

# Changes Occurring in Protein Body Structure and $\alpha$ -Zein During Cornflake Processing<sup>1</sup>

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## ABSTRACT

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Zeins, which comprise the majority of proteins in corn, are located in spherical organelles called protein bodies. Changes in protein body shape and release of encapsulated  $\alpha$ -zeins as a result of cornflake processing (conventional pressed or extrusion flaking) were investigated. Size-exclusion chromatography, SDS-PAGE, and protein solubility tests showed that, upon cooking, zein proteins form large, disulfide-bound polymers, many of which were insoluble in nonreducing solvents. Transmission electron microscopy with immunogold staining revealed that cooking had no effect on protein body structure in corn, but after processing to cornflakes, protein body structure was altered. In conven-

tional pressed cornflakes, the protein bodies were flattened, partially fused together, and  $\alpha$ -zeins were to some degree released, whereas in the extruded flakes, protein bodies were completely disrupted and  $\alpha$ -zeins dispersed. These results suggest that zeins in cornflakes, particularly extruded ones, are not confined to rigid protein bodies but can interact with each other and other components in the system. The disruption of protein bodies, zein release, and the chemical changes that proteins undergo during processing are speculated to be determinants of texture in ready-to-eat corn-based breakfast cereals.

Corn is the main ingredient in numerous ready-to-eat (RTE) breakfast cereals, of which one of the most widely consumed is cornflakes. Despite numerous advances and improvements in cornflake processing, manufacturers still have some difficulties making consistent, high quality cornflakes.

Cornflake quality is largely determined by texture. Textural studies have mostly focused on starch (Wasserman et al 1992), which constitutes the majority of the flake. However, proteins may also play a significant role in texture formation. Proteins are interactive molecules that are susceptible to numerous conformational and chemical changes during processing. They are major determinants of functional as well as textural properties in processed wheat products and in many other food systems (Kinsella 1978).

Corn generally contains 8–9% protein, of which  $\approx$ 60% are zeins (Hamaker et al 1995), the storage protein of corn. Zeins are prolamins located in spherical organelles called protein bodies with a peripheral disulfide-bound region (Lending and Larkins 1989). They are subdivided into three main classes,  $\alpha$ ,  $\beta$ , and  $\gamma$ , which differ in molecular weight, location within protein bodies, and amino acid sequence.  $\alpha$ -Zein, which constitutes 75–85% of the total zein, is located in the central portion of the protein body and is made up of two polypeptides of  $M_r$  22,000 and 19,000 (Larkins et al 1989).  $\alpha$ -Zein contains large amounts of the amino acids glutamine and leucine, as well as other hydrophobic amino acids.  $\beta$ -Zein, which represents 10–15% of the zein, is made of a methionine-rich  $M_r$  14,000 polypeptide.  $\gamma$ -Zein, which constitutes 5–10% of the zein, is made of two proline-rich polypeptides of  $M_r$  27,000 and 16,000.  $\beta$  and  $\gamma$ -Zeins are located at the periphery of protein bodies (Lending and Larkins 1989) and contain smaller amounts of leucine and other hydrophobic amino acids, and much higher amounts of cysteine than does  $\alpha$ -zein. Zeins have many unique characteristics based mainly on their highly hydrophobic nature. They can form water-resistant films and are used to a limited extent industrially. It is not known how zeins influence textural parameters in breakfast cereal products or in other processed foods.

Lawton (1992) showed that by mixing isolated zein, cornstarch, and water at temperatures  $>30^\circ\text{C}$ , a viscoelastic dough formed that contained extensive protein fiber networks similar to wheat

dough. If similar networks are formed during the production of cornflakes (from flaking grits or extruded pellets), they may affect the texture and quality of the final product. To form such a network, however, zeins need to be released from the constraints of protein bodies, complex together, and form viscoelastic polymers during the course of processing.

The objectives of this study were to investigate the potential of protein body breakdown during processing and to localize  $\alpha$ -zeins in unprocessed and cooked corn, and in conventional (pressed) and extruded cornflakes. Additional studies were done to identify chemical changes in zeins that occur during heat treatment to complement physical changes visualized by microscopy.

