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Rheological Studies Utilizing Various Lots of Zein in N,N-Dimethylformamide Solutions

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Rheological studies were carried out on solutions of zein in *N,N*-dimethylformamide (DMF), where the specific lot of zein, concentration, time, and temperature were varied. DMF is a good solvent for zein, giving clear, relatively low viscosity solutions. It was found that all of the zein solutions behaved in a non-Newtonian fashion. At high concentration and elevated temperature, zein solutions will increase in viscosity with time. A temperature study on the rate of viscosity rise illustrated that at temperatures above 40 °C, the rate of viscosity rise increased in a non-Arrhenius fashion. There can be significant lot to lot variations in commercially obtained zein that gives rise to differences in viscosity and rate of viscosity rises. With the samples studied, viscosity was found to double from one lot of zein to another. Size exclusion chromatography suggests that compositional differences between the lots drive the observed differences in viscosity.

KEYWORDS: Zein; rheology; lot variation

INTRODUCTION

Zein, the main protein from corn, has the potential to be one of the main coproducts of the bioethanol industry. It represents approximately 25% of the nonstarch fraction of the kernel. Zein is currently not being isolated on a large scale and is instead a major component in corn gluten meal or distillers dried grains, both of which are used primarily as animal feeds. Historically, zein was commercially isolated and used in the fibers and coating industries. Chemical modification of the zein was required to reduce the impact of humidity on physical properties. This was accomplished predominantly through the use of formaldehyde (*1*, *2*). The use of zein in these markets was greatly reduced when petroleum-based products became more economical and provided improved overall properties.

With the growing need for products that are biodegradable and/or utilize renewable raw materials, developing methods to better utilize zein has become more important. Efforts are in progress to develop improved methods of isolating zein from either corn gluten meal or ground corn (*3*−*7*).

To deliver a product capable of meeting the needs of the market, chemical modifications of zein will be required. Commercial formaldehyde treatments require significant investment so that they can be performed safely. Alternative solution chemistries have been studied that utilize reagents that are not suspected carcinogens (*8*−*11*). Defining how a zein solution responds to shear and temperature is very important to develop a successful commercial process for modifying zein, where pumps and agitators need to be sized for the appropriate solution viscosity. If the pumps are not appropriately sized, the resulting pressure drop may be such that the desired flow rates may not be achievable. If the agitators and their drives are not of the appropriate size, the solution may not be adequately mixed so that inhomogeneities may result.

Rheological studies have been carried out on zein in ethanol (EtOH)−water solutions (*12*, *13*). On examining the primary structure of zein, the functional group with a reasonable reactivity and in the greatest abundance is the hydroxyl moiety of serine and threonine (*14*−*16*). Unfortunately, using EtOH−water as the solvent greatly limits the number of reagents that can be used to modify the hydroxyl groups of zein without reacting with the solvent. Efforts have been put forth in using other solvents, such as dimethylformamide (DMF), to dissolve the zein and other proteins for treatment with various chemical reagents (*8*, *11*, *17*−*19*).

While information is available concerning the structure of zein in various nonaqueous solvents including DMF (*20*, *21*), no rheological information is available for zein in DMF. As a part of optimizing the reactions of zein with desirable reagents in DMF, research was undertaken to define the rheological

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properties of zein in DMF at various temperatures and concentrations.

In addition, there have been no published studies to date examining the effects of zein lot to lot variability on properties where the zein was isolated utilizing the same procedures. One study of zein films has been performed where the zein was isolated using different methods, resulting in varying amounts of starch carryover, which impacted water vapor permeability (*22*). Given that variation of certain raw material properties will have a significant impact on final product properties, the rheological properties of multiple lots of zein were also examined.

