Chemical and Physical Properties of Proteins in Wet-Milled Corn Gluten[1]

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ABSTRACT

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The chemical and physical properties of proteins in wet-milled corn gluten were investigated to establish possible food uses. Commercially produced wet corn gluten (WCG) (28% protein, 60% moisture) and commercially dried corn gluten meal (DCGM) (62% protein, 10% moisture) were sequentially extracted with 0.5*M* NaCl and 70% ethanol, and then with 0.5% sodium dodecyl sulfate (SDS) in pH 10 buffer and 0.5% SDS containing 0.6% 2-mercaptoethanol (2-ME) in pH 10 buffer or 70% ethanol containing 0.6% 2-ME and 0.5% SDS plus 0.6% 2-ME in pH 10 buffer. The yields of proteins soluble in 70% ethanol and 0.5% SDS in pH 10 buffer are much greater from gluten than from native corn, demonstrating that SO_2 in the steepwater from wet milling cleaves protein disulfide bonds. Analysis for sulfhydryl and disulfide content, total cysteine-cystine compounds as cysteic acid and total sulfur showed that much of the cystine is converted to other derivatives of cysteine during steeping. In contrast to WCG, DCGM had no cysteine, indicating that sulfhydryls were oxidized during drying of gluten. Wet corn gluten (pH 3.6) and WCG adjusted to pH 7.0 form doughs when mixed at proper moisture levels, but DCGM does not.

Corn is wet-milled to separate germ, fiber, protein, and starch constituents from the kernel. The separation is facilitated by an initial steeping of the whole grain in solutions containing SO_2, which softens the kernel to aid removal of germ and fiber. Steeping in SO_2 also cleaves protein disulfide cross-links so as to disrupt the endosperm protein matrix and enable the separation of starch and insoluble protein components (Wall and Paulis 1978). Corn gluten meal is a valuable, dry concentrated protein by-product of the wet-milling process.

The steeping process modifies corn endosperm protein from its native state through reduction of cystine residues by SO_2 to form cysteine and S-sulfo derivatives (R-S-SO_3^-) of cysteine and by the action of endogenous proteases (Wall and Paulis 1978). The cysteine residues formed can then be oxidized by air to produce new disulfides. Thus, many of the disulfide bonds in the endosperm protein matrix may be converted to S-sulfo derivatives of cysteine (James et al 1969).

Watson and Yahl (1967) reported that commercial corn gluten meal protein contained 1.2% globulins, 68% zein, and 28% glutelin when sequentially fractionated by saline, 70% ethanol, and 0.1*N* NaOH solutions. The yield of ethanol-soluble zein is greater than that extracted from normal corn endosperm protein with 70% EtOH-0.5% NaOAc (46% of total nitrogen) by Wall et al (1975). This increase in proportion of alcohol-soluble protein may be due, in part, to the loss of some water- and salt-soluble protein to the steepwater used in production of corn gluten and to the formation and solubilization by SO_2 of alcohol-soluble reduced glutelin that is bound by disulfides in native corn protein (Landry and Moureaux 1970, Paulis and Wall 1971).

In commercial practice, the wet corn gluten press cake (about 60% moisture) is dried at a high temperature. Wall et al (1975) found that whole corn dried from 25 to 15% moisture at 143°C showed a substantial decrease in salt-soluble protein and a small decrease in alcohol-soluble protein. After removal of these fractions, increases were observed in protein solubilized by the protein dissociating agent, sodium dodecyl sulfate (SDS) buffered at pH 10.0, and SDS plus the reducing agent, 2-mercaptoethanol (2-ME). They also observed a decrease in sulfhydryl content with increasing heat treatment. These results suggest that extensive heat treatment of native whole corn denatures protein and causes molecular aggregation through noncovalent hydrophobic interactions and covalently through formation of new intermolecular disulfide cross-links that contribute to protein insolubility.

