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An Acidic Method of Zein Extraction from DDGS

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Zein with a higher intrinsic viscosity and phosphorus content, similar protein content, lower yellowness, and at potentially much lower cost than commercially available zein was obtained from distillers dried grains with solubles (DDGS). A novel extraction method using acidic conditions in the presence of a reducing agent has been used to obtain about 10% aqueous ethanol soluble zein from DDGS. The optimum pH, time, temperature, and amount of reducing agent that can produce zein with high quality and yield have been developed. In addition to the zein, about 17% oil based on the dry weight of DDGS has also been obtained during zein extraction. The zein obtained from this research is expected to be suitable for use as fibers, films, and binders and in paints.

KEYWORDS: DDGS; zein; extraction; protein; oil

INTRODUCTION

The increasing price and demand for petroleum products has led to the development of alternative fuel sources such as ethanol and biodiesel. Cereal crops such as corn and soybeans are currently being used as sources for biofuels. About 20% of corn produced in the United States is used to produce about 5 billion gallons of ethanol every year. The production of ethanol is also expected to generate about 10 million tons of DDGS in 2006 (*1*). Currently, there are limited uses of DDGS in food and feed applications and about one-tenth of the DDGS produced is being exported (*1*). However, DDGS is rich in protein, fat, and crude fiber (29.7, 8.8, and 9.3% based on dry weight, respectively) that can be used to produce valuable products (*2*).

Zein, which is insoluble in water but soluble in aqueous alcohols, is the main protein in corn and corn byproducts such as DDGS. The relatively low hydrophilicity, good elasticity, and excellent film-forming capabilities make zein a preferred protein source for several applications (*3*). Zein has been used as fibers, films, and adhesives (*1*−*6*). Currently, commercial zein is mainly extracted from corn gluten meal and reported to cost about $8−10 per pound. Therefore, attempts have been made to obtain zein at lower costs from corn processing byproducts such as DDGS using various methods (*7*−*12*). However, the previous methods used to obtain zein from corn processing byproducts such as DDGS have not been successful to obtain high-quality zein with high yields and low cost.

In a study on extracting zein from corn distillers grains, Wolf and Lawton used sodium hydroxide in the presence of a reducing agent (dithiothreitol) and surfactant (sodium dodecyl sulfate) and obtained about 1.5−6.6% crude zein based on the total weight of the DDGS, but the proteins obtained had low purity of 37−57% (*7*, *9*, *11*). In addition, except for the yield and the composition of the proteins obtained, these studies have not reported the quality (for example the molecular weight) of the zein obtained. Also, all of the previous methods used to extract zein from corn, and corn processing byproducts have used aqueous alcohols under alkaline conditions either with or without a reducing agent (*7*−*12*). No reports are available on the use of alcohols under acidic conditions for zein extraction. Because proteins are more readily hydrolyzed under alkaline conditions, especially under high temperatures, using acidic conditions instead of the alkaline conditions are expected to produce better quality zein.

Obtaining high-quality zein with low cost is expected to make zein a useful protein source for fiber, film, adhesive, and other applications. The increasing availability of DDGS as byproduct of ethanol production could help to obtain zein at low cost and facilitate the development of industrial applications for zein. Developing industrial applications with large market and high value addition for DDGS and zein would also help to decrease the cost of ethanol production. Fibrous applications offer both the large market and high value addition for the proteins and other products obtained from DDGS. Several other agricultural byproducts are also being considered for fiber production in an effort to increase the value of agricultural crops (*13*−*19*).

In this research, we have developed a new method of extracting high-quality zein from DDGS using acidic conditions in the presence of a reducing agent. We recognize that the zein extracted from DDGS is not pure zein and may contain some carbohydrates and lipid complex moieties. However, we will refer to this zein complex as zein since previous reports on zein extraction from wet milling had about 80−85% protein, 15−20% lipids, and less than 0.25% starch (*8*). The extraction conditions such as the pH, concentration of reducing agent, time, and temperature were optimized to obtain zein with better quality and higher yield than previously reported. The yield, composi-

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tion, viscosity, and color of the zein obtained at various extraction conditions have been determined.

EXPERIMENTAL PROCEDURES

Materials. DDGS was supplied by Abengoa BioEnergy Corporation, York, NE. The DDGS had about 25% protein and was yellow in color. Commercially available fiber-grade zein (F4000) with a molecular weight of 35 000 was obtained from Freeman Industries LLC, Tuckahoe, NY (*20*). The Freeman zein will be referred to as commercial zein throughout the manuscript. Anhydrous sodium sulfite (98% ACS grade) and hydrochloric acid were purchased from VWR International, Bristol, CT.

