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Processing Scale-Up of Sicklepod (*Senna obtusifolia* L.) Seed

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Sicklepod (*Senna obtusifolia* L.) is an invasive weed species especially of soybean and other field crops in the southeastern United States. The seeds contain a small amount (5−7%) of a highly colored fat as well as various phenolics, proteins, and galactomannans. The color of sicklepod seed oil is such that the presence of a small amount of the weed seed in a soybean crush lowers the quality of the soybean oil. Sicklepod is very prolific, and even volunteer stands yield >1000 lb of seed per acre, and prudence calls for tapping the potential of this weed as an alternative economic crop in the affected region. Pursuant to this, we have shown in laboratory-scale work the feasibility of separating the components of sicklepod seed. However, at kilogram and higher processing quantities, difficulties arise leading to modification of the earlier approach in order to efficiently separate components of the defatted seed meal. In a version for cleanly separating the proteins, the defatted meal was extracted with 0.5 M NaCl solution to remove globular proteins. Prolamins were extracted from the pellet left after salt extraction using 80% ethanol, and glutelins were then obtained in 0.1 N alkali from the residual solids left from ethanol treatment. In a pilot-scale version for water-soluble polysaccharides, the defatted meal was stirred with deionized water (DI) and centrifuged. The pooled centrifugates were heated to 92 °C (20−25 min), filtered, cooled to room temperature, and passed through a column of Amberlite XAD-4 to separate the polysaccharides from the anthraquinones. *Senna obtusifolia* L. is a one-stop-shop of a seed (from food components to medicinals).

KEYWORDS: *Senna obtusifolia*; albumins; globulins; glutelins; prolamins; glycoproteins; DSC characterization; carbohydrates; anthraquinones

INTRODUCTION

Sicklepod (*Senna obtusifolia* L) is a leguminous plant that thrives unwanted in crop fields such as soybean and grain farms and is therefore described as a weed in much of southeastern United States where it is endemic. Although the recent introduction of Round-Up-ready soybeans mitigates infestation of the beans by sicklepod, the latter is so prolific that even volunteer stands yield over 1000 lb of seed per acre. The composition of sicklepod seed has been reported to include anthraquinones 1−2%, fats 5−7%, proteins 14−19%, and carbohydrates 66−69% (*1*−*5*). Sicklepod flour imported from India is already in the market as a hydrocolloid additive in pet foods because of its unique galactomannan ratio, which enhances flexibility in the range of formulation compositions ((*6*) and Nicholson, J. J., personal communication). Agricultural prudence calls for tapping the potential of this domestic plant as an alternative economic crop in the area of its occurrence, and some co-operative effort by the Agricultural Research Service, farmers, academics, and industry has been expended in exploring the promise of this plant. In this regard, we have previously reported a laboratory-scale process for partitioning the crushed seed to separate the four main classes of components in the seed (*7*). To effect a scale-up of that process to the kilogram and higher quantities, technical considerations led to the development of a two-pronged approach for enhanced isolation of the different seed components into their corresponding classes. In both approaches, water is the principal solvent used except for defatting the meal. To isolate the various protein groups, we have used solubility as the guiding principle (*8*−*10*). Thus, saline treatment was used to solubilize the globular proteins. For the sparingly water-soluble proteins, aqueous ethanol and aqueous alkali were respectively used to effect complete extraction of prolamins and glutelins. This study investigated scaled-up procedures for the extraction of sicklepod seed components, mainly fat, carbohydrates, phenolics, and

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proteins, and a partial characterization of the carbohydrate and protein fractions by FTIR, ^{13}C NMR, and MDSC techniques.

MATERIALS AND METHODS

Materials and Reagents. Sicklepod seeds were obtained from Wilder Farm, Raleigh, NC via NC State Department of Agriculture, NC State University. Petroleum ether was purchased from Sigma-Aldrich Co. (St. Louis, MO); Amberlite XAD-4 was obtained from Supelco, Bellefonte, PA; dialysis tubing (regenerated cellulose, MWCO 3,500), sodium chloride, sodium hydroxide, and acetic anhydride were obtained from Fisher Scientific (Chicago, IL). Sample centrifugations were achieved using for laboratory scale a J2-HS centrifuge (Beckman, Fullerton CA) and for kilogram scale a Clinton Separators Inc. centrifuge (Warminster, PA). Volume reduction of dilute extracts was attained using Reverse Osmosis Concentrator, Seprotech Systems, Inc. (Ottawa, Canada).

