Impact of Thiocyanate Salts on Physical, Thermal, and Rheological Properties of Zein Films

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ABSTRACT

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A new class of zein additives was investigated, thiocyanate salts. Ammonium, potassium, guanidine (GTC), and magnesium thiocyanate salts were added to solutions of zein in with various amounts of tri(ethylene glycol) (TEG), cast as films, and then tested to determine the impact that each salt had on properties. The presence of these salts affected solution rheology and intrinsic viscosity, demonstrating that the salts interacted with the protein. It was found that these salts acted as plasticizers, as they lowered the glass transition temperature of zein when evaluated with differential scanning calorimetry. In zein films in which TEG was present, these salts increased elongation and reduced tensile strength. However, unlike traditional plasticizers (such as TEG), when the salts were used as the only additive, elongation was not increased and tensile strength was not decreased. Of the salts tested, GTC in combination with TEG was found to increase elongation the most. The impact of salts on elongation was greatly affected by the relative humidity in which the samples were stored.

With the production of ethanol from corn, whether production is done with dry- or wet-milling processes, a great deal of proteinaceous coproduct is generated (Lawton 2002). The dominant protein present in the dry- or wet-milling coproduct is zein. Developing improved end uses for zein will improve the economics of the ethanol production industry. In the early 1930s, zein was used in the textile fiber market as a wool alternative (Swallen 1939; Croston et al 1945; Evans and Croston 1949). For use in this market, the zein was treated with formaldehyde to impart improved physical properties and solvent durability. With the introduction of textile fibers based on petroleum feeds, the use of zein in the textile fiber market disappeared. To replace products that use nonrenewable materials, zein products need improved elongation and improved solvent resistance. Various nonformaldehyde routes have been investigated to improve the solvent resistance of zein, including glyoxal (Selling et al 2012), glutaraldehyde (Sessa et al 2008), multivalent (e.g., citric) acids (Reddy et al 2009; Jiang and Yang 2011), and diisocyanates (Wu et al 2003; Yao et al 2007). These reagents typically lead to branching and/or cross-linking and lower elongation. Techniques have been developed to improve the elongation of zein, albeit with reduced tensile strength, but these techniques typically involve the use of plasticizers such as diols, triols, and long-chain carboxylic acids (Padua and Santosa 1999; Paramawati et al 2001; Lawton 2004; Gao et al 2006; Ghanbarzadeh et al 2007; Gillgren and Stading 2008; Gillgren et al 2009; Wu et al 2010). These additives suffer from moisture sensitivity, migration, and loss of plasticizer resulting from deposit formation or volatility. There is a need for developing additional methods to improve the elongation of zein.

The Hofmeister series (abbreviated listing in Fig. 1) is a set of guidelines that can be used to determine if a particular salt will increase protein solubility in water (salt in, chaotropes, to the right in Fig. 1) or cause a protein to decrease in solubility in water (salt out, kosmotropes, to the left in Fig. 1) (Whitford 2005; Cho et al 2008; Rembert et al 2012).

Inorganic salts are known to interact with proteins, altering the protein structure or changing their solubility characteristics (Baldwin 1996). Salts such as calcium chloride and sulfate have been shown to alter the physical properties of soy protein films through the cation by apparently forming calcium bridges between proteins (Park et al 2001). However, calcium cations and chloride anions are known to increase its solubility, whereas sulfate anions will decrease its solubility. Sodium lactate was shown to reduce tensile strength and increase elongation of whey protein films (Zinoviadou et al 2010); however, in that study only the one salt was evaluated, and both the sodium and lactate ions would be in the middle of the Hofmeister series in terms of their impact on a protein. Salts have also been used to modify the properties of zein. Alkali salts typically having long-chain fatty acid anions have been shown to alter the solubility of zein (James 1945). However, these long-chain fatty acids have been shown to have compatibility issues with zein (Santosa and Padua 2000). Other researchers have used salts such as sodium chloride, sodium sulfate, zinc sulfate, ammonium sulfate, ammonium phosphate, and so on to modify the properties of zein cross-linked with formaldehyde (Cline 1949; Croston and Evans 1949; Jenkins and Magee 1958). In these studies, the impact of salts was not detailed in a controlled fashion, nor were the singular impacts of the salts on zein properties determined. Ammonium thiocyanate (ATC) has been used in conjunction with *p*-toluenesulfonic acid, monochloroacetic acid, and formaldehyde to provide durable zein fibers (Huppert 1946). It was proposed in a patent by Huppert (1946) that the purpose of the ATC was to convert the globular zein protein into a fibrous structure; however, no data was provided to support this claim. A systematic study with salts that fit the series has not been undertaken.

The effect that Hofmeister salts have on proteins has almost exclusively been studied in water. With zein not being soluble in water, it is not clear what effect these salts will have on zein. The salts in this series have been evaluated in conjunction with polymers other than proteins (Heuvingh et al 2005), albeit in water solution. Thiocyanate salts have been used in zein systems (Huppert 1946), and based on the Hofmeister series, the thiocya-

$$F^- \approx SO_4^{-2} > \text{acetate} > NO_3^- > Br^- > SCN^-$$

$$NH_4^+ > K^+ > Li^+ > Mg^{+2} > \text{guanidinium}$$

Fig. 1. Abbreviated Hofmeister series.

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nate anion is among the strongest anions for impacting the properties of a protein in water. Although typically the anion has a larger effect on protein solubility in water systems (Rembert et al 2012), the cation, as detailed earlier involving calcium ions, can play a role as well. In addition, many of these protein–salt studies were performed in solution; it is not clear whether the cation or anion will have a larger effect on a protein in the solid state. Select thiocyanate salts can be found that are soluble in 90% ethanol/water. If thiocyanate changes the structure of a protein while in solution, then the structure that the protein has while drying may be different relative to the control and perhaps give increased elongation. Although the cation may have a lesser role on protein properties per the Hofmeister series, it is not clear how cation selection will impact zein properties. To define the impact that thiocyanate salts have on zein, various thiocyanate salts were incorporated into zein solutions. Cations were selected to provide solubility in the solvent used, as well as to allow the investigation of protein–cation interactions according to the Hofmeister series. Investigations in which the anion is varied are in progress and will be reported at a later date. The zein thiocyanate salt formulations had some of their solution properties characterized, and then films were cast, dried, and characterized.

