DOI:10.1021/jf901713w

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Comparison of Methods for Extracting Kafirin Proteins from Sorghum Distillers Dried Grains with Solubles

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Use of coproducts generated during fermentation is important to the overall economics of biofuel production. The main coproduct from grain-based ethanol production is distillers dried grains with solubles (DDGS). High in protein, DDGS is a potential source of protein for many bioindustrial applications such as adhesives and resins. The objective of this research was to characterize the composition as well as chemical and physical properties of kafirin proteins from sorghum DDGS with various extraction methods including use of acetic acid, HCl−ethanol and NaOH−ethanol under reducing conditions. Extraction conditions affected purity and thermal properties of the extracted kafirin proteins. Extraction yields of 44.2, 24.2, and 56.8% were achieved by using acetic acid, HCl−ethanol and NaOH−ethanol, respectively. Acetic acid and NaOH−ethanol produced protein with higher purity than kafirins extracted with the HCl−ethanol protocol. The acetic acid extraction protocol produced protein with the highest purity, 98.9%. Several techniques were used to evaluate structural, molecular and thermal properties of kairin extracts. FTIR showed α-helix dominated in all three samples, with only a small portion of β-sheet present. Electrophoresis results showed α_1, α_2 band and β kafirins were present in all three extracts. Glass transition peaks of the extracts were shown by DSC to be approximately 230 °C. Kafirin degraded at 270−290 °C. Size exclusion chromatography revealed that the acetic acid and HCl−ethanol based extraction methods tended to extract more high molecular weight protein than the NaOH−ethanol based method. Reversed phase high-performance liquid chromatography showed that the γ kafirins were found only in extracts from the NaOH−ethanol extraction method.

KEYWORDS: DDGS; DSC; extraction; FTIR; kafirin; LCE; protein; RP-HPLC; sorghum; SEC; TGA

INTRODUCTION

In the United States, sorghum is an important crop in the Central Plains and ranks behind only wheat and maize in production. Sorghum is used worldwide as both animal feed and human food, but its major use in the United States is animal feed. However, increasing amounts are being used for fuel production; more than 15% of the U.S. sorghum crop is used for ethanol production, which produces more than 450 kilotons of DDGS, the major coproduct generated from the fermentation of grain (*1*). Currently, sorghum DDGS is used as a high-protein, non-animal-based livestock feed supplement (*2*). As more ethanol plants are built in response to demand for fuel ethanol, there will be an increasing supply of DDGS (*3*) with potential oversupply in regions with a high density of ethanol plants. Therefore, there is a need to develop alternative uses for DDGS in food or bioindustrial products. DDGS is the residue left after starch is converted to ethanol; thus, it is high in protein (~30−40% on average) (*4*) and an inexpensive source of protein (*5*). From 450 kilotons of sorghum DDGS, more than 136 kilotons of sorghum protein (on average) is available for use.

When heated in the presence of water, sorghum proteins extensively cross-link to a greater degree than similar proteins in maize and rice (*6*). Cross-linking of sorghum proteins with phenolic compounds has been exploited to produce high-quality protein films (*7*). Sorghum proteins may also be more hydrophobic than similar proteins in maize (*6*). These unique properties make sorghum proteins of interest for industrial applications.

Several methods have been investigated for extracting kafirins from sorghum flour or bran for food and other uses. Emmambux and Taylor (*8*) adapted a method widely used for zein extraction and studied the interaction between isolated kafirins and phenolic compounds. Taylor et al. (*9*) also developed a novel method to extract sorghum proteins with acetic acid, desirable because of its low dielectric constant, noninflammable property and acceptability to certain religions and because it is a food-compatible solvent. Wong et al. (*10*) and El Nour et al. (*11*) extracted kafirin with 2-propanol, and several other groups have used tertiary

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 Published on Web 08/24/2009

butanol (*12*−*14*). Park and Bean (*15*) optimized the use of alkaline sodium borate/SDS buffers with reducing agents to extract sorghum protein based on the method of Hamaker et al. (*16*). Zhao et al. (*13*) and Ioerger et al. (*17*) combined sonication with alkaline sodium borate/SDS buffers to extract sorghum proteins without reducing agents as first reported by El Nour et al. (*11*). Tesso et al. (*18*) combined pepsin with sodium tetraborate to extract proteins from sorghum. Bean et al. (*19*) compared different extraction and precipitation conditions for isolating sorghum proteins from flour. Da-Silva and Taylor (*20*) extracted kafirin from sorghum bran left over from sorghum flour milling with ethanol combined with reducing reagent. Gao et al. (*21*) used the same method to extract kafirin to study the secondary structure and biofilm formation. Some research has investigated extracting maize proteins (zein) from maize-based DDGS (*22*−*24*).

