CORN PROTEINS: CHEMICAL AND PHYSICAL CHANGES DURING DRYING OF GRAIN¹

J. S. WALL, C. JAMES, and G. L. DONALDSON, Northern Regional Research Laboratory², Peoria, IL 61604

ABSTRACT

Changes in proteins in high-moisture corn dried at elevated temperatures were investigated to determine reasons for previously reported reduced grain quality for seed, milling, and feed uses. Grain harvested at 25% moisture was dried to about 15% moisture by heating at air temperatures ranging from 15° to 143°C in a forced-air dryer. Proteins were sequentially extracted from defatted grain or endosperm meals with various solvents. Amounts of proteins extracted with 0.5N NaCl were markedly reduced in meals heated to 143°C, and their electrophoretic patterns changed significantly. A smaller

decrease occurred in yield of zein extracted with 70% ethanol-0.5% sodium acetate. Buffer containing 0.5% sodium dodecyl sulfate (SDS) dissolved some of the unsolubilized protein. Increased solubility in 0.5% SDS solution containing mercaptoethanol of proteins of corn dried with 143°C air indicated that intermolecular disulfide bonds are formed during heating. The number of sulfhydryl groups decreased during heating of whole grain; a parallel decrease occurred in grain viability. Lysine and available lysine contents were reduced slightly by heating at the highest temperature.

Most corn is currently harvested at 22 to 30% moisture to facilitate use of the picker-sheller combine, but it must be dried to less than 15.5% moisture to ensure safe storage. Modern continuous dryers can dry grain safely at uniformly moderate temperatures; but when these facilities are taxed, drying may be accelerated by operating them at higher temperatures. High-temperature drying may be detrimental to many uses of grain. A corn sample dried above 82° C gave poor starch-protein separation during wet milling (1). Kernels dried at elevated temperatures exhibited excessive stress cracks and friability, which resulted in poor separation of germ and hull and which reduced yield of prime grits in dry milling (2). Drying high-moisture corn above 83° C may reduce the nutritional value of its protein according to Hathaway et al. (3), but Jensen et al. (4) observed no change in nutritional value of grain dried at 104° C providing that ample air flow accelerated drying time.

Many of these defects in quality of artificially dried corn are attributed to changes in the properties of their proteins. McGuire and Earle (5) found that the yields of proteins extracted by water, saline, and alkaline solutions are reduced as corn is dried at successively higher temperatures. Both moisture-binding capacity of the grain protein (6) and enzymatic activity (7) diminish in corn dried at elevated temperatures.

Heat-induced denaturation and chemical changes in proteins are complex and proceed in steps depending on severity of heating and the environment of the proteins. Functional group modification, chain unfolding, disulfide interchange, and possibly covalent bond formation may occur on heating proteins in cereals.

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²Agricultural Research Service, U.S. Department of Agriculture, Peoria, IL 61604.

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We have explored the nature of the changes in corn proteins that can occur during drying of high-moisture grain at various temperatures in a commercial dryer. A complex relation between inlet air temperature, initial and final moisture of grain, air flow and dryer design, and time of exposure determines grain heating in commercial dryers. Optimum air inlet temperatures will thus vary among different types of dryers. However, the information obtained in this study may be useful in testing for overheated grain and may provide a basis for improving performance of artificially dried grain for many applications.

MATERIALS AND METHODS

Artificial Drying of Corn

Corn for these experiments was provided by G. C. Shove of the Department of Agricultural Engineering, University of Illinois. The corn was a commercial double cross hybrid (B57TMS × H64) × (8443REX × AG19) grown in 1968 in Urbana, Ill. The grain was harvested by picker-sheller at a moisture content of about 25% and dried to around 15% moisture. One portion was bin-dried with ambient air at about 15° C. Five other portions of grain were dried in a gas-fired 8-bu capacity fluidized bed dryer at air temperatures ranging from 32° to 143° C. Time of heating, approximate maximum kernel temperature, and final moisture content are given in Table I. None of the corn samples exhibited marked browning or discoloration. The dried corn was the same as that used by Brekke et al. (2) in dry-milling investigations. The corn had been stored for 9 months at 4° C after drying.