MATERIALS AND METHODS

Materials and Equipment

Zein was obtained from Freeman Industries (Tuckahoe, NY, U.S.A.), lot #40006124 (14.4% nitrogen, 4.8% moisture). Ethyl alcohol (EtOH, Sigma-Aldrich, Saint Louis, MO, U.S.A.) and deionized water were used to generate the solvent in which the zein and salts were dissolved. Tri(ethylene glycol) (TEG) was used as received from Sigma-Aldrich. Ammonium (ATC), potassium (KTC), guanidine (GTC), and magnesium thiocyanate (MTC) salts were obtained from J. T. Baker Chemical (Austin, TX, U.S.A.), Sigma-Aldrich, Sigma-Aldrich, and Pfaltz and Bauer (Waterbury, CT, U.S.A.), respectively. Sample thicknesses of cast films were measured at five different locations with a micrometer (model 49-63, Testing Machines, Amityville, NY, U.S.A.). Constant relative humidity (50 and 70% rh) at 23°C was obtained by storing samples in a constant temperature and humidity room. Constant relative humidity of 15% rh at 23°C was obtained with a saturated lithium chloride solution and a desiccator stored in the constant temperature room. Moisture contents were measured with an Ohaus MR45 moisture analyzer at 105°C for 20 min on 1 g of sample. Tensile strength (MPa, TS), Young's modulus (MPa, YM), elongation to break (%Elo), and toughness (MPa) were evaluated for each sample with an Instron universal testing machine model 4201 and an ASTM D638 type V test. Physical properties were determined with a crosshead speed of 10 mm/min, a gauge length of 7.62 mm, and either a 1 kN load cell or a 100 N load cell, depending on the strength of the samples. Toughness was determined by dividing the energy to break by the volume of the sample (gauge length × width × depth). Toughness would be analogous to the area underneath the stress–strain curve. NMR spectra were run on a Bruker 500 MHz NMR (Billerica, MA, U.S.A.) in 90% ethanol-d_6/10% deuterium oxide (Cambridge Isotope, Andover, MA, U.S.A.). Differential scanning calorimetry (DSC) was performed to determine the glass transition temperatures of zein control films, zein/TEG control films, and zein/TEG/thiocyanate salt films using a TA Instruments model Q2000 DSC (New Castle, DE, U.S.A.). Solution rheology experiments were done on a TA Instruments ARES LS-1 controlled strain rheometer. Dynamic mechanical analyses (DMA) were performed with a TA Instruments ARES LS-2 controlled strain rheometer.

Solution Preparation

To 27 g of 90% EtOH/10% water (m/m; hereafter 90% EtOH/water) was added the TEG. After 2 min of stirring, the zein was added, and then the solution was stirred for 1 hr. For example, if the sample was to be composed of zein having 17.5% TEG, then 1.86 g of TEG would be added to 27 g of 90% EtOH/water, and then 8.75 g of zein would be added. For salt formulations after the addition of TEG and the 2 min of stirring, the salt was added. After the salt had completely dissolved, the zein was added to the solution, followed by stirring for 1 hr. For example, if the sample was to be composed of zein having 17.5% TEG and 8% ATC, then 2.06 g of TEG was added to 27 g of 90% EtOH/water, and then 0.94 g of ATC was added. After dissolution, 8.75 g of zein was added. For one formulation (sample 8-ATC-17.5 31.3%), which was used for rheological testing only, the amount of 90% EtOH/water used was 25.80 g so as to increase the relative amount of zein for solution rheological testing. The zein solutions were then degassed under vacuum, poured into a gasket mold, and allowed to dry in a chemical hood for a week. The mold was a rubber gasket having an opening in the center with dimensions of approximately 13 × 26 cm that was placed on a Teflon-coated glass plate. After three days, the rubber gasket was removed, and the film was carefully lifted up and turned over to help the film to dry completely.

Sample Evaluation

Tensile property samples were stored at 23°C and 15, 50, and 70% rh for five days before testing physical properties. TS, YM, and %Elo data are presented as the calculated means and standard deviations for a minimum of five samples. The model that was used to predict TS versus total plasticizer present was developed on Minitab 15 (Minitab, State College, PA, U.S.A.). The ^{1}H NMR spectra were obtained after dissolution in 90% ethanol-d_6/10% deuterium oxide at room temperature with and without the addition of TEG. Intrinsic viscosity (η) measurements were made with a Schott AVS 360 injection pump, CT 52 water bath (set to 30°C), Dilut4 controlling software, and Schott 53110 capillary viscometer tube (Schott Instruments, College Station, TX, U.S.A.). Intrinsic viscosity solutions were prepared as follows: to 30 g of sample was added 100 mL of 90% EtOH/water; it was then stirred for 1 hr at room temperature and filtered through a Whatman nylon 0.45 micron syringe filter. A sample of filtered solution was used to determine concentration by drying overnight at room temperature and then in an oven at 120°C overnight. Filtered solution (15 mL) was used in the intrinsic viscosity instrument, for which five time measurements were made on each solution. Five dilutions were then automatically made (addition of 5, 5, 5, 7, and 9 mL), for which a time measurement was made after addition of each aliquot of 90% EtOH/water. From the intrinsic viscosity values, the radius of gyration and mean end-to-end distance could be calculated (Seymour and Carraher 1992). The DSC scans were conducted on samples that had been stored at 50% rh for five days to minimize the effects of moisture. The samples were run through two cycles on the DSC: first from –88 to 160°C, then back to –88°C, and finally up to 270°C. The DSC traces reported were from the second heating cycle. Solution rheology studies in which the strain rate was varied from 1 to 100 sec^{-1} were averaged to obtain the viscosity of these Newtonian zein solutions. The geometry used for these viscosity measurements was a 50 mm titanium cone and plate with a 0.046 mm gap and an angle of 0.0401 rad. The temperature was controlled via a Peltier plate at a temperature of 25 ± 0.1°C, and a humidity cover was used to prevent solution evaporation. Zein films with dimensions of 0.3–0.4 mm thickness, 5–10 mm width, and 10–15 mm length, depending on the zein films provided, were submitted for DMA analyses. Dynamic temperature ramps were run on these films from 25 to 200°C at 1°C/min with a strain of 0.5% and a frequency of 1 rad/sec. A constant normal force of 0 ± 10 g was placed on the sample as it was heated; in this fashion, if the sample expanded or contracted during the analysis, it could be monitored. Some samples when heated to temperatures above 140°C would fail before reaching final temperature (200°C).