Still, little to no research has been conducted on extraction of sorghum proteins from DDGS. Objectives of this study were to compare various methods for extracting sorghum kafirin proteins from DDGS and characterize the chemical composition as well as physical and thermal properties of the isolated proteins. Such information should benefit future studies on functional properties of sorghum protein and use of sorghum protein from DDGS for industrial applications.

MATERIALS AND METHODS

Materials. Sorghum DDGS (moisture content 14.4%) was provided by White Energy (Russell, KS). Sodium metabisulfite, sodium sulfite, glacial acetic acid, HCl, ethyl alcohol, NaOH and petroleum ether were purchased from Fisher Scientific (Pittsburgh, PA).

Kafirin Extraction Using Acetic Acid Method. Three different protein extraction methods were used to prepare kafirin protein isolates for testing. The acetic acid method was that of Taylor et al. (*9*). Sorghum DDGS was milled through a 1.0 mm screen with a Magic Mill III Plus Grain Mill (Salt Lake City, UT), and then 75 g was presoaked in four volumes of 0.5% (w/w) sodium metabisulfite for 16 h at 70 rpm in a Gyromax 939 XL incubator shaker (Amerex Instruments, Inc., Lafayette, CA). The sodium metabisulfite solutions were removed by sieve filtration (US #200 with 7.4×10^{-5} m opening) combined with vacuum filtration (vacuum 9.5×10^{4} Pa, Whatman grade 4 filter paper). The filtrate was then mixed with five volumes of glacial acetic acid and stirred for 1 h. The mixture was centrifuged at 1000*g* for 10 min (Thermo IEC, Needham Heights, MA). The supernatant was decanted through a six-layer cheesecloth to remove the oil layer on top. The pH of the supernatant was slowly adjusted to 5.0 with 50% (w/v) NaOH in a beaker placed in an ice water bath. The mixture was kept overnight at 4 °C and then centrifuged at 3500*g* for 10 min. The sediment was rinsed and washed with distilled water by centrifuging at 3500*g* for 10 min three times and then freeze-dried. The protein was defatted three times by mixing with a 5-fold weight of petroleum ether followed by shaking in the incubator shaker at 70 rpm for 5 min and centrifuging at 3500*g* for 10 min and then kept under a fume hood overnight to evaporate the solvent.

Kafirin Extraction Using HCl−Ethanol Method. The HCl−ethanol method was referenced from Xu et al. (*23*). Milled sorghum DDGS was defatted three times as described for the acetic acid method. The defatted sample (75 g) was mixed with 10-fold 70% ethanol, the pH was adjusted to 2.0 with 20% (v/v) HCl, and 0.25% (w/v) sodium sulfite was added. The mixture was put in a water bath (Precision 280 Series, Winchester, VA) at 78 °C and stirred for 2 h. The mixture was centrifuged at 3500*g* for 10 min. The supernatant was collected and reduced by a rotary evaporator (RE 111 Rotavapor, type KRvr TD 65/45, BUCHI, Switzerland) under low pressure at 40 °C. The remaining solvent was dried in a Blue M Stabil-Therm oven (model OV-500C-2, Blue M Electric Co., Blue Island, IL) at 49 °C overnight. Because visible oil was observed on the isolated proteins, the sample was defatted a second time as described previously. The final product was milled into a powder with a Udy cyclone mill (model 3010-018, UDY Corp., Fort Collins, CO).

Kafirin Extraction Using NaOH−Ethanol Method. The extraction method described by Emmambux and Taylor (*8*) was used for the NaOH−ethanol method. Milled sorghum DDGS (75 g) was mixed with 10-fold 70% ethanol, 0.35% NaOH (w/v) and 0.5% sodium metabisulfite (w/v). The mixture was placed in a water bath at 70 °C, stirred for 1 h, and then centrifuged at 3500*g* for 10 min. The ethanol content in the supernatant was diluted to 40%, and the suspension was put in a freezer at −20 °C overnight. The suspension was centrifuged at 3500*g* for 10 min, and the sediment was rinsed and washed 3 times with distilled water and dried in the oven at 49 °C overnight. The product was defatted following the procedure described previously and milled into a powder.

Chemical Analysis of Kafirin Protein. Protein content was measured using nitrogen combustion via a Leco FP-2000 nitrogen determinator (St. Joseph, MI) according to AOAC method 990.03 (*25*). Nitrogen was converted to protein using a factor of 6.25. Fat content was determined by using the Soxhlet petroleum ether extraction method according to AOAC method 920.39C for cereal fat and expressed as the weight percentage on dry basis (*26*). Crude fiber was determined according to AOCS approved procedure Ba 6a-05 (*27*).