Extraction of Corn Proteins

Portions of each sample of corn were soaked for 5 min in water and then manually separated into endosperm, germ, and pericarp. The whole corn and endosperm samples were ground in a hammer mill to pass a 40-mesh screen. Meals (100 g) were then extracted at 4°C for 1 hr with 200 ml petroleum ether, washed with additional petroleum ether on a Buchner funnel, and air-dried.

Albumins and globulins were extracted from 50 g of defatted meal or endosperm by stirring with 250 ml 0.5M NaCl for 1 hr at 4°C. After

TABLE I Conditions for Drying Shelled Corn

Drying Air Temperature °C	Approximate Maximum Kernel Temperature °C	Final Corn Moisture %	Drying Time hr	% Nitrogen (db)
15	15	15.8	48	1.45
32	32	16.0	7	1.45
60	57	16.5	2.5	1.50
88	82	15.5	1.2	1.49
116	88	14.4	0.8	1.49
143 ^a	104	14.2	0.5	1.53

^aOperating limit of dryer.

centrifugation of the mixture, the residue was reextracted with 250 ml 0.5 M NaCl under similar conditions. The solid was then washed with 75 ml of additional cold 0.5 M NaCl, and supernatants and washings were combined.

To remove zein, the corn and endosperm residues were next extracted 3 hr with 250 ml 70% ethanol-0.5% sodium acetate at room temperature (8). The dispersion was centrifuged and supernatant solution decanted. Extraction was repeated twice on the solid, and the zein extracts were combined.

Nitrogen contents of the extracts were determined. The extracts were then dialyzed extensively against water at 4°C. Globulins, which precipitated on dialysis of the 0.5M NaCl extract, were separated from supernatant solution containing albumins. Both extracts and solids were freeze-dried. The meal residues were also washed with water and freeze-dried.

To investigate the action of denaturing and reducing agents on protein solubility, extraction procedures based on those of Landry and Moreaux (9) were used. Whole corn meal residue (5 g) was extracted twice with 50 ml of 0.025M borate buffer, pH 10, containing 0.5% sodium dodecyl sulfate (SDS) at room temperature. The supernatants after centrifugation were combined. The solid residue was then extracted two times with 50 ml of the SDS-borate solvent to which 0.6% β -mercaptoethanol was added to break disulfide bonds. Again, supernatants after centrifugation were combined for analysis.

Proteins in corn meal residues (obtained after extraction with the saline and ethanol-acetate solutions) were concentrated by destarching the residue with α -amylase as described by Paulis *et al.* (8). To facilitate study of its subunits by starch gel electrophoresis, the protein, consisting primarily of corn glutelin, was reduced and alkylated by the method of Paulis and Wall (10). The destarched residue, as a 1.0% dispersion in pH 8.0 tris buffer containing 6M guanidine hydrochloride (GHCl), was reacted with 0.2M β -mercaptoethanol for 16 hr at room temperature. Sulfhydryl groups were next alkylated by addition of acrylonitrile to 0.4M and reaction for 1 hr. The reaction mixture was centrifuged and the solids were washed with buffer containing 6M GHCl; the combined supernatants were dialyzed and lyophilized.

Analytical Procedures

Aliquots of extracts and portions of solid protein isolates were assayed for nitrogen by a semimicro-Kjeldahl method.

For amino acid analysis, corn meal containing 1.0 mg nitrogen was refluxed in 6N HCl (2 ml/mg sample) for 24 hr. Amino acids in duplicate hydrolysates of each corn sample were quantitatively determined with a Beckman Model 121 automatic amino acid analyzer with the manufacturer's recommended modification of the procedure of Benson and Patterson (11). The results were integrated on Infotronics Model CH 210 integrator and calculated according to a computer program similar to that of Cavins and Friedman (12). Cystine was determined as cysteic acid after performic acid oxidation of protein (13).

Available lysine was measured in the corn meals by the method of Finley and Friedman (14). Corn meal (100 mg) was reacted in 10 ml 0.1 M borate buffer with 0.5 ml methyl acrylate for 24 hr. The reaction mixture was dialyzed and lyophilized. A portion was acid-hydrolyzed and its content of nonalkylated lysine (unavailable lysine) was determined with the amino acid analyzer. This value was subtracted from the total lysine in the initial corn since dialyzed control

samples (no methyl acrylate) had slightly higher lysine contents.