RESULTS AND DISCUSSION

Shown in Table I are the zein formulations tested in this work. Included is a column detailing the total amount of plasticizer (in mol/kg of zein) present in the sample, which makes it easier for comparisons between samples. Based on DSC and DMA analysis discussed later, the thiocyanate salts were found to be plasticizers for zein. To make a good comparison on the effectiveness of these thiocyanate salts to plasticize zein, a known zein plasticizer, TEG (Lawton 2004; Selling et al 2004), was used as an additional control. Various amounts of TEG were used to establish trends. These were coded as samples C0–C30, for which the C represents control and the number represents the amount of TEG (in % of the amount of zein) used in that sample. For example, formulation C17.5 was a sample made from zein and sufficient TEG to give 17.5% in the sample. Five levels of ATC were evaluated with 17.5% TEG used as a coplasticizer. In addition, one level of ATC, 4%, was evaluated with various amounts of TEG to determine if synergism was present. For example, formulation 4-ATC-17.5 was a sample made from zein, ATC, and TEG to give a sample having 4% ATC and 17.5% TEG. Additional thiocyanate salts, KTC (K^+ is kosmotropic), GTC (guanidinium ion is very chaotropic), and MTC (Mg^{+2} is chaotropic), were evaluated at single levels of TEG. When evaluating MTC it was understood that in addition to it having a different cation, the cation also had a charge of +2. Also, because of the rather limited number of volatile solvents that zein is soluble in, the choice of potential salts was also somewhat limited, as salts could only be chosen if they were soluble in the same solvent used to dissolve the protein zein. All films were of good quality with no obvious defects.

Solution Rheology and Intrinsic Viscosity

A brief study was undertaken to determine if these types of salts had a large impact on the structure of zein in solution, as determined with rheological techniques. It has been shown previously that the addition of salts can affect the solution rheological properties of polyamides (Shashkina et al 2005), and it was of interest to determine if the thiocyanate salts being studied here affected the rheology of zein in solution. Three solutions were prepared, C17.5, 8-ATC-17.5, and 8-ATC-17.5 31.3%. In sample 8-ATC-17.5 31.3%, the total solvent was adjusted so that the overall protein % solids was the same as the control. This was done because the salt being added may act as a diluent, and if the viscosity was reduced (with the addition of 8% ATC, the % protein solids was 22.6 instead of 23.3%), it may have been because the amount of protein was reduced. Shown in Figure 2 is the viscosity of these solutions versus shear rate. With the addition of the salt, there was a modest reduction in viscosity. This reduction was still observed when the % protein solids of the solution was increased to match that of the control. This observation suggested that there was an interaction between the protein and the salt.

With the observed differences in solution rheology, intrinsic viscosity studies were also undertaken. To perform intrinsic viscosity, it is important that the overall % solids be known. This value is typically determined with oven drying. Given that TEG is somewhat volatile, it was not used in this study, as it would introduce error in the solids measurements. The intrinsic viscosity of zein in 90% EtOH/water was determined to be 0.147 dL/g (standard deviation = 0.007 dL/g). For the sample having 8% ATC, the intrinsic viscosity was 0.168 dL/g (standard deviation 0.011 dL/g). These results suggested that in the presence of ATC, the zein protein opened up, leading to a higher mean end-to-end distance and a higher radius of gyration. The mean end-to-end distance and radius of gyration were, respectively, 115.5 and 47.1 Å for the control and 120.7 and 49.3 Å for the sample having 8% ATC. Both the intrinsic viscosity and solution rheology results suggested that ATC interacted with the zein protein.

^{1}H NMR

Although infrared spectroscopy (IR) can be used to discern changes in protein structure, for the films made here, spectral changes (beyond addition of the S=C=N stretch in the IR spectra with added ATC) were not observed with the addition of the thio-

TABLE I
Zein–Thiocyanate Salt Formulations

Formulation	Zein (g)	% TEG	TEG (g)	TEG (mol/kg of zein)	Salt	% Salt	Salt (g)	Salt (mol/kg of zein)	Total Plasticizer (mol/kg of zein)
C0	8.75	0.0	0.00	0.00	…	…	…	…	0.00
C5	8.75	5.0	0.46	0.35	…	…	…	…	0.35
C10	8.75	10.0	0.97	0.74	…	…	…	…	0.74
C13	8.75	13.0	1.31	1.00	…	…	…	…	1.00
C15	8.75	15.0	1.54	1.18	…	…	…	…	1.18
C17.5	8.75	17.5	1.86	1.41	…	…	…	…	1.41
C20	8.75	20.0	2.19	1.66	…	…	…	…	1.66
C25	8.75	25.0	2.92	2.22	…	…	…	…	2.22
C30	8.75	30.0	3.75	2.85	…	…	…	…	2.85
0.5-ATC-17.5	8.75	17.5	1.87	1.42	ATC	0.5	0.05	0.08	1.50
1-ATC-17.5	8.75	17.5	1.88	1.43	ATC	1.0	0.11	0.16	1.59
2-ATC-17.5	8.75	17.5	1.90	1.45	ATC	2.0	0.22	0.33	1.77
4-ATC-17.5	8.75	17.5	1.95	1.48	ATC	4.0	0.45	0.66	2.14
8-ATC-17.5	8.75	17.5	2.06	1.56	ATC	8.0	0.94	1.41	2.97
8-ATC-17.5 31.3%[a]	8.75	17.5	2.06	1.56	ATC	8.0	0.94	1.41	2.97
4-ATC-0	8.75	0.0	0.00	0.00	ATC	4.0	0.36	0.55	0.55
4-ATC-2.5	8.75	2.5	0.23	0.18	ATC	4.0	0.37	0.56	0.74
4-ATC-5	8.75	5.0	0.48	0.37	ATC	4.0	0.38	0.58	0.94
4-ATC-10	8.75	10.0	1.02	0.77	ATC	4.0	0.41	0.61	1.39
4-ATC-13	8.75	13.0	1.37	1.04	ATC	4.0	0.42	0.63	1.68
4-ATC-15	8.75	15.0	1.62	1.23	ATC	4.0	0.43	0.65	1.88
4-ATC-17.5	8.75	17.5	1.95	1.48	ATC	4.0	0.45	0.67	2.15
6-GTC-17.5	8.75	17.5	2.00	1.52	GTC	6.0	0.69	0.66	2.18
5-KTC-17.5	8.75	17.5	1.98	1.50	KTC	5.0	0.56	0.66	2.16
7-MTC-17.5	8.75	17.5	2.03	1.54	MTC	7.0	0.81	0.66	2.20

