Influence of Commercial Processing on Composition and Properties of Corn Zein[1]

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ABSTRACT

The chemical and physical properties of two commercial preparations of corn zein were compared with those of zein prepared in the laboratory by direct extraction of the grain with 70% ethanol. The three zeins differed in content of sulfur amino acids, disulfide sulfur, and total sulfur and in their electrophoretic mobilities on starch gel. Evidently, commercial steeping of corn grain in sulfur dioxide before milling cleaves disulfide bonds, thereby disrupting disulfide-linked intermolecular aggregates in native zeins. Sulfurous acid-treated zein may contain S-sulfocysteine residues, as indicated by high sulfur content and low electrophoretic mobility. A commercial zein subjected to alkaline treatment so as to render its solutions more stable had less cystine and total sulfur than the native protein.

Zein, the 70%-ethanol-soluble protein of corn, has been widely studied because it is a readily available by-product of corn wet-milling and because of its unique properties (1). Zein is produced commercially by extracting corn gluten with 86% aqueous isopropyl alcohol. Since starch is the main product from wet-milling of corn, little attention has been paid to how processing affects proteins. This study was made to determine the alterations the protein might undergo.

Zein contains disulfide-linked molecular aggregates (2). These intermolecular disulfides are cleaved by reducing or oxidizing agents, so that the average molecular weight of the protein is decreased and its electrophoretic characteristics are changed.

In commercial wet-milling, corn is steeped in a sulfur-dioxide solution at elevated temperatures (50°–60°C.) for approximately 40–44 hr. The sulfurous acid acts as a mild reducing agent. The properties of zein processed commercially from corn gluten (the residue after SO_2 steeping, milling, and starch separation) were compared to those of zein extracted directly from the grain to establish whether steeping and drying modify the protein. Properties of commercial zein, further stabilized by an alkaline treatment, were also investigated. Analyses were made on cystine-cysteine, methionine, sulfur, sulfhydryl, and disulfide contents in the different zeins.

MATERIALS AND METHODS

Unmodified Zein

Zein was prepared in the laboratory from a normal dent variety corn, Schwenk U.S. 13, which contained 1.43% nitrogen and 0.59% sulfur. The corn grains were cracked in a burr mill and ground in a hammer mill to pass a 1/16-in. screen. The meal was defatted with cold (4°C.) pentane-hexane for 1 hr., rinsed with fresh cold pentane-hexane, and air-dried on a

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Büchner funnel. The zein was then extracted from the ground meal with 70% ethanol (v./v.) in a solvent-to-meal ratio of 5:1 for 5 hr. at room temperature. The alcoholic zein solutions were dialyzed against water to precipitate the protein, and the precipitate was lyophilized (3); it assayed 14–15% nitrogen and formed clear yellow solutions in 70% ethanol.

Modified Zein

The S-sulfonate derivative of zein was prepared by dissolving 200 mg. of unmodified zein in 10 ml. of 0.05*M* sodium sulfite-8*M* urea and allowing the solution to stand at room temperature for 24 hr. The modified protein was then precipitated from the reaction mixture with water, washed with water, and frozen until required for assay.

S-sulfo zein was also made according to the procedures of Mertz and Bressani (4) with copper sulfite as reagent. The unmodified zein was dissolved in 0.006*M* sodium sulfite-0.02*M* copper sulfate, pH 12, for 24 hr. at room temperature. The reaction mixture was dialyzed against 0.02*N* HCl and then water at 5°C., and lyophilized.

The disulfide bonds of zein were also cleaved by the method of Reichmann and Calvin (5) with performic acid. The zein was then precipitated from the reaction medium with water and lyophilized.

Another preparation of zein was made by extraction of commercial corn gluten (supplied by Corn Products Co.) with 70% ethanol (v./v.) in the same manner as for the unmodified zein from corn meal.

Two commercial zeins, HV-9 and G-200 (supplied by Corn Products Co.), were assayed as received. Zein HV-9, no longer produced, is believed not to have had any alkaline treatment, whereas zein G-200, in current production, has. An alkaline treatment of zein HV-9 was made as described by Morris and Wilson (6) with calcium hydroxide solution at 75°C. for 15 min. Presumably this treatment is similar to that given G-200 zein.

Starch Gel Electrophoresis

The starch gel preparation and electrophoretic conditions were as described previously (2).

Sulfur and Nitrogen Determination

Sulfur was determined colorimetrically according to White (7), and nitrogen, by the micro-Kjeldahl method in which mercuric oxide is used as the catalyst.