Sulfhydryl groups were analyzed in 200 mg freshly ground whole corn meal by amperometric titration with 0.001M AgNO₃ in 5 ml buffer with the microapparatus described by Rothfus (15). Buffer for titration was 0.01M tris pH 8.0 containing 0.01M KCl, $10^{-5}M$ EDTA, and 8M urea (16). The electrode cell consisted of mercury-saturated mercuric oxide and barium hydroxide prepared as described by Benesch *et al.* (16).

Germination tests were conducted on the grain by the Illinois Crop Improvement Association, Urbana, according to procedures of the Association of Official Seed Analysts (17).

RESULTS

Changes in Solubility of Proteins

In agreement with the conclusions of McGuire and Earle (5), the yields of nitrogenous materials extracted with 0.5 M NaCl are reduced by heating the grain (Fig. 1). However, in contrast to their findings, the decrease in yield does not follow a simple linear relationship to temperature. At higher drying temperatures than employed in their study, the incremental decrease in solubility becomes

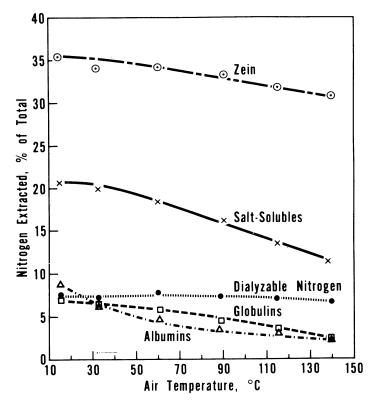


Fig. 1. Effect of drying air temperatures on extraction of proteins from whole corn.

greater at higher temperatures, despite the reduced time required for drying. Yields of albumins declined slightly more than globulins at the lower temperatures (Fig. 1). The dialyzable nitrogen content was not changed by the temperature of heating. The yield of dialyzable nitrogen is similar to that of nonprotein nitrogen soluble in trichloroacetic acid extracts of corn obtained by McGuire and Earle (5), but probably varies from it in composition. Also consistent with their findings was the small decrease in zein extracted with 70% ethanol-0.5% sodium acetate with increased drying temperature as shown in Fig.

TABLE II
Extraction of Proteins from Endosperm of Corn Dried at Different Temperatures

Drying Air	% Endosperm	Nitrogen		ogen in , % of Total
Temperature °C	in Grain	(db) %	0.5M NaCl	70% Ethanol- 0.5% NaOAc
15	85.0	1.42	7.4	45.8
60	85.1	1.46	7.0	42.2
143	85.7	1.50	2.9	38.9

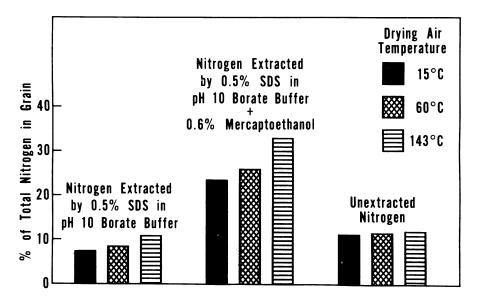


Fig. 2. Yields of protein nitrogen solubilized by sodium dodecyl sulfate (SDS) and mercaptoethanol from residues of previously extracted meals of corns dried at different temperatures. Meal residues were from defatted corn extracted with 0.5M NaCl and 70% ethanol-0.5% sodium acetate.

1. Evidently zein is not as readily heat-denatured as albumins and globulins.

Proteins were extracted from three samples of endosperm isolated from high-moisture corn dried under different conditions (Table II). In whole grain, germ makes a major contribution to the content of salt-soluble proteins in the kernel, and the effect of drying germ could mask concurrent changes in endosperm protein. The percentage decrease in solubility of proteins in 0.5 M NaCl from endosperm (Table II) of corn dried at 143°C is greater than that from the whole kernel (Fig. 1). The loss of solubility of endosperm salt-soluble proteins is important since changes in endosperm influence protein-starch separation in wet milling. Zein is exclusively located in endosperm, and the yields of zein and its decreased solubility were proportional to those observed in the whole kernel.