Fourier Transform Infrared Spectroscopy (FTIR). The FTIR data were collected in the region of 400 to 4000 cm^{-1} with a Nicolet Nexus 670 FTIR spectrometer (Thermo Electron Corp., Madison, WI). About 4 mg of sample was ground thoroughly with about 150 mg of KBr (Acros, IR grade, +99%) in a smooth mortar. The transmission spectra of 50 scans of each sample were collected at a resolution of 4 cm^{-1} in the reflectance mode.

Lab-on-a-Chip Electrophoresis (LCE). Soluble fractions of protein samples (150 mg total weight of sample) were extracted in 1 mL of 50% isopropanol with 2% SDS (w/v) and 2% beta-mercaptoethanol (2BME) (w/v). Mixtures were then vortexed for 30 min (Daigger vortex genie 2 model G-560, Bohemia, NY), and centrifuged (Eppendorf centrifuge 5415, Westbury, NY) for 5 min at 13400*g*, and supernatants were collected and used for protein analysis. Samples were then loaded to a Protein 230 chip for analysis on the Bioanalyzer 2100 lab-on-a-chip equipment (Agilent Technologies, Palo Alto, CA) according to the manufacturer's instructions. The molecular weight markers (ladder) of the LabChip Kit were applied for the molecular weight determination of the unknown proteins. Evaluation of the results was performed with Protein 230 assay software.

Size Exclusion Chromatography (SEC). The size distribution analysis was conducted as described by Ioerger et al. (*17*). Briefly, a high-performance liquid chromatography (HPLC) system (1100, Agilent, Palo Alto, CA) with a 300 mm × 7.8 mm BioSep-3000 column and security guard columns (Phenomonex, Torrance, CA) was used. The mobile phase was a pH 7 sodium phosphate buffer (50 mM) with 1% SDS added. Column temperature was maintained at 40 °C, and flow rate was 1 mL/min. Soluble fractions of protein samples were extracted from 100 mg of sample in 1 mL of a pH 10 Tris buffer (50 mM) with 2% SDS. Mixtures were then vortexed for 30 min, heat deactivated for 2 min at 80 °C and centrifuged for 4 min at 9300*g*, and supernatants were transferred to HPLC vials and injected as samples. Standard proteins thyroglobulin (669 kDa), BSA (66 kDa), and carbonic anhydrase (29 kDa) were analyzed to estimate the molecular weight distribution of the sorghum proteins separated by SEC.

Differential Scanning Calorimetry (DSC). Thermal transition properties of protein samples were measured with a Perkin-Elmer DSC 7 instrument (Perkin-Elmer, Norwalk, CT) that was calibrated with indium and zinc before official measurements. Samples of modified proteins weighing approximately 5 mg were measured in a large DSC pan under a nitrogen atmosphere. All samples were held at 25 °C for 1 min and then scanned from 25 to 280 °C at a heating rate of 10 °C/min. All experiments were performed in duplicate.

Reversed Phase High-Performance Liquid Chromatography (RP-HPLC). An HPLC system (1100, Agilent, Palo Alto, CA) with a 2.1 mm × 75 mm Poroshell 300 SB-C18 column and security guard columns (Agilent, Palo Alto, CA) was used. Soluble fractions of protein samples (100 mg) were extracted in 1 mL of 60% *tert*-butanol with 2% 2BME and 0.5% sodium acetate. Mixtures were then vortexed for 30 min, alkylated with 4-vinylpyridine and centrifuged for 4 min at 9300*g*, and supernatants were transferred to HPLC vials and injected as samples. Solvents A and B were water/0.1% trifluoroacetic acid and acetonitrile/0.1% trifluoroacetic acid (w/v). The gradient used was that B% reached 20−40−60−20% by 18 min elution. Column temperature was maintained at 55 °C, and flow rate was 0.7 mL/min.

Thermal Stability. The thermal stability of extracted proteins was characterized by thermal gravimetric analysis (TGA) (Perkin-Elmer TGA Pyris 7, Norwalk, CT). Around 10 mg of sample was examined at a heating rate of 10 °C/min from 25 to 800 °C under a dry nitrogen flux.

