Endosperm Fragmentation of Ordinary and High-Lysine Corn

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ABSTRACT

Upon treatment with isotonic buffer, the endosperm of either hand-dissected corn or selected dry-milled products–grits, meal, and flour–fragmented into fine particles that gave improved protein shifting upon air classification. Release of adhering protein from starch granules was demonstrated by optical and scanning electron microscopy. More than 50% of the total protein was shifted into two high-protein fractions by air classification of treated flours from ordinary and high-lysine corns compared to 20 to 33% for untreated flours. Protein concentrates from endosperm products of high-lysine corn have potential for use in low-cost foods. Low-protein fractions may be suitable for industrial applications.

After corn kernels dehydrate during maturation, starch granules and protein adhere so tightly together in agglomerated particles that they cannot be separated by dry grinding. Only after steeping, as in wet milling, can they be separated.

An economical way to separate protein and starch from corn endosperm by dry milling is desirable if high-protein fractions are to be produced for food and feeds, and if low-protein flours are to become available for industrial uses. New high-lysine

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varieties of corn, which have improved levels and balance of essential amino acids (1,2) and some of which have a higher protein content (3), offer an opportunity to produce protein concentrates of greater nutritional value.

Air classification has been extensively studied as a means for separating protein-rich fractions from finely ground cereal flours. High-protein preparations obtained by air classification of wheat flour or by dry milling of wheat millfeeds offer improved functional or nutritional properties (4,5,6). However, efforts to air-classify corn flours thus far have not been productive, presumably owing to the close association of protein and starch (7).

A new approach has been taken to disrupt native agglomerates of starch and protein in ordinary and high-lysine corn and to facilitate their separation by air classification or other methods. By this new procedure, endosperm particles are first hydrated and then dried at low temperatures before fine grinding. The degree of breakdown of particles in corn flours was evaluated by screening and by air classification. The magnitude of protein shift was taken as an indication of how much protein was released from starch by water or buffer treatments.

MATERIALS AND METHODS

Endosperm Fragmentation

Hand-dissected endosperms from ordinary or high-lysine (*opaque-2*) corns ground through 60 mesh U.S. were hydrated with either water or isotonic buffer (0.01M KH_2PO_4, 0.006M $MgCl_2 \cdot 6H_2O$ adjusted to pH 7.2 with NaOH) by stirring at a gram:milliliter ratio of 1:10 for 24 hr. at 4°C. After gentle stirring, the whole mix was then dried to 3 to 5% moisture by lyophilization. Particle-size reduction was measured by comparing amounts of material that passed through a 200-mesh screen (−75 μ material) before and after treatment. Particle-size distributions of the −75 μ materials were determined by air sedimentation in a Micromerograph apparatus. Influence of treatment on disruption of particles in flours could be demonstrated by these means.

Fragmentation of Dry-Milled Products

Break flour from ordinary corn was obtained from General Foods, Kankakee, Ill. This flour comes only from break rolls and is not representative of a blended standard corn flour. The grit product was milled at this Laboratory from a single lot of dent corn. High-lysine corn flour was from a composite of high-lysine corns grown experimentally at the University of Illinois. Another experimental high-lysine corn also studied was grown at Purdue University and had an initial protein content of 15%. This high-protein, high-lysine corn was selected for study because of its improved kernel hardness and potential as a commercial variety. The high-lysine corns were dry milled at this laboratory.[2]

Dry-milled products were ground and stirred with isotonic buffer under conditions similar to those described for whole hand-dissected endosperm. The amount of material passing a 200-mesh screen after buffer treatment was compared with the amount of material obtained by screening the original dry-milled product.

Microscopic Examination

Disrupted endosperm particles resulting from treating dry-milled products from

[2]Pfeifer, V. F. Unpublished results.

high-lysine and ordinary corn with buffers were examined under the microscope. Scanning electron microscopy served as an excellent means to study association and disruption of protein-starch agglomerates in endosperm particles. Air-classified fractions were also examined microscopically to establish concentrations of free starch granules and protein particles in the various fractions. Protein and starch were readily distinguishable after being stained with iodine vapor for 3 min. Protein particles were stained lightly yellow; starch granules were stained heavily blue.