The purpose of the present study was to determine the effect of steeping and commercial drying on the proteins of commercially produced wet-milled corn gluten. We studied protein solubility, as determined by sequential extraction with various solvents, to better understand the types of molecular associations that are responsible for some of the physical properties of corn gluten protein. We analyzed sulfhydryl-disulfide content to establish how steeping in SO_2 solution and commercial drying affects the protein's disulfide cross-links. Mixing properties of nonheat-treated corn gluten meal (CGM) were studied in an effort to relate the chemical data to some observed physical-rheological properties of the protein. Better understanding of the chemical and physical properties of CGM could increase its utilization as a food ingredient.

MATERIALS AND METHODS

Corn Gluten

Wet corn gluten (WCG) press cake and commercially hot-air-dried corn gluten meal (DCGM) were obtained from a large commercial corn wet-milling operation from the same day's operation of a single processing line and were of similar dry matter composition. Samples were stored at −20°C before analyses.

Proximate Analysis

Moisture contents were determined by vacuum oven drying, by AOAC method 14.003 (AOAC 1980). All nitrogen determinations were made by micro-Kjeldahl, AOAC method 47.021 (AOAC 1980), using a nitrogen to protein conversion factor of 6.25. Oil, crude fiber, and ash contents were determined by AOCS official methods Ba 3-38, Ba 6-61, and Ba 5-49, respectively (AOCS 1973). Starch was determined by a polarimetric method (Corn Industries Research Foundation 1957).

Protein Extraction

Protein was sequentially extracted from WCG and DCGM by two sequences depicted in Fig. 1. Each sequence was done in duplicate. Extractions were done on 5.0-g (db) samples by constant magnetic stirring with the appropriate solvent for a designated time in the same 250-ml centrifuge bottles throughout each sequence. After each extraction, samples were centrifuged at 10,000 × *g* for 20 min at the stated extraction temperature. Each sample was

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extracted twice with each solvent, and the supernatants of both extractions were combined to comprise the fraction. An aliquot of each fraction was analyzed for protein.

The samples were extracted first with 150 ml of 0.5*M* NaCl for 1 hr at 4° C. The residues were washed to remove salt with 100 ml of cold distilled water, then were centrifuged, decanted, and washed again with 50 ml of cold distilled water, centrifuged, and decanted. Water washes were combined with the 0.5*M* NaCl fraction. The residues were then extracted with 200 ml of 70% EtOH for 3 hr at room temperature (rt).

After extraction of salt-soluble and ethanol-soluble protein from both sequences, the residues from sequence I were extracted with 100 ml of 70% EtOH containing 0.6% (w/v) 2-ME for 1 hr at rt. The residues from sequence II were extracted with 100 ml of 0.5% SDS in 0.025*M* borate buffer (pH 10.0) for 3 hr at rt. Residues from sequences I and II were then extracted with 100 ml of 0.5% SDS in 0.025*M* borate buffer (pH 10.0) containing 0.6% (w/v) 2-ME for 1 hr at rt.

The insoluble residues left after sequential extraction were suspended in distilled water, lyophilized, and weighed, and their protein contents were determined.

Protein recoveries were greater than 96% of the total protein for all samples. Results are expressed as percent of total recovered protein.

Total Sulfur

Total sulfur of lyophilized WCG and DCGM was determined by Galbraith Laboratories, Knoxville, TN, on a LECO No. 132 sulfur analyzer.

Cysteine-Cystine

Total cysteine-cystine content was determined as cysteic acid after oxidation of the sample with performic acid as described by Moore (1963). Cysteic acid was separated from 6*N* HCl hydrolysates of the protein and quantitated by a Dionex D-300 amino acid analyzer.

Amperometric Titration

Free sulfhydryls of cysteine and sulfhydryls liberated after reduction of cystine with excess sodium sulfite were detected by amperometric titration with silver ion according to the method of Rothfus (1966), using the apparatus described by Rosenberg et al (1950). Samples (10–20 mg) were dispersed by constant magnetic stirring in 5 ml of pH 7.4 tris(hydroxymethyl)aminomethane buffer containing 8*M* urea and titrated with standardized 0.001*M* $AgNO_3$. The titrating solution was purged with nitrogen, and a few drops of silicon-based antifoaming agent were added to prevent frothing. The titer was determined by plotting the scale reading of the galvanometer against the milliliters of 0.001*N* $AgNO_3$ titrant dispensed into the solution. Linear regression of the last four data points was used to determine the intersection of the X axis, which corresponded to the titer of the solution. Reagent blanks were titrated and found to equal zero. Results are the average of at least duplicate titrations.

