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Increasing and Stabilizing β-Sheet Structure of Maize Zein Causes Improvement in Its Rheological Properties

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ABSTRACT: Wheat gluten proteins are considered to have the unique ability to form viscoelastic matrices that are essential for breadmaking. This study shows that maize seed storage protein (zein), if properly treated, can be made to function similarly to gluten at the protein secondary structure level with concomitant improved viscoelasticity. Here, we propose the concept of a small amount of coprotein (high molecular weight glutenin or casein) acting to stabilize a build-up of β-sheet structure in a zein-based dough, thus creating a viscoelastic matrix that is retained over time. This discovery is relevant to the need for gluten replacement viscoelastic proteins for wheat intolerant individuals and as well opens possibilities of creating wheatlike cereal varieties that could more cheaply substitute for wheat imports in developing countries.

KEYWORDS: *maize, zein, protein structure, viscoelasticity, FTIR*

INTRODUCTION

Wheat gluten forms viscoelastic networks able to retain CO_2 produced from yeast fermentation that allows for the production of leavened baked products.[1,2] It is considered the only protein, cereal-based or otherwise, with the proper functionality to produce high-quality breads and associated baked products. High molecular weight (HMW) glutenins, a group of proteins unique to wheat gluten, are thought to be mainly responsible for the formation of an extended network of intermolecular β-sheet structures that upon extension allow gluten-hydrated polymers to hold CO_2 over an extended period of time, thus letting a wheat flour dough withstand the leavening and baking processes.[3,4] Although corn zein, when hydrated and at a slightly elevated temperature (above its glass transition temperature), can also form viscoelastic polymers when shear is applied,[5] the nature of these polymers is unstable, and they largely collapse after their formation, coinciding with a gain and subsequent rapid loss of β-sheet content.[6]

Nuclear magnetic resonance (NMR) and Fourier transform infrared (FT-IR) spectroscopy analyses showed that β-sheet structure of HMW glutenin subunits increases when the proteins are in a mixed doughy hydrated state.[7,8] This structural change was not only related to gluten viscoelasticity but also to its stability.[3,4] It is thought that HMW subunits of glutenin, initially present in a loop conformation, are extended during gluten fibril formation and form polymeric alignments in which high proportions of β-sheet structures are favored at the expense of β-turns. For this reason, such polymers are believed to have a high resistance to extension. The number and pattern of disulfide cross-links in wheat glutenin polymers also affect dough strength.[2] Thus, β-sheet and disulfide bridge properties of gluten contribute to the stabilization of viscoelastic polymers during dough mixing and proofing.[3]

Maize zein can be made viscoelastic when hydrated and brought above its glass transition temperature of ∼28 °C.[5] We reported that zein also gains in β-sheet structure during this process when shear mixing is applied, although quickly loses β-sheet structure in the absence of shear.[6] A reasonable hypothesis to explain this loss was the lack of analogous HMW subunits in maize zein that might provide stability to the β-sheet alignments. To make zein more glutenlike for breadmaking, the addition of similar small amounts of HMW glutenins or another protein capable of stabilizing β-sheet structures was proposed to increase stability and relaxation time of its viscoelastic fibrils. This report shows successful use of such "coproteins" to change the structure and improve the viscoelastic properties of zein polymers. Understanding the structural basis behind the formation and stabilization of viscoelastic polymers is the basis of creating gluten-free dough-like polymers and generally of changing the functionality of nonwheat cereal proteins.

EXPERIMENTAL SECTION

Materials and Methods. Commercial zein and wheat gluten were purchased from Sigma-Aldrich (St. Louis, MO). Native normal maize starch was obtained from Tate & Lyle (Decatur, IL). HMWG was isolated with aqueous 1-propanol mixtures of wheat gluten.[9,10] Composites of starch and proteins (10% of total composite weight) were made into viscoelastic polymers[5] and allowed to relax for up to 6 min at 25 °C. Wheat gluten, zein, isolated HMW glutenin, and casein were used as protein sources. The secondary structure of the proteins in the polymers was compared using FT-IR spectroscopy. Viscoelastic properties and relaxation rates were evaluated simultaneously using a novel rheological technique developed in our group[11] and stress relaxation tests using a texture analyzer.