Air Classifications

The various flours were separated into sized fractions by fine grinding and air classification, a method applicable to both pilot-plant and commercial operations. Grinding was done with an Alpine 160 Z Kolloplex pin mill and separations were made with a Pillsbury laboratory-model air classifier which divided the material into two parts, one finer and one coarser than the adjustable cut points. The general processing method consisted of grinding the material followed by air-classification sizing, the procedure being repeated one or more cycles for some flours. For a five-fraction classification, four successive passes were made at cut points of 15, 18, 24, and 30 μ. The fine materials from these cuts correspond to fractions 1, 2, 3, and 4, respectively. Fraction 5 is the remaining, unclassified coarse material.

Specifically, the buffer-treated, high-lysine flour was ground by one pass through the pin mill at 14,000 r.p.m. and classified at the 15 and 18 μ cut points into three parts: fine fractions 1 and 2, and a coarse residue. With this residue as a starting material, the above procedure was repeated for the second cycle. For the final, or third, cycle the residue from cycle 2 was pin milled by one pass at 14,000 r.p.m. and classified into five fractions at the cut points specified in the preceding paragraph. The total yield for each fraction, such as fraction 1, was the sum of all subfractions removed at the same classifier setting or cut point.

The untreated high-lysine corn flour was given three consecutive passes through the pin mill at 14,000 r.p.m. and then fractionated into five parts .

For both untreated and treated ordinary corn flours, the material was pin-mill ground by one pass at 14,000 r.p.m. and classified into five parts. Fractions 3 and 4 were combined, ground by two passes through the pin mill at 14,000 r.p.m., and classified into four fractions at cut points of 15, 18, and 24 μ. Appropriate subfractions were then combined.

Protein shifts were estimated from Kjeldahl analyses of the air-classified fractions (N X 6.25). Amino acid compositions of the air-classified fractions were determined on an automatic Phoenix analyzer after hydrolysis by refluxing in 6N HCl for 24 hr. Results were calculated by computer according to the method of Cavins and Friedman (8). Tryptophan after pronase digestion (9) was analyzed by the colorimetric method of Opieńska-Blauth et al. (10).

RESULTS

Endosperm Fragmentation

The effects of buffer and water treatments on endosperm particle disruption are illustrated in Table I. The amount of material passing the screen after buffer treatment was compared with the amount of material obtained from original coarsely ground endosperm and water-treated endosperm. Only 14% of ground

TABLE I. PARTICLE-SIZE REDUCTION BY TREATMENT OF WHOLE ENDOSPERM

Endosperm	−75 μ Particle-Size Weight[a] %
Ordinary corn[b]	
Field-dried:	
Control (12% moisture)	14
Water-treated	16
Buffer-treated	67
Picker-shelled:	
Control (21% moisture)	52
High-lysine corn[c]	
Field-dried:	
Control	39
Water-treated	94
Buffer-treated	96

[a]Percentage of total sample.

[b]Holmes dent hybrid.

[c]W64Ao_2 high-lysine.

endosperm from ordinary corn could be screened to material less than 75 μ in diameter. This fragmentation increased only slightly after treatment with water. However, when the coarsely ground endosperm was equilibrated with buffer, almost 70% of the particles were reduced in size. The buffer treatment is more effective than water treatment in disrupting both starchy and horny endosperms of ordinary corn. Fragmentation of untreated high-moisture corn harvested by a picker-sheller gave a high yield of material less than 75 μ in size. The increased fragmentation of high-moisture grain indicates that when grain is dried the susceptibility of endosperm to disruption during milling diminishes.