MATERIALS AND METHODS

Raw Materials and Equipment. Three lots of F4000 zein from Freeman Industries LLC (Tuckahoe, NY) were used as received: F40003121C (89.0% protein, 3.0% moisture, 5.0% fat, 0.04% fiber, and 0.05% ash) herein designated as lot 3121, F40003111C (83.1% protein, 4.3% moisture, 7.1% fat, 0.42% fiber, and 1.6% ash) herein designated as lot 3111, and F40002371C (90.1% protein, 3.3% moisture, 1.9% fat, 0.1% fiber, and 1.5% ash) herein designated as lot 2371. Zein analyzes to measure % protein, % moisture, % fat, % fiber, and % ash were carried out by the University of Missouri, Experimental Station Chem. Lab (Columbia, MO). EtOH was used as received from Aaper Chemical Co. (Shelbyville, KY). DMF, ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA-Na_2), methanol, and acetic acid were used as received from Aldrich Chemical Co. (Milwaukee, WI). Coomassie Brilliant Blue, ethylenediamine tetraacetic acid, potassium chloride (KCl), sodium borate, *tert*-butanol, and the size exclusion chromatography M_w standards thyroglobulin (669 kDa), β-amylase (200 kDa), carbonic anhydrase (29 kDa), and α-lactalbumin (14.2 kDa) were obtained from Sigma (St. Louis, MO). Tris(hydroxymethyl)aminomethane (TRIS) was used as received from Fisher Chemicals (Fair Lawn, NJ) for the sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) experiments from Sigma. Dithiothreitol (DTT) was used as received from Molecular Probes (Eugene, OR). SDS was used as received from Bio-Rad Laboratories (Hercules, CA). 4-Morpholineethanesulfonic acid monohydrate (MES) was used as received from Acros Organics (Pittsburgh, PA). Acetonitrile was high-performance liquid chromatography (HPLC) grade and used as received from Burdick and Jackson (can be purchased through VWR, West Chester, PA). Ultrapure water was produced using a NANOpure system (Barnstead International, Dubuque, IA); all other water used was deionized water. Dialysis tubing was Spectra/Por 7 with an 8000 molecular mass cutoff. For SDS-PAGE electrophoresis, X-Cell Surelock Mini cell, NuPage 4−12% Bis-Tris gels, NuPage LDS sample buffer, and Mark 12 MW standard were obtained from Invitrogen (Carlsbad, CA). A scanner was used to record SDS-PAGE gels (Hewlet Packard HP psc 2175, Palo Alto, CA). Those materials requiring centrifugation were performed using a Beckman centrifuge J2-21M (Fullerton, CA) with a JA-10 rotor. A Brookfield DV-III+ rheometer (Middleboro, MA) using the SSA 18/13R spindle (1.77 cm diameter) and water-jacketed sample cup (1.88 cm diameter) was used to determine the solution's rheological properties. The temperature of the water being circulated through the jacket was controlled using a Brookfield TC-601 constant-temperature bath. Rheometer temperatures reported are those of the circulating water. HPLC separations were performed using an Agilent 1100 HPLC system with UV detection at 200 nm using a BioSep SEC-S3000 column (Phenomenex) with 50% acetonitrile/0.1% TFA as the mobile phase (isocratic conditions) with a column temperature maintained at 40 °C with a flow rate of 1.0 mL/min.

Defatting. To 200 g of zein was added 500 mL of ethyl ether. This mixture was stirred for 30 min at room temperature. The ethyl ether was decanted. This procedure was repeated two more times. The zein was allowed to air dry.

Deamidation. Zein was deamidated using a method previously described (*23*). To 15 g of NaOH was added 1500 mL of cold water (5 °C). Then, 100 g of lot 3121 zein was added to the chilled solution. The mixture was heated at 80 °C for 8 h. The solution was cooled, and the pH was adjusted to 7 using concentrated HCl. The zein mixture was centrifuged at 11325*g* for 20 min. The supernatant was recovered, and the pH was adjusted to 3 using concentrated HCl. The precipitate was collected and washed with 0.001 M HCl. The solid was dispersed into water using an overhead stirrer, and the pH was adjusted to 7.0 using aqueous 10% NaOH. After the solid dissolved, the resulting opaque solution was centrifuged at 11325*g* for 20 min during which an oily layer was formed. The mixture was carefully poured into a separatory funnel and left overnight. The water layer was recovered and dialyzed against deionized water. The material was then lyophilized to deliver 63 g of deamidated zein (42%) that was soluble in pH 7 water.