Extracting Oil from DDGS. The DDGS was powdered in a laboratory scale Wiley mill to pass through a 20 mesh dispenser. The oil in the powdered DDGS was extracted using anhydrous ethanol in a Soxhlet extraction apparatus until the extracted liquids were colorless. The oil suspension was centrifuged to separate the components after vaporizing the ethanol. Some of the proteins in DDGS were also extracted with the oil. These proteins were collected by vaporizing the anhydrous ethanol at 40 °C under low pressure. The precipitate collected was centrifuged at 10 000 rpm for 20 min to obtain the proteins. The proteins obtained were dried and weighed.

Extracting Zein from DDGS. After extracting the oil and pigment in the first step, zein was extracted with 70% (w/w) ethanol in the presence of a specified quantity of sodium sulfite. All concentrations of the chemicals used were based on the total weight of the extracted liquids. The pH of the solution was adjusted using 20% (v/v) hydrochloric acid or 20% (w/w) aqueous sodium hydroxide at the beginning of the extraction before adding sodium sulfite. This was done because sodium sulfite does not completely dissolve in 70% (w/w) ethanol at room temperature, and the pH of the solution changes gradually as the temperature of the solution increases. The solution was heated to the desired temperature in about 10 min and held at various temperatures and time. After this extraction, the solution was centrifuged at 10 000 rpm for 5 min to separate the alcohol solution from the other components in DDGS. The ethanol solution containing the dissolved proteins was then vaporized under low pressure at 40 °C to obtain the proteins. The collected proteins were washed in distilled water, centrifuged at 10 000 rpm for 5 min, dried, and weighed. All experiments were repeated at least twice, and the averages and error bars with ±1 standard deviations are reported.

CIE Yellowness Index (YI). The commercial zein and proteins extracted from DDGS were made into circular pellets 2 cm in diameter and about 5 mm in thickness to measure the color of the proteins. The color of the proteins was measured in terms of the CIE Yellowness Index (YI) using a Hunterlab spectrophotometer (Model: Ultrascan, XE) with a 0.25 in. viewing area and D65/10° observer. Three readings were taken, and the average is reported.

Intrinsic Viscosity. The intrinsic viscosities of the commercial zein and proteins obtained from DDGS at various conditions were determined according to ASTM standard D 2857 at a temperature of 25.0 ± 0.1 °C.

FTIR Measurement. The protein samples made into pellets were used to obtain the infrared spectrum. The FTIR spectrums of the protein pellets and oil were obtained on a Thermo Electron Corporation FTIR (model Nicolet 380). Sixty-four scans were recorded for each sample at a resolution of 32 cm^{-1} in the reflectance mode.

Compositional Analysis. The phosphorus content in the zein extracted from DDGS was determined according to the Bray and Kurtz method (*21*). The nitrogen contents of commercial zein and zein extracted from DDGS were analyzed using the Dumas method (Elementar Rapid N) (*22*). The protein contents were calculated by multiplying the nitrogen content by a factor of 6.5 (*8*).

SDS-PAGE Analysis. SDS-PAGE electrophoresis was performed on the commercial zein and proteins obtained from DDGS with and without reduction. About 10 mg of commercial zein and proteins extracted from DDGS were powdered and mixed with 200 μL of SDS-PAGE 1× sample buffer (0.83 mM Tris−HCl, 2% SDS, 2% β-mercaptoethanol, 10% glycerol, and double-distilled water, ddH_2O) to observe the electrophoretic patterns in the reduced state. The unreduced samples were prepared using about 10 mg of commercial zein and proteins extracted from DDGS. The samples were powdered and mixed with 200 μL of SDS-PAGE 1× sample buffer without reducing agent (0.83 mM Tris−HCl, 2% SDS, 10% glycerol, ddH_2O, respectively). The four samples were kept at room temperature for 20 min. The samples were then centrifuged and the clear top layer was collected and the protein concentrations in the solution were measured by the BioRad protein assay method. The samples were then diluted using a 1× reducing and nonreducing buffer for each of the commercial zein and zein obtained from DDGS to obtain a concentration of 40 μg of proteins per 30 μL of the solution. Each sample was heated for 1 min at boil and then loaded, one sample per lane, in the gel. After electrophoresis, the gel was washed twice and stained with Coomassie Brilliant Blue G-250. After standing overnight, the gel was flushed with deionized water and put in a destained liquid until a clear background was formed. The molecular weights of the protein standard mixture ranged from 10 to 250 kDa and were obtained from BioRad Chemical Co.