High-lysine endosperm responds to treatment much differently than ordinary corn endosperm. Much more of the untreated endosperm can be reduced to fine particles by simple coarse grinding, since this endosperm is of a flour type. Both water and buffer treatments disrupt more than 90% of the endosperm into fine particles.

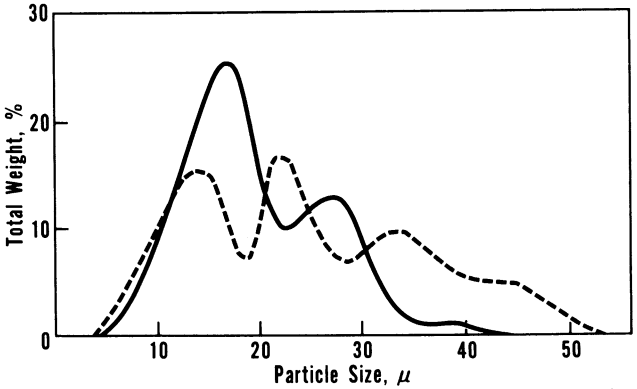

Fig. 1. Particle-size distribution of −75 μ agglomerates of treated endosperms (hand-dissected). Line = high-lysine corn; dashes = ordinary corn.

Micromerograph curves of the −75 μ material from buffer-treated ordinary and high-lysine corn endosperm further demonstrate significant differences in particle-size distribution (Fig. 1). Changes in distribution of particles reflect differences in the morphology of the endosperm of ordinary and high-lysine corns. The largest percentage (almost 60%) of the total weight of high-lysine endosperm is less than 20 μ, whereas fragments of ordinary corn endosperm vary randomly over a range of 10 to 50 μ.

Fragmentation of Buffer-Treated Dry-Milled Fractions

Yields and protein contents of dry-milled products from ordinary, high-lysine composite, and the high-protein high-lysine corn are given in Table II. Grits yields

TABLE II. YIELDS OF DRY-MILLED PRODUCTS AND THEIR PARTICLE-SIZE REDUCTION BY BUFFER TREATMENT

Corn	Yield %	Protein %	−75 μ Particle-Size Weight[a] Control %	−75 μ Particle-Size Weight[a] Treated %
Ordinary dent				
Grits[b]	48	7.5	8	44
Break flour	4	5.5	25	93
High-lysine (composite) dry-milled products				
Grits[c]	8	9.6	...	...
Coarse meal	8	12.3	28	96
Flour	30	6.3	36	98
High-lysine (high-protein) dry-milled products				
Grits[c]	28	12.4	15	97
Meal	15	12.3	74	98
Flour	16	10.0	60	96

[a]Percentage of total sample.
[b]−4+6 grits milled from artificially dried ordinary dent corn ground in a Wiley mill through a 60-mesh screen.
[c]Ground in a hammer mill through a 0.027-in. mesh screen before sieving.

in high-protein, high-lysine corn were as much as threefold greater than those from composite regular high-lysine corn. Protein content of the grit is also improved over both composite high-lysine and ordinary corns. The high-protein content of coarse meal from composite high-lysine corn could be due to the increased presence of germ particles.

After buffer treatment, ordinary corn grits showed a fivefold increase in particles reduced to −75 μ. Break flour was almost completely reduced to particles less than 75 μ in size. In the high-protein, high-lysine, and the composite high-lysine corn dry-milled products, agglomerates fragmented almost completely upon buffer treatment. These data provided evidence for disruption of particles by buffer treatment and the potential for subsequent separation of protein and starch by air classification.

Microscopic Examination

Scanning electron microscopy vividly demonstrates the release of protein from

starch upon buffer treatment of high-lysine corn flour. Figure 2 contains photomicrographs of starch-protein agglomerates of high-lysine flour. Before treatment, protein adhered to the starch granules in layers. During treatment, the protein slides off the starch granule in sheaths and leaves the starch granules free and smooth. These protein sheaths were larger in size than starch granules and can be partially separated by sieving.