Viscosity Measurements. Concentrations reported are in % protein. Initial zein dissolution was done at room temperature for at least 10 min. For tests conducted at 25 °C, the solutions were allowed to equilibrate for 5 min while stirring at 1.32 cm^{-1}. Five 15% zein protein solutions were evaluated to obtain an estimate of error. It was found that the standard deviation for the viscosity measurement was 0.0016 Pa s at a shear rate of 13.2 s^{-1} with a mean viscosity of 0.0350 Pa s. This amount of variation is representative for other shear rates and samples. For solutions with a concentration of more than 20%, the solutions were tested within 15 min after preparation. Solutions tested at 10, 15, and 40 °C were allowed to equilibrate for 15 min at 1.32 cm^{-1}. A different sample was used for each temperature. Viscosity measurements were taken at the desired shear rate when the reading had stabilized. If desired, the shear rate was then increased by the desired amount and the process was repeated until either the highest desired shear rate was reached or the motor reached its maximum torque. When it was desired to monitor the change in viscosity with time at temperatures of 25, 40, 48, and 55 °C, the shear rate was set to 2.64 cm^{-1}, and viscosity readings were taken at various time intervals without an equilibration time.

SDS-PAGE Procedure. DMF was added to zein to give a 2% protein solution. To 15 μL of this solution was added 10 μL of 50 mM DTT, 25 μL of NuPage LDS sample buffer, and 50 μL of water. This solution was then placed in boiling water for 5 min. Into the wells, 10 μL of each sample was added and the Mini cell was filled with running buffer (9.76 g of MES, 6.0 g of Tris, 1.0 g of SDS, and 0.3 g of EDTA-Na_2 in 1 L of water). The amperage was set to 100 mA by adjusting voltage. Gels were run until the dye front reached the desired location. Gels were then rinsed with ultrapure water and stained with Coomassie Brilliant Blue solution (1 g of Coomassie Brilliant Blue, 500 mL of methanol, 120 mL of acetic acid, and 380 mL of water) for 1 h. The gel was destained (using solution of 180 mL of EtOH, 80 mL of acetic acid, and 740 mL of ultrapure water) overnight and then scanned.

Size Exclusion Chromatograph (SEC) Procedure. Zeins were weighed out to contain 2 mg of protein (masses adjusted for the protein content of each sample). Samples were first extracteded with 50 mM Tris-HCl (pH 7.8) + 100 mM KCl and 5 mM EDTA two times for 5 min each followed by a 5 min extraction with deionized water to remove water and salt soluble proteins or contaminants. Zeins were then resuspended in 60% *tert*-butanol + 12.5 mM sodium borate buffer (pH 10) and then submitted to SEC using 50% aqueous acetonitrile and 0.1% trifluoroacetic acid mobile phases. Samples were filtered prior to injection. For determination of M_n and M_w, Polymer Laboratories' PL Caliber Explorer software was used to convert the SEC chromatograms to a file format readable in PL Caliber version 7.04. Chromatogram baselines were manually created, and individual peaks were isolated for molecular mass calculations based on a multilinear calibration equation developed by using four molecular mass standards dissolved directly in the SEC mobile phase with known molecular masses: 669 kDa (5.15 min), 200 kDa (5.95 min), 29 kDa (7.50 min), and 14 kDa (9.85 min).