## MATERIALS AND METHODS

### Corn

Corn (cultivar W64A+) was ground using an abrasive disc mill (model MLI-204, Buhler-Miag, Minneapolis, MN) and then passed through a cyclone mill (Cyclotec 1093, Tecator, Höganäs, Sweden) using a 0.5-mm screen. Flour was washed with petroleum ether and decanted three times to defat. Corn flour was cooked with water (1:10, w/v) by placing in a boiling water bath for 20 min. Conventional (flaked) and extruded cornflake samples (Fig. 1) were provided by Kellogg Co. (Battle Creek, MI).

### Transmission Electron Microscopy

Defatted uncooked and cooked corn flour, and conventional and extruded cornflakes were fixed in a 1% glutaraldehyde, 4% paraformaldehyde in 0.05M potassium phosphate buffer, pH 6.8, for 16 hr at  $4^\circ\text{C}$ . The fixing solution was removed by rinsing with the 0.05M potassium phosphate buffer, and the samples were dehydrated in a graded ethanol series 15 min each in 10, 30, 50, 70, 90, 95, and 100% ethanol. The samples were infiltrated for one day each in 20, 40, 60, and 80% LR White resin (Electron Microscopy Science, Ft. Washington, PA) in ethanol, and then for four weeks in 100% LR white resin. The samples were placed in polyethylene capsules and polymerized for one day in a nitrogen-purged oven at  $55^\circ\text{C}$ . Specimens were thin-sectioned with a diamond knife on a Sorvall Porter-Blum MT-2-B ultramicrotome (Research and Manufacturing Co., Tucson, AZ). Sections were collected onto formvar-coated 300-mesh copper grids.

Immunochemistry was done by floating grids on successive drops of reagents on dental wax in petri dishes. Grids were first floated in 20 mM Tris, 500 mM sodium chloride, 0.3% Tween-20, pH 8.2 (TBS-T) for 15 min. The grids were then incubated at  $4^\circ\text{C}$  overnight in 50- $\mu\text{L}$  drops of  $\alpha$ -zein-specific rabbit polyclonal anti-

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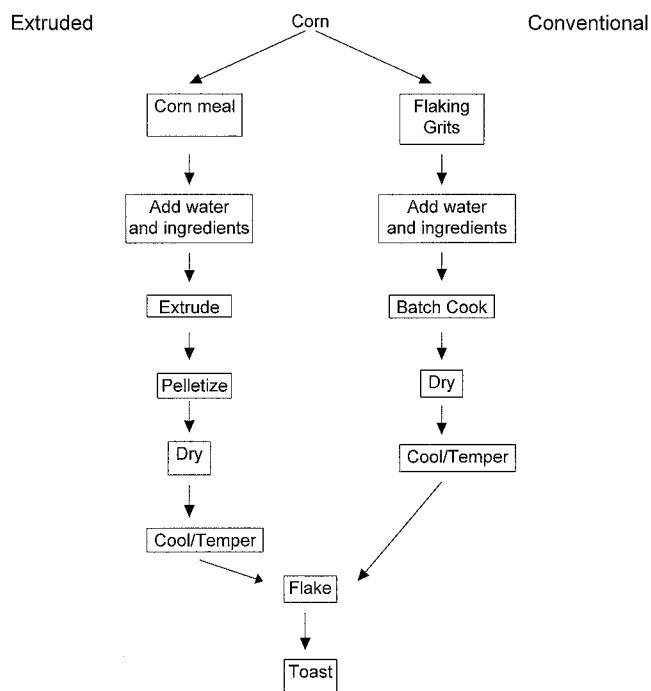
serum (1:500 dilution) (provided by B. A. Larkins, University of Arizona). After rinsing with TBS-T containing 1% (w/v) bovine serum albumin (TBS-TB) for 10 min, the grids were floated for 1 hr in 10-nm gold conjugated to goat-antirabbit IgG (Electron Microscopy Sciences, Ft. Washington, PA) at a 1:25 dilution in TBS-TB. The grids were then rinsed with distilled water and placed on a filter paper to dry. Finally, the grids were stained with 2.5% (w/v) uranyl acetate for 2 min followed by 0.1% (w/v) lead citrate for 10 sec. Sections were viewed on a Philips EM 200 transmission electron microscope (Philips Electronic Instruments Co., Eindhoven, Netherlands) at 60 kV.