FT-IR Analysis. Spectra were obtained with a FT-IR spectrometer (Thermo Nicolet 670, Madison, WI) equipped with a diamond attenuated total reflectance (ATR) cell with a 45° aperture angle, a liquid nitrogen-cooled MCTA detector, and OMNIC software. All spectra were collected at 25 °C using a Diamond ATR cell and 4 cm^{-1} resolution. Spectra of starch and water were subtracted prior to

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analysis. Curve deconvolution, fitting, and peak assignment[4,12] were done with PeakFit software (v4.11 Systat Software Inc., Point Richmond, CA) to quantify protein secondary structure (α-helix at 1652−1657 cm^{-1} and β-sheet at 1620−1637 cm^{-1}) from the resolved spectra.[3,4,6,12,13] A Gaussian shape was assumed for resolved components. Full-width at half-maxima was 6.75 and kept constant for all peaks during deconvolution. The accuracy of the fitting procedure was evaluated by their correlation coefficients ($R^2 > 0.90$), residual plots, and fit standard errors. Samples were analyzed in triplicate. Integrated areas were calculated for the assigned peaks that correspond to the structural elements of the protein. To monitor changes in the secondary structure of the dough systems during the first minutes of sample relaxation, zein, gluten, zein HMW glutenin, and zein casein dough systems that were mixed for 5 min at 35 °C were then allowed to relax at 25 °C for up to 6 min. FT-IR spectra of the polymers were taken after 0, 3, and 6 min of relaxation.

Analysis of Viscous and Elastic Components. This method was based on the squeezing flow viscometry methodology.[14−16] This technique applies small amplitude oscillations at random frequencies of up to 20 kHz. The design uses a Piezo-electric crystal stack attached to an impedance head (type 8001, Bruel and Kjaer, Korea). Upon the application of voltage, the upper plate oscillates, and force and acceleration values are obtained by the impedance head and transformed into a frequency response specific for the sample. The frequency response data were obtained using SigLab-MatLab VNA software program (DSP Siglab, Dynamic Signal Analysis, System, Spectral Dynamics, San Jose, CA). Data that included the force and the velocity of the moving plate were converted through Fourier transformation to the frequency field. The mechanical impedance was then calculated as the ratio of the transformed forces and velocities in the MatLab environment. The calculated complex mechanical impedance was then used to model the mechanical properties of the material by traditional dashpot and spring components. This model enables the calculation of the viscous and elastic components in terms of sample's damping and stiffness. Damping is associated with amplitude of resonance frequencies. Higher amplitude values imply lower mobility where resonance occurs or samples with less liquidlike behavior (viscous component). Conversely, values of resonance frequency are directly associated with stiffness or the ability of the material to store energy (elastic component). Thus, higher resonance frequencies indicate stiffer materials.

Measurements were done in compression (2 N) using a Texture Analyzer (TA-XT2i, Texture Technologies, NY) with a parallel flat plate attachment mounted on a digital dry bath (Accublock Digital Dry Bath, Labnet International Inc., NJ) that maintained the temperature of the polymers at 23 or 35 °C. The Texture Analyzer also allows for the simultaneous measurement of force relaxation curves. Force relaxation curves represent the decrease of the force or stress when the sample is subjected to a fixed deformation or strain. The slope at which stress decreases over time can be calculated and named the same relaxation rate. A large slope indicates a fast relaxation behavior.

Statistical Analysis. To analyze the effects of the different proteins in the polymers and the relaxation time on the protein's secondary structure, a two-way analysis of variance was done with α-helix or β-sheet content (%) as dependent variables. The factors analyzed were the type of protein used in the polymer (zein, gluten, and zein HMW glutenin) and relaxation time (0, 3, and 6 min). The significance level used was $\alpha \leq 0.05$, and the population (n) was equal to 27. A comparison was also done on the effects of HMW glutenin and casein addition to zein on secondary structure and relaxation duration. The significance level used was also $\alpha \leq 0.05$, and the population (n) was equal to 18. Analysis of Variance on the General Linear Models Procedure from the Statistical Analysis System (SAS) (v8.2e, 2001, Cary, NC) was used to detect significant differences. The Tukey−Kramer multiple comparison procedure at a significance level of 0.05 was used to compare differences observed. The program utilized for these studies was also SAS. Rheological tests were done by triplicate, and data are reported as mean values and error bars considering a 95% confidence interval.