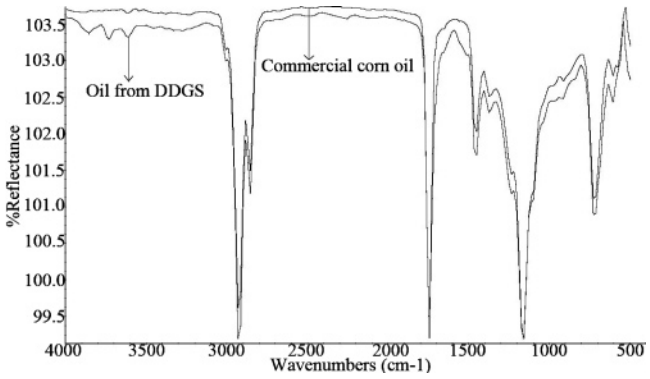

Figure 1. FTIR spectrum of oil extracted from DDGS compared with commercially available corn oil.

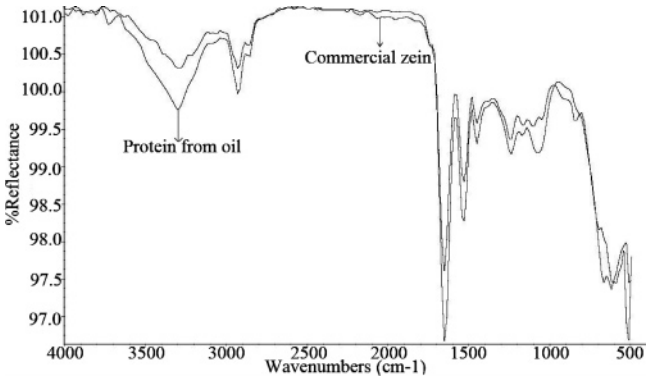

Figure 2. FTIR spectrum of solids obtained with the oil after the first extraction step have similar composition to the commercial zein.

Statistical Analysis. Statistical analysis to determine the statistical significance between the different treatment conditions was performed using the software program SAS 9.1 TS Level 1M3. A Tukey adjustment test was done with a 95% confidence interval ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Extraction and Characterization of Oil from DDGS. About 17% oil based on the dry weight of DDGS and about 1% solids were obtained with the oil during the first extraction step. The oil obtained from DDGS has similar composition to that of corn oil available on the market, as seen from the FTIR spectrum in **Figure 1**. The solids extracted with the oil are mostly proteins and have similar spectrum to the commercial zein as seen from **Figure 2**.

Effect of Extraction Conditions on the Yield of Zein. *Effect of Initial pH.* The amount of zein extracted from DDGS is highly

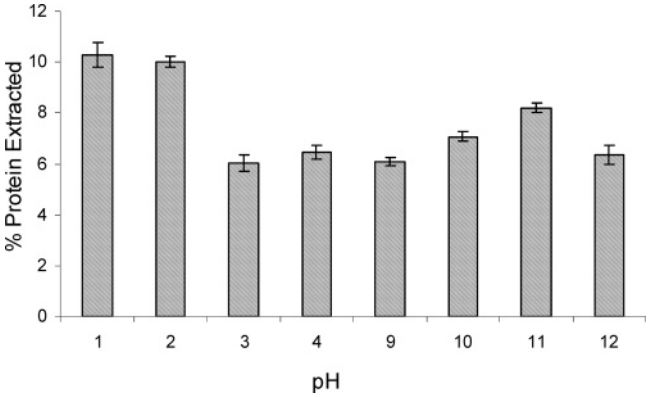

Figure 3. Effect of pH on the yield of zein (% based on the dry weight of DDGS used). The effect of pH was studied using 70% (w/w) ethanol at boil for 2 h with a solvent to solids ratio of 10:1 in the presence of 0.25% sodium sulfite.

dependent on pH, as seen from **Figure 3**. About 10% zein is obtained at pH 1 and pH 2, whereas the highest yield obtained under alkaline conditions (pH 11) is about 8%. The treatments at pH 2 and pH 11 were statistically significantly different with a p value of <0.0001. The yield of zein obtained under weak acidic or weak alkaline conditions (between pH 3 and 10) is relatively low and is in the range of 6−7%. However, the yield of zein obtained under the optimum conditions developed in this research is high at about 44% of the total proteins in the DDGS.