### Column Chromatography

Proteins were extracted from uncooked and cooked defatted corn flour with 2% sodium dodecyl sulfate (SDS), 6M urea, 0.01M Tris buffer (pH 8) by shaking a suspension (1:20, w/v) for 3 hr. Following centrifugation, 5-mL samples were loaded onto a Sephacryl S-300 (Pharmacia, Uppsala, Sweden) column (60 × 2.5 cm) and eluted at a flow rate of 20 mL/hr at room temperature with an eluent consisting of 0.1% SDS, 6M urea, 0.01 Tris (pH 8), 0.001M EDTA, and 0.01% sodium azide. The outflow was detected at 280 nm and collected in 5-mL fractions. Fractions conforming to chromatographic peaks were pooled, dialyzed against water at 4°C, and lyophilized.

### SDS-PAGE

SDS-PAGE was done on a vertical gel system (model V15.17, Gibco BRL Life Technologies, Gaithersburg, MD) using a 10–15% acrylamide gradient separation gel with a 4% stacking gel. Portions of the lyophilized materials from chromatography fractions collected above were solubilized in SDS-PAGE sample solvent (0.05% bromophenol blue, 10% glycerol, 0.5M Tris, 10% SDS) both reduced (with 4% 2-mercaptoethanol [2-ME]) and unreduced. Samples were placed in a boiling water bath for 3 min, and a portion was loaded into sample wells. Electrophoresis was performed at 40V for 18 hr. Gels were stained with 0.25% Coomassie Brilliant Blue R-250 in 46% methanol and 8% acetic acid and destained with 20% ethanol and 10% glacial acetic acid. A broad range molecular weight standard (6,500–200,000 kDa) was used (BioRad, Richmond, CA).



**Fig. 1.** Diagram of the production of cornflakes by extrusion and conventional methods.

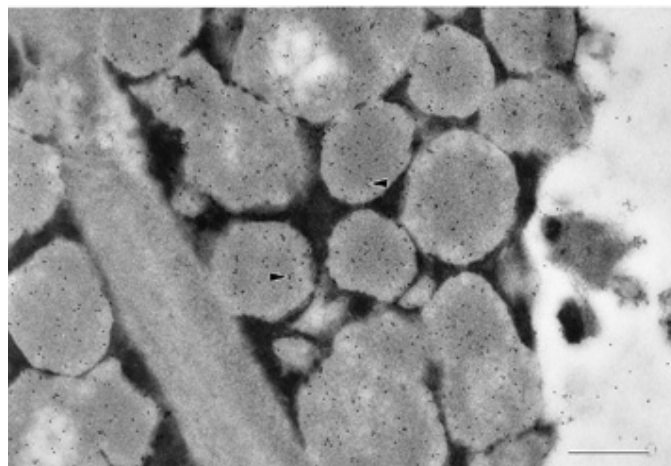
### Protein Determination

Uncooked defatted flour and the same flour cooked for 1, 3, 5, 10, and 20 min at 20, 40, 50, 60, 70, and 100°C were extracted with 0.01M Tris buffer (pH 8), 2% SDS with and without the addition of 2% 2-ME (at a ratio of 1:15, flour to solvent). Following centrifugation, supernatants were placed in microKjeldahl tubes and dried overnight at 80°C. Protein of extracts and flour samples was determined by the microKjeldahl procedure (AACC 1995) using the conversion of N × 5.7 for corn.