RESULTS AND DISCUSSION

Protein Secondary Structure. Figure 1 shows the deconvoluted spectra of zein, gluten, and zein HMWG after the viscoelastic polymers were formed at 35 °C and after 6 min of relaxation at 25 °C. The integrated intensities that

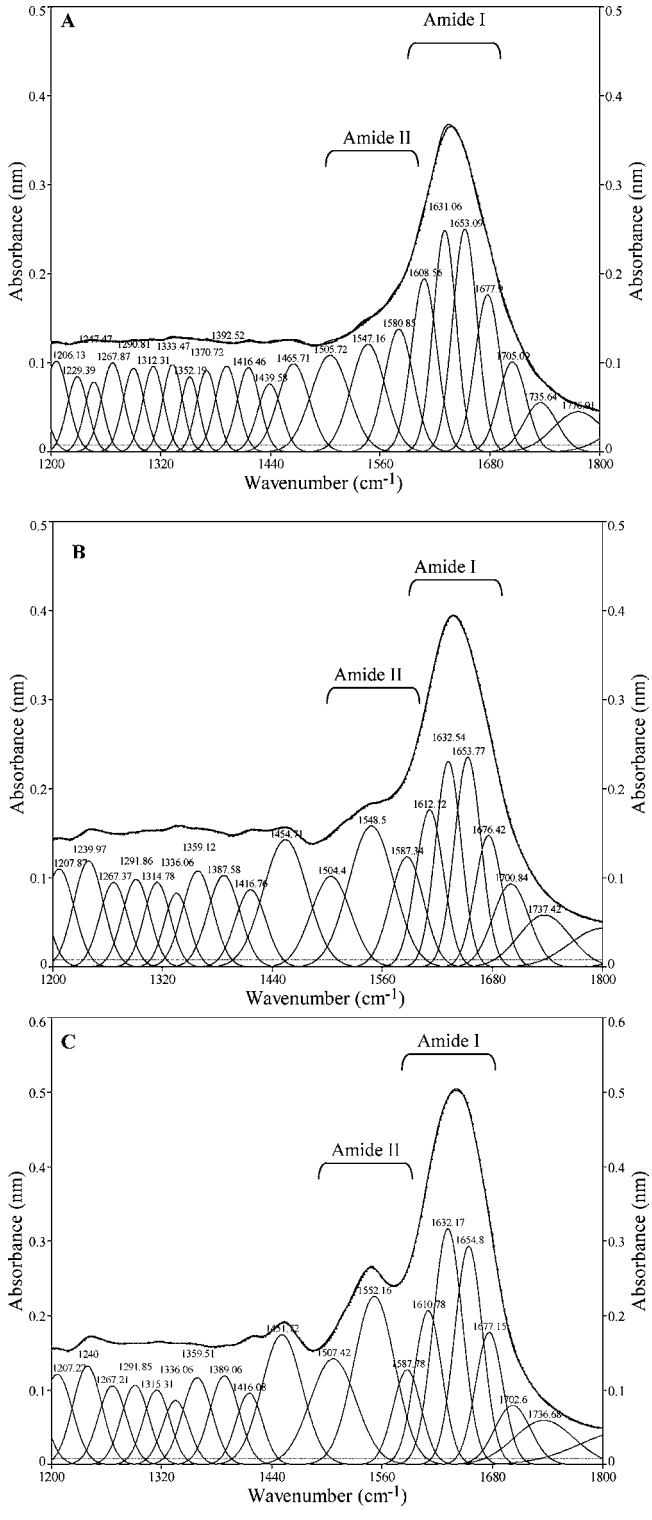

Figure 1. Representative deconvoluted spectra of zein (A), gluten (B), and zein HMW glutenins (C) in a viscoelastic system conditioned and mixed at 35 °C after 6 min of relaxation at 25 °C. Amides I and II regions are noted.