The isoelectric point of zein is 6.2, and therefore zein will have relatively low solubility around the isoelectic point at pHs 3, 4, and 9 (*6*). Increasing or decreasing pHs will increase the net negative or positive charges on the proteins, respectively, leading to higher protein solubility and yield. Also, the ester or ether linkages between the carboxyl or hydroxyl groups in the proteins and the hydroxyl groups in the polysaccharides (cellulose, hemicellulose, and starch) in DDGS are probably more readily broken under strong acidic or alkaline conditions. The hydrolysis of the ester or ether linkages between the proteins and polysaccharides will provide more proteins that are soluble in ethanol/water. This increases the yield of proteins under strong acidic or alkaline conditions. However, strong alkaline conditions cause severe hydrolysis of the proteins, and the hydrolyzed proteins will dissolve when washed in water after extraction. Therefore, the yield of proteins in strong alkaline conditions is lower than the yield obtained under strong acidic conditions. On the basis of the conditions used in this study, pHs of 1 and 2 are most suitable for obtaining a high yield of proteins from DDGS. However, a pH of 2 is more desirable than pH 1 due to the higher viscosity of the proteins obtained at pH 2 compared to pH 1, as will be discussed later.

The zein obtained at pH 1 has an intrinsic viscosity of about 9.6 compared to a viscosity of 31.5 for the zein obtained at pH 2 and a viscosity of 25.8 for the commercial zein. Because having high viscosity is a crucial factor for better strength, elongation, and flexibility of the zein products such as fibers and films, a pH of 2 is the most desirable pH for extracting the zein. Although pH 1 gives the highest yield, most of the proteins extracted at this pH have lower molecular weights compared to the zein obtained at pH 2. Because proteins are hydrolyzed more readily at pH 1 than at pH 2, the molecular weight and therefore the viscosity of zein decreases at pH 1. Similarly, proteins are hydrolyzed more readily under strong alkaline conditions, which lead to low viscosities. Commercial zein is extracted under alkaline conditions and therefore has lower viscosities than the zein obtained from DDGS at pH 2.

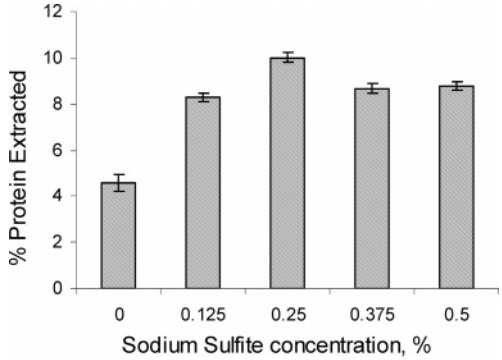

Figure 4. Effect of adding sodium sulfite on the yield of zein (% based on the dry weight of DDGS used). The effect of sodium sulfite was studied using 70% (w/w) ethanol at boil for 2 h at a pH of 2 and a solvent to solids ratio of 10:1.

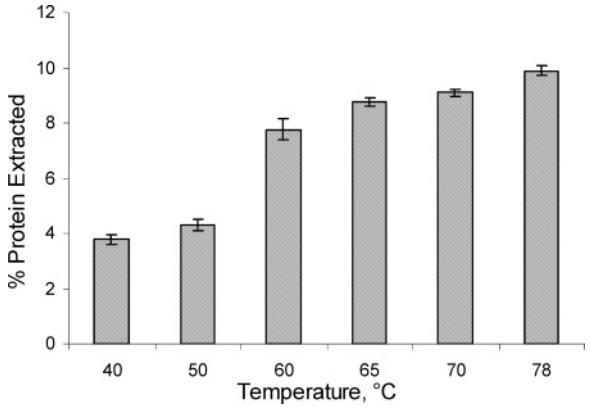

Figure 5. Effect of temperature on the yield of zein. The effect of temperature was studied using 70% (w/w) ethanol and a sodium sulfite concentration of 0.25% for 2 h and solvent to solids ratio of 10:1 and a pH of 2.