RESULTS AND DISCUSSION

The typical variables for a rheological investigation are shear rate, concentration, temperature, and time. For most synthetic polymers, the primary backbone composition of the polymer is well-controlled. Molecular mass control, branching, and impurities are typically the attributes that lead to variations in the

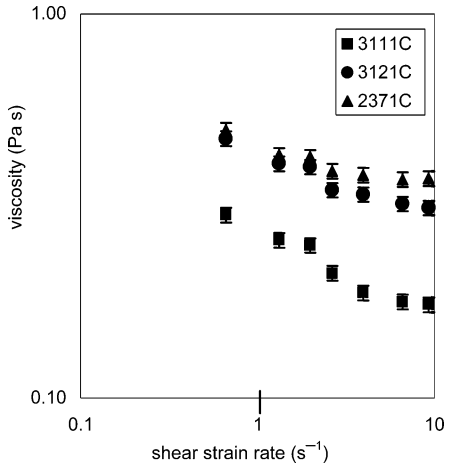

Figure 1. Viscosity curves on log–log axes for zein samples in DMF at 25% protein and 25 °C at various shear rates.

Table 1. Consistency (K, Pa s^m) and Shear Thinning Index (m) Values for 10–25% Zein Protein Solutions at 25 °C and Shear Rates between 0.66 and 132 s^{-1} [a]

protein	lot 3121		lot 3111		lot 2371	
concn (%)	K	m	K	m	K	m
10	0.015	0.848	0.011	0.877	0.015	0.890
15	0.039	0.879	0.027	0.903	0.039	0.936
20	0.113	0.892	0.063	0.959	0.096	0.971
25	0.432	0.840	0.273	0.779	0.454	0.891

[a] K values are ±0.001.

polymer's rheology and give rise to differences in polymer processing or properties. For polymers based on agricultural proteinaceous materials, there can be significant variation in the primary backbone composition due to a number of variables that were present in the environment during plant growth. These could include variables such as the corn hybrid, weather conditions, and method of zein isolation. In addition to these primary differences, the rheological properties of the renewable materials would also be dependent on the degree of branching and impurities. Given this, it is more important than in synthetic polymers to study the rheology of different samples of zein.

Effect of Shear Rate. Figure 1 shows the viscosity of three lots of zein at 25% protein in DMF vs shear rate at 25 °C. It can be seen that lots 3121 and 2371 zein are more similar than lot 3111. The lot 3111 zein had a much lower viscosity at this and all other concentrations. The viscosity of the solutions is not constant with shear rate, and thus, they are by definition a non-Newtonian weakly shear thinning solution (*24*). The same trend is observed at other concentrations (data not shown). The shear rates were not run low enough to measure the zero-shear viscosity. Given that the solutions are shear thinning, their viscosities can be modeled using the Ostwald-de Waele model $\eta = K\gamma^{m-1}$ where η is the apparent viscosity (Pa s), K is the consistency index (Pa s^m), γ is the shear rate (s^{m-1}), and m is the shear thinning index. For solutions of zein, the m and K values are detailed in **Table 1**. Given that the shear thinning index numbers are slightly less than 1, these zein solutions are mildly shear thinning. It can be seen that the K indexes of lots 2371 and 3121 are higher than those of lot 3111.

Effect of Concentration. Shown in **Figure 2** is the relationship between % protein and viscosity for the zein samples. As with most polymers, viscosity increases in a power fashion with polymer concentration (*12*). The relationship between % protein and viscosity at 25 °C for the three lots of zein is similar with

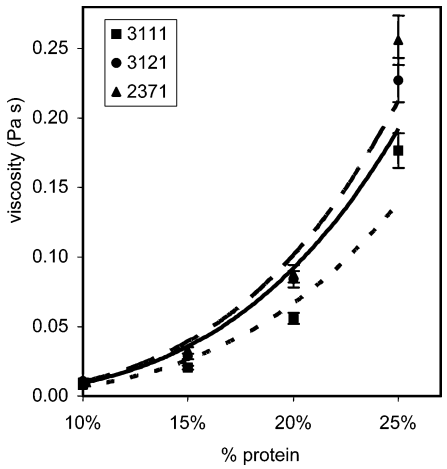

Figure 2. Viscosity vs % protein for zeins at 25 °C in DMF at 13.2 s^{-1}. Equations for fitted lines are as follows: lot 3111 (dotted line) is $y = 13.40$ (% $\text{protein}^{3.29}$) and $R^2 = 0.97$; lot 3121 (solid line) is $y = 18.62$ (% $\text{protein}^{3.30}$) and $R^2 = 0.98$; and lot 2371 (dashed line) is $y = 20.98$ (% $\text{protein}^{3.31}$) and $R^2 = 0.97$.