Instrumentation. The ^{13}C NMR spectra were recorded on a Bruker ARX-500 spectrometer with a 5-mm dual proton/carbon probe (Bruker Spectrospin, Billerica, MA); the internal standard was tetramethylsilane. Protein analysis was performed using a Truspec CHN 2000 analyzer, St. Joseph, MI.

Fourier Transform Infrared (FTIR) Spectrometry. FTIR spectra were measured on an Arid Zone FTIR spectrometer (ABB MB-Series, Houston, TX) equipped with a DTGS detector. Dried test samples of isolated solids were pulverized with KBr (1/300 mg) and pressed at 24,000 psi to generate transparent discs for FTIR analysis. Liquid derivatives were pressed between two NaCl discs (25 mm × 5 mm) to give thin transparent oil films for analysis by FTIR spectrometry. Absorbance spectra were acquired at 4 cm^{-1} resolution and signal-averaged over 32 scans. Interferograms were Fourier transformed using cosine apodization for optimum linear response. Spectra were baseline corrected, adjusted for mass differences and normalized to the methylene peak at 2927 cm^{-1}.

Thermal Analysis. Moisture content of each sample was obtained using the isohume method on a Q5000SA Dynamic Sorption analyzer (TA Instruments, New Castle, DE). Samples were analyzed using a Q2000 MDSC (TA Instruments, New Castle, DE).

Methods. *Isolation of Proteins. Albumins and Globulins.* Dehulled and petroleum ether defatted sicklepod meal (100 g), as described previously (*7*), was stirred into 0.5 M sodium chloride solution (1.0 L) at room temperature (25 min). The resulting mixture was centrifuged (12,000*g*) for 20 min. and the centrifugate saved. The resulting pellet was resuspended and stirred in a fresh equivalent volume of sodium chloride solution for the same time interval as that described above followed by centrifugation. This procedure was repeated a third time so as to exhaustively extract the albumin and globulin components of the sample. The pooled supernatants (centrifugates) were then dialyzed against deionized water using regenerated cellulose tubing, molecular weight cut off (3,500). The dialysis was carried out at 5 °C. The dialysis retentate comprised the soluble albumins and precipitated globulins, which were separated and freeze-dried to give 5.10 g, 5.1% albumin and 11.40 g, 11.4% globulin (**Figure 1**).

Prolamins. The solids (pellet) from the sodium chloride treatment were then triturated with 80% ethanol (1.0 L) for 30 min at room temperature and centrifuged (12,000*g*) for 20 min. The supernatant was saved while the pellet was treated two more times with the same volumes of fresh 80% ethanol. The combined ethanol extracts were dialyzed against deionized water at 5 °C and the retentate lyophilized to give the prolamin component (5.88 g, 5.9%). The FTIR spectrum of this component is shown in **Figure 2A**.

Glutelins Fraction. The glutelin component was extracted from the lyophilized pellet (61.2 g) left over from the prolamin extraction. This solid was stirred into 0.1 M sodium hydroxide solution (1.0 L × 3) for 30 min in each extraction. Each batch was centrifuged for 20 min at 12,000*g* and the centrifugate saved, whereas the final pellet was triturated with the alkali solution and filtered. The combined NaOH extracts were treated with 6.0 M HCl to pH 6.5−6.9 and then dialyzed against deionized water at 5 °C. The dialysis retentate comprised a supernatant and a precipitate; the mixture was centrifuged as described above for 20 min. The supernatants were filtered and the filtrate