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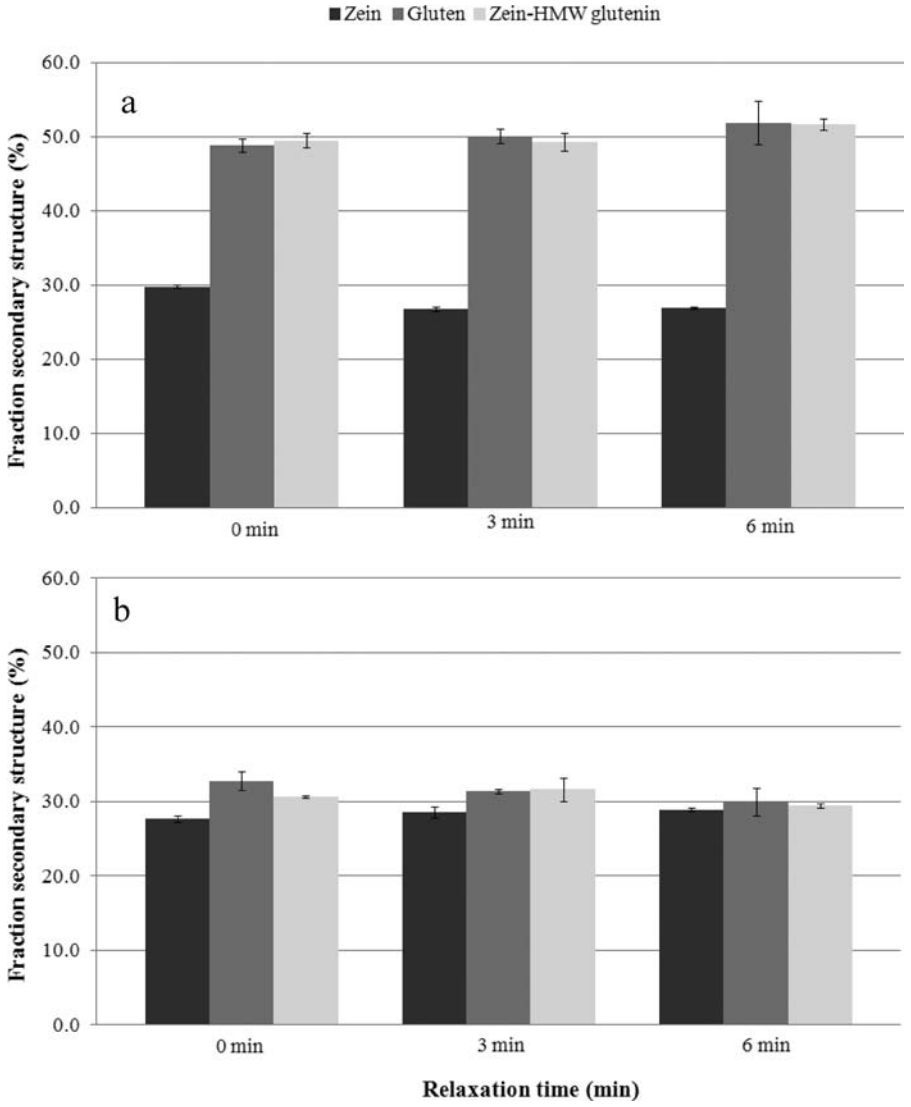

Figure 2. FTIR-determined secondary structure of protein polymers during relaxation. β-Sheet (1620−1637) (a) and α-helix (1652−1657) (b) contents of zein, gluten, and zein HMW glutenin in viscoelastic polymers during relaxation at 25 °C. Error bars represent standard deviation among replicates.

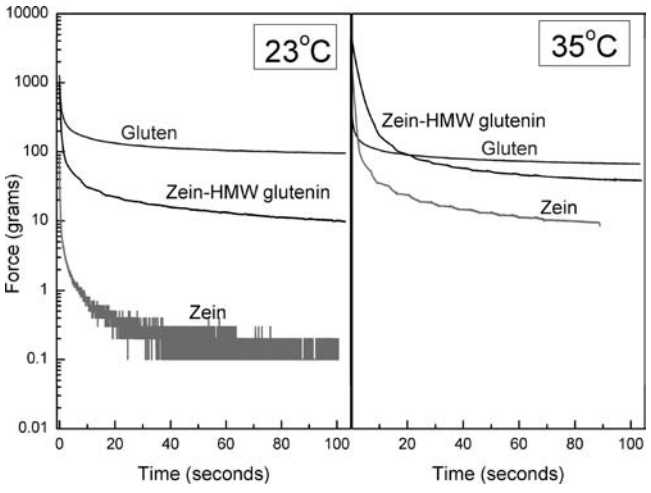

Figure 3. Relaxation tests on the viscoelastic polymers of zein, gluten, and zein-HMW glutenin at 23 and 35 °C during the first 100 s of relaxation after 5 min of mixing at 35 °C.