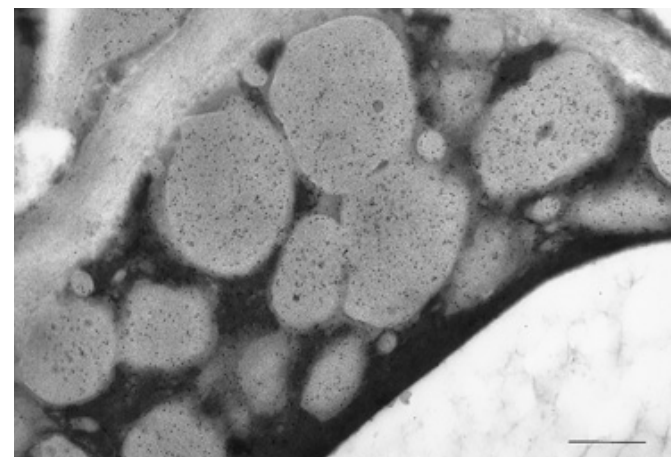
## RESULTS AND DISCUSSION

### Protein Body Microstructure

Figures 2–5 show transmission electron microscopy (TEM) micrographs of uncooked and cooked corn flour, and conventional (pressed grit) and extruded (pressed extruded pellet) cornflakes. Uncooked corn flour shows typical protein bodies with round, spherical structures ≈1 μm in diameter (Fig. 2). Immunolocalization of α-zein (black dots indicate α-zein antibody binding sites) shows its location to be throughout the protein body. Lending and Larkins (1989) showed that α-zein is located in the large central portion of the protein body with β- and γ-zeins surrounding it on the periphery. The microstructure of cooked corn flour also



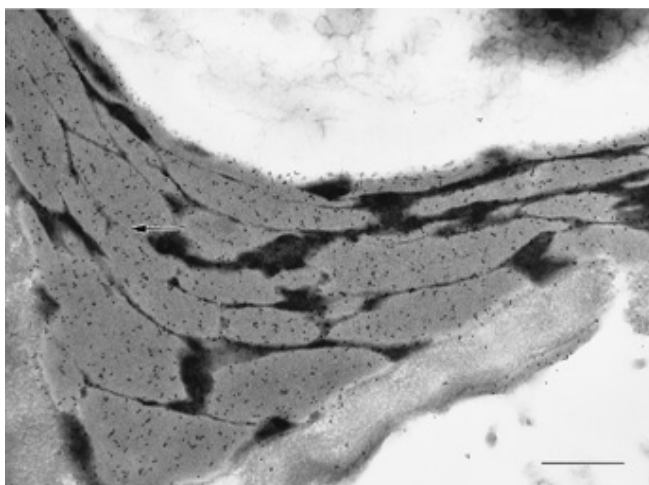
**Fig. 2.** Transmission electron micrograph showing protein bodies in corn flour. α-Zeins immunolabeled with colloidal gold label (black dots, arrows show examples) are contained within the protein bodies. Size bar = 0.5 μm.



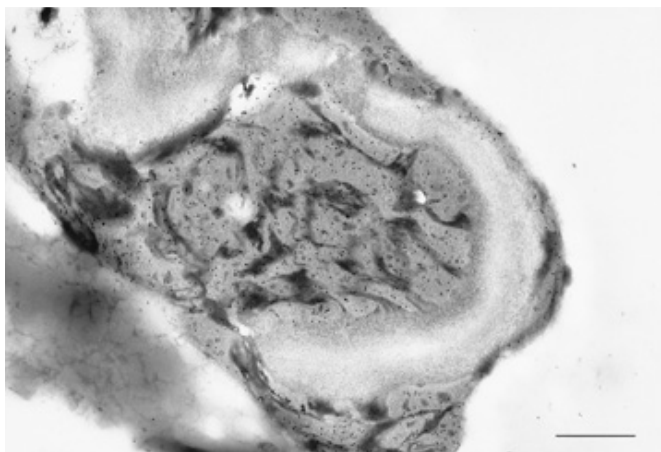
**Fig. 3.** Transmission electron micrograph showing protein bodies in cooked corn. α-Zeins immunolabeled with colloidal gold (black dots) are contained within the protein bodies. Size bar = 0.5 μm.

revealed round, spherical protein bodies with  $\alpha$ -zeins located in the center, similar to the uncooked sample (Fig. 3). Cooking alone did not affect protein body shape, nor was zein released. Similarly, Rom et al (1992) observed by scanning electron microscopy (SEM) that cooking sorghum (closely related to corn), did not alter the shape of the protein bodies.