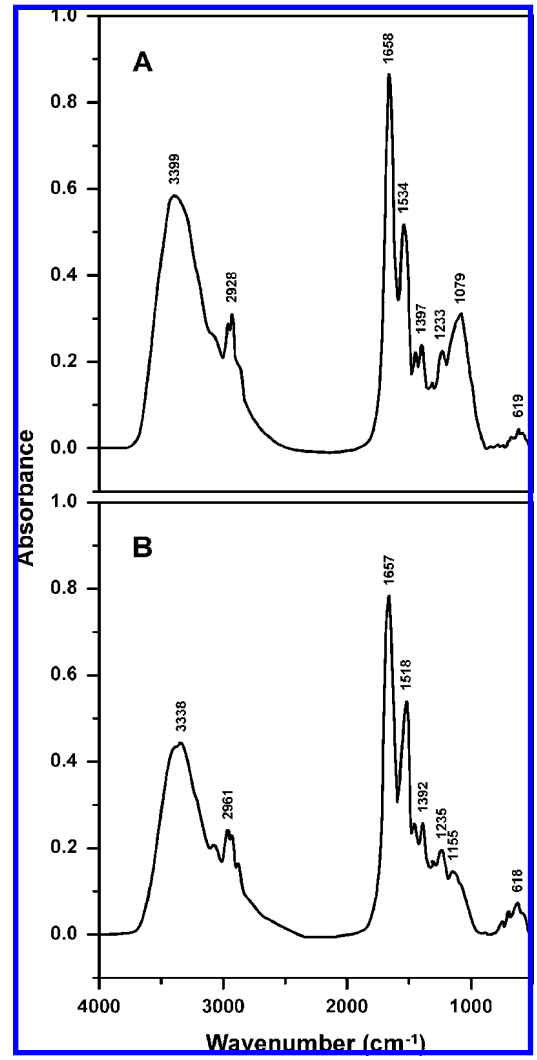

Figure 1. FTIR Spectra of (**A**) Sicklepod albumin fraction; (**B**) its globulin fraction.

lyophilized separately from the combined filter cake. Thus, two glutelin components were obtained from alkali extraction, namely, a water-soluble fraction, β-glutelin (7.4 g, 7.4% relative to starting material) and a water-insoluble fraction, α-glutelin (12.2 g, 12.2% overall, respectively). FTIR spectra of the freeze-dried β-glutelin ν_{KBr} (cm^{-1}) (3413 bvs, 2930 m, 1630 s, 1540 m, 1414 m, 1390 w-m, 1239 w, 1093 s, 1046 s, 634 w) and that of α-glutelin is shown in **Figure 2B**.

Sodium Hydroxide-Insoluble Residue. The remaining material was recalcitrant to NaOH treatment. A suspension of this component in deionized water had a pH of 11.5; on acidulation (HCl) to pH 6.5−7.0 followed by dialysis against deionized water, the resulting retentate contained a mother liquor and a large precipitate. These were separated and freeze-dried to give a minor component from the supernatant (1.0 g, 1.0%) and a major component from the precipitate (36.0 g, 36% overall). The light-brown major product gave an IR spectrum as seen in **Figure 2C**, whereas the freeze-dried supernatant, minor fraction (1.0 g), gave an IR spectrum (cm^{-1}): 3413 vs, 2930 m, 1630 s, 1540 m, 1414 m, 1093 s-vs, 1045 s-vs, 634 w.

Thermal Analysis Procedures. Samples (15−20 mg) were weighed into stainless steel DSC pans. Distilled water was added to each sample as follows: 40% (w/w) for freezable water analysis; 20%, 30%, 40%, and 50% for albumin peak kinetics; and 40%, 50%, 60%, and 70% for globulin peak kinetics. For the freezable water analysis, each sample was cooled to −90 °C and then heated 5 °C/min with modulation up to 170 °C. For the effect of moisture content on the thermal properties of each fraction, each sample was heated at 5 °C/min with modulation from 10 to 180 °C. Freezable water was calculated by dividing the enthalpy of the melting peak of water in the sample at 40% H_2O added