correspond to the amide II region (1500−1600 cm^{-1}) of the zein HMW glutenin spectra (Figure 1C) were higher than the zein spectra (Figure 1A) but lower than the gluten spectra (Figure 1B). Integrated intensities of the amide II region have been reported to increase linearly with the increase of water content in the sample.[17] Thus, results of this study suggest that the protein mixture of zein HMW glutenin presented an amide II region related to a more hydrated polymer than in the case of zein by itself, although gluten showed the greatest ability to become hydrated as a viscoelastic polymer. In this study, only the amide I region was considered for protein secondary structural analysis, due to amide II region high sensitivity to hydration.[17]

Figure 2 shows differences in the β-sheet and α-helix contents of zein, gluten, and the zein HMW glutenin mixture in the viscoelastic polymers during relaxation at 25 °C after conditioning and mixing at 35 °C. The β-sheet content of zein polymers was substantially lower ($P < 0.05$) when compared to gluten or zein HMW glutenin and at each relaxation time. Thus, during the first 6 min of the polymers' relaxation at 25 °C, the β-sheet content of the zein polymer with added HMW

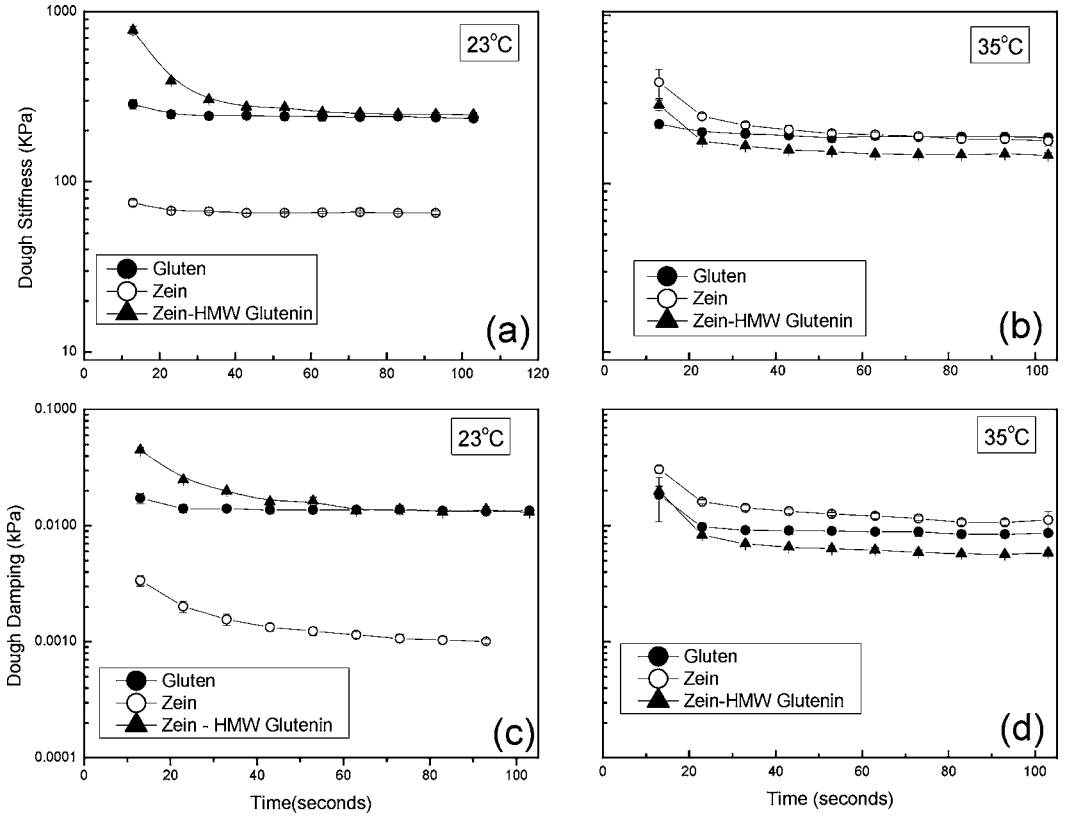

Figure 4. Comparison of viscoelastic properties of zein, gluten, and zein HMW glutenin at 23 and 35 °C during the first 100 s of relaxation after 5 min of mixing at 35 °C. Viscoelastic parameters were obtained with the squeezing flow technique. (a) Dough stiffness at 23 °C, (b) dough stiffness at 35 °C, (c) dough damping at 23 °C, and (d) dough damping at 35 °C.

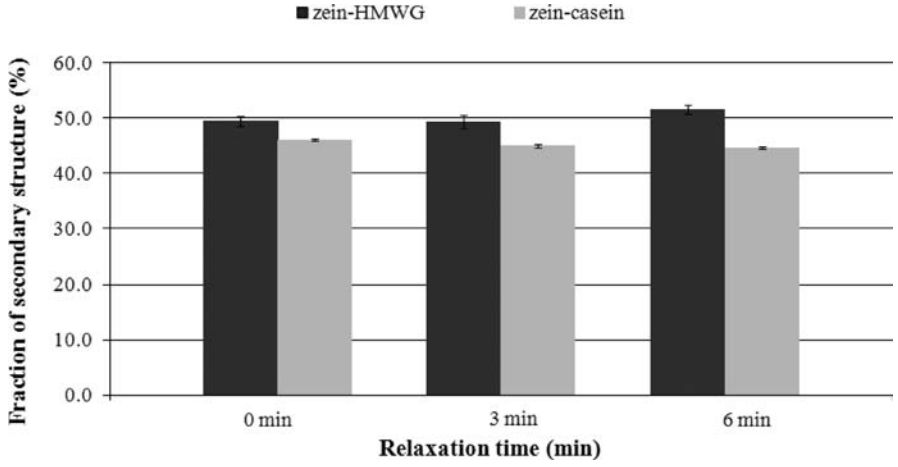