RESULTS AND DISCUSSION

Extraction methods affect the yield, purity and properties of kafirin proteins. The chemical content of DDGS and kafirin extracts is listed in **Table 1** along with extraction yields. Acetic acid and NaOH−ethanol methods resulted in higher yields and purity of kafirn extraction than HCl−ethanol method. Extraction rates of 44.2, 24.2, and 56.8% were achieved by using acetic acid, HCl−ethanol and NaOH−ethanol, respectively. This result is similar to the result of protein extracted from corn DDGS which is 44% of total protein (*28*). The HCl−ethanol method was not able to extract kafirin with purity as high as that in kafirin extracted by Xu et al. (*23*). This is probably due to the proteins in DDGS being mostly denatured and HCl−ethanol not being strong enough to dissolve the denatured proteins. HCl−ethanol solution did not have the right polarity to dissolve sorghum protein as compared to the acetic acid solution and the NaOH−ethanol in the acetic acid and NaOH−ethanol methods. In addition, reduction of disulfide bonds is not as effective at low pH. With the acetic acid method, samples were presoaked for extended times in the presence of reducing agents; this was not the case with the HCl based method.

Table 1. Chemical Composition of Sorghum DDGS and Extracted Kafirin Protein

	chemical composition (%) and kafirin extraction rate (% db)			
source of materials	protein	extraction rate	lipid	crude fiber
DDGS	35.47		3.88	10.39
method I[a]	98.94 a[b]	44.1b	2.79a	1.03b
method II[a]	42.32 b	24.2c	0.87b	0.80c
method III[a]	94.88 a	56.8a	0.80b	1.54a

[a] Method I: Kafirin was extracted using acetic acid under reducing conditions. Method II: Kafirin was extracted using HCl−ethanol under reducing conditions. Method III: Kafirin was extracted using NaOH−ethanol under reducing conditions. [b] Values followed by the same letter in the same column are not significantly different ($p < 0.05$).

Fourier Transform Infrared Spectroscopy. The FTIR spectra of kafirin proteins extracted by the acetic acid and NaOH−ethanol methods were similar (**Figure 1**). Examination of the carbohydrate region (1250−1000 cm^{-1}) indicated this region for kafirin extracted by HCl−ethanol has increased intensity relative to the same region for the kafirin extracted by NaOH−ethanol, followed by the absorptions for kafirin extracted by acetic acid, suggesting a higher carbohydrate content in kafirin extracted by HCl−ethanol, followed by the NaOH−ethanol and then acetic acid methods. This result is in agreement with the gross composition of the samples; lower purity protein had higher impurities, which could include carbohydrates (*29*).

The amide A, A′, I, II, and III bands are frequently used protein characteristic absorption bands (*30*). The N−H stretch is a localized mode that appears as a doublet called amide A and amide A′. Amide A originates from N−H stretching, and amide A′ originates from the Fermi resonance of either the N−H stretching with the overtone of the amide II band in *trans*-amides or N−H stretching with the combination band of C═O stretching and N−H in-plane binding in *cis*-amides (*31*). Amides A and A′ appeared in the curves at 3300 cm^{-1} and 3070 cm^{-1} in **Figure 1** (*32*, *33*). The amide I band arises primarily from C═O stretching vibrations of the peptide backbone and possibly from C−N stretching and C(C−) deformation and appeared at 1660 cm^{-1}. The amide II band arises from an out-of-phase combination of largely N−H in plane bending and C−N stretching and smaller contributions from C═O in plane bending and N−C stretching and appeared at 1540 cm^{-1} (*34*). The amide III band arises from the in-phase combination of N−H in plane bending and C−N stretching and some contribution from C−C stretching and C−O bending (*33*). Amide III is a relatively complex and weak band and appears at 1240 cm^{-1} (*35*, *36*).

The locations of amide I, II and III bands are sensitive to the secondary structures. Because of vibration of various turns and random coil, secondary structures induce overlapped broad absorbance (*33*, *37*). Several numerical calculations can increase the apparent resolution of the amide I band so secondary