Protein bodies of conventional (pressed) cornflakes were deformed, flattened, and disrupted (Fig. 4). Such deformations are presumably due to the pressure of the flaking rolls compressing the protein body structure during processing. Some  $\alpha$ -zein release was observed, as well as some joining among protein bodies (Fig. 4, arrow). Such continuity among protein bodies suggests that some zeins are released from the constraints of the protein body and merge together, which may be indicative of zeins fusing together to create fibrils or a partial protein matrix in conventional cornflakes. The micrograph of the extruded flake shows that the original, spherical shape of the protein bodies can no longer be identified (Fig. 5). The protein bodies, therefore, were completely disrupted during extrusion and  $\alpha$ -zeins (black dots) dispersed. The  $\alpha$ -zeins appear to have merged together, and if conceptualized in the third dimension, form a continuous network. Such putative zein interactions in extruded, flaked samples appear to be much more extensive than in conventional flakes, and it is likely that a structural protein matrix or fibrils formed during the extrusion and flaking process.



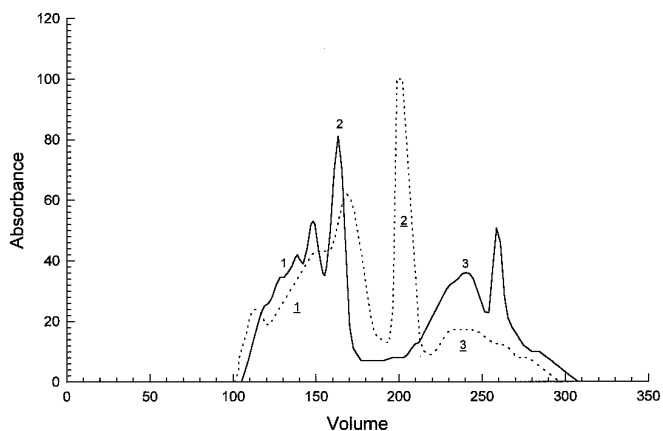
**Fig. 4.** Transmission electron micrograph of protein bodies in conventional cornflakes. Arrow shows fusing among some protein bodies and partial release of  $\alpha$ -zeins (black dots). Size bar = 0.5  $\mu$ m.



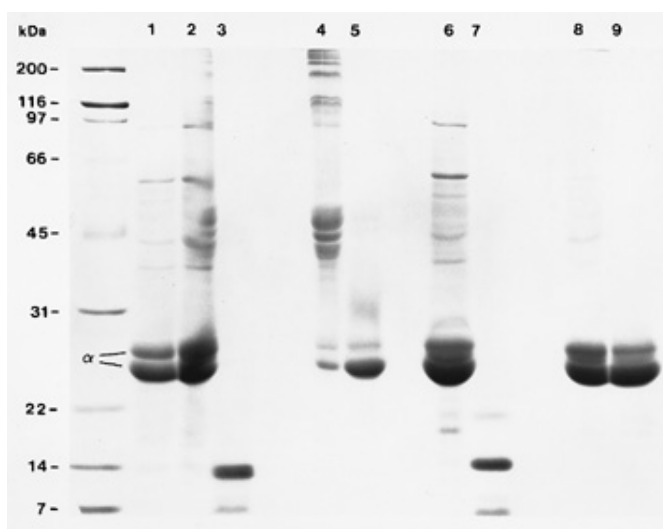
**Fig. 5.** Transmission electron micrograph showing proteins in extruded cornflakes. Individual protein bodies can not be identified.  $\alpha$ -Zeins immunolabeled with colloidal gold (black dots) are dispersed in the flake. Size bar = 0.5  $\mu$ m.