[a] Used 25.8 g of 90% EtOH/water. TEG = tri(ethylene glycol); ATC = ammonium thiocyanate; GTC = guanidine thiocyanate; KTC = potassium thiocyanate; and MTC = magnesium thiocyanate.

cyanate salts (data not shown). Protein structural changes have been observed with NMR (Tamiola and Mulder 2012). To determine if the addition of these salts altered the structure of zein, NMR spectroscopy was employed. Shown in Figure 3 are the NMR spectra for zein with 17.5% TEG (Fig. 3A) and zein, 8% ATC, and 17.5% TEG (Fig. 3B). The overall solids were reduced so that the viscosity of the solution was such as to make obtaining the NMR spectra possible. The peaks located between 4.3 and 5.7 were different for the two samples (Fig. 3). Spectra of TEG with and without ATC were also run. The peaks for the CH_2 groups α to the oxygens were unchanged with the addition of ATC to the TEG, showing that the differences observed were not because of TEG/ATC interaction (data not shown). In addition, zein and zein/ATC spectra were also obtained without TEG, and the same pattern was observed (data not shown). The peaks at 3.6 and 3.7 resulted from the added TEG as well as residual protio peaks for the NMR solvent (CD_3CD_2OD). There, sharpness was because as small molecules they would freely rotate in the solvent and the peaks would not be broadened. Peaks located between 4.3 and 5.7 are typically attributed to hydrogen atoms on carbon that are α to electron-withdrawing groups such as hydroxyl (Silverstein et al 1981). Given that zein is a mixture of proteins (Paraman and Lamsal 2011), it was not surprising that the peaks were broad. On examination of these two spectra, it could be noted that the shape of the peak in this region changed with the addition of ATC. This observation suggested that the environment around these hydrogen atoms changed because of the presence of the salt. The impact of thiocyanate salts on the NMR spectra of proteins has been studied previously, and it was found to change the position of methylene protons on the protein backbone (Rembert et al 2012).

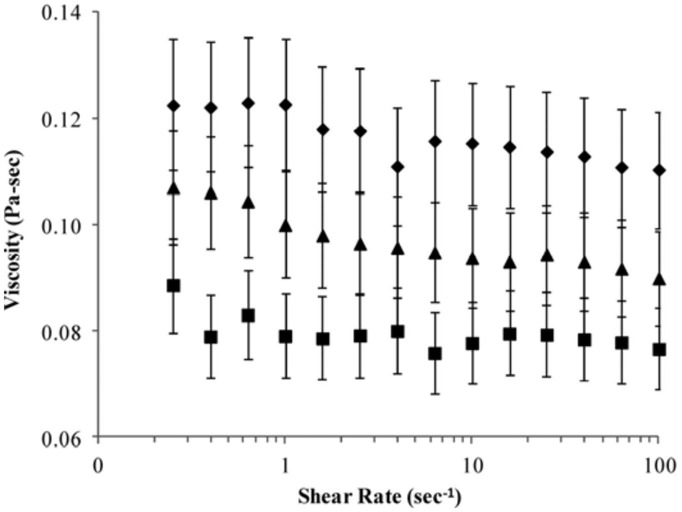

Fig. 2. Viscosity of select zein solutions at various shear rates, ◆ = C17.5; ■ = 8-ATC-17.5; and ▲ = 8-ATC-17.5 31.3%.

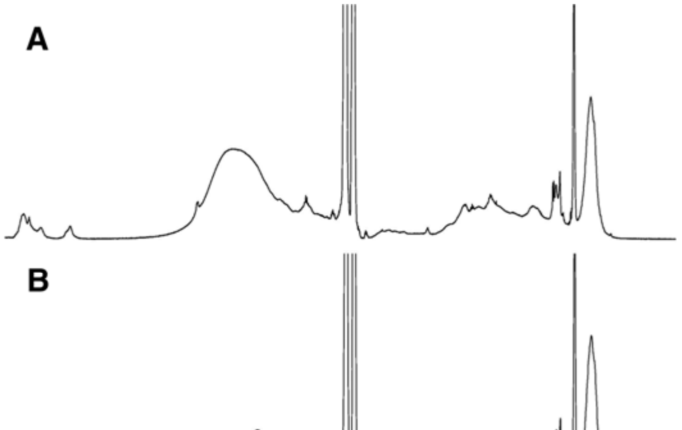

Fig. 3. ^{1}H NMR spectra. **A,** zein with 17.5% tri(ethylene glycol) (TEG); and **B,** zein, 8% ammonium thiocyanate, and 17.5% TEG.