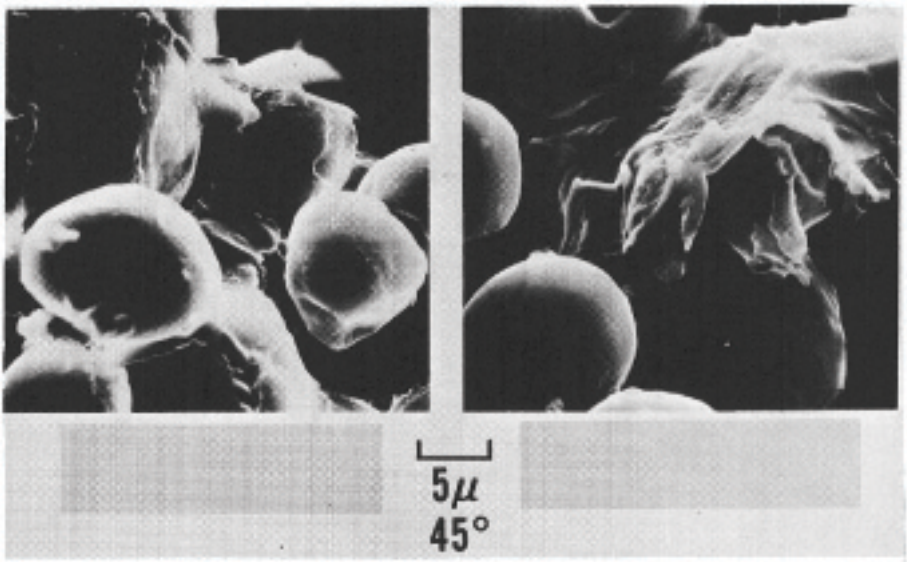

Fig. 2. Scanning electron micrographs illustrating disruption of protein from starch granules upon treatment of high-lysine corn flour. Left, before treatment; right, after treatment with buffer.

Air Classification of Ordinary and High-Lysine Corn Flours

Size and composition of agglomerates in ordinary break flour are changed upon treatment with buffer. These changes can be demonstrated by air classification since protein and weight shifts differ from those of untreated flour. These changes are not solely the result of pin milling. The untreated and treated flours were pin milled and air classified under similar conditions.

Figure 3 compares the separations achieved by air classification of treated and untreated ordinary break flours. The protein content of intermediate fractions 3 and 4 from the treated flour was reduced to 2.7%. More than half of the original flour weight was classified to these intermediate fractions. The same fractions in untreated flour were higher in protein (4.7%). Because endosperm particles in ordinary corn flour resisted pin milling, almost 40% of the weight remained in coarse fraction 5. The percentage protein in fractions 1 and 2 are comparable for both treated and untreated flour. However, 50% of the total protein in treated corn flour shifts to fractions 1 and 2. This percentage compares with only 28% in untreated flour. With this improved protein shifting in treated flours, a starch product suitable for industrial use can be obtained from the low-protein fraction. Regrinding fraction 5 of ordinary corn flour lowers the weight yield from that

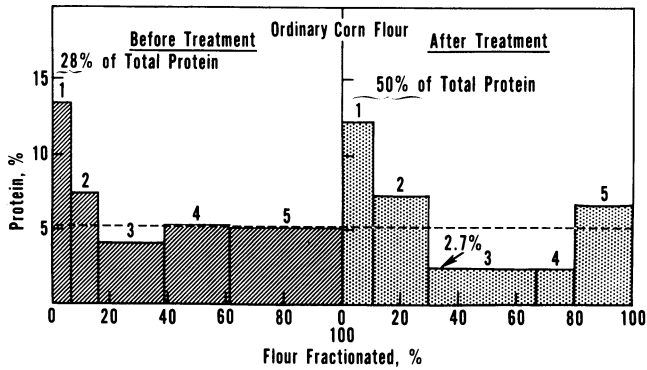

Fig. 3. Fractionation of pin-milled normal corn break flour before and after treatment with isotonic buffer. Initial protein concentration, 5.2%. Untreated and treated flours were air-classified into five fractions after pin milling once at 14,000 r.p.m. Fractions 3 and 4 were recycled after a second pin milling at 14,000 r.p.m. Results shown for fractions 1 to 4 are cumulative from the second cycle.

fraction, but the protein level remains about the same; therefore, this fraction was not recycled, owing to its vitreous nature.