Cysteine was determined from the molar concentration of the sulfhydryl titer. Cystine was determined by reducing the sample with excess sodium sulfite (30 mol/mole of cysteic acid) and titrating the liberated sulfhydryls. The molar concentration of cysteine in the sample was subtracted from the titer, and the result was multiplied by two to obtain the molar half-cystine content of the sample.

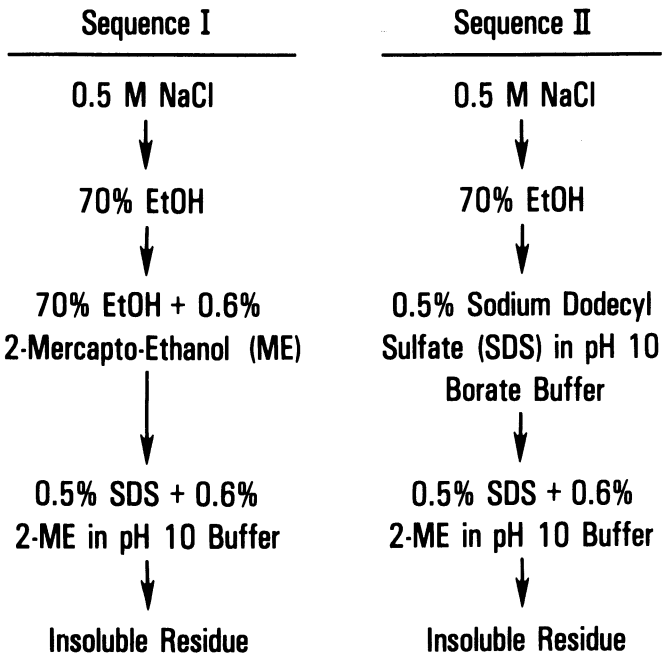

Fig. 1. Scheme for sequential extraction of protein from corn gluten meal.

Mixograms

Samples of lyophilized WCG (pH 3.6) and WCG adjusted to pH 7.0 while dispersed in water were lyophilized. Mixograms of these lyophilized samples were obtained using a 10.0-g Swanson-Working mixograph (National Mfg. Co., Lincoln, NE) as described by Finney and Shogren (1972). Sample weight was adjusted from 10.0 to 8.0 g (db). Enough distilled water was added to the WCG and to the WCG samples adjusted to pH 7.0 to give dough moistures of 62–63%, respectively. These dough moistures were selected because they maximized peak height for each sample and were similar to that of the wet gluten cake. Mixograms were terminated after approximately 5 min of mixing.

RESULTS AND DISCUSSION

Composition and Fractionation

Proximate compositions of WCG and DCGM are given in Table I. The WCG press cake has approximately 60% moisture and an intense yellow color because of high xanthophyll content (Wall and Paulis 1978). Wet corn gluten has a pH around 3.6 and a flavor that can be described as acrid or sour. Its consistency resembles that of moist clay. Dried corn gluten meal has 10% moisture and is similar in color, pH, and flavor to WCG. The appearance of DCGM is differentiated from that of WCG by the presence of browned particles approximately 0.5–1.0 mm in diameter mixed in with the gritty, yellow flour.

Fraction yields from sequential extraction of protein from WCG and DCGM are shown in Table II. Wet corn gluten contains more salt-soluble protein than does DCGM; however, both contain more than that reported by Watson and Yahl (1967). More than 60% of the protein of either WCG or DCGM is soluble in 70% ethanol. The yield is slightly less from DCGM than from WCG, and both yields are less than that reported in the literature (Watson and Yahl 1967). These small differences may reflect differences in grain supplies, extraction conditions, or processing conditions.

