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Viscoelastic properties of concentrated aqueous ethanol suspensions of α-zein

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ABSTRACT

Viscoelastic properties of α-zein dispersed in aqueous ethanol were studied using oscillatory strain rheometry. In 55–80% v/v aqueous ethanol, zein was only partially soluble, forming a gel at a sufficiently high zein concentration. The strain dependence of the storage modulus and the loss modulus of gelled systems exhibited features characteristic to closely-packed swollen particles. Close-packing was found to occur at a lower zein concentration with increasing ethanol concentration as the threshold for gelation decreased from a zein concentration of ca. 29 to 20% w/v with increasing ethanol concentration from 55 to 80% v/v. A contrasting trend in the effect of the solvent quality was revealed at a constant zein concentration of 30% w/v, at which the storage modulus decreased from ca. 12,000–700 Pa with increasing ethanol concentration from 55 to 80% v/v. The two major factors determining viscoelastic properties of the partially solvated zein systems were identified to be: (1) the degree of dissolution of zein into the continuous phase that was negatively correlated with the volume fraction of the dispersed phase and positively correlated with the osmotic pressure of the continuous phase; and (2) the degree of swelling of partially solvated zein particles that was positively and negatively correlated with the volume fraction and the storage modulus of the dispersed phase, respectively.

1. Introduction

According to Osborne's classification, prolamins are a class of alcohol-soluble storage proteins in grains. This group of proteins in corn kernels is named as zein, and four types of zein - α, β, γ, and δ – have been identified (Coleman & Larkins, 1999), each with different molecular weight, amino acid sequence, and solubility (McKinney, 1958). ~70% of corn zein is the α type that has two major bands corresponding to molecular weights of 19 and 22 kDa based on gel electrophoresis (Thompson & Larkins, 1989). Aqueous alcohol is used to extract α-zein directly, whereas the extraction of β, γ, and δ-zeins requires a reducing agent (Thompson & Larkins, 1989). Possible disulfide linkages between β- and α-zein molecules were also suggested (Pomes, 1971). Another study reports the existence of other three types of zein in the fraction rich in β-zein (Lawton, 2002).

Corn gluten meal is a co-product of corn wet milling and is a common source for commercial production of zein (Shukla & Cheryan, 2001; Zhu, Kale, & Cheryan, 2007). Commercial zein products are usually a mixture of different types of zein and their composition and purity depend on raw materials and conditions used for extraction and purification (Shukla & Cheryan, 2001). Zein products with a high purity currently are relatively expensive as food ingredients - $10–40 per kg or ~$5–20 per pound (Shukla & Cheryan, 2001). Nevertheless, the cost can be decreased by improved separation technology or substituted by less pure zein if no apparent changes in product quality are expected. With the growing ethanol industry, zein can be recovered as a co-product with an estimated cost of $4.4 per kg or ~$2 per pound when purified to a ~90% purity (Kale, Zhu, & Cheryan, 2007). It was estimated that approximately 13,000 tons of zein could be recovered when 50 million gallons of ethanol are produced (Kale et al., 2007; Shukla & Cheryan, 2001; Xu, Reddy, & Yang, 2007).

As water-insoluble, hydrophobic prolamins, zein has been researched for numerous applications. The ability of zein to form films and coatings is well-known (Dawson, Hirt, Rieck, Acton, & Sotthibandhu, 2003; Wang, Lin, Liu, Sheng, & Wang, 2005). Recently, treatment of zein films using UV/ozone was observed to have resulted in varying surface hydrophobicity (Shi, Kokini, & Huang, 2009). Zein films have also been studied as intervention systems to enhance microbial safety of semi-solid foods by incorporating antimicrobials such as nisin (Janes, Kooshesh, & Johnson, 2002; Ku & Bin Song, 2007; Lungu & Johnson, 2005), lysozyme (Mecitoglu et al., 2006), and plant essential oil thymol (Del Nobile, Conte, Incoronato, & Panza, 2008). The solubility characteristics of zein are also used to produce particulates for delivery of bioactive

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compounds. Commonly, zein is dissolved in aqueous alcohol that is then dispersed into another solvent to precipitate zein because the eventual solvent becomes as a non-solvent (anti-solvent) with respect to zein. The anti-solvents include an aqueous system where a stock solution of zein in aqueous ethanol is sheared into another solution with no or lowered ethanol (Hurtado-Lopez & Murdan, 2006; Liu, Sun, Wang, Zhang, & Wang, 2005; Parris, Cooke, & Hicks, 2005; Zhong & Jin, 2009b), air as in the case of spray drying (Zhong & Jin, 2009a), or supercritical carbon dioxide (Zhong, Jin, Davidson, & Zivanovic, 2009; Zhong, Jin, Xiao, Tian, & Zhang, 2008).