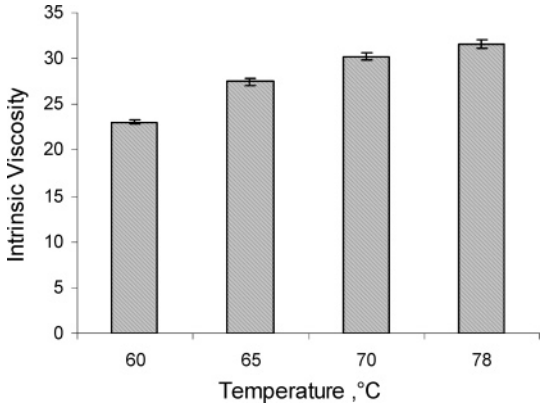

Figure 6. Effect of temperature on the intrinsic viscosity of the zein obtained. The effect of temperature was studied using 70% (w/w) ethanol and a sodium sulfite concentration of 0.25% for 2 h and solvent to solids ratio of 10:1 and a pH of 2

Effect of Sodium Sulfite. About 5% of zein is extracted from the DDGS without adding any sulfite, but adding 0.125−0.5% sodium sulfite increases the amounts of zein obtained, as seen from **Figure 4**. A sodium sulfite concentration of 0.25% gives the highest yield of zein among the conditions studied as seen from **Figure 4**. Variations in the amount of sodium sulfite used were statistically different, with a p value of <0.0001 between all the treatments except between 0.375 and 0.5%. As a reducing

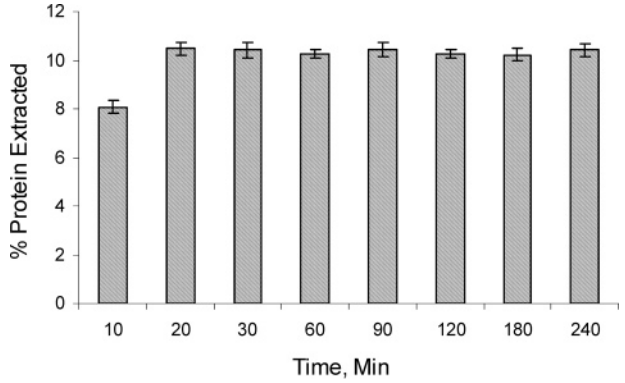

Figure 7. Effect of time on the yield of zein. The effect of time was studied using 70% (w/w) ethanol at boil with a solvent to solids ratio of 10:1 and sodium sulfite concentration of 0.25% at pH 2.

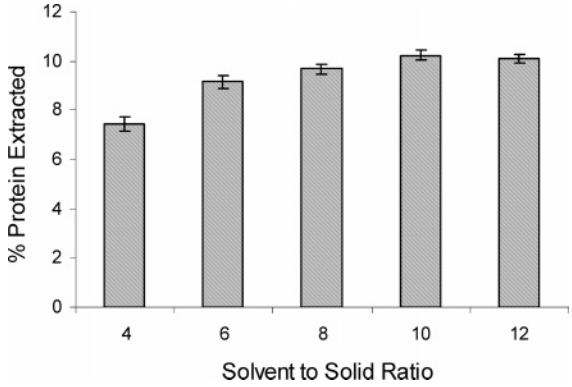

Figure 8. Effect of solvent to solids ratio on the yield of zein. The effect of solvent to solids ratio was studied using 70% (w/w) ethanol at boil for 2 h and sodium sulfite concentration of 0.25% at pH 2.

agent, sodium sulfite breaks the disulfide bonds between proteins and makes the proteins more soluble. At very low concentrations of sulfite, not enough disulfide bonds are broken, and therefore the yield of zein obtained is low. However, higher sulfite concentrations should be avoided because excessive breakage of the disulfide bonds will substantially decrease the size of the proteins. Some of the low-molecular-weight proteins would be removed during washing, resulting in lower yield. The increase in the yield of zein in the presence of a reducing agent was also observed by others (*8*).

Effect of Temperature. Increasing temperature increases the yield of the zein as seen from **Figure 5**. The yield of the zein is similar at 40 and 50 °C with a *p* value 0.1623, but there is nearly a 1-fold increase in yield when the temperature is increased from 50 to 60 °C. The yields at the temperatures 50 and 60 °C are statistically significantly different, with a *p* value <0.0001. The highest yield is obtained at the boiling temperature of ethanol solution, 78 °C. The treatment at 78 °C is significantly different than the treatments at 40, 50, 60, 65, and 70 °C, with *p* values of <0.0001, <0.0001, <0.0001, <0.0292, and <0.0490, respectively.