DSC and DMA

A standard method for determining if a polymer additive is a plasticizer is through the use of DSC (Lawton 2004). Shown in Figure 4 are the DSC traces for C0, C17.5, 8-ATC-0, and 8-ATC-

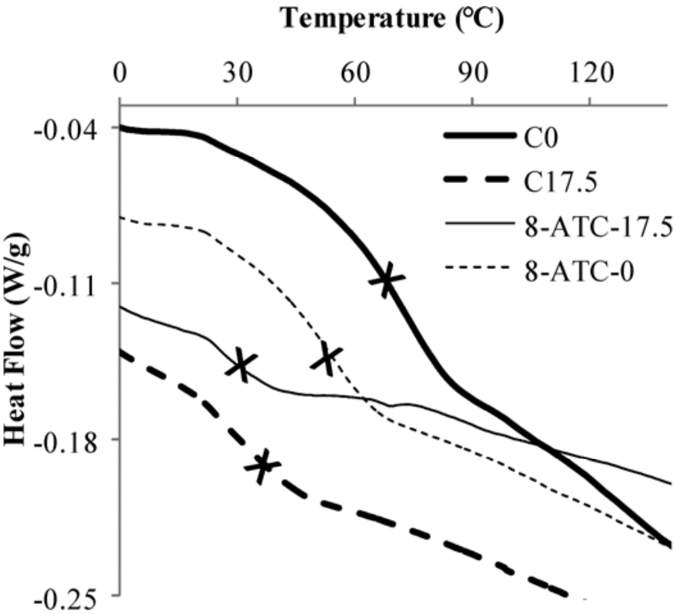

Fig. 4. Differential scanning calorimetry trace of C0, C17.5, 8-ATC-17.5, and 8-ATC-0. X indicates the T_g of the respective samples.

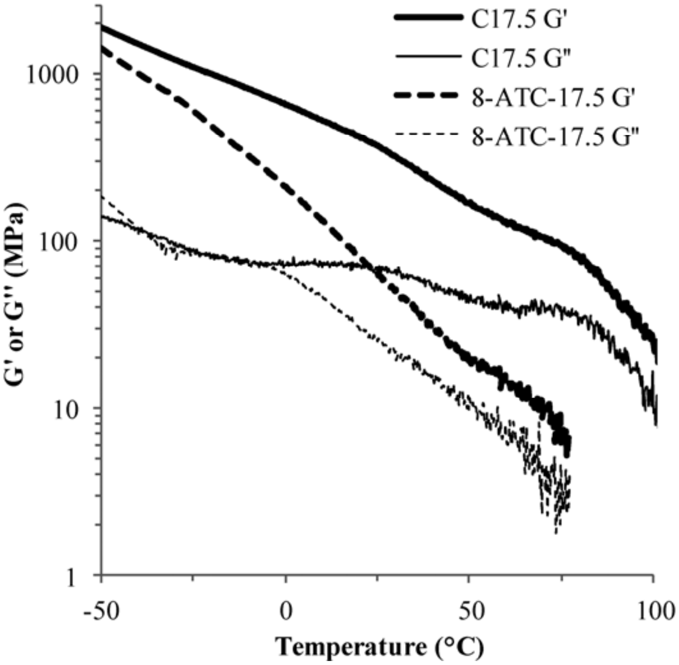

Fig. 5. Dynamic mechanical analysis scans of C17.5 and 8-ATC-17.5 from –50 to 100°C. Sample 8-ATC-17.5 failed at 60°C.

17.5. With the addition of TEG or ATC, the T_g decreased (X in the figure indicates the T_g) from 67°C for the control to 37, 58, and 30°C, respectively. The lowest T_g was for the sample having both additives. This same trend was observed for the other salts; with the addition of more or less ATC, the T_g increased or decreased, respectively.

To complement DSC, DMA is often used. In this technique, the sample is flexed at a defined strain with varying temperature while the stress is monitored. From these measurements, the elastic modulus (*G*′) and viscous modulus (*G*″) can be measured. Figure 5 shows the DMA traces for C17.5 and 8-ATC-17.5 from –50 to 100°C. *G*′ for the samples with ATC was lower than that of the control at all temperatures. This result was to be expected for a plasticizer. The *G*″ of the ATC sample was more similar to the control at very low temperatures. Above –5°C, *G*″ of 8-ATC-17.5 began to decline rapidly. When the temperature reached 60°C, the 8-ATC-17.5 sample failed. Shown in Table II are the *G*′ and *G*″ values for select samples at 40°C. It is interesting to note that at low levels of ATC, the modulus was actually higher than the control at 40°C. Over 2% ATC was required for the *G*′ and *G*″ to be lower than the control's *G*′ and *G*″. At 4 and 8% ATC, *G*′ and *G*″ were much lower than the control's *G*′ and *G*″.

TABLE II
***G*′ and *G*″ for Select Formulations at 40°C**

Sample	*G*′ (MPa)	*G*″ (MPa)
C17.5	223	51
0.5-ATC-17.5	553	69
1-ATC-17.5	492	96
2-ATC-17.5	398	92
4-ATC-17.5	83	31
8-ATC-17.5	28	14

Physical Properties—ATC

Shown in Table III are the physical properties of films after storing at 50% rh. For the ATC salt, two sets of results are shown: using variable amounts (0.5–8%) of ATC at the same % of TEG (17.5%) and using 4% ATC with variable amounts of TEG (0–17.5%). In examining the series of samples with various amounts of ATC (##-ATC-17.5), it could be seen that as the amount of ATC increased, %Elo increased and TS and YM decreased, demonstrating that it affected physical properties like a plasticizer. In the series in which the amount of ATC was fixed at 4% and the amount of TEG varied, it could be observed that ATC was not an effective plasticizer when used on its own. When 4% ATC was the

TABLE III
Physical Properties of Zein Films with Various Amounts of TEG and Thiocyanate Salts, Stored at 50% rh[a]