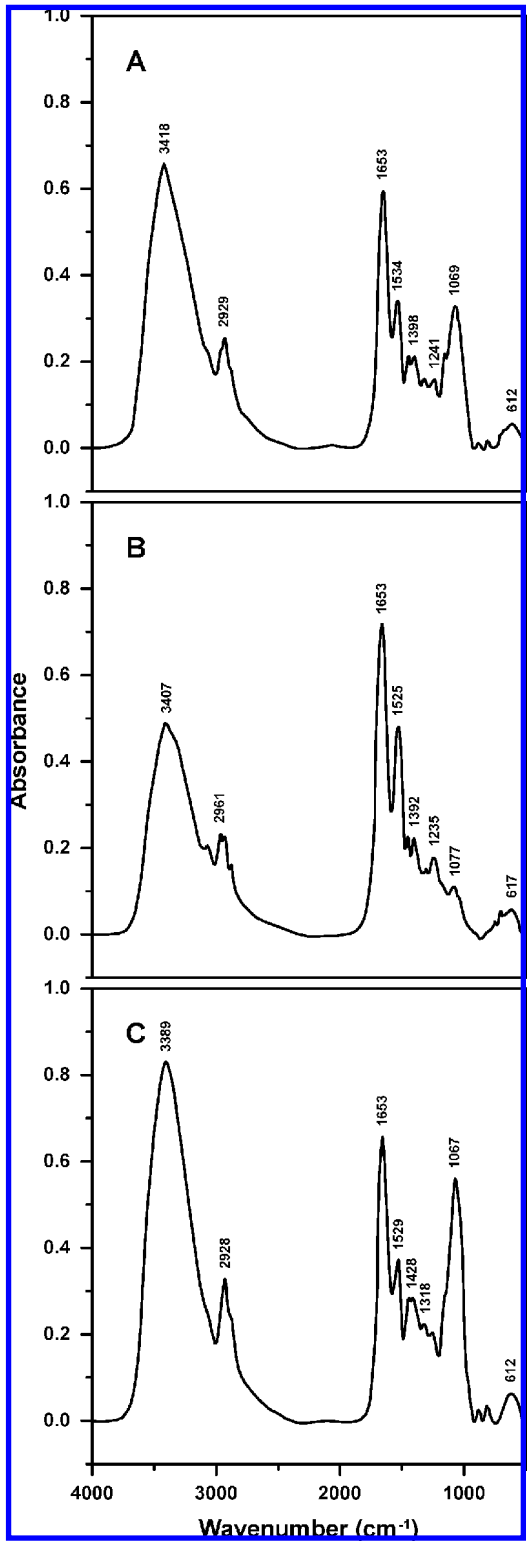

Figure 2. FTIR Spectra of other sicklepod protein fractions: (**A**) prolamin; (**B**) α-glutelin; and (**C**) glycoprotein.

by the heat of fusion of pure water (333.55 J/g). Unfreezable water was calculated as the difference between total moisture content of the sample and freezable water. Protein samples (20−25 mg) were placed in aluminum pans and hermetically sealed and quench cooled to −90 °C using the refrigeration system connected to the DSC. Throughout the run, the DSC cell was conditioned with nitrogen flow at a rate of 24 cm^3/min. Samples were then heated from −90 to 170 at 5 °C/min. The onset and peak temperatures were determined by the tangent method utilized by the instrument software, which minimizes operator error in determining the onset temperature.

Isolation of Carbohydrates and Anthraquinones. Defatted sicklepod meal (2.40 kg) was stirred into 70 L of deionized water (ca. 1 h) then pumped into the centrifuge (Clinton Separators, Inc.) running at 5,000−6,000 rpm. The centrifugates were recycled until clear supernatants were obtained and saved. The resulting pellet was removed from the centrifuge bowl and stirred with a fresh volume of deionized water (ca. 70 L) for the same amount of time as before and centrifuged to a clear supernatant that was again saved. The resulting pellet comprising water-insoluble proteins and nonwater-soluble polysaccharides was removed from the centrifuge drum and freeze-dried to 1.80 kg. Meanwhile, a portion of the combined centrifugates (3 L) was heated to 92−93 °C for 25 min. and filtered through a pad of Celite in a medium porosity sintered glass funnel; portions of the cooled filtrate were passed through a column bed of Amberlite XAD-4 (3180.9 mL) at a flow rate of about 15 mL/min. The column was rinsed with deionized water and the rinse volume combined with the earlier carbohydrate eluate. This dilute (0.3−0.7% solids) eluate was concentrated to 3.0% solids for lyophilization or spray drying. The bulk of the carbohydrate extract was decolorized with a mixture of clay and charcoal and lyophilized to a colorless powder (∼365 g). A sample of this solid gave an IR spectrum ν_{KBr} (cm^{-1}): 3418 vs,b, 2923 m, 1624 m, 1413 m, 1074 vs, 1034 vs, 812 w. ^{13}C NMR (DMSO-d_6) δ (ppm): [104.5, 103.6, 99.54, 97.26, 92.21, 92.08] anomerics; [83.00, 82.83, 77.56, 77.50, 77.31, 77.06,] C-4; [74.78, 74.72, 73.38, 73.33, 73.28, 72.95, 72.10, 71.94, 71.69, 71.38, 70.74, 70.32, 69.93, 69.25, 69.02, 67.16] C2, C3, C5; [63.53, 62.66, 62.61, 62.55, 60.99, 60.96] C-6.