Following extraction of salt-soluble and ethanol-soluble protein, the residues from sequence I were extracted with 70% EtOH containing 2-ME to determine the amount of protein soluble in ethanol after reduction of disulfide bonds. An additional 3.9 and 3.2% of the total nitrogen were extracted by this solvent from WCG and from DCGM, respectively. Paulis and Wall (1977) extracted

TABLE I
Proximate Composition of WCG and DCGM (db)[a]

Sample[a]	Protein	Fat	Fiber	Ash	Starch	Other Carbohydrate[b]
WCG	70.4	6.7	...[c]	1.3	12.0	9.6
DCGM	71.4	4.1	0.8	1.2	12.4	10.1

[a] WCG = wet corn gluten; DCGM = commercially dried corn gluten meal.
[b] Determined by difference.
[c] Too low to be determined by method.

20% of the total nitrogen from defatted corn endosperm meal (after removal of salt-solubles and zein) with 70% EtOH-0.5% NaOAc containing 0.1*M* 2-ME. They designated this fraction alcohol-soluble reduced glutelin (ASG). The lower yield of ASG from wet-milled corn gluten may be due, in part, to its solubilization in the previously extracted zein fraction (70% EtOH) as a result of the cleavage of intermolecular disulfide bonds during the steeping process.

Following extraction of salt-soluble and ethanol-soluble protein, the residues from sequence II were extracted with the protein-dissociating agent, SDS (0.5% SDS in pH 10 buffer). Sodium dodecyl sulfate should solubilize protein aggregated by noncovalent hydrophobic bonds. As shown in Table II, 24.5% of the nitrogen in WCG is extracted by this solvent, compared to 18.8% for DCGM. The residues were then extracted with the same solvent containing 2-ME, to see how much of the remaining protein could be solubilized after cleavage of disulfide cross-links. In both WCG and DCGM, nearly all of the remaining nitrogen was extracted by this solvent. The yield of this fraction from WCG was only 5.0%, compared with 14.2% for DCGM. The decrease in protein soluble in SDS-pH 10 buffer and the increase in protein yield in the same solvent containing 2-ME indicate that intermolecular disulfide bonds are formed during high-temperature drying of CGM.

Amino Acid, Sulfur, and Sulfhydryl Contents

Total sulfur content was determined to investigate whether there was enough sulfur in excess of methionine and cystine sulfur to account for a significant quantity of the cysteine-cystine content present as S-sulfocysteine. Wet corn gluten and DCGM each contained 1.1% total sulfur (db). This value is more than enough sulfur in excess of sulfur amino acids (SAA) content to account for all the cysteine-cystine present as S-sulfocysteine (Table III). Amperometric titration indicates the presence of cystine and cysteine; therefore wet-milled corn gluten must contain, in addition to SAA, sulfur compounds that were naturally present in the grain or were formed during the steeping process, such as sulfite salts of basic amino acids.

S-sulfocysteine cannot be determined directly because it is unstable to hydrolytic procedures that liberate amino acids (Bailey and Cole 1959). Relative lability of S-sulfocysteine makes it uncertain that it remains in this form throughout processing. Therefore, it was referred to as other derivatives of cysteine (ODCys) and estimated indirectly, by subtracting the cysteine-cystine content determined by amperometric titration from the cysteine-cystine-ODCys content determined as cysteic acid from amino acid analysis of performic acid-oxidized samples. Wet corn gluten and DCGM contain the same amount of ODCys but differ in their cysteine and cystine contents (Table IV). Almost 10% of WCG compounds yielding cysteic acid is cysteine (Table IV).

Amperometric titration data of Wall et al (1975) indicate that corn endosperm protein contains only low levels of free sulfhydryls (less than 3.3% of total cysteine-cystine content of the whole grain). The presence of appreciable quantities of cysteine in WCG compared to native endosperm protein would indicate that some of the sulfhydryls formed from the reduction of cystine with SO_2 are not subsequently oxidized to form disulfides. The difference suggests that either the process is sufficiently mild as to limit the oxidation of all sulfhydryls formed or that inaccessibility of sulfhydryls to one another prevents their oxidation.