No previous studies have focused on the effects of extrusion processing on corn protein bodies or how protein body disruption affects the functionality of the final product. Researchers have, however, examined the effect of extrusion processing on legume protein bodies. A study done on pea flour demonstrated that during the last one-third of the extrusion process, the effects of shear, heat, and pressure had caused its protein bodies to join, forming a protein matrix (Ben-Hdech et al 1991). Similar studies performed in soy also showed that during extrusion, protein bodies melted and fused to create a continuous protein matrix (Kazemzadeh et al 1982, Noguchi 1989). These protein bodies are fundamentally different from those of the coarse cereals in that they form within vacuoles and, because no structural proteins have been identified at their periphery, they would be expected to have weak structures when compared to corn protein bodies. Zein-containing protein bodies in corn are produced within the endoplasmic reticulum (Fukushima 1991, Richard et al 1996). Despite these structural differences, it appears as though protein bodies in corn may react similarly during extruded cornflake processing.

Cornflake quality is judged by flavor, color, texture, overall appearance (Midden 1989), and "bowl life" (Levine 1994), the latter being the amount of time before the product becomes soggy after



**Fig. 6.** Size-exclusion chromatography of uncooked (---) and cooked (—) corn flour proteins extracted in a nonreducing solvent.



**Fig. 7.** SDS-PAGE of protein peaks 1 and 2 obtained by size-exclusion chromatography from uncooked and cooked corn flour samples. Lanes 1–5 are unreduced samples; Lanes 6–9 are reduced samples. Lanes: 1, corn flour control; 2, peak 1 of uncooked; 3, peak 2 of uncooked; 4, peak 1 of cooked; 5, peak 2 of cooked; 6, peak 1 of uncooked; 7, peak 2 of uncooked; 8, peak 1 of cooked; 9, peak 2 of cooked corn flour samples.

the addition of milk. In a past study (Tan and Chinnaswamy 1993), most of these quality parameters were attributed to starch, but zeins could also significantly affect quality due to their hydrophobic character and ability to form polymers, the latter indicated by SEM (Lawton 1992) and through chemical analysis in this work. In this study, zein proteins were partially or completely released from the protein bodies in cornflakes and, therefore, are free to form polymers as well as react with other components in the system. The severity of disruption of protein bodies and redistribution of zeins may partially explain textural quality differences between cornflakes processed by the two methods as well as differences in flavor and appearance.

### Polymerization of Zeins During Cooking

Size-exclusion chromatograms (Fig. 6) showed that the pattern of the cooked corn sample (solid line) shifted slightly left, compared to the uncooked sample (dashed line). This indicates that protein aggregates formed upon cooking, resulting in faster elution through the size-exclusion column. Chromatographed proteins were extracted in a dissociating solvent with no reducing agent, suggesting that disulfide-bound polymers were formed during cooking.

SDS-PAGE analysis of proteins in the major chromatographic peaks 1 and 2 of the uncooked and cooked corn samples revealed that there was extensive disulfide-mediated polymerization of zein during cooking (Fig. 7). Chromatographic peak 1 from the uncooked extract, nonreduced (lane 2) and reduced (lane 6), showed  $\alpha$ -zein in the monomer (23 and 25 kDa) and dimer (40–60 kDa) forms. Chromatographic peak 2 from the uncooked extract ap-

peared as two small bands of <14 kDa in the native (lane 3) and reduced (lane 7) forms. Chromatographic peak 1 of the cooked extract showed the formation of multiple higher order polymers (>100 kDa) upon cooking (lane 4). When reduced to the monomer, these polymers were identified as  $\alpha$ -zein (lane 8). Chromatographic peak 2 from the cooked extract, nonreduced (lane 5) and reduced (lane 9), appeared mostly as the monomer form of  $\alpha$ -zein at 23 and 25 kDa. SDS-PAGE analysis thus showed the formation of large, disulfide-bound, protein polymers upon cooking. The formation of such protein aggregates suggests that zeins are not static, but rather that they can change considerably during cooking. Although cooking alone caused polymerization of proteins, there were no observed physical deformations of the protein bodies in cooked corn (Fig. 3).