Formulation	Total Plasticizer (mol/kg of zein)	TS	TS (St. Dev.)	%Elo	%Elo (St. Dev.)	YM	YM (St. Dev.)	Toughness	Toughness (St. Dev.)
C0	0.00	30.7	0.8	6.7	0.2	595	8	1.1	0.0
C5	0.35	25.9	1.1	5.6	0.5	527	12	0.8	0.1
C10	0.74	24.8	1.1	5.6	0.5	527	12	0.8	0.1
C13	1.00	23.9	1.1	7.7	0.7	404	19	0.9	0.1
C15	1.18	19.9	1.2	10	1	295	8	1.1	0.3
C17.5	1.41	10.2	0.7	52	12	120	11	2.9	0.3
C20	1.66	4.6	0.3	286	17	36	3	10.0	0.4
C25	2.22	1.2	0.1	980	26	2	1	8.4	0.4
C30	2.85	0.5	0.0	1,048	35	0	0	3.2	0.2
0.5-ATC-17.5	1.50	10.1	0.5	85	19	100	4	6.3	1.2
1-ATC-17.5	1.59	8.4	0.5	101	17	78	6	6.5	1.0
2-ATC-17.5	1.77	4.2	0.1	223	74	37	4	8.1	2.5
4-ATC-17.5	2.14	1.6	0.1	499	54	7	1	7.1	0.7
8-ATC-17.5	2.97	0.1	0.0	3,368	126	0	0	1.6	0.5
4-ATC-0	0.55	27.1	2.1	6.8	0.3	515	42	0.9	0.0
4-ATC-2.5	0.74	24.2	0.6	6.3	1.3	484	13	0.8	0.3
4-ATC-5	0.94	23.8	1.1	13	2	392	19	1.9	0.4
4-ATC-10	1.39	16.6	0.8	28	4	205	16	3.5	0.4
4-ATC-13	1.68	9.6	0.6	71	5	107	8	4.4	0.7
4-ATC-15	1.88	5.8	0.1	169	10	51	4	8.3	0.6
4-ATC-17.5	2.15	1.6	0.1	499	54	7	1	7.1	0.7
6-GTC-17.5	2.18	0.7	0.1	1,203	91	2	0	7.3	1.2
5-KTC-17.5	2.16	1.1	0.0	879	11	9	6	6.6	0.7
7-MTC-17.5	2.20	2.2	0.4	399	56	13	4	8.2	1.7

[a] TEG = tri(ethylene glycol); TS = tensile strength; %Elo = elongation to break; YM = Young's modulus; ATC = ammonium thiocyanate; GTC = guanidine thiocyanate; KTC = potassium thiocyanate; and MTC = magnesium thiocyanate.

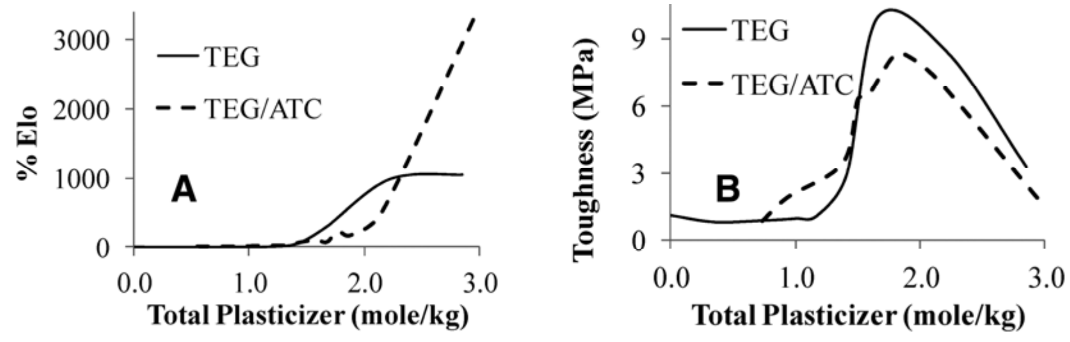

Fig. 6. A, Elongation to break (%Elo) versus total plasticizer; and **B,** toughness versus total plasticizer.

only additive, the %Elo was the same as the control for which no plasticizer was present. Between 5 and 10% TEG was needed before an effect on %Elo was observed. Apparently, the ATC was not capable of interacting with the protein in the solid state to the extent needed without the presence of an additional plasticizer to allow increased polymer chain mobility. Therefore, ATC did not function in the role of a classic plasticizer, and it did depress the T_g of the protein; physical properties were little changed when it was used alone. A number of TEG controls (C0–C30) were also prepared to better gauge the effects of ATC as a plasticizer. These samples clearly demonstrated the effect of increasing plasticizer; as the level of TEG was increased from 0% TEG (C0) to 30% TEG (C30), TS and YM decreased and %Elo increased. When the total amount of plasticizer (measured in moles of plasticizer per kilogram of material) was plotted versus %Elo (Fig. 6A), it could be seen that at lower levels of plasticizer, TEG alone provided higher %Elo. At the higher levels of plasticizer when TEG and ATC were used, the combination of plasticizers gave a higher %Elo. Toughness is a measure of the area underneath a stress–strain curve. When toughness was plotted against total plasticizer content (Fig. 6B), it could be seen that at lower levels of ATC, the toughness was improved relative to the use of TEG alone. An interesting trend was observed when examining the amount of each plasticizer versus TS (Fig. 7). The experimental results could be placed into a model developed through Minitab 15, in which the two additives as well as their cross-term were selected for the model. Given that a certain amount of TEG was needed for the ATC to impact properties, the cross-term was chosen as well. In this model, as expected, both TEG and ATC reduced TS. The predicted TSs agreed fairly well (R^2 = 0.89) with those observed experimentally. In this way, a given TS could be achieved through using various amounts of each plasticizer.

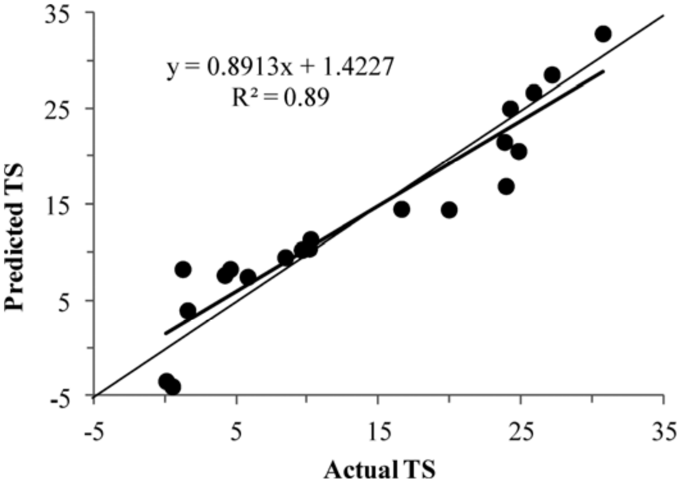

Fig. 7. Predicted tensile strength (TS) of various ammonium thiocyanate (ATC) formulations versus actual TS using the following equation: TS = 32.85 + (–1.23 × %TEG) + (–1.08 × %ATC) + (–0.04 × %TEG × %ATC). Bold line represents fitted equation, and thin line represents ideal relationship of predicted TS = actual TS. TEG = tri(ethylene glycol).