Acetylation of a sample of this solid with neat acetic anhydride and Et_3N as catalyst gave a light yellow viscous acetate derivative. Its IR ν_{film} on NaCl disk (cm^{-1}): 3482 vw, 2960 m (CH_3 asym.), 2944 m (CH_2 asym.), 2855 w (CH_3 sym.), 1750 vs (C═O), 1434 m (CH_2 deform), 1370 s (CH_3 deform), 1224 vs (C-C-O), 1040 s-vs (C-CH_2-O), 907 m, 601 m. ^{13}C NMR $CDCl_3$ δ (ppm): [170.5, 170.1, 170.0, 169.7, 169.6, 169.5, 169.3, 169.27, 169.2, 169.11, 169.1, 169.06, 168.9, 168.55] C═Os; [100.27, 100.20, 95.95, 95.34, 92.78, 91.79, 91.35, 91.28, 89.87, 89.78, 88.72] anomerics; [80.64, 79.85, 78.96, 78.81, 78.20, 78.08] C-4; [72.39, 72.28, 70.07, 69.56, 69.33, 68.26, 68.09, 67.64] C-2, C-3, C-5; [63.38, 63.27, 63.13, 62.58, 61.96, 61.83, 61.62, 61.38, 61.31, 60.96, 59.93] C-6s; [20.54, 20.38, 20.33, 20.30, 20.27, 20.25, 20.22, 20.18, 20.12, 20.10, 20.07, 20.02, 19.99] -CH_3 of the acetoxy groups.

The column bed was further rinsed with deionized water, and this rinse was discarded. The resin bed was then eluted with 0.1 M ammonium hydroxide in ethanol to recover the anthraquinones as a reddish solution, which was concentrated in vaccuo to give 3.0 g of a red residue. The column bed was conditioned for further use by several washings with aqueous ethanol and finally with dilute ammoniacal ethanol until the light pink color faded at ca. pH 10.

RESULTS AND DISCUSSION

In contrast to the use of acetone−water, ethanol−water, 2-propanol−water, and so forth as extracting solvents to isolate sicklepod flour from the endosperm (*6*), the use of 0.5 M sodium chloride solution as a triturating solvent for the defatted endosperm allowed the removal of globular proteins from the meal (*7*, *8*), thus obviating the filtration difficulties usually encountered in the former process. This was intended to circumvent the filtration difficulties brought about by the fouling of filter systems by water-soluble proteins in the extraction and decolorization steps of water-soluble carbohydrate isolation from the meal. In processing small quantities of sicklepod meal for carbohydrates, soluble proteins were a manageable problem; however, the problem becomes unacceptable at the kilogram scale. An additional advantage of the current process is provision of a means of separating the biologically active proteins (albumins and globulins) from the purely storage types in the seed meal. The many steps involved in the overall process allowed the separation of the various classes of proteins in reasonable purity as shown in **Table 1**. Analysis of the albumin

Table 1. Analysis of Dry Protein Content of Sicklepod Protein Isolates

sample	% dry protein	amount extracted (g)	actual protein content (g)
albumin	64.59	5.10	3.30
globulin	92.24	11.40	10.50
prolamin	49.94	5.88	2.50
β-glutelin	56.11	7.40	4.20
α-glutelin (ppt)	99.47	12.00	11.90
residue (sol)	23.77	1.00	0.20
residue (ppt)	36.00	33.30	12.00
total grams protein from 100 g defatted meal sample			~44.59