Solvent extractability differences were shown with and without the presence of a reducing agent, also indicating that large, insoluble disulfide-bound polymers were formed during the cooking of corn. The amount of protein extracted in a nonreducing solvent after heating to 100°C was nearly half the amount extracted from samples heated at  $\leq 70^\circ\text{C}$  (Fig. 8A). Similarly, after only 5 min of heating, the protein extracted in a nonreducing solvent was less than half the amount extracted in uncooked corn flour (Fig. 8B). The addition of 2-ME to break disulfide bonds, markedly increased the amount of extractable protein in flour suspensions, especially those that had been heated for longer times. Most processing temperatures are  $>70^\circ\text{C}$ , which suggests that disulfide bonds are produced at very early stages of food manufacturing processes that involve heat treatment as an initial step.

### CONCLUSIONS

Protein bodies in corn changed shape and  $\alpha$ -zeins were released during two types of cornflake processing. In the conventional flaking process, protein bodies appeared flattened or misshaped but mostly still intact, and  $\alpha$ -zeins were only partially released. In the extruded cornflakes, the protein bodies were completely destroyed and  $\alpha$ -zein was dispersed. Therefore, the extrusion process appeared to be much harsher than the conventional flaking process. Cooking alone did not alter protein body shape, indicating that shear or pressure during further processing can physically deform or disrupt protein bodies causing the release of zein. Cooking corn caused large, disulfide-bound protein polymers to form. Released zein may form a viscoelastic protein network or matrix that could influence texture and quality of the final product. These results demonstrate that zeins are not static in the processing of corn products, nor are they always confined to rigid protein bodies. Rather they likely undergo chemical and physical changes that could affect the quality of corn-based RTE breakfast cereals.

### ACKNOWLEDGMENTS

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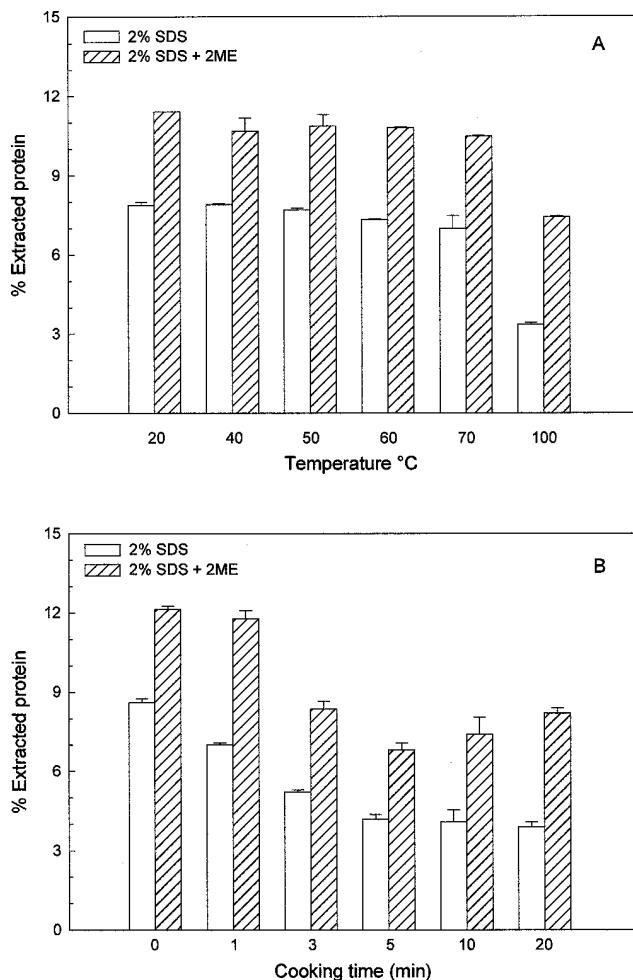


Fig. 8. Protein extracted in nonreducing and reducing solvents from corn flour cooked at various temperatures (A) and times (B).

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