Effect of Cation Selection

Although the anion may typically have a larger effect on proteins, the cation can play a role as well (Heuvingh et al 2005; Whitford 2005). To define what impact changes in cation have on the protein, other thiocyanate salts were tested. Based on protein solubility in water-based systems, the ammonium salt tested (ATC) should destabilize the zein in solution. The remaining salts would begin to stabilize the protein, and the order of increasing stability would be the potassium salt (KTC), the magnesium salt (MTC), and finally the guanidinium salt (GTC). The GTC would stabilize the zein to the largest extent in a water solution if the Hofmeister series trends can be applied here. Films containing these salts were produced and their physical properties evaluated. When compared with ATC (Table III), GTC (tested in 6-GTC-17.5) had higher %Elo and lower TS. The impact KTC (tested in 5-KTC-17.5) had on properties was less than that of GTC; however, relative to ATC, it too had higher %Elo and lower TS. Both of the cations in these salts were to the right of ammonium in the Hofmeister series. Magnesium was to the right as well; however, MTC (tested as 7-MTC-17.5) had lower %Elo and higher TS than ATC. As previously mentioned, the Hofmeister series was developed to predict properties in water solution, and so its applicability may be different here. MTC has a cation with a different charge (+2), which may be a factor when defining the impact cations have on physical properties.

Effect of Relative Humidity—ATC

Although the effect that ATC has on physical properties, such as %Elo and the T_g of the zein article containing ATC (discussed earlier), suggest that ATC is a plasticizer of zein, for the increased elongation to be observed some amount of water must be present. Shown in Table IV are the physical properties of select formulations at different relative humidities. For the control (C17.5), as the relative humidity increased, %Elo and toughness increased and TS and YM decreased. For the salts, at the lowest relative humidity tested the %Elo was less than or equal to the control, suggesting that the thiocyanate salts were not efficient traditional

TABLE IV
Impact of % Relative Humidity on Physical Properties of Zein Films Having 17.5% TEG with Various Thiocyanate Salts[a]

Formulation	% Relative Humidity	% Moisture	TS	TS (St. Dev.)	%Elo	%Elo (St. Dev.)	YM	YM (St. Dev.)	Toughness	Toughness (St. Dev.)
C17.5	15	3.2	25.2	0.2	10	1.1	447	20	1.4	0.1
C17.5	50	6.0	10.2	0.7	52	12	120	11	2.9	0.3
C17.5	70	10.4	3.7	0.6	328	24	21.7	12.3	9.7	2.2
4-ATC-17.5	15	2.2	19.8	2.2	7	0.3	334	39	0.7	0.1
4-ATC-17.5	50	5.6	1.6	0.1	499	54	7	1	7.1	0.7
4-ATC-17.5	70	8.3	1.1	0.2	648	2	0.9	0.4	4.5	0.6
6-GTC-17.5	15	4.1	18.1	1.2	9	0.6	283	20	0.8	0.1
6-GTC-17.5	50	5.8	0.8	0.1	1,203	91	2	0	7.3	1.2
6-GTC-17.5	70	11.0	0.7	0.1	865	40	0.4	0.1	4.2	0.4
5-KTC-17.5	15	1.7	17.5	1.5	7	0.4	303	17	0.6	0.1
5-KTC-17.5	50	6.1	1.1	0.0	879	11	9	6	6.6	0.7
5-KTC-17.5	70	9.0	0.3	0.0	1,738	5	0.2	0.1	3.1	0.2
7-MTC-17.5	15	2.9	22.3	1.2	5	0.6	512	22	0.5	0.1
7-MTC-17.5	50	7.1	2.2	0.4	399	56	13	4	8.2	1.7
7-MTC-17.5	70	9.2	0.2	0.1	985	117	0.9	0.5	1.7	0.7

[a] TEG = tri(ethylene glycol); TS = tensile strength; %Elo = elongation to break; YM = Young's modulus; ATC = ammonium thiocyanate; GTC = guanidine thiocyanate; KTC = potassium thiocyanate; and MTC = magnesium thiocyanate.

plasticizers. However, as the relative humidity increased, the %Elo increased and TS decreased quickly. Water was necessary for these salts to affect the physical properties of zein. This effect was independent of the cation chosen. Water is a known plasticizer for zein (Lawton 2004; Selling et al 2004), and as a plasticizer, it will increase the free volume within the protein. Apparently, increased free volume must be present for the salts to affect the physical properties of zein. The large changes in properties with changes in humidity may have value in certain end uses such as humidity sensors.

CONCLUSIONS

A new class of plasticizer for zein was developed using nonvolatile thiocyanate salts. It was found that ATC, KTC, and GTC salts can be used to increase the elongation and reduce the modulus of zein films. Toughness also increased in these formulations that included ATC. The salts required the use of TEG and moderate humidity to increase elongation. At low humidity the film samples were brittle, and at high humidity the samples were so weak that they would fail at only moderately higher elongation. Thiocyanate salts were shown to interact with the zein proteins in solution. Additional studies are in progress examining the effects of other anions and cations and how they affect the properties detailed in this study.

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LITERATURE CITED

Baldwin, R. L. 1996. How Hofmeister ion interactions affect protein stability. Biophysical J. 71:2056-2063.

Cho, Y., Zhang, Y., Christensen, T., Sagle, L. B., Chilkoti, A., and Cremer, P. S. 2008. Effects of Hofmeister anions on the phase transition temperature of elastin-like polypeptides. J. Phys. Chem. B 112:13765-13771.

Cline, E. T. 1949. Continuous spinning and stretching of zein filaments. U.S. patent 2475879.

Croston, C. B., and Evans, C. D. 1949. Method of producing prolamine filaments. U.S. patent 2478248.