and globulin isolates by IR spectrometry show characteristic amide A and B peptide absorption bands, N−H stretching modes or Fermi resonance usually seen around 3280 $cm^{-1}$and 3090 cm^{-1}, but observed here at 3399 cm^{-1}, 3077 cm^{-1} for albumins (**Figure 1A**) and 3338 cm^{-1} and 3068 cm^{-1} for globulins (**Figure 1B**). The main diagnostic protein bands in the IR spectra, i.e., the strong amide I (C═O stretch) and amide II (N−H in-plane bending) bands are observed at 1656 cm^{-1} and 1534 cm^{-1}, respectively, for the albumin fraction, which also shows a broad, medium to strong band at 1079 cm^{-1}(C−C−O stretch of 2° alcohol moiety out-of-phase stretch). The globulin fraction gives strong amide I and II absorption bands at 1657 cm^{-1} and 1518 cm^{-1}, respectively. Analysis of percent N in the globulin component gave a value corresponding to 92.2% dry protein content compared to 64.6% dry protein for the albumin fraction. The relatively stronger infrared absorbance bands at 3399 cm^{-1} (overlapping OH and NH stretching modes) and 1079 cm^{-1} (C−C−O stretch) in the albumin fraction compared to that of globulin may indicate the presence of occluded or bound carbohydrate moiety in the albumin that could not be removed in the dialysis process. The spectrum of the aqueous ethanol-soluble prolamin fraction, **Figure 2A**, shows a very strong, broadband centered at 3418 cm^{-1}, which overlaps the amide A absorption. The frequency and intensity of this band is reminiscent of a hydrogen bonded OH/NH stretch. Other infrared spectral features of this sample include strong amide I and II absorption bands at 1653 cm^{-1} and 1534 cm^{-1}, respectively. In addition, the spectrum exhibits a reasonably strong (C−C−O) band at 1069 cm^{-1} attributable to an out-of-phase stretch for 1° alcohol function on the protein surface. The water-insoluble α-glutelin fraction from NaOH treatment readily precipitated out of the dialysis retentate leaving the more soluble β-glutelin in the supernatant solution as had been observed by earlier workers in wheat, barley, and rye flours (*8*−*10*). The IR spectrum, **Figure 2B**, of the freeze-dried α-glutelin (12.2%) overall yield from the defatted meal gave a typical protein absorption spectrum. A broad amide A and B absorbance centered at 3423 cm^{-1} and 3077 cm^{-1}, a very strong amide I band at 1653 cm^{-1}, and a strong amide II at 1525 cm^{-1}. The analysis for percent N in this isolate gave a value equivalent to 99.5% dry protein content, whereas the material that was recalcitrant to sodium hydroxide treatment turned out to be complex. Its IR spectrum, **Figure 2C**, gave very strong absorbances at 3389 cm^{-1}(Fermi resonance), a strong amide I band at 1653 cm^{-1}, a medium to strong amide II band at 1529 cm^{-1}, and a strong band at 1067 cm^{-1}. The intensities and frequencies of these IR bands (3389 and 1067 cm^{-1}) indicate a material with both protein and carbohydrate characteristics and therefore could best be described as a glycoprotein. It comprised 36% of the defatted starting meal. Interestingly, the IR spectral characteristics of this isolate are exactly identical to that of an earlier water-insoluble residue we obtained from exhaustively extracting defatted sicklepod meal with warm water.

Differential scanning calorimetry testing of all the protein fractions indicated the presence of glass transition for the prolamin and β-glutelin, whereas albumin and globulin each showed an endothermic transition signifying their denaturation under the experimental conditions. The glycoprotein and α-glutelin showed no transition under the conditions of the experiment. Prolamin and β-glutelin, however, exhibited glass transition (*Tg*) at 57 °C (0.123 J/g/° C) and 52.2 °C (0.237 J/g/° C), respectively, which is consistent with the *Tg* values of grain proteins (*11*). Because of their globular structure, albumin and globulin exhibited denaturation transition, indicating that their structure was not influenced by the isolation method. Although both samples were freeze-dried before thermal analysis testing, albumin had twice the moisture content (5.8%) compared to that of globulin (2.16%). This difference was also reflected in the amount of moisture needed to achieve denaturation. Albumin denatured after the moisture content was raised to 25.8%, whereas globulin showed the corresponding transition at 42.2% moisture content. This indicates structural difference between the two fractions, where the higher ΔH of globulin (12.1 J/g) signifies a more compact structure than that of albumin (6.05 J/g) at the same moisture content. The high moisture content significantly reduced the peak temperature and the ΔH for both albumin and globulin (**Figure 3A** and **B**). Because of its more compact structure, globulin showed a stepwise degradation mechanism (**Figure 3B**), whereas albumin exhibited a steep drop in peak temperature and ΔH (**Figure 3A**).

The amount of freezable water in the protein was determined from the DSC data in accordance with the method of Vittadini and Vodovotz (*12*). Freezable water is one of the characteristics of proteins that can be used to test their effectiveness in some applications such as food products. Of these protein fractions, albumin and β-glutelin exhibited the lowest freezable-water content; they also showed the lowest melting temperature during the heating cycle (**Figure 4**). The melting of the freezable water in albumin and β-glutelin peaked at −7.4 and −18.2 °C, respectively, while the other samples melted between −0.04 and 1.66 °C. In **Figure 5**, the percent moisture content and the freezable water for each protein fraction is presented. Although the moisture content of all fractions was almost the same, the freezable water showed significant variation. Once again, globulin behaved differently relative to the other fractions. The high unfreezable water content of a sample indicates the ability of the protein to bind the water, where the water interaction with the surface of the protein is stronger than that of the water−water interaction. This interfacial interaction prevents water from freezing.