The larger amount of disulfide (cystine) and absence of cysteine in DCGM must be due to the quantitative oxidation of cysteine to cystine during heat denaturation of the protein. The increase in

TABLE II
Yields of Protein Fractions Obtained from Solvent Sequences I and II for WCG and DCGM[a]

	Percent of Total Recovered Protein Extraction Sequence (avg of duplicates)			
	Sequence I		Sequence II	
	WCG	DCGM	WCG	DCGM
0.5*M* NaCl	6.2	3.8	6.1	4.3
70% EtOH	63.4	62.1	63.5	61.8
70% EtOH + 0.6% 2-ME	3.9	3.2	...	...
0.5% SDS[b] in pH 10 buffer	...	...	24.5	18.8
0.5% SDS[b] in pH 10 buffer + 0.6% 2-ME[b]	25.6	29.9	5.0	14.2
Insoluble residue	1.0	1.0	0.9	0.9

[a]WCG = wet corn gluten; DCGM = commercially dried corn gluten meal.
[b]SDS = sodium dodecyl sulfate; 2-ME = 2-mercaptoethanol.

TABLE III
Sulfur Amino Acids (SAA) Content of WCG and DCGM as a Mole Percent of Total Sulfur[a] (mol amino acid/mol total S × 100%)

Sulfur Distribution	WCG (%)[a]	DCGM (%)[a]
Half-cystine	33.8	33.9
Methionine	26.5	26.8
Total SAA	60.3	60.7
Sulfur not accounted for by SAA	39.7	39.3

[a]WCGM = wet corn gluten; DCGM = commercially dried corn gluten meal.

TABLE IV
Cysteine, Cystine, and Other Derivatives of Cysteine (ODCys) Content of WCG and DCGM[a]

	Percent of Determined Cysteic Acid[b]		
Sample[b]	Cysteine	Half-Cystine	ODCys
Lyophilized WCG, pH 3.6	9.9	41.4	48.7
DCGM	0.0	51.2	48.8
Lyophilized WCG, pH 7	5.3	45.4	49.3

[a]WCG = wet corn gluten; DCGM = commercially dried corn gluten meal.
[b]Average of two determinations.

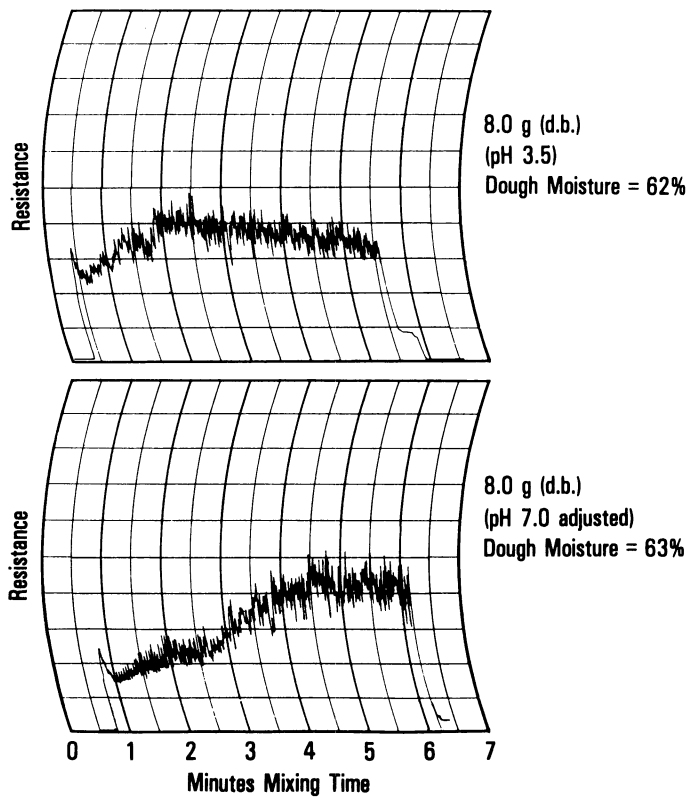

Fig. 2. Mixograms of lyophilized wet corn gluten (pH 3.6) and lyophilized wet corn gluten adjusted to pH 7.

protein yield of extraction sequence II SDS + 2-ME fraction (Table II) may be directly related to the increase in disulfide content that occurs as a result of commercial drying.