Croston, C. B., Evans, C. D., and Smith, A. K. 1945. Zein fibers—Preparation by wet spinning. J. Ind. Eng. Chem. (Washington, D.C.) 37:1194-1198.

Evans, C. D., and Croston, C. B. 1949. Formaldehyde curing of zein fibers. Textile Res. J. 19:202-211.

Gao, C., Stading, M., Wellner, N., Parker, M. L., Noel, T. R., Mills, E. N. C., and Belton, P. S. 2006. Plasticization of a protein-based film by glycerol: A spectroscopic, mechanical, and thermal study. J. Agric. Food Chem. 54:4611-4616.

Ghanbarzadeh, B., Oromiehie, A. R., Musavi, M., Falcone, P. M., Jomeh, Z. E., and Rad, E. R. 2007. Study of mechanical properties, oxygen permeability and AFM topography of zein films plasticized by polyols. Packaging Technol. Sci. 20:155-163.

Gillgren, T., and Stading, M. 2008. Mechanical and barrier properties of avenin, kafirin, and zein films. Food Biophys. 3:287-294.

Gillgren, T., Barker, S. A., Belton, P. S., Georget, D. M. R., and Stading, M. 2009. Plasticization of zein: A thermomechanical, FTIR, and dielectric study. Biomacromolecules 10:1135-1139.

Heuvingh, J., Zappa, M., and Fery, A. 2005. Salt softening of polyelectrolyte multilayer capsules. Langmuir 21:3165-3171.

Huppert, O. 1946. Artificial fibers from globular and fibrous proteins. U.S. patent 2413501.

James, A. L. 1945. Zein dispersions. U.S. patent 2377237.

Jenkins, H. S., and Magee, J. R. 1958. Manufacture of protein fibers. U.S. patent 2845362.

Jiang, Q., and Yang, Y. 2011. Water-stable electrospun zein fibers for potential drug delivery. J. Biomat. Sci. Poly. Ed. 22:1393-1408.

Lawton, J. W. 2002. Zein: A history of processing and use. Cereal Chem. 79:1-18.

Lawton, J. W. 2004. Plasticizers for zein: Their effect on tensile properties and water absorption of zein films. Cereal Chem. 81:1-5.

Padua, G. W., and Santosa, F. X. B. 1999. Tensile properties and water absorption of zein sheets plasticized with oleic and linoleic acids. J. Agric. Food Chem. 47:2070-2074.

Paraman, I., and Lamsal, B. P. 2011. Recovery and characterization of α-zein from corn fermentation coproducts. J. Agric. Food Chem. 59:3071-3077.

Paramawati, R., Yoshino, T., and Isobe, S. 2001. Properties of plasticized-zein film as affected by plasticizer treatments. Food Sci. Technol. Res. 7:191-194.

Park, S. K., Rhee, C. O., Bae, D. H., and Hettiarachchy, N. S. 2001. Mechanical properties and water-vapor permeability of soy-protein films affected by calcium salts and glucono-δ-lactone. J. Agric. Food Chem. 49:2308-2312.

Reddy, N., Li, Y., and Yang, Y. 2009. Alkali-catalyzed low temperature wet crosslinking of plant proteins using carboxylic acids. Biotechnol. Prog. 25:139-146.

Rembert, K. B., Paterová, J., Heyda, J., Hilty, C., Jungwirth, P., and Cremer, P. S. 2012. Molecular mechanisms of ion-specific effects on proteins. J. Am. Chem. Soc. 134:10039-10046.

Santosa, F. X. B., and Padua, G. W. 2000. Thermal behavior of zein sheets plasticized with oleic acid. Cereal Chem. 77:459-462.

Selling, G. W., Sessa, D. J., and Palmquist, D. E. 2004. Effect of water and tri(ethylene) glycol on the rheological properties of zein. Polymer 45:4249-4255.

Selling, G. W., Woods, K. K., and Biswas, A. 2012. Electrospun zein fibers using glyoxal as the crosslinking reagent. J. Appl. Poly. Sci. 123:2651-2661.

Sessa, D. J., Mohamed, A., and Byars, J. A. 2008. Chemistry and physical properties of melt-processed and solution-cross-linked corn zein. J. Agric. Food Chem. 56:7067-7075.

Seymour, R. B., and Carraher, C. E. 1992. Rheology and solubility. Pages 61-70 in: Polymer Chemistry: An Introduction, 3rd Ed. Marcel Dekker: New York.

Shashkina, J. A., Philippova, O. E., Zaroslov, Y. D., Khokhlov, A. R., Pryakhina, T. A., and Blagodatskikh, I. V. 2005. Rheology of viscoelastic solutions of cationic surfactant. Effect of added associating polymer. Langmuir 21:1524-1530.

Silverstein, R. M., Bassler, G. C., and Morrill, T. C. 1981. Proton NMR. Page 220 in: Spectrometric Identification of Organic Compounds. Wiley: New York.

Swallen, L. C. 1939. Zein filaments. U.S. patent 2156929.

Tamiola, K., and Mulder, F. A. A. 2012. Using NMR chemical shifts to calculate the propensity for structural order and disorder in proteins. Biochem. Soc. Trans. 40:1014-1020.

Whitford, D. 2005. Protein expression, purification and characterization. Pages 325-326 in: Proteins: Structure and Function. Wiley: Hoboken, NJ.

Wu, L., Wen, Q., and Yang, X. 2010. Effect of plasticizer on surface and mechanical properties of zein films. Huagong Xuebao/CIESC J. 61:137-145.

Wu, Q., Sakabe, H., and Isobe, S. 2003. Studies on the toughness and water resistance of zein-based polymers by modification. Polymer 44:3901-3908.

Yao, C., Li, X., and Song, T. 2007. Electrospinning and crosslinking of zein nanofiber mats. J. Appl. Poly. Sci. 103:380-385.

Zinoviadou, K. G., Koutsoumanis, K. P., and Biliaderis, C. G. 2010. Physical and thermo-mechanical properties of whey protein isolate films containing antimicrobials, and their effect against spoilage flora of fresh beef. Food Hydrocolloids 24:49-59.

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