Figure 5. Comparison of β-sheet structure of zein HMW glutenin and zein casein during relaxation of polymers. β-Sheet contents of zein HMW glutenin and zein casein in the viscoelastic polymers during relaxation at 25 °C. Error bars represent standard deviation among replicates.

glutenin was not only stable but was statistically the same as the β-sheet content of the gluten polymer (Figure 2a). In our previous paper,[6] it was shown that zein exhibited comparable β-sheet content to gluten after the formation of viscoelastic polymers at 35 °C. This structure was lost in zein polymers upon removal of stress. This suggested that, unlike gluten, temperature and shear were necessary for the β-sheet stability of zein polymers, which could be related to the rapid relaxation that only the zein polymer exhibited. The present study shows that the small addition of HMW glutenin to the zein polymers caused an increased stability of the β-sheet content of zein present in the polymer that resulted in maintenance of high β-sheet content during relaxation. The addition of the HMW glutenin to zein not only significantly increased the β-sheet content but also slightly increased the α-helix content in the polymers (Figure 2b).

Thus, when zein (9% dwb) and HMW glutenin (1% dwb) were combined in a model system with starch (90% dwb), incubated (for 24 h), mixed at 35 °C, and allowed to relax at 25 °C, an increase and stabilization of the β-sheet content were found to be similar to that of gluten protein. Moreover, an increase in β-sheet structure was maintained below its glass

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transition temperature (~28 °C). As little change was observed in α-helix content in the polymers and the total amount of secondary structure did not decrease significantly, the increase in β-sheet structure was suggested to be at the expense of β-turns and unordered structures as noted by Belton.[3]