Greater protein shifts were achieved after air classification of buffer-treated high-lysine flour when compared to those for untreated flour (Fig. 4). Since 33% of

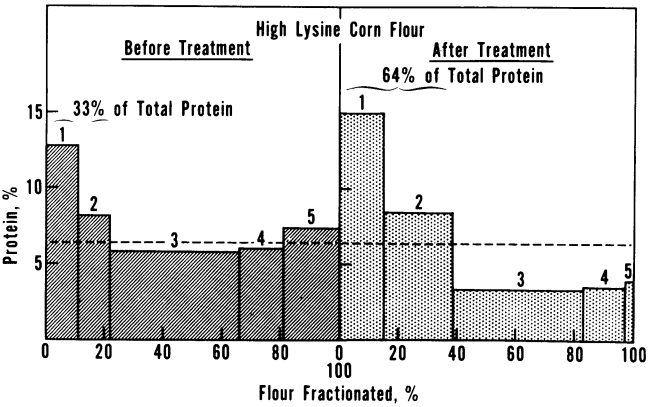

Fig. 4. Fractionation of pin-milled high-lysine corn flour before and after treatment with isotonic buffer. Initial protein concentration, 6.3%. Untreated and treated flours were air classified into five fractions after pin milling once at 14,000 r.p.m. Fractions 3 to 5 were combined and pin milled once at 14,000 r.p.m. to obtain further protein shifting to fractions 1 and 2. Fine fractions 1 and 2 are cumulative from the second cycle.

the total protein was shifted to fine fractions 1 and 2, whereas only 20% was shifted in ordinary corn (Fig. 3), protein shifting is slightly more efficient with untreated high-lysine flour. Upon treatment of high-lysine corn flour with buffer, 64% of the total protein shifts during air classification to fine fractions 1 and 2 (Fig. 4). Fraction 5 was recycled with fractions 3 and 4, since this residue is less vitreous than fraction 5 of ordinary corn flour. The low-protein product (fractions

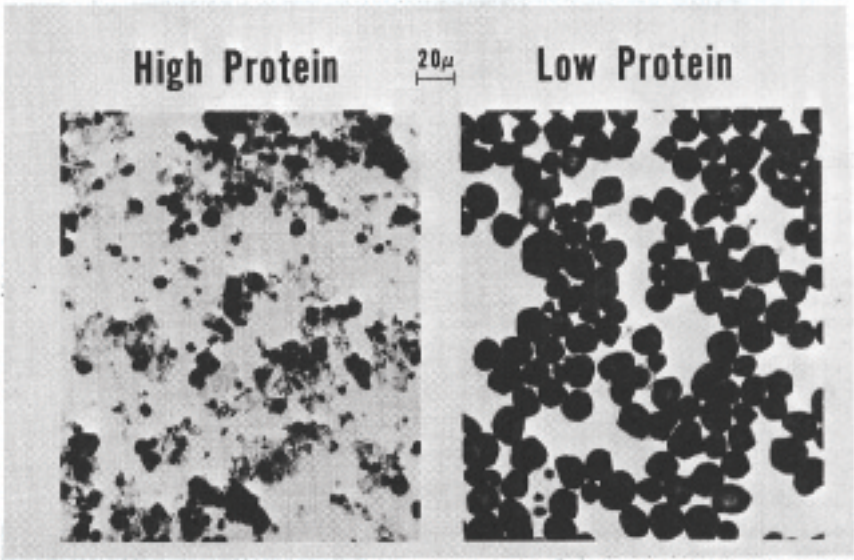

Fig. 5. Photomicrographs of high- and low-protein air-classified fractions from buffer-treated high-lysine corn flour.