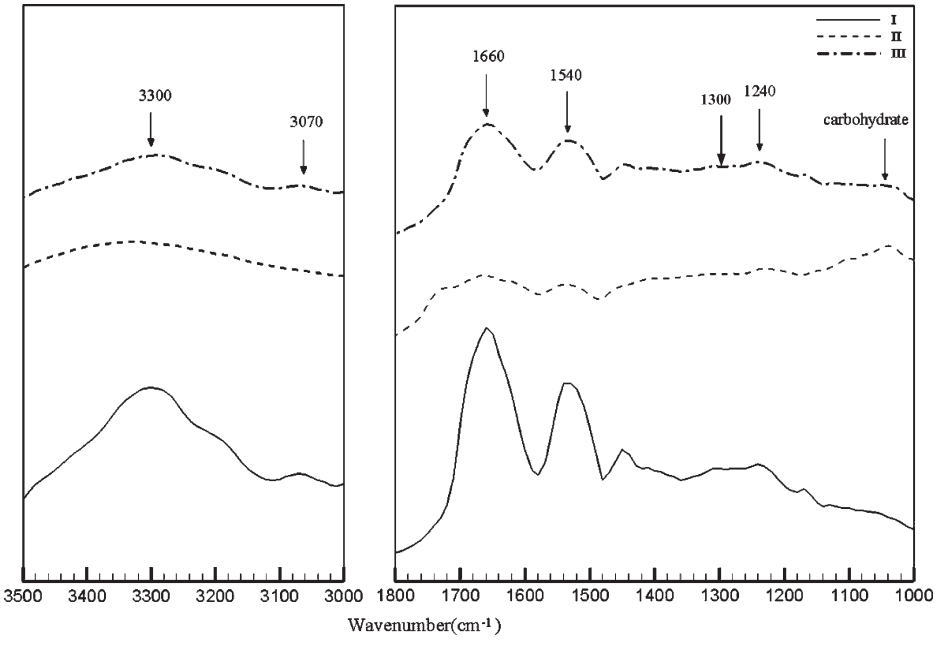

Figure 1. Fourier transform infrared spectra of the kafirin extracts. I, II and III denote the kafirin extracted with method I using acetic acid under reducing conditions, method II using HCl−ethanol under reducing conditions, and method III using NaOH−ethanol under reducing conditions, respectively.

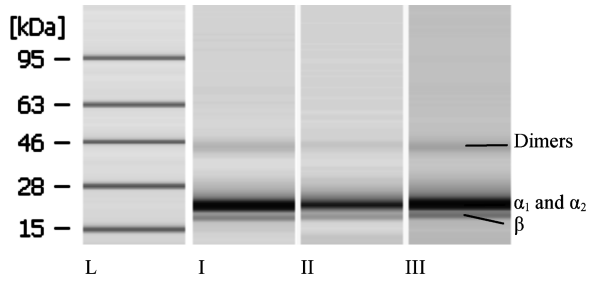

Figure 2. Molecular weight analysis. Lanes L, I, II and III denote the protein ladder and kafirin extracted with method I using acetic acid under reducing conditions, method II using HCl−ethanol under reducing conditions, and method III using NaOH−ethanol under reducing conditions, respectively.

structures can be estimated (*38*). The amide I band centered around 1660 cm^{-1} is considered to represent α-helix in all three sample curves, whereas the characteristic band of *β*-sheet at 1620 cm^{-1} is only shown in the curve for the kafirin extracted using HCl−ethanol. The higher extraction temperature and the reducing agent (sodium sulfite) in the kafirin extracted using HCl−ethanol might be the reason that more proteins were coagulated and more *β*-sheet conformation was present at the expense of some α-helix (*39*, *40*). The characteristic band of α-helix in the amide II band is shown at 1540 cm^{-1} in all three sample curves, and the band of *β*-sheet at 1530 cm^{-1} might have overlapped with the band at 1540 cm^{-1}. This requires certain numerical methods to increase the resolution of the band to make the estimation possible. Vibration of α-helix and random coil conformation in amide III are present at 1300 cm^{-1} and 1240 cm^{-1} in all three sample curves; greater intensity is shown in curves for kafirin extracted using acetic acid and NaOH−ethanol than for the kafirin extracted using HCl−ethanol (*33*, *39*, *41*). Investigation of secondary structure showed that α-helix dominated in all three samples, and a small portion of *β*-sheet was present.

Lab-on-a-Chip Electrophoresis. Electrophoresis patterns of protein extracts (**Figure 2**) separated with the lab-on-a-chip are similar to SDS−PAGE patterns reported in El Nour et al. (*11*) and Byaruhanga et al. (*42*). The α kafirins were resolved by electrophoresis into two closely spaced bands of about 25 kDa and 23 kDa (α_1 and α_2). The *β* kafirins have a lower molecular weight than α kafirins and were resolved at 18 kDa as indicated by the first peak in the molecular weight analysis curve. Only faint bands for the *γ* kafirins were seen with a molecular weight of 28 kDa, but previous research has shown that *γ* kafirins may not be detected well in the LCE system used (unpublished data). Bands with a molecular weight of 46 kDa were kafirin dimer (*42*). The band with molecular weight of 46 kDa did not appear in extracts using HCl−ethanol with the same intensity. This may be due to lower protein in the extracts using HCl−ethanol. All three extracts contained α, *β* and kafirin dimers; the intensity of bands in kafirin extracted using acetic acid is similar to that in kafirin extracted using NaOH−ethanol, whereas that in kafirin extracted by HCl−ethanol is smaller probably because of the low purity of the protein.