At low temperatures, there is not enough energy in the system to break the disulfide bonds and other interactions within the proteins and between the proteins and polysaccharides to solubilize the proteins. Therefore, most of the high-molecular-weight proteins cannot dissolve at low temperatures and the protein yield is low. Increasing temperature not only breaks a great number of disulfide bonds and other interactions but also improves the solubility of the high-molecular-weight proteins, leading to high yields. However, the molecular weight of the proteins obtained is more important than the total yield obtained as discussed earlier.

The intrinsic viscosity of the proteins obtained at various temperatures is shown in **Figure 6**. Increasing temperature not only increases the yield but also the intrinsic viscosity of the protein, indicating the increase in average molecular weight. This reiterates the finding that relatively low temperatures do not allow for the breakage of the disulfide bonds and other interactions in some of the higher-molecular-weight proteins. On the basis of the yield and viscosity of the zein obtained, a temperature of 78 °C is believed to be the most optimum temperature for extraction among the conditions studied.

Effect of Holding Time. **Figure 7** depicts the effect of increasing extraction time on the percentage of extractives obtained. The time shown here is the holding time approximately 10 min after the solution took to reach the boiling point. As seen from the figure, about 8% yield is obtained just after 10 min of holding at boil, and there is a significant increases in yield to about 10% after 20 min of boiling with a *p* value <0.0001. There is no further significant increase in yield from 20 min to 4 h, with *p* values from 0.8981 to 1.0000. The high yield of zein obtained in this research at relatively short extraction times compared to the extraction times used in previous researches should mainly be due to the low pH and higher temperature used in this research. Previously, crude zein was extracted from DDGS in three steps of 30 min each at 60 °C at a pH of 10 (*7*). The amount of pure zein obtained in that research was about 3.3% (based on weight of DDGS used), much lower than the yield of zein obtained in this research.

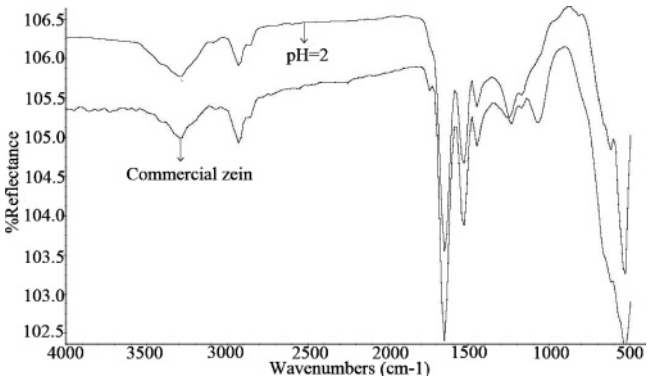

Figure 9. FTIR spectrum of crude zein obtained at pH 2 compared to commercial zein. The spectrums for the zein obtained at other pH conditions studied were similar.

Effect of Solvent-to-Solid Ratio. A solvent-to-DDGS ratio of 10:1 or 12:1 produces the highest yield of zein using a sodium sulfite concentration of 0.25%, as seen from **Figure 8**. At low solvent-to-solid ratios such as 4:1, the total amount of the zein dissolved in the solvent decreases because there is limited availability of the solvent, and therefore the yield of proteins decreases. However, varying the solvent-to-solids ratios gave yields with statistically significant differences, with *p* values of <0.0001 between 4:1 and 6:1, <0.0107 between 6:1 and 8:1, and <0.0204 between 8:1 and 10:1. However, the yield obtained with solids-to-solvents ratio between 10:1 and 12:1 did not produce significant differences with a *p* value of 0.7467.