3, 4, and 5) constitutes almost 65% of the flour and is suitable for industrial use as a low-grade starch. Yield of these fractions from untreated flour was 78%, with an average protein content of 6.1%.

This significant shift in protein after air classification of treated high-lysine corn flour can be viewed under the microscope. In Fig. 5 are photomicrographs of fine, high-protein fractions 1 and 2 (combined) and of low-protein fraction 3. The major portion of the starch in fraction 3 is now free starch granules. Little protein remains attached to these granules. The left photomicrograph of the protein-rich fractions shows the fine protein fragments. These fractions are contaminated with very small starch granules, the granules that reduce the protein content of fractions 1 and 2 in treated flour. The protein concentration of fractions 1 and 2 combined is 10.7% (weighted average), in contrast to 6.3% in the original flour. These fractions contain 64% of the total protein.

Amino Acid Analysis of Air-Classified Fractions from High-Lysine Flour

Protein-rich concentrates obtained by air classification contained as much as 15% protein (fraction 1, Fig. 4). Amino acid composition of the protein in the two fine fractions (1 and 2) obtained by air classification of treated high-lysine corn is shown in Table III. Results are expressed as milligrams per gram total essential amino acids. Although slight differences exist in the amino acid composition of the fractions, there is no preferential shifting of different forms of protein, as indicated by the similarity in amino acid composition of the fractions. This finding is important because concentration of less desirable protein, such as zein, in the protein-rich fractions would reduce their potential nutritional value. Protein concentrates maintain a good essential amino acid balance and would be suitable for food preparations where good quality proteins are required.

TABLE III. ESSENTIAL AMINO ACID PATTERNS IN PROTEIN CONCENTRATES[a] FROM HIGH-LYSINE CORN[b]

	mg./g. Essential Amino Acids					
		Protein concentrates				
		Original flour		Coarse residue[c]		
Amino Acids	High-lysine corn flour	Fraction 1	Fraction 2	Fraction 1	Fraction 2	Hen's Egg
Isoleucine	91	89	97	85	88	129
Leucine	248	244	267	246	232	172
Lysine	77	81	64	87	103	125
Tyrosine	100	96	72	97	96	81
Phenylalanine	102	99	71	101	101	114
Cystine	54	64	68	66	52	46
Methionine	37	47	52	47	43	61
Threonine	97	92	104	90	94	99
Tryptophan	45	46	48	45	61	31
Valine	148	144	160	135	131	141
Protein, %	6.3	15.0	8.5	13.3	6.6	...
Total protein, %	...	25.0	16.4	11.2	13.8	...
Recovered product, %	...	11.2	13.1	3.1	7.7	...

[a]Protein concentrates were obtained by air classification of a buffer-treated flour.

[b]Flour milled from a composite of high-lysine varieties from the University of Illinois, 1967 crop.

[c]Coarse-residue fractions 3 to 5 from original flour, pin milled and reclassified to produce fine fractions 1 and 2.

CONCLUSIONS

A method has been developed for disruption by isotonic buffer conditioning of starch-protein agglomerates in endosperms of ordinary and high-lysine corns. Disruption is achieved with both the floury and horny portions of ordinary dent corn. In high-lysine corn, fragmentation is almost complete. The exact action of the isotonic buffer in promoting disruption of the protein from starch is not known, but the disruption does facilitate concentration of proteins by air classification.

Experimental results show that it is possible to fragment corn dry-milled products and obtain protein-enriched fractions by air classification. Such techniques improve the potential use of dry-milled corn products for food uses. High-lysine corn is especially suitable for milling, fragmenting, and air classifying to gain protein concentrates of good nutritional quality.

Acknowledgments

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