The scale-up of sicklepod meal extraction was achieved by stirring of the dry defatted meal into water allowing for solubilization of the water-soluble polysaccharides and globular proteins and some anthraquinones. The process allowed monitoring of the centrifugate for particulates so that the eluate could be recycled until free of solids. The particulate-free centrifugate was saved, and the pellet in the centrifuge bowl was fed back into the holding tank with a fresh volume of water for a second pass through the centrifuge. Portions of the extract were heated as indicated to coagulate soluble proteins for removal from the polysaccharide by filtration. The cooled filtrate was then passed through the (Amberlite XAD-4) resin bed to separate the polysaccharides from the anthraquinones that are retained on the column bed. Samples of the freeze-dried polysaccharide gave an IR spectrum typical of carbohydrates (**Figure 6A**). A very strong OH stretch broadened due to hydrogen bonding and centered at 3423 $cm^{-1}$was observed, while a much weaker alkyl

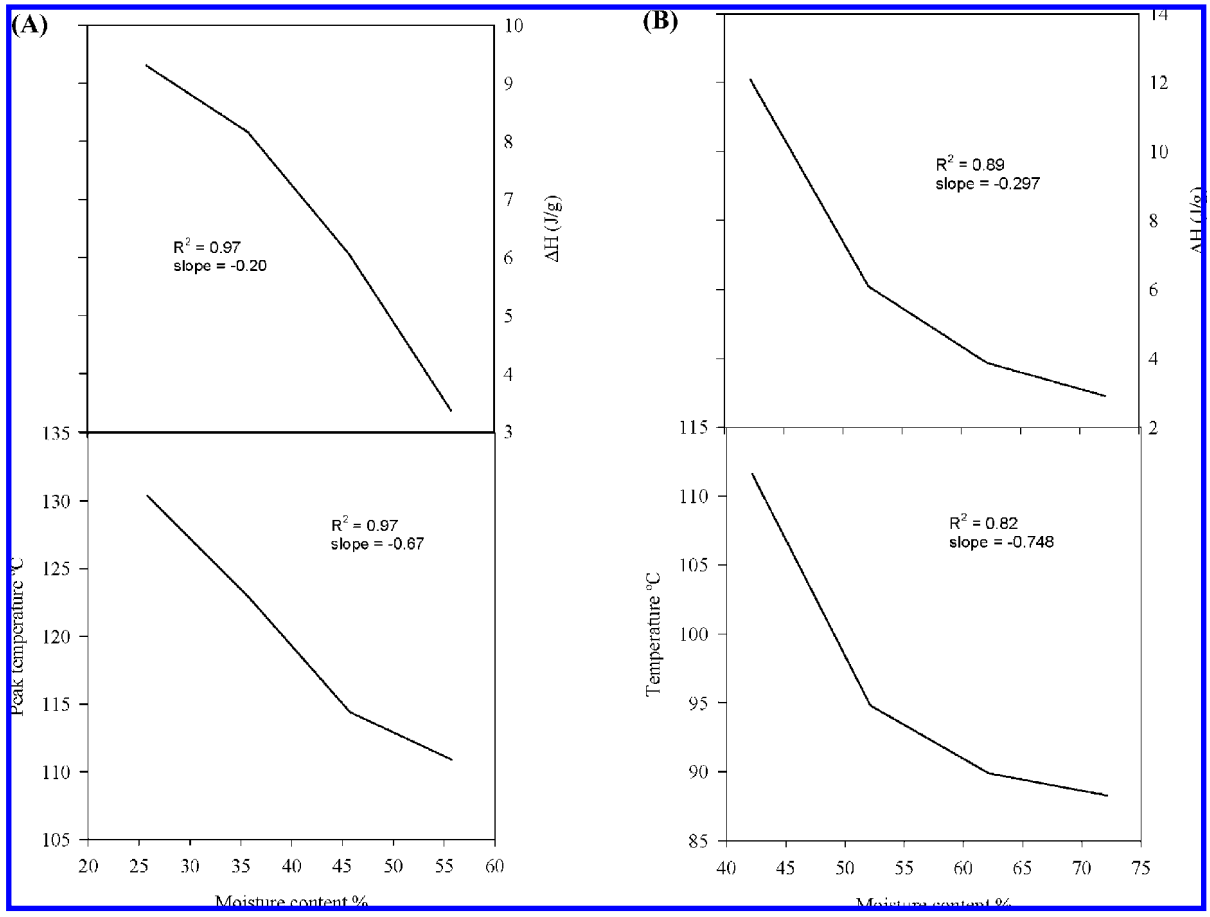

Figure 3. (**A**) Effect of moisture content on the peak temperature and ΔH value of albumin. (**B**) Effect of moisture content on the peak temperature and ΔH value of globulin.