Force Relaxation. Favorable changes in secondary structure of zein with the HMW glutenin coprotein translated to enhanced polymer viscoelasticity. Viscoelasticity of polymers is often characterized by performing relaxation tests. These tests consist of applying a constant deformation (or strain) to the sample while observing changes on the relaxation force (or stress) necessary to sustain that deformation. The rate at which the relaxation force changes with time is a clear indication of a material's structure. During the first 100 s of relaxation at 23 °C, the zein polymer exhibited a viscoelastic behavior that is characterized by a short relaxation time (Figure 3) or high relaxation rate (measured by the slope of the curve) relating to its inability to sustain a force, represented in the *Y*-axis of the figure in logarithmic coordinates, upon the imposition of a deformation or a strain. The figure illustrates that for the dough prepared with zein alone at 23 °C, the forces needed to sustain the deformation/strain of the sample decrease significantly up to the limit of sensitivity of the instrument as shown by the noise in the data, clearly indicating the lack of the elasticity of this dough. Conversely, gluten showed a slow relaxation (slower relaxation rate) and sustained force while the strain was applied, a viscoelastic behavior that is typical of elastic-like polymers. The addition of the small amount of HMW glutenin to zein caused a higher resistance to the deformation applied (higher forces) and slower relaxation time at 23 °C that resembles that of the gluten and at the same time paralleled its retention of β-sheet structure under the same conditions. At 35 °C, the addition of HMW glutenin to the zein polymer caused a relaxation curve that closely resembled that of the wheat gluten polymer. Zein polymers held at 35 °C exhibited an improved elastic behavior, although still inferior to the zein HMW glutenin complex or gluten. Oom et al.[18] showed that zein, as well as sorghum kafirin, has extensional rheological properties similar to wheat gluten dough.

Analysis of Viscous and Elastic Components (Squeezing Flow). Values of the damping (viscous component) and stiffness (elastic component) measured using the squeezing flow technique are illustrated in Figure 4 in logarithmic coordinates as a function of resting/relaxation time. During the first 100 s of relaxation at 23 °C, the zein polymer exhibited the lowest damping (viscous component) and the lowest stiffness (elastic component) values (Figure 4a,c), which indicates that the zein polymer has a lower viscosity and more importantly the least ability to store energy. However, when zein polymers were held at 35 °C, they had similar viscous and elastic components as gluten (Figure 4b,d). This has been previously reported[5] and is likely as a result of the structural rearrangement observed in the maintenance of high β-sheet structure above its zein glass transition. At 23 °C, in contrast, the addition of HMW glutenin to a zein polymer increased the measured damping and stiffness to values that were similar to those of gluten polymer and were completely different than its base protein polymer of zein. Thus, incremental increase and stabilization of β-sheet in a zein polymer, through the addition of HMW glutenin, was directly related to its improved viscoelastic properties and its relaxation rate. This increased resistance to extension may be associated with the formation of a viscoelastic doughlike polymer able to withstand CO_2 pressure during fermentation and baking. If so, a product similar to wheat bread might be made with a mixture of zein and HMW glutenin or other protein capable of forming stable β-sheets.

Alternative Gluten-Free Coprotein. For a gluten-free product, there was a need to search for an alternative coprotein to HMW glutenin. Casein was found comparable in retaining β-sheet structure (Figure 5), even at levels as low as 3% of total protein (data not shown). Our data support the view that the addition of the coproteins HMW glutenin or casein to zein (or other like cereal prolamins) causes β-sheet alignments to become favorable by increasing their overall ability to form hydrogen bonds necessary to maintain the structure of polymers during relaxation at 25 °C. The observation that a small amount of coprotein can alter the structural and functional properties of the parent protein is an important one and may have broader implications in the larger field of protein structure−function relationships.

This investigation shows that the addition of a small amount of coprotein to maize zein produces an extended and stable network of β-sheet structures that, at least in the case of the tested HMW glutenin, conferred it similar viscoelastic properties to wheat gluten polymers. We believe that this is directly due to conformational change in the structure of zein, because the amount of increase in β-sheet observed is more than that which can be expected from the small amount of coprotein added. Thus, stable β-sheets in the polymer appear directly related to their viscoelastic properties and relaxation rate. Beyond providing a glutenlike protein for celiac patients, these findings suggest that maize, or its botanically related cereals sorghum and millet, ultimately may be developed to partially replace wheat. The obvious application of such technological development would be in tropical developing countries, particularly in Africa, that have considerable imports of wheat and could benefit from higher incorporation of local cereals in breads and other baked products.

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Notes

The authors declare no competing financial interest.

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