Solubilization of Corn Protein with SDS and Reducing Agent

Heat denaturation of proteins may involve changes from stable conformations to more random structures that permit hydrogen-bonding or hydrophobic functional groups to interact noncovalently and to produce molecular aggregation and insolubility. Such insoluble proteins should yield to extraction by a protein dissociating agent such as SDS. As shown in Fig. 2, extraction of corn meal residues (previously extracted with saline and ethanol solutions) with 0.5% SDS in pH 10 borate buffer yielded additional protein. The SDS solution dissolved the most protein from the residue of the corn heated to the highest temperature during drying. Evidently, some protein that was insoluble in the previous solvents due to heating was extracted with SDS.

Since SDS may function primarily by disrupting hydrophobic bonds, GHCl, reputed to break hydrogen bonds, was also tested. Because 6M GHCl disrupts starch granules and impairs protein extraction from whole grain, its effect was studied on destarched meal residues. Yields of proteins in dialyzed extracts were confirmed by amino acid analysis because complete removal of GHCl was difficult and uncertain. Results were similar to those obtained by SDS, but a slightly smaller increase in extraction of protein was noted from the residues from corn heated at the higher temperature.

Disulfide bonds connecting polypeptide chains are an integral part of the glutelin proteins of corn and contribute to their insolubility (8,10). New intermolecular disulfide bonds may be formed when sulfhydryl groups are oxidized or intramolecular disulfide bonds may be converted to intermolecular bonds. To determine if grain heating generates additional intermolecular disulfide bonds, the meal residue previously extracted with SDS in buffer was next extracted with the SDS buffer solution but containing 0.6% β -mercaptoethanol. As shown in Fig. 2, the residues of corn subjected to higher temperatures of drying yield more protein than samples dried at ambient temperature. Results were similar when destarched residues were extracted with mercaptoethanol in 6M GHCl.

The amount of protein not extracted by all these solvents from the corns dried at various temperatures differed little (Fig. 2). Evidently the heat-insolubilized albumins, globulins, and zein are almost completely extracted by SDS and SDS-mercaptoethanol solutions. Apparently conformational changes and intermolecular disulfide bonds account for most of the heat insolubilization of these proteins.

Starch-Gel Electrophoretic Patterns of Proteins from Heated Corn

Changes in albumin and globulin proteins due to heating of corn were observed in the starch-gel electrophoretic patterns of the extracts. Equal concentrations of protein were dissolved in the solutions applied to the paper strips. In Fig. 3, the patterns of the albumins from corn dried with 143°C air showed loss of total protein indicating that protein may be aggregated and could not migrate into the gel. Some specific bands are diminished in intensity suggesting that these proteins may be more susceptible to the denaturing action of heat. Changes in the electrophoretic band intensities of specific globulin proteins are not so great at 143°C, but more protein appears to have been retained at the origin in this sample.

Electrophoretic patterns of reduced-alkylated glutelin proteins from corn dried at three temperatures are illustrated in Fig. 4. The 143°C corn glutelin shows a major band (indicated by the arrow) that is deficient in protein of corn dried at 15° or 60°C. Disulfide cross-linking possibly resulted in bonding a new protein component to the glutelin. This component has mobility similar to slow-moving albumin proteins that are diminished in the 143°C pattern in Fig. 3.

Loss of Sulfhydryl Groups on Heating Grain

Sulfhydryl groups on proteins, peptides, or other substances may undergo oxidation or react with substances, such as carbohydrates, when grain is dried at elevated temperatures. A decrease in silver-titratable groups in corn occurs with increased drying temperature (Fig. 5). The measurement of sulfhydryl groups in endosperm gave low values (ca. 0.5 μ eq per g corn). Evidently much of the sulfhydryl groups of the grain must reside in the germ.

As shown in Fig. 5, loss of seed viability or germinability is exhibited by samples of corn heated to various temperatures. Seed viability is the most sensitive test for grain heating. The parallel loss of sulfhydryl groups may be related to decreased germination of heated seeds.