the exponents for all three fitted curves being approximately 3.3. The main difference is in the preexponential. The results for lot 2371 are similar to those of lot 3121 zein. The consistency indexes, K (**Table 1**), for the solutions increases in an exponential fashion as the protein concentration is increased. While the power to which the % protein is raised is very similar for the three zein lots, the consistency factor for lot 3111 is ~35% lower than the other zeins demonstrating that lot 3111 has a lower viscosity at all concentrations. As the protein concentration is increased from 10 to 20%, the shear thinning index is also increasing. At 25%, this trend does not continue. Work is in progress to better understand zein solutions at higher concentrations. It is well-known that as the concentration increases, gel forms more quickly, and it could be that those processes that are occurring at this concentration and these may give rise to higher viscosity and make the solution more shear thinning.

The main component in zein that would contribute to viscosity would be the protein. As detailed above, there are significant differences in protein concentration between the three lots of zein (protein contents for lots 3121, 3111, and 2371 of zeins were, respectively, 89.0, 83.1, and 90.1%). If % solids were used as the metric for concentration, then the amount of protein could be off by almost 10%, which would give significantly different viscosities at equivalent solids.

It has been suggested that zein concentration in solution can be determined from measuring only the viscosity at a given shear rate after developing the relationship between protein concentration and viscosity (*12*). When working with a single lot of zein, this approach will work. However, if different lots of zein are used, measurement of viscosity is not sufficient to predict protein concentration.

Effect of Temperature. When 25% protein solutions were produced using these lots and the viscosity was measured at different temperatures, it was found that the viscosity differences were magnified at lower temperatures (**Table 2**). This concentration was used so as to generate easily measured viscosity values at all temperatures tested. It was not possible to accurately measure the viscosity of zein solutions at 25% protein content and at temperatures of 48 °C and higher as the viscosity increased during the equilibration period. Once again, the viscosities of lot 3121 and lot 2371 were more similar than that of lot 3111.

Table 2. Viscosity (Pa s) of 25% Protein DMF Solutions at 2.64 s^{-1} at Various Temperatures

	°C			
lot	10	15	25	40
3121	0.975	0.611	0.308	0.204
3111	0.366	0.291	0.212	0.120
2371	0.953	0.678	0.333	0.177

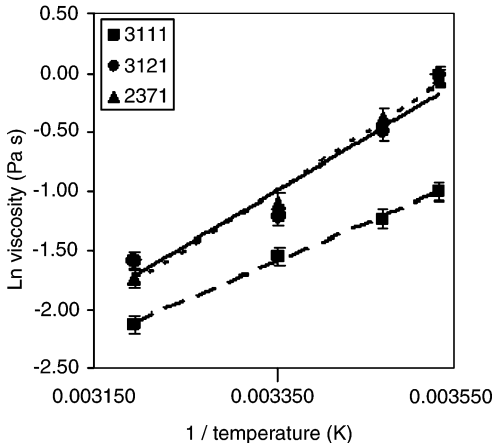

Figure 3. Viscosity vs temperature for 25% zein DMF solutions after 15 min at specific temperatures at 2.64 s^{-1}. Equations for fitted lines are as follows: lot 3121 (solid line) is $y = 4575$ (1/K) − 16.3 and $R^2 = 0.94$; lot 2371 (dotted line) is $y = 5010$ (1/K) − 17.8 and $R^2 = 0.99$; and lot 3111 (dashed line) is $y = 3240$ (1/K) − 12.5 and $R^2 = 0.99$.