Differential Scanning Calorimetry. No peak was observed in the DSC thermograms until around 230 °C (**Figure 3**). The peaks, for which onset temperatures are 233.75, 227.94, and 231.35 for kafirins extracted by the acetic acid, HCl−ethanol, and NaOH−ethanol methods, respectively, are speculated to be glass transition peaks. Nanda and Nayak (*43*) observed a similar thermal property of urea modified soy protein isolate, and Ghanbarzadeh and Oromiehi (*44*) observed it in zein film. The high temperature caused protein denaturation including disulfide cross-linking, which leads to the lowered digestibility of cooked

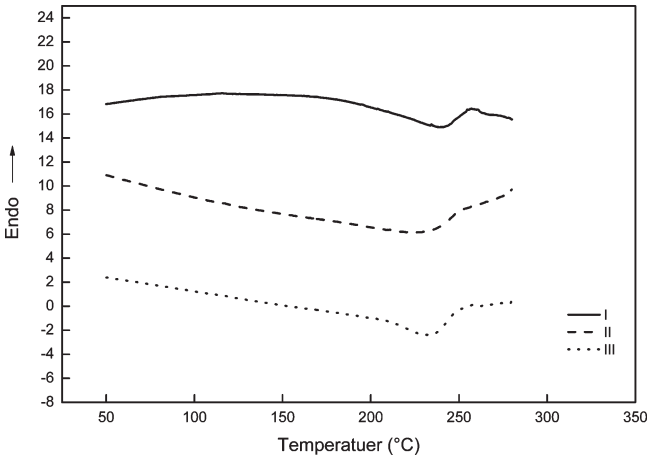

Figure 3. Thermograms of the kafirin extracts. I, II and III denote the kafirin extracted with method I using acetic acid under reducing conditions, method II using HCl−ethanol under reducing conditions, and method III using NaOH−ethanol under reducing conditions, respectively.

kafirins (*6*). This could explain why the glass transition temperature was higher than the 150 °C reported by Gao et al. (*45*). There was no denaturation peak (endothermic) detected, indicating the protein was denatured from heating in cooking and distillation procedures during fermentation. Zhao et al. (*46*) suggested sorghum proteins cross-link and denature after fermentation. The heat energy was probably used to break the disulfide bonds in protein molecules because the disulfide bond was reported cleaved at 230−250 °C (*47*). This is also the temperature at which Osaki (*48*) reported pyrolysis of spider silk thread occurs.

Size Exclusion Chromatography. The SEC chromatograms of protein extracts from the different methods are shown in **Figure 4**. Chromatograms of the protein extracts were very similar in the 6.6−10 min elution range. More proteins with a molecular weight >669 kDa were present in kafirins extracted by acetic acid and HCl−ethanol than in the kafirin extracted by NaOH−ethanol. The acetic acid and HCl−ethanol methods tend to extract more high molecular weight protein fractions than the NaOH−ethanol method. This protein fraction has a molecular weight much larger than individual kafirins (≈20−30 kDa), indicating the presence of large polymeric proteins, despite the fact that reducing agents were used in all extraction methods. These high molecular weight peaks occur naturally in sorghum flour (*11*, *17*) but would also be expected to result from heating during the fermentation procedure (*46*). The fact that not all the larger polymers were broken down during extraction, even in the presence of reducing agent, could indicate either reduction-resistant polymers or polymers not formed via disulfide bonds. Additionally, the pH of the extraction procedure may influence the effectiveness of the reduction reaction. Because the reducing reaction is much more effective in an alkaline environment than in an acidic environment, the NaOH−ethanol method might have induced more disulfide bond reduction, as in Cayot et al.'s research (*49*). Bean et al. (*19*) also observed chromatograms similar to the acetic acid and HCl−ethanol methods without this high molecular weight peak in analysis of protein extracted from sorghum grain with an acidic procedure similar to acetic acid and HCl−ethanol. The peak shown around 66 kDa was probably the dimer of the kafirin monomers (**Figure 2**).

Reversed Phase High-Performance Liquid Chromatography. The RP-HPLC patterns of protein extracted from various extraction conditions are shown in **Figure 5**. Patterns from kafirin extracts using acetic acid and HCl−ethanol were similar to the