Effect of Drving Temperature on Amino Acids

Differences in amino acid contents of corn samples dried at different temperatures in this study are small. Amino acid values in Table III are means of the analyses performed on duplicate hydrolysates of each corn sample. The analytical differences observed between duplicate hydrolysates are small, as shown by the standard deviation(s) of each amino acid in the duplicates. Analysis of variance indicates significant variation of amino acid content among samples heated to various temperatures only for lysine and arginine. But at the highest drying temperature, lysine content is only 7% less than that for ambient temperature. The variation in arginine does not appear highly correlated with temperature.

Lysine in grain protein may, on heating, form compounds with sugars that render it nutritionally unavailable but that decompose on acid hydrolysis and are determined as lysine. These biologically unavailable lysine derivatives in proteins do not react with such alkylating agents as methyl acrylate. Effects of alkylating meals of corn heated to different temperatures upon yields of lysine are shown in Table IV. In ambient temperature-dried corn, 0.32 g of lysine per 16 g N does not react with methyl acrylate while at 143°C the value is increased to only 0.51. Total available lysine is reduced from 2.81 to 2.38 g per 16 g N as a result of both

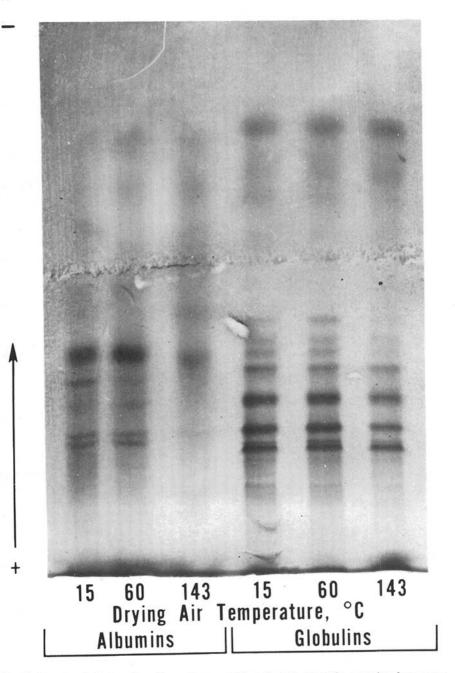


Fig. 3. Starch-gel electrophoretic patterns of albumin and globulin proteins from corn dried at different temperatures. Gels are 16 g starch per 100 ml buffer. Buffer is $0.008\,M$ aluminum lactate, $0.5\,M$ lactic acid, and $8\,M$ urea, pH 3.1. Protein applied in 5% solution on filter paper strips inserted in gel. Electrophoresis at $600\,V$ at $60\,m$ for $4\,h$ r.

lysine destruction and derivatization due to drying of grain by 143°C air.

DISCUSSION

The change in solubility of the saline-soluble proteins in corn heated to temperatures above 80°C, during artificial drying, is probably related to physical changes in the grain that affect wet and dry milling. A rapid procedure for

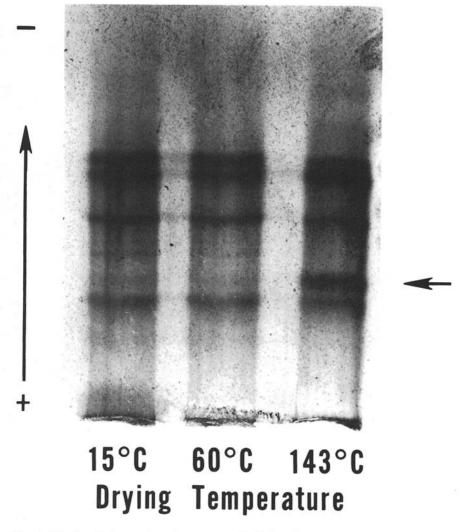


Fig. 4. Starch-gel electrophoretic patterns of alkylated-reduced glutelin proteins from corn dried at different temperatures. Conditions of electrophoresis similar to those in Fig. 3. Horizontal arrow points to enhanced band in 143°C corn protein.

quantitating extractable saline-soluble proteins in corn has been used to assess heat denaturation of corn proteins (18).