Table 3. Viscosity (Pa s) at 13.2 s^{-1} for 15% Lot 3121 Zein in DMF Stored at Room Temperature for Various Time Periods

	time (h)				
lot	0	24	48	72	144
2371	0.0033	0.0033	0.0033	0.0033	0.0034
3111	0.002	0.002	0.002	0.002	0.0022
3121	0.0028	0.004	0.0047	0.0047	0.0056

By plotting the natural logarithm of the viscosity data detailed in **Table 2** vs reciprocal temperature (K), the E_{act} of flow will be the slope of the line after multiplying by negative 1, the gas constant, and correcting for the units (**Figure 3**) (*25*, *26*). When this is performed, the resulting E_{act} values of flow for the three zein samples are as follows: Lot 3121 is 38.0 kJ/mol, lot 2371 is 41.7 kJ/mol, and lot 3111 is 26.9 kJ/mol. Clearly, lot 3111 zein is different than the other two materials. Previous work in a 60% EtOH−water solution gave an E_{act} of flow of 22.1 kJ/mol for a 12% zein solids solution (*12*).

Effect of Time. It has been shown previously that at higher zein concentrations, zein solutions will increase in viscosity or gel (*27*−*30*). To gain a better understanding of how aging impacts viscosity, a lower concentration of zein was selected. When the viscosity of zein solutions using these lots at 15% protein was monitored with time and no shear at room temperature, the viscosities of lots 2371 and 3111 were found to be much more stable than lot 3121 zein (**Table 3**). The viscosity of the 15% lot 3121 zein solution grew from 0.0028 to 0.0056 Pa s after 144 h. Over the same time period, the viscosity of the other zein samples did not change. While stable polymer viscosity solutions have certain commercial processing advantages, if a polymer solution is unstable, knowing the rate of its viscosity rise can be useful in developing strategies to improve processing. At higher concentrations, all of the zein samples will gel if allowed to stand long enough at room temperature.

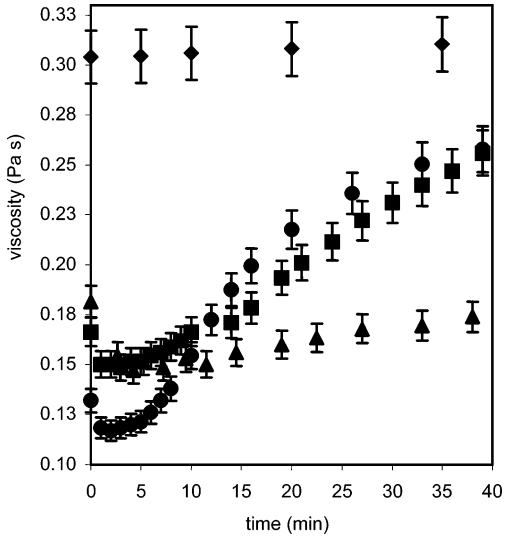

Figure 4. Viscosity of 25% lot 3121 zein in DMF solutions at 2.64 s^{-1} measured at various times at different temperatures: ◆, 25 °C; ▲, 40 °C; ■, 48 °C; and ●, 55 °C.

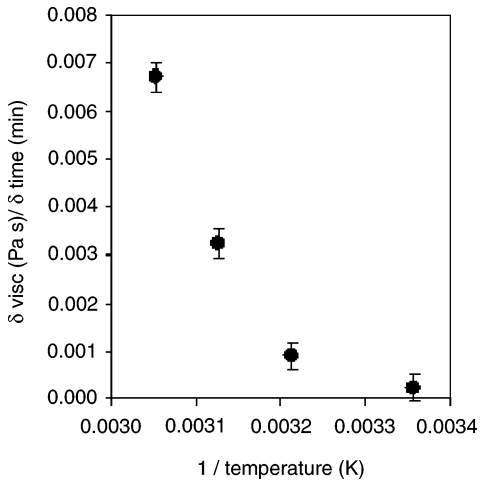

Figure 5. Plot of rate of viscosity increase for 25% lot 3121 zein in DMF solutions vs reciprocal temperature (K).