Amino Acid Assay

The zeins were oxidized by Moore's (8) method of converting cystine to cysteic acid. They were then hydrolyzed in constant-boiling hydrochloric acid in sealed tubes for 24 hr., and the solutions were assayed for amino acids on a Phoenix automatic amino acid analyzer, Model K-8000. Methionine was calculated from the sulfones; total cysteine and ½ cystine from cysteic acid.

Sulfhydryl and Disulfide Groups

Sulfhydryl content of the zeins was determined on 100-mg. zein samples by the amperometric titration method of Benesch *et al.* (9) as adapted for wheat gliadin proteins by Beckwith *et al.* (10). Disulfides were determined

by amperometric titration of sulfhydryls formed after reaction with an excess of sulfite (10).

RESULTS

Amino Acid Composition

Data on total nitrogen contents for an unmodified laboratory-prepared zein, an old type of commercial zein no longer in production (HV-9), and an alkali-treated commercial zein (G-200) are given in Table I. The laboratory-prepared zein is lowest in nitrogen content (14.3%) and may contain nonprotein contaminants. Since both commercial-type zeins are about 16% nitrogen, deamidation by alkaline treatment of the G-200 zein was minimal.

TABLE I

DISTRIBUTION OF SULFUR AND TOTAL NITROGEN IN DIFFERENT ZEINS

Composition	Zein		
		Commercial	
	Native Unmodified	HV-9	Alkali-Treated G-200
	% wt.	% wt.	% wt.
Total nitrogen	14.3	15.8	16.1
Sulfhydryl	0	0	0
Disulfide sulfur	0.24	0.09	0.06
Cysteic acid sulfur	0.25	0.24	0.06
Methionine sulfur	0.37	0.36	0.48
Cystine and methionine sulfur	0.62	0.54	0.54
Total sulfur	0.59	0.66	0.52
Unaccounted-for sulfur	.....	0.12	.....

The amino acid contents after performic acid oxidation of three zein preparations are tabulated in Table II. The performic acid oxidation permitted quantitative analysis for cystine as cysteic acid and methionine as the sulfone derivative. Because of its known lability to reducing agents and alkali, cystine was of major interest in this study. The cysteic acid content per 16 g. nitrogen of the unmodified laboratory zein is 8.8 mM, whereas that of HV-9 from commercial corn gluten is 7.9 mM. In contrast, the alkali-treated zein G-200 showed a considerable decrease in cysteic acid to 2.0 mM per 16 g. nitrogen. Methionine contents varied in the three zeins; the HV-9 had the lowest. Amounts of other amino acids reported did not vary appreciably among the three zeins. Tyrosine and histidine values are not given because of destruction of these amino acids during performate oxidation (8); also,

TABLE II

AMINO ACID COMPOSITION OF PERFORMIC ACID-OXIDIZED ZEINS

Amino Acids	Unmodified	HV-9	G-200
	mM/16 g. N	mM/16 g. N	mM/16 g. N
Cysteic acid	8.8	7.9	2.0
Methionine	13.2	9.4	14.9
Aspartic acid	42.2	45.7	46.9
Threonine	27.0	27.3	25.4
Serine	65.9	60.1	54.6
Glutamic acid	176.0	181.5	177.1
Proline	95.5	83.4	94.2
Glycine	18.5	16.6	17.5
Alanine	119.6	125.4	120.5
Valine	27.4	34.3	33.6
Isoleucine	24.0	33.6	30.4
Leucine	156.9	170.6	169.9
Phenylalanine	44.7	47.1	54.0
Arginine	9.2	8.9	8.3
Ammonia	258.9	249.1	258.0

lysine and tryptophan are not reported since zein contains only negligible amounts (1).

Sulfhydryl, Disulfide, and Total Sulfur Analyses

To establish whether the sulfur-containing amino acid accounted for all the sulfur in the three zein preparations, their total sulfur was determined. As listed in Table I, the sum of cystine and methionine sulfur of the laboratory-prepared zein, 0.62%, is closely equivalent to the total sulfur analysis of the protein. Also, in the alkali-treated zein the sulfur content, 0.52%, was accounted for by the cystine and methionine. In contrast, the HV-9 zein had more sulfur, 0.66%, than that present in the amino acids, 0.54%. Probably some sulfurous acid from the steeping process remains associated with the HV-9 zein forming the S-sulfo derivative of cysteine.