The solubilization of additional protein by mercaptoethanol in SDS solution from meals of corn heated to the highest temperatures employed here indicates that formation of new intermolecular disulfide bonds contribute to the loss of solubility of the protein. However, McGuire and Earle (5) and Watson and Hirata (1) found that when heated corn was steeped in SO₂, which should disrupt some disulfide bonds, the yield of soluble nitrogen was less than from similarly treated corn dried at low temperatures. Two factors may contribute to this situation: a) Chain unfolding in heated grains decreases solubility of the proteins and may require, as in our experiment, denaturing agents to dissociate proteins and permit disulfide cleavage. b) Heating the grain inactivates acid proteases that accelerate release of nitrogen from corn during steeping (19).

Sulfhydryl groups in corn may be oxidized to disulfides during drying. If titration with silver ions measures sulfhydryl groups exclusively, there are 2.8 μ eq per g corn. Amino acid analysis indicates 15.2 μ eq half-cystine per g corn. Thus oxidation of 1.5 μ eq sulfhydryls per g corn by drying at 143°C can generate appreciable new disulfide bonds. Loss of sulfhydryl groups could contribute to impaired activity of proteases, reductases, and other enzymes essential for seed germination. The relation between reduction in silver-titratable groups, sulfhydryl content, and seed germination in corn that was stored or artificially dried is being studied further at the Northern Laboratory.

Many conflicting reports have appeared about the effect of artificial drying on nutritional value of protein in corn. In our studies, due to rapid drying with high air flow, the maximum kernel temperature did not exceed 104°C, and only

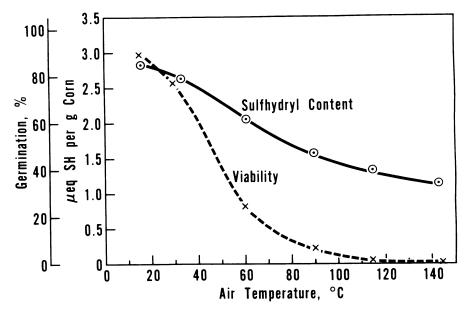


Fig. 5. Sulfhydryl content and seed viability of corn dried at different temperatures.

moderate changes in lysine availability occurred. At grain temperatures below 100°C apparently little or no damage to amino acids takes place during drying. These results are consistent with only small losses of protein feed value in corns heated to 104°C (3). Heating corn to higher temperatures, 160°C or above, for drying or roasting may cause marked browning, indicative of extensive Maillard reactions, with appreciable loss of lysine and lowered protein nutrient value (20,21).

TABLE III
Amino Acid Analyses of Corn Grain Dried at Different Temperatures^a

Amino Acid	Temperature of Drying Air				s Standard Deviation per Hydrolysate within Groups
	15° C	60° C	116° C	143° C	
		g per	16 g N		
Lysine ^b	3.09	3.13	2.89	2.89	0.04
Histidine	3.07	3.10	2.95	3.07	0.06
Ammonia	2.94	2.93	3.05	2.72	0.21
Arginine ^c	5.28	5.31	4.93	5.16	0.08
Aspartic acid	6.43	6.45	6.49	6.57	0.20
Threonine	3.58	3.60	3.58	3.66	0.05
Serine	4.80	4.84	4.92	4.78	0.19
Glutamic acid	18.39	18.20	18.30	18.73	0.46
Proline	8.08	7.80	8.09	7.65	0.85
Glycine	3.82	3.86	3.72	3.93	0.06
Alanine	7.30	7.36	7.30	7.57	0.13
Half-cystine	2.10	2.10	1.96	2.07	•••
Valine	4.76	4.90	4.95	4.89	0.20
Methionine	2.79	2.84	2.74	2.86	•••
Isoleucine	3.6	3.69	3.58	3.70	0.08
Leucine	12.07	11.96	12.21	12.43	0.36
Tyrosine	4.42	4.45	4.35	4.54	0.08
Phenylalanine	4.80	4.86	4.80	4.99	0.16

^aResults are means of analysis of duplicate hydrolysates.

TABLE IV
Available Lysine in Corn Dried at Different Temperatures

Air Drying Temperature °C	Total Lysine g/16 g N	Available Lysine g/16 g N
15	3.09	2.77
32	3.10	2.77
60	3.13	2.80
88	2.98	2.63
116	2.89	2.50
143	2.89	2.38

^bDifferences significant at 5% level by R test.

Differences significant at 10% level by R test.

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