Characterizing the Zein Extracted from DDGS. *Zein Composition.* The proteins obtained from DDGS at various conditions have similar functional groups to that of commercial zein, as seen from the FTIR spectrum in **Figure 9**. The spectra of the proteins obtained at all conditions were similar, and only the spectrum for pH 2 is shown for clarity. The major difference in the peaks of proteins obtained in this experiment compared

Table 1. Comparison between Zein from DDGS and Commercial Zein

properties	zein from DDGS	commercial zein
extraction conditions	acidic	alkaline
protein content, %	90.4	92.4
color (CIE YI)	54	95
phosphorus content, %	0.08	0.02
intrinsic viscosity	31.6	25.1

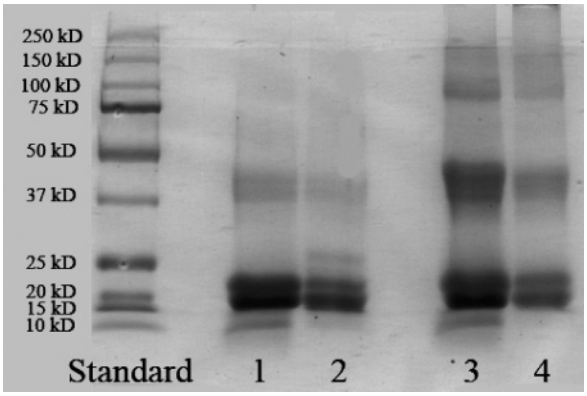

Figure 10. SDS-PAGE of proteins extracted from DDGS and commercial zein. Lanes 1 and 3 are commercial zein in reduced and unreduced forms, respectively. Lanes 2 and 4 are proteins from DDGS in reduced and unreduced forms, respectively.

to that of commercial zein is the presence of a sharp peak in the proteins obtained from DDGS at about 1100 cm^{-1}. This peak at 1100 cm^{-1} should be from the phosphoryl group, which reportedly has a peak at that position (*23*). Commercial zein has about 0.02% phosphorus compared to 0.08% in the proteins extracted from DDGS, and therefore the proteins obtained from DDGS gives a stronger phosphorus peak in the spectrum. Higher phosphorus content in the proteins obtained from DDGS may provide them with better flame resistance than that of commercial zein (*24*).

The zein extracted from DDGS has about 2% lower protein content than commercial zein, as seen from **Table 1**. Carbohydrates and lipid complex moieties probably account for the remaining 10%. Zein previously extracted from wet milled corn had 80−85% protein, 15−20% lipids, and less than 0.25% starch (*8*).

SDS-PAGE. SDS-PAGE was performed to compare the similarities in the electrophoresis patterns between the zein extracted from DDGS and commercial zein. Lanes 1 and 2 in the gel in **Figure 10** are the commercial zein and zein obtained form DDGS, respectively, in the reduced form, and lanes 3 and 4 are the commercial zein and zein obtained form DDGS in the unreduced form, respectively. As seen from **Figure 10**, the unreduced commercial zein (Lane 3) and zein from DDGS (Lane 4) have similar molecular weight bands in the 15−150 kD range. However, the DDGS zein has some higher-molecular-weight proteins (>250 kD), whereas the commercial zein has lower-molecular-weight proteins (<10 kD). The high-molecular-weight proteins in the DDGS zein are mostly likely due to the crosslinking of proteins during production of DDGS. The lower-molecular-weight proteins (<10 kD) in the DDGS zein are probably removed during the ethanol fermentation process and/or defatting and washing during zein extraction and are therefore not seen in lane 4 in the gel. In the reduced form, the DDGS zein has some proteins around 25 kD (lane 2) not seen in the commercial zein (lane 1), whereas the commercial zein has lower-molecular-weight proteins in the 15−10 kD range that are not present in the DDGS zein. The proteins above 75 kD seen in both the commercial and DDGS zein in the unreduced form disappear after reduction mainly due to the reduction of the disulfide bonds in the proteins. However, the presence of nondisulfide bonds in the DDGS zein should be the main reason for the presence of some proteins around 25 kD, as seen from lane 2 in **Figure 10**.

Overall Comparison of Zein from DDGS and Commercial Zein. Table 1 summarizes the major differences between the zein obtained from this research compared to commercial zein. As shown, the zein from DDGS obtained using acidic conditions in the presence of a reducing agent has higher yield, viscosity, phosphorus content, and reduced color than commercially available zein from wet milling. The protein content of zein from DDGS is also similar to that of commercial zein. The reduced color of the zein is most likely due to the defatting during oil extraction from DDGS.

A novel process of using acidic conditions in the presence of a reducing agent has been optimized to obtain high quality zein with about 90% proteins and a yield of about 44% of the proteins in DDGS. The zein obtained in this research has higher viscosity and is whiter than commercially available zein. In addition to the zein, about 17% oil similar in composition to commercially available corn oil has also been obtained. The zein obtained also has high phosphorus content, which may provide the proteins with relatively high flame resistance.

ACKNOWLEDGMENT

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