Amperometric sulfhydryl and disulfide determinations were made on the different zeins to establish the oxidation state of the cystine sulfur (Table I). No free sulfhydryl groups were present in any of the zeins. Essentially all the cystine-cysteine in the laboratory-prepared zein was in the disulfide form, 4.3 mM disulfide per 16 g. nitrogen. The smaller amount of cystine-cysteine in the alkali-treated G-200 was also entirely in the disulfide form, 1.4 mM disulfide per 16 g. nitrogen. The HV-9 zein had only a small portion of its cystine-cysteine sulfur present as disulfide, 1.4 mM; the remaining cysteine determined as cysteic acid could be present as the S-sulfo derivative or as cysteic acid. Because the S-sulfocysteine does not complex with silver ion, it cannot be measured during amperometric titration of sulfhydryls formed by reaction of disulfides with sulfite.

Starch Gel Electrophoresis

Behavior of the zeins on starch gel electrophoresis provided further information on changes in their properties during industrial processing or various chemical treatments. The electrophoretic patterns of various zein preparations are compared in Fig. 1. The unmodified laboratory-

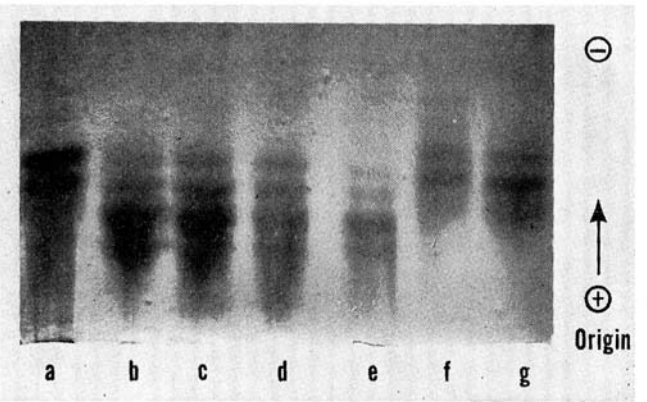

Fig. 1. Starch gel electrophoretic patterns of zeins (a) unmodified, (b) HV-9, (c) copper-sulfite treated, (d) 70% ethanol extract of gluten, (e) sulfite-treated, unmodified, (f) G-200, and (g) HV-9 calcium hydroxide-treated. Electrophoresis was conducted in pH 3.5, aluminum lactate-8*M* urea buffer.

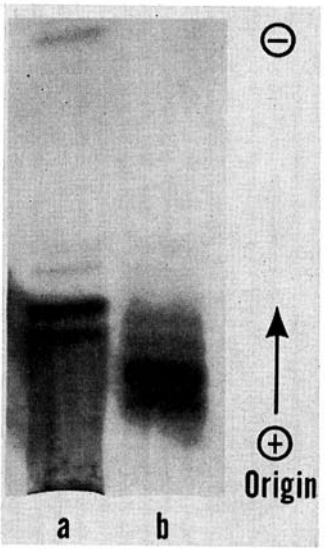

Fig. 2. Starch gel electrophoretic pattern of zeins: (a) unmodified and (b) performic acid-oxidized. Electrophoresis was conducted in pH 3.5, aluminum lactate-8*M* urea buffer.

prepared zein exhibits two major bands, two minor ones of faster mobility, and immobile material retained at the origin. The minor bands are more prominent when the protein is applied at a higher concentration, as in Fig. 2. Turner *et al.* (2) demonstrated that the immobile material consists of high-molecular-weight protein containing disulfide bonds linking polypeptide chains. Reduction of the disulfide bonds cleaves the protein into fragments that migrate into the gel.

The electrophoretic pattern of the commercial zein HV-9 (Fig. 1, b) also exhibits four bands but little material at the origin. Sulfurous acid steeping disrupted intermolecular disulfide bonds in this protein. Another major difference between the HV-9 and the native zein patterns is the mobility of their component proteins; the HV-9 proteins migrate more slowly through the gel than the native proteins.