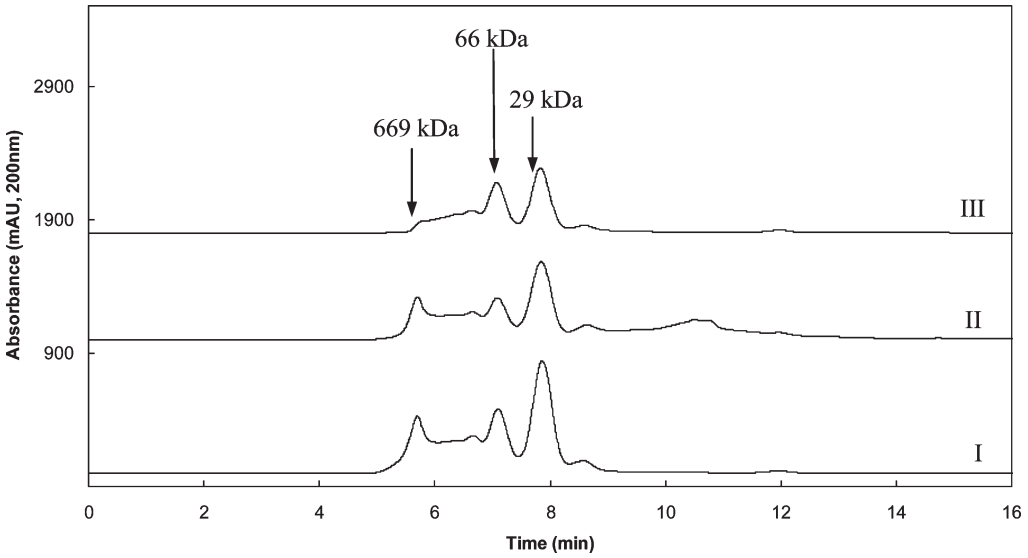

Figure 4. Size exclusion chromatography separations of kafirin extracts. I, II and III denote the kafirin extracted with method I using acetic acid under reducing conditions, method II using HCl−ethanol under reducing conditions, and method III using NaOH−ethanol under reducing conditions, respectively. Arrows indicate elution position of standard proteins used as molecular weight markers.

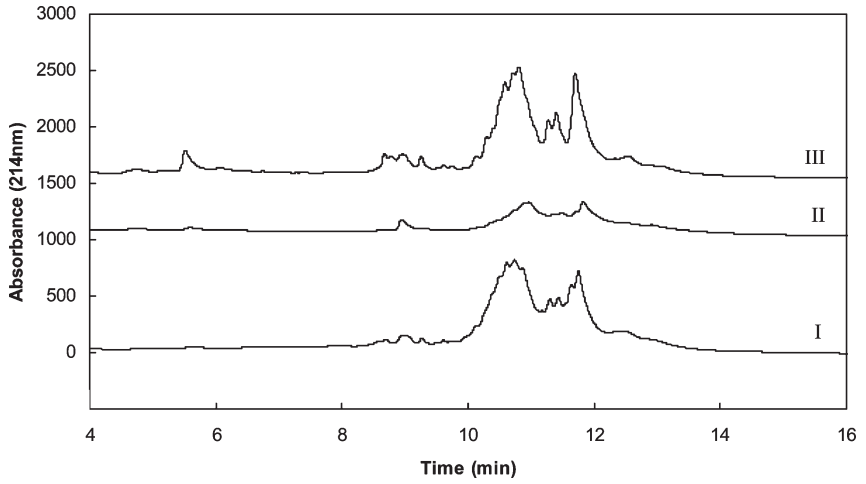

Figure 5. Reversed phase high-performance liquid chromatography separations of kafirin extracts. I, II and III denote the kafirin extracted with method I using acetic acid under reducing conditions, method II using HCl−ethanol under reducing conditions, and method III using NaOH−ethanol under reducing conditions, respectively.

kafirin patterns reported by Park and Bean (*15*) and Ioerger et al. (*17*). The α/β kafirin peaks elute at ~8−13 min and were seen in all extracts. The γ kafirins (~5.7 min) were observed only in kafirin extract using NaOH−ethanol, which is similar to studies by Ioerger et al., (*17*) and Zhao et al., (*13*) in which the extraction was done with alkaline sodium borate and 60% *tert*-butanol, respectively. Parris et al. (*50*) observed γ zeins in similar extraction conditions (60% ethanol and 0.1 N NaOH). The overall peak heights in kafirin extract using HCl−ethanol are not as large as in the other two samples because of the lower purity from this extraction method. Minor qualitative differences of α/β kafirins were noted between kafirin extracted by acetic acid and kafirin extracted by NaOH−ethanol because of the different extraction procedure. This kind of difference could exist in kafirin samples from the different precipitation step alone (*19*). The RP-HPLC patterns of kafirins from DDGS were not as sharp as patterns typically seen in sorghum kafirins extracted directly from sorghum grain (*17*, *51*) indicating proteins might have been degraded by the extreme extraction conditions or modified through reactions including deamidation (*50*).