While there are growing interests in utilizing zein for novel applications, rheological properties of zein are less studied. Viscosities of zein dispersed in 50–90% aqueous ethanol were characterized at 10–60 °C (Fu & Weller, 1999). Zein dispersions behaved like Newtonian fluids and had a higher viscosity at a lower temperature, lower concentration of ethanol, and a lower zein concentration. The temperature dependence of viscosity was satisfactorily described by the Arrehenius-type model. An exponential correlation between viscosity and zein concentration was also observed. In another study (Selling et al., 2005), zein was dissolved at 10–25% in N,N-dimethylformamide, a good solvent that dissolved zein to low viscosity systems. In contrast to dispersions in aqueous ethanol, zein solutions showed shear-thinning behavior. A higher viscosity was observed at a lower temperature between 10 and 40 °C, but the correlation of viscosity and temperature did not follow the Arrhenius-type relationship. Further, when the zein solutions were continuously sheared, solution viscosities increased over time, indicating rheopectic properties, and a higher increase rate of viscosity was observed at a higher temperature, which eventually resulted in a higher viscosity at 48 and 55 °C than that at 40 °C.

In early literature (Evans & Manley, 1943; Manley & Evans, 1943), solutions and dispersions of zein were observed to gel with time and heat, especially at high concentrations. Further, although a variety of solvents have been used to dissolve zein (Lawton, 2002), viscoelastic properties of zein have not been reported. The objective of this work was to characterize viscoelasticity of zein in aqueous ethanol because the binary solvent mixture is generally recognized as safe and is most commonly used for food applications.

2. Materials and methods

2.1. Materials

Purified α-zein and ethanol (200 proof) were purchased from Acros Organics (Morris Plains, NJ). Protein assay reagents were obtained from Pierce Biotechnology (Rockford, IL).

2.2. Visual inspection of gel formation at room temperature

Zein was dispersed to a concentration between 22 and 30% w/v in 2 mL water/ethanol mixtures (55–90% v/v ethanol) contained in 4 mL vials. The dispersion was continuously agitated overnight using an end-to-end shaker (Laboratory Industries Inc., Berkeley, CA) and then incubated at room temperature for 14 days to fully equilibrate samples. The aged samples were photographed, and samples that flew after inverting the vials were determined to be non-gel forming samples.

2.3. Solubility analysis

Solutions were prepared by dispersing zein in 55–90% ethanol at 20% w/v and continuously mixed at room temperature overnight using the above end-to-end shaker. Samples were centrifuged at 14,400 *g* for 30 min (model MiniSpin Personal, Eppendorf, Westbury, NY), and the supernatant was transferred and diluted 150 times in 70% v/v ethanol for protein assays using bicinchoninic acid (BCA) method (Li et al., 2005). Our preliminary data showed that samples had much lower absorbance (sensitivity) when assayed by the Bradford dye-binding method and zein samples had lower absorbance than solutions of bovine serum albumin (standards in BCA and Bradford methods) with same mass concentrations. A standard curve was established from a series of zein solutions in 70% v/v ethanol at 0–10 mg/mL. The remaining zein concentration in the supernatant was estimated based on the standard curve and percentages of soluble zein were determined after normalization to the mass concentration before centrifugation.

Samples before and after centrifugation were also assayed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using a 15% Tris–HCl gel from Bio-Rad Laboratories (Hercules, CA). The instrument was a Protean II xi 2D Cell (Bio-Rad), and the separation was operated at 200 V until the indicator dye reached the gel bottom. The gels were stained by Coomassie® blue dye.

2.4. Sample preparation for rheological tests

Zein was dispersed to a concentration between 22 and 30% w/v in 20 mL water/ethanol mixtures (55–90% v/v ethanol). The dispersion was continuously agitated overnight using a stirring plate and subsequently incubated for a total of 48 h before rheological tests. Upon incubation, samples showed significant variations in visual appearance with ethanol concentrations. At 55% ethanol, macroscopic syneresis was evident at zein concentrations $< 30\%$, leading to a brittle bottom phase. At 90% of ethanol, dispersions still flowed even at a zein concentration of 30% and were not tested. At 70% ethanol, dispersions did not consistently form a gel (judged by whether or not the system flowed after inverting the container).

2.5. Rheological measurements

Dynamic rheological tests were performed with an AR2000 rheometer (TA Instruments, New Castle, DE) using a parallel-plate setup (plate diameter = 40 mm, gap = 1 mm). After positioning the top plate and removing the excess sample, a layer of mineral oil was applied onto the plate edge and a sealing cap was used to minimize the solvent loss during measurements. A strain amplitude of 0.001 (within the linear viscoelasticity regime) was used during the following steps: (1) A frequency sweep step at 37 °C, (2) a cooling step to 20 °C and a frequency sweep step at 20 °C, (3) a cooling step to 5 °C and a frequency sweep step at 5 °C, (4) a linear heating step from 5 to 40 °C at 1 °C/min, and (5) a linear cooling step from 40 to 5 °C at 1 °C/min. In addition, strain sweep steps were performed after step (5) using shear strain amplitudes ranging from 0.0001 to 1 at 40 °C. A frequency of 1 Hz was used in steps of (4) and (5). Before the start of each step, a sample was equilibrated for 2 min after reaching the target temperature. Each sample was tested in triplicate and results are reported as averages and 95% confidence intervals from three tests.

3. Results and discussion

3.1. Visual inspection of gelation properties

Photographs of samples after aging for 14 d are shown in Fig. 1. Samples prepared with 55% ethanol showed macroscopic syneresis at 22–28% zein, as evidenced by a layer of solvent. At 30% zein, no apparent liquid layer was evident. At other ethanol concentrations, samples did not show phase separation. After inverting vials,