Shown in **Figure 4** is the relationship between viscosity and time as the solution was being sheared at various temperatures. Each trace represents a new sample being sheared at the indicated temperature, with the viscosity being recorded at various times. It can be seen that over the time period studied that only small changes in viscosity were seen at 25 °C. At 40 °C and higher temperatures, there was initially a rapid decrease in viscosity as the sample warmed to the desired temperature. At 40 °C, there was a moderate increase in viscosity with time. At 48 and 55 °C after the initial decrease in viscosity, the rate of viscosity rise was significantly higher than that observed at 40 °C. If the rate of viscosity rise of the linear portions of these curves is plotted vs reciprocal temperature (K), a straight line with good correlation cannot be obtained. An Arrhenius relationship is not present (**Figure 5**). A possible reason for the increased rate of viscosity rise taking place above 40 °C includes the presence of an additional reaction pathway (such as denaturation followed by either aggregation or disulfide branching) taking place at higher temperatures. Another possibility is that with the reduction in viscosity that takes place at

elevated temperatures, the reaction path that leads to elevated viscosity at lower temperatures is greatly increased in the lower viscosity present at elevated temperatures. Additional work is in progress to define the dominant mechanism for viscosity growth at different temperatures.

Impact of Zein Molecular Mass Distribution. When examined individually, each of the zein samples gave solutions that behaved in typical polymer fashion. Their viscosities increased in a power law fashion with concentration and decreased exponentially with increased temperature. The solutions were shear thinning. To develop a successful commercial operation, it is important that variation of key raw material attributes be either eliminated or reduced. If this is not possible, then control strategies need to be developed to accommodate the variations. Zein lot 3121 had a relatively high viscosity that changed significantly with time at room temperature (RT). Zein lot 3111 had relatively low viscosity that did not change significantly with time at RT. Zein lot 2371 had a relatively high viscosity that did not change significantly with time at RT. Various tests were performed on the zein to attempt to find the key attribute(s) that need to be monitored that drives these rheological properties.

On examining the composition of the three lots as analyzed by an external lab, one of the large differences observed was the fat content of the three lots. The oil content of the lot 3111 was significantly higher than that of the other two materials. This oil could act as a lubricant, which could reduce the imposed shear on the solution and reduce the observed viscosity. To determine if this contributes to the observed differences in viscosity, lot 3111 was extracted with ethyl ether to remove residual fat (*31*). After extraction, a 25% protein solution was prepared and its viscosity was measured at 40 °C and 2.64 s^{-1}. The viscosity of defatted lot 3111 zein was 0.114 Pa s. Untreated lot 3111 zein had a viscosity of 0.120 Pa s at these same conditions. This suggests that fat content does not significantly contribute to the apparent differences in solution viscosity.

The published procedures for commercial production of zein involve heating a corn gluten meal slurry at relatively high pH (*31*−*33*). Some of the glutamines or asparagines could be hydrolyzed under these conditions. In addition, some of the primary structure may be lost as well. To determine if small amounts of deamidation or hydrolysis play a role in the observed viscosity differences, some of the standard lot 3121 zein was replaced with deamidated lot 3121 zein. It was understood that adding a separately treated zein to standard zein may give somewhat different results than utilizing a zein that has been processed differently. However, we believe that this test would provide some insight into whether deamidated or hydrolyzed product plays a large role in the viscosity differences observed. A 25% protein solution utilizing lot 3121 zein was made where 10% of the lot 3121 zein was replaced with deamidated lot 3121 zein (*23*). At 25 °C and 6.16 s^{-1}, the viscosity of this solution was 0.218 Pa s. The viscosity of the standard lot 3121 solution at these conditions was 0.204 Pa s. This result suggests that mild amounts of hydrolysis are not responsible for the differences in viscosity.