Thermal Degradation. Thermal degradation properties of kafirin extracts from different extraction procedures were different (**Figure 6**). The degradation of samples went through three stages. The first stage (weight loss from room temperature to around 124 °C) is ascribed to water evaporation (*52*, *53*). Wang et al. (*54*) had a similar explanation for their results with soy protein. The second stage corresponds to the degradation of sorghum protein. The degradation of protein undergoes multiple mass loss peaks (**Figure 6B**), suggesting compositions of different thermal stability in protein. Degradation rate of kafirin extracted by acetic acid was similar to that of kafirin extracted by NaOH−ethanol. Sorghum proteins could have associated with nonprotein components like phenolic compounds, nonstarch polysaccharides in the pericarp and endosperm cell walls and phytate in the pericarp and germ (*6*). The HCl−ethanol method produced kafirin extracts with the lowest purity; the association between protein and nonprotein components might have survived the extraction procedure and resulted in the degradation peak around 200 °C. Kafirin extracted by NaOH−ethanol has less impurity and shows a small peak while kafirin extracted by acetic

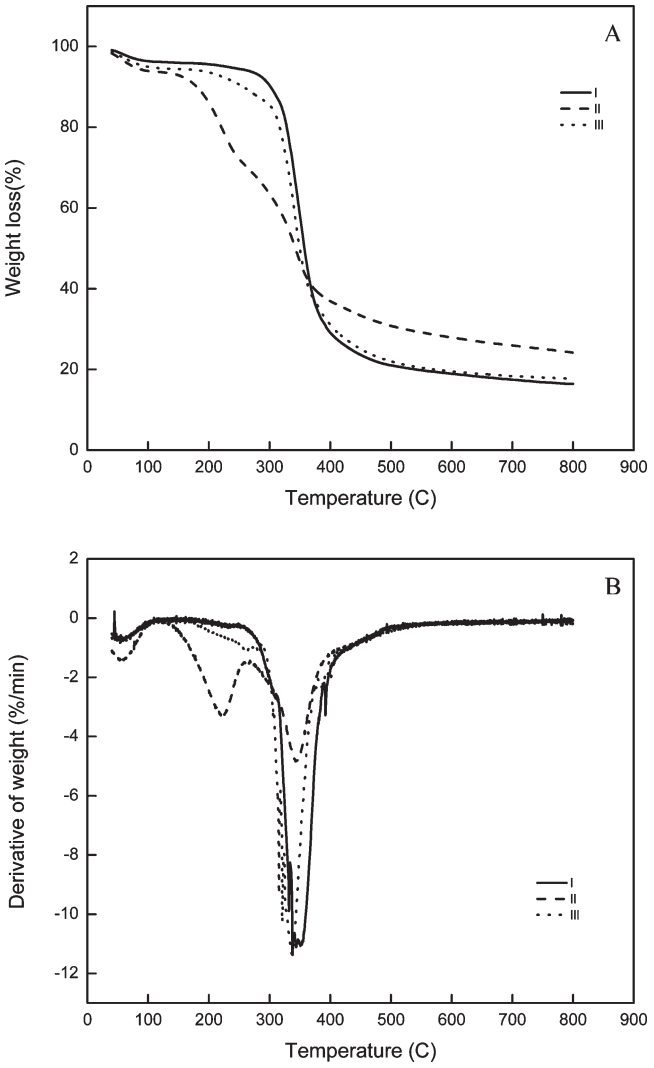

Figure 6. Thermal gravimetric analysis (**A**) and derivative thermal gravimetric analysis DTGA (**B**). Thermal stability of sorghum kafirin extracted with different methods. I, II and III denote the kafirin extracted with method I using acetic acid under reducing conditions, method II using HCl−ethanol under reducing conditions, and method III using NaOH−ethanol under reducing conditions, respectively.

acid almost did not have any peak. This result is in accordance with the chemical contents of these three protein extracts. The third stage, starting around 270−290 °C, is believed to be protein thermal degradation peak, similar to the thermal degradation of zein at 250−280 °C (*54*, *55*) and soy protein at 292 °C (*57*). For sorghum protein, the thermal degradation was completed at temperature around 580 °C. Above 580 °C the sample loss was not significant and the solids left were primarily ash.

Protein functionality is derived from the protein's structure. Understanding structural, molecular and thermal properties of the protein extracted from DDGS may be of use in characterizing the functional properties of the isolated proteins. Research is in progress to test these isolated protein fractions in bioindustrial applications, and basic material characterization is necessary and important to fully utilize proteins isolated from DDGS.

ACKNOWLEDGMENT

The authors extend their gratitude to Dr. Keith Hohn for providing results of the infrared spectroscopy. The authors also thank Dave Trumble for carrying out the chemical analysis. Contribution No. 09-330-J from the Kansas Agricultural Experiment Station, Manhattan, KS 66506.

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Received May 21, 2009. Revised manuscript received July 31, 2009. Accepted August 4, 2009.