The electrophoretic pattern of the HV-9 zein closely resembles that of the sulfite-treated native zein (Fig. 1, e), the copper sulfite-treated zein (Fig. 1, c), and the zein isolated by ethanol extractions in the laboratory from commercial corn gluten prepared after sulfur dioxide steeping of the grain (Fig. 1, d). The alkaline sulfite treatment cleaves disulfide bonds in the protein and forms equivalent amounts of residues of cysteine and S-sulfo derivative of cysteine. In the presence of air, cysteine is oxidized further to cystine and the process is repeated. Copper catalyzes the oxidation of cysteine and hastens the complete conversion of cystine to S-sulfocysteine residues in the protein. The similarities in mobilities of the patterns in Fig. 1, b to e, indicate that the cysteine in these proteins is primarily in the S-sulfo form. The absence in these patterns of immobile protein at the origin is due to cleavage by the sulfite ions of disulfide bonds in intermolecularly cross-linked proteins.

The diminished mobility of these proteins relative to the native zein is explained by the more negative isoelectric point due to the added S-sulfo groups. Figure 2 shows the starch gel pattern of the performic acid-oxidized zein. Introducing sulfonic acid groups into the protein by cystine-to-cysteic-acid conversion results in a similar decrease in protein mobilities in the acidic electrophoretic media.

Alkali treatment further changes the electrophoretic characteristics of sulfurous acid-modified zeins. The mobilities of the components of the alkali-treated G-200 zein (Fig. 1, f) are similar to those of the native protein. A diffuse protein band below the two major components may be due to retention of some cysteine derivative in the protein. No immobile component is present in this zein. That the changes in mobilities in G-200 proteins are due to alkali treatment is demonstrated by the effect of alkali on electrophoretic properties of HV-9. After the HV-9 was exposed to a pH 9 calcium hydroxide solution for a short period at elevated temperatures, it exhibited the same pattern as G-200 (Fig. 1, g). These results indicate that alkali treatment desulfurizes the protein but that the loss of S-sulfo groups restores the isoelectric point of the protein to nearly that of the native.

DISCUSSION

This investigation provides further evidence on the important contribution of sulfur dioxide steeping to corn wet-milling. Sulfur dioxide serves as an agent which cleaves disulfide bonds between polypeptide chains of zein. In 1944 Cox *et al.* (11) observed that sulfurous acid solutions disrupted the protein matrix of corn sections and promoted efficient separation of starch and protein.

Commercial steeping is done at acid pH to minimize growth of undesirable microorganisms and to favor a lactic acid fermentation. Neutral or alkaline conditions would be more effective for disulfide cleavage, since the sulfite ion is the effective cleaving agent. Long steeping periods at elevated temperature are required for protein rupture under acid conditions. A major portion of protein cystine is converted to S-sulfocysteine by sulfurous acid under these conditions. The conversion is indicated by the high sulfur content of HV-9 zein and its electrophoretic properties which resemble those of the sulfite-treated zein.

Amperometric titration reveals that HV-9 zein still retains about 30% of its cystine sulfur as disulfide. These bonds must be mostly intramolecular, because little material is retained at the origin during electrophoresis. The disulfides could result from reoxidation of sulfhydryls during drying of the zein.

The S-sulfocysteine present in early commercial zeins, such as HV-9, may have caused gelation of alcohol solutions containing high concentrations of protein. S-sulfocysteine decomposes to yield cysteine, which may be oxidized or undergo interchange to result again in disulfide bonds, which form intermolecular cross-links. G-200 zein was stabilized by removing much of the cysteine sulfur by the action of alkali. Gelation of G-200 zein under

some conditions may result from unfolding of the molecules and formation of noncovalent associations.

Many theories have been proposed for the action of alkali in desulfurizing cystine and its derivatives. Strong evidence that beta elimination, leading to dehydroalanine, is the predominant reaction is summarized by Cecil and McPhee (12). Lanthionine, isolated from alkali-treated wool, is believed to come from the condensation of dehydroalanine and cysteine. Lanthionine sulfoxide elutes in the general area of methionine oxidation products during chromatography and may account for the high methionine values in the alkali-treated G-200. Lysine also reacts with dehydroalanine formed from the treatment of cystine with alkali to yield a new amino acid (13). Lysine content of zein is normally low, however, and is not reported here because of difficulties in accurate determination in performic acid-oxidized proteins.

The sulfurous acid or sulfurous acid-alkali treatment of corn did not modify most of the other amino acids in the zeins. Also, the mild conditions of alkali treatment of G-200 did not produce significant deamidation of glutamine residues, because the ammonia content in its hydrolysate is comparable to that of the other two proteins.

It must be stressed that some properties of zeins extracted directly from corn with alcoholic solutions cannot be observed in currently available commercial zeins because of disruption of intermolecular disulfide bonds and decreased cystine content.

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