Zein is a complex mix of proteins with the main fractions designated as α, β, and γ, which are located in protein bodies dispersed in the endosperm of the kernel (*28*). The dominant zein fraction present in corn is α zein. The α zein has one or two cysteines, which could give either dimerization or chain extension (*14*−*16*). The β and γ zeins are believed to exist in the perimeter of the protein bodies and are present in smaller amounts. The β and γ zeins contain many cysteine residues

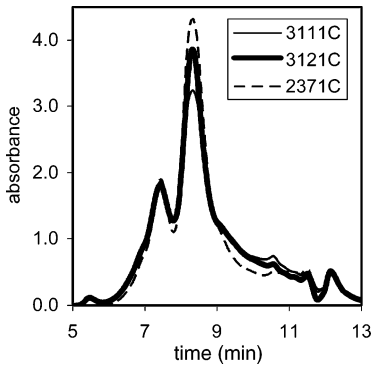

Figure 6. SEC analysis of zein lots. Traces were normalized so that the areas under the curves are the same. Lot 3121 was the only material that had material eluting from the column in less than 6 min.

(*16*, *34*−*36*). Variations in the amount of the β and γ zeins, which can readily cross-link, could have a large impact on the rheology of fresh and aged zein solutions.

SDS-PAGE experiments were carried out in the presence and absence of DTT. In this fashion, the amount of disulfide bonds can be assessed. SDS-PAGE results obtained without the use of DTT suggested that there was slightly more high molecular mass material in lot 3121. There is very little difference between lot 3111 and lot 2371 in this analysis. Treatment with DTT followed by SDS-PAGE reduced the difference between the three lots of zein and eliminated the bands above 45000 Da. This suggests that the proteins with molecular masses more than 45000 were mainly disulfide bridged proteins.

On the basis of the SDS-PAGE results, additional analytical techniques were employed that are more quantitative. Capillary electrophoresis and reverse phase HPLC did not show significant differences between the zein lots. SEC of the zein lots as received illustrated some important differences. Shown in **Figure 6** are the SEC traces for the three zeins, where the traces were normalized so that the areas under the curves are equal. Lot 3121 had an overall M_n of 23.4 kDa and an M_w of 44.5 kDa giving a dispersity of 1.90. Lot 3121 also had a high molecular mass fraction making up approximately 1% of the total mass (based on relative absorbance), which had an M_n in excess of 1000 kDa. This high molecular mass material of lot 3121 may be due to significant amounts of chain extension and branching. Lot 3111 had an overall M_n of 23.0 kDa and an M_w of 34.1 kDa giving a dispersity of 1.49. Lot 2371 had an overall M_n of 23.4 kDa and an M_w of 30.8 kDa giving a dispersity of 1.31. Lot 2371 had a narrower molecular mass distribution relative to either lot 3121 or lot 3111. The high molecular mass fraction in the lot 3121 would increase its initial viscosity, and its presence could modify the viscosity stability of this zein. Lot 3111 had the highest amount of low molecular mass material; this would contribute to some of the rheological differences. The narrow molecular mass distribution of lot 2371 will also contribute to the observed differences in rheology. Work is in progress to understand if the differences in molecular mass are due to how the zein was processed or if they arose due to differences in the feedstock.

In conclusion, zein gives shear thinning solutions in DMF. The viscosity of these solutions increases exponentially with increased protein concentration and decreases exponentially with increased temperature. The rate at which the viscosity increases with elevated temperatures, when plotted in an Arrhenius fashion, does not give a straight line. Thus, the dominant mechanism for viscosity rise changes above a certain temperature. There are significant lot to lot variations in zein. These

differences are seen in as-is viscosity as well as how the viscosity changes with time. These differences do not appear to be due to other components present in the zein or to various amounts of deamidated protein. Through SDS-PAGE and SEC, it was found that there were differences in molecular mass distribution. SEC was able to better quantify the data and illustrated that one of the lots had a high molecular mass fraction, another had a narrower distribution, and a third had more low molecular mass material. With these differences in composition, it is to be expected that the rheology of these lots of zein would be dissimilar. Additional work is required to understand how these differences arose and how to modify the zein or develop strategies to control rheology.

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