The size of those zein bodies was about 0.1 μ (24).

Tightly packed starch with tightly adhering protein matrix would not be expected to give under external stress, and thus would be responsible for the hard character of this type of endosperm.

Shape of the Starch Granules. The difference in shape of starch granules from floury and those from horny endosperms has been noted previously (21). Why a single kernel of grain should have those two types of starch is an intriguing question. One possible explanation is that during the natural drying process the protein loses water and shrinks. If the strength of adhesion within the protein is strong enough the starch granules are pulled closer and closer together. If the starch granules still contain enough water to be somewhat flexible at this stage the result would be polygonal shaped, tightly packed starch granules. This would be the case in hard endosperm. In the soft endosperm the data shows protein

distribution and amino acid composition to be quite different. If that distribution gives a weaker adhesion and the protein—protein bonds rupture during drying, the result would be intergranular air spaces and round starch granules.

Starch Damage. If sufficient force is applied, the kernel fractures. If the strength of adhesion between protein and starch is less than that within the starch granule the fracture should leave intact starch granules and exposed protein surfaces. This is the case of soft endosperms either normal, opaque-2 or modified as shown by the photomicrographs (4 bottom, 6 top and bottom). Examination of hard endosperms photomicrographs (4 top, 5 top and bottom) reveals a predominance of fractured starch granules. Thus the strength of adhesion between protein and starch would be stronger than that within the starch granules.

The degree of starch damage shown above would raise questions about the amount of starch damage in dry mill corn products. Examination of corn grits with the scanning electron microscope reveals the same fracture patterns as in Fig. 6. Thus the degree of starch damage increases tremendously as the particle size decreases. Corn flour has a very limited market, possible due, at least in part, to its high degree of starch damage. In the wet milling process corn is soaked in acid solution which weakens the protein-starch bond and allows the starch to be separated.

Summary

Modified opaque-2 corn had different endosperm protein distribution from that in completely opaque corn endosperm. There was a loss in protein quality in modified opaque-2 compared to the opaque phenotype opaque-2 since modified endosperm protein was lower in lysine than opaque endosperm protein. The modified phenotypes were low in alcohol soluble protein (14%) compared to normal (40%) and only slightly higher than the opaque (10%). However, the modified phenotypes were high in ME soluble protein (29%) compared to both the normal (17%) and the opaque (21%). Thus it appears there is a shift from alcohol soluble protein to ME soluble protein upon conversion to the modified phenotype.

When modified kernels were hand dissected into hard and soft endosperm portion and analyzed separately it was found that the protein distribution of soft endosperm was similar to that of the opaque phenotype endosperm. The protein distribution in the hard portion of modified endosperm was lower in W plus S and higher in alcohol soluble fractions, and thus lower in lysine, than the opaque phenotype. The hard portion of the modified endosperm was then responsible for the loss in protein quality. However, the hard portion of the modified endosperm was lower in alcohol soluble and higher in W plus S soluble fractions and thus higher in lysine, than the normal corn endosperm.

The HL46 genotype kernels did not follow the general protein distribution pattern. The W plus S soluble fraction was not as high as in the other modified materials and more in the range of normal genotypes. The alcohol soluble fraction was significantly lower than for normal kernels

but higher than for the rest of the modified genotypes. The ME soluble fraction did not show increased values with respect to normal endosperms. It would appear that the genotype influences the protein distribution in modified materials.

Amino acid composition of protein extracted by various solvents was essentially constant for each solvent and not affected by the genotype of the material extracted. Thus, the protein distribution gives useful information as to the amino acid composition of the samples.

Scanning electron photomicrographs agreed with the protein and amino acid data. Hard normal endosperms, which had a high alcohol soluble fraction, showed zein bodies embedded in the protein matrix. Hard modified endosperms, though having the same general endosperm structure, polygonal shaped, tightly packed starch granules, and a continuous protein matrix, did not show any zein bodies. Modified hard endosperms were found to be much lower in alcohol soluble protein than normal corn endosperm. The figures were about 40% for normal and 14% for hard modified endosperms.

The data suggests that translucent phenotypes of opaque-2 corn would have a substantially better protein quality than normal corn. However, the protein quality of those phenotypes may not be as high as that of opaque types. Since the modified endosperms were shown to contain approximately 2% more protein than the opaque endosperms, therefore actual amount of lysine per unit weight of endosperm would be nearly the same in both phenotypes.

Acknowledgements

The author wishes to express his thanks to:

Dr. Carl Hoseney, for his supervision and helpful suggestions in the execution and writing of this work.

Dr. Clyde Wassom, who not only furnished part of the corn samples, but also gave valuable suggestions.

Dr. Charles Deyoe and Debbie Roney, for performing the amino acid analysis.

Dr. Cliff Watson, who made possible the use of facilities and pieces of equipment at the USDA Grain Marketing Research Center Labs. Acknowledgement is also given to many other staff members.

Paul Enos, from the KSU Scanning Electron Microscopy Lab. The scanning electron microscopy work was supported in part by NIH Biomedical Sciences Support Grant RR-07036 and NSF Equipment Grant GB032296.

Dr. Karl Finney, from the USDA Hard Winter Wheat Quality Lab, for the use of some pieces of equipment. Acknowledgement is also given to some other staff members.

Drs. Harry Pfost and S. Ruliffson for serving on the advisory committee and reviewing this manuscript.

The Ford Foundation, which supported in part this research.

CIMMYT (International Wheat and Maize Improvement Center) which furnished part of the corn samples.

Drs. Arlin Ward and Eugene Farrell, who furnished part of the samples.

The author wishes also to express his gratitude to his wife Maria for her encouragement and moral support and to his children Marcelo and Carolina.

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PROTEIN DISTRIBUTION, AMINO ACID COMPOSITION AND MICROSTRUCTURE OF MODIFIED OPAQUE-2 CORN ENDOSPERMS

by

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AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1973

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 also 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. Certain kernels were transversely cut and viewed in a ETEC scanning electron microscope.

The protein content of opaque-2 endosperms was less than that of their normal counterpart. The modified opaque-2 endosperms had protein contents equal to or higher than the normal ones. Hard and soft portions of modified endosperms had equal protein contents.

The opaque-2 materials had an altered protein distribution with higher amounts of water plus salt soluble protein and lower amounts of alcohol soluble protein when compared to normal materials. Modified endosperms had protein distributions that, though closer to opaque than to normal patterns, were significantly different from opaque-2 endosperms. The hard endosperm portion was responsible for that difference.

Opaque-2 materials had increased levels of lysine, arginine, glycine, and aspartic acid and decreased levels of alanine leucine, and phenylalanine when compared to normal genotypes. 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 nor opaque genotypes, but, in general, was intermediate between the two types.

Scanning electron micrographs of soft endosperms from normal, opaque-2, or modified opaque-2 corn showed loosely packed, nearly round, starch granules associated with thin sheets of protein and many intergranular air spaces. The hard endosperms had tightly packed, polygonal shaped starch granules associated with a continuous protein matrix, and no intergranular air spaces. Normal hard endosperms had zein bodies embedded in the protein matrix, whereas modified hard endosperms did not.