The S-sulfo linkage is stable at neutral and mildly acidic pH; however, it is relatively labile under oxidative conditions and heat (Glazer et al 1975, Johnson and Bick 1951, Swan 1959). Since ODCys (Table IV) and total sulfur content of WCG and DCGM are the same, ODCys is not degraded by commercial drying. The thiosulfuric acid group has a pK of 1.7 (Margolis 1966); thus, it can be expected to be ionized at the acid pH of corn gluten. Lindley (1959) reported that the thiosulfuric acid group of S-sulfocysteine in sulfite-treated wool was stabilized at acid pH, possibly by electrostatic bonds with positively charged nonpeptide amino groups. It may be possible that the S-sulfo group in WCG is protected from decomposition during commercial drying by electrostatic interactions with positively charged functional groups of basic amino acids and/or polar lipids.

Mixing Characteristics of CGM

Wet corn gluten (60% moisture) mixed in a shear stress environment in the laboratory developed a cohesive, somewhat elastic dough. Dried corn gluten meal did not exhibit this property. Wet corn gluten dough-forming properties were investigated further, using a mixograph. The mixograph measures the changes in the resistance of the hydrated gluten dough with time during mixing. Wet corn gluten was adjusted to pH 7 so that we would see how changes in ionic character and concomitant changes in protein conformation and solubility affected its mixing properties. As shown in Fig. 2, dough development is much slower and more gradual for pH 7 WCG, and maximum peak height is greater than that attained by WCG (pH 3.6).

The WCG doughs did not resemble wheat gluten doughs because they had less strength and elasticity. Wheat flour doughs contain much less protein than WCG and exhibit much higher maximum resistance to mixing, as demonstrated by Finney and Shogren (1972). The molecular associations and interactions responsible for their development, however, may be similar. Hydrophobic forces, sulfhydryl-disulfide interchange, polar lipid-protein associations, and shear stresses may play important roles in dough development of WCG. Shear forces during mixing may reshape and align protein molecules and possibly expose free sulfhydryls to air oxidation, leading to formation of new inter- and intramolecular disulfide bonds. This process could also catalyze sulfhydryl-disulfide interchange and contribute, in part, to dough cohesiveness and elasticity.

Table IV shows the cysteine, cystine, and ODCys content of WCG when adjusted to pH 7. Adjustment of pH caused a decrease in free sulfhydryl groups (cysteine) that was accompanied by an increase in disulfide content (cystine) of similar magnitude. ODCys content was unchanged. Changes in protein conformation as a result of pH adjustment probably exposed some sulfhydryls to air oxidation and resulted in the formation of new disulfide bonds. The longer development time and greater dough strength of the pH 7 WCG may be related, in part, to the decrease in sulfhydryl groups. Tsen and Bushuk (1968) reported that wheat flours that yield strong doughs with long development times had fewer sulfhydryl groups than did weak flours.

CONCLUSIONS

During steeping of corn, cleavage of disulfide bonds by SO_2 solution results in the conversion of almost half of the total cysteine-cystine content of the gluten protein to S-sulfocysteine (ODCys) and produces some sulfhydryls that are protected from oxidation back to the disulfide (cystine). Reduction of intermolecular disulfide bonds makes it possible to solubilize 94% of WCG protein in nonreducing solvents. The disruption of the disulfide cross-links in native corn glutelin during SO_2 steeping facilitates starch and protein separation in wet-milling. Commercial drying of corn gluten denatures the protein and oxidizes cysteine to cystine, but ODCys are preserved during the process. The oxidation of cysteine to cystine reduces protein solubility in nonreducing solvents to slightly less than 85% of DCGM protein.

Wet corn gluten and pH 7 WCGM when mixed at the proper moisture level exhibit dough-forming characteristics unlike those of DCGM. Dough behavior and strength appear related to the location and content of disulfide bonds in the WCG protein. Dough development is but one functional property of nonheat-treated CGM that may be attributed, at least in part, to the chemical changes in the protein during the process used to produce it from corn.

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