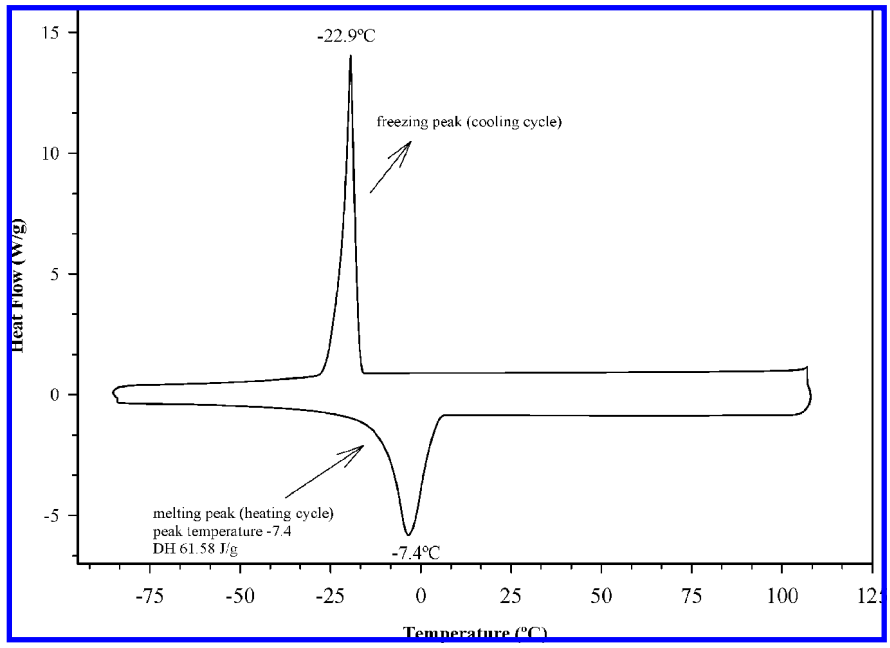

Figure 4. DSC profile of the sicklepod albumin fraction.

band (CH_2 stretch) occurred at 2923 cm^{-1}; a broad, medium intensity band was seen at 1624 cm^{-1} overlapping the region where the OH bending mode of water usually occurs. This band could be due to a trace amount of anthraquinone trapped in and coeluted with the polysaccharide. A weak broadband at 1409 cm^{-1} is assignable to the CH_2 deformation, whereas very strong C−C−O stretching modes are observed at 1074 and 1034 cm^{-1}, which are characteristic stretching modes of secondary and primary hydroxylated carbons, respectively. Acetylation of a sample of this solid gave an IR spectrum, **Figure 6B**, with new features: notably, the disappearance of the very strong OH band of the starting material as expected from substitution of the OH proton in the acylated moiety and the appearance of a very strong 1750 cm^{-1} C═O stretching mode of the ester; a strong

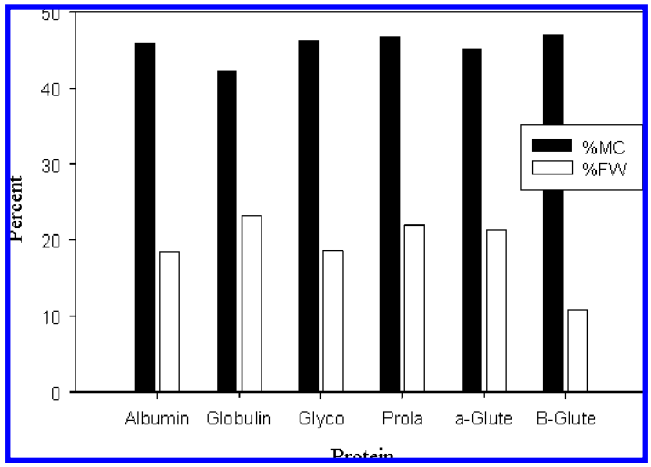

Figure 5. Effect of total water content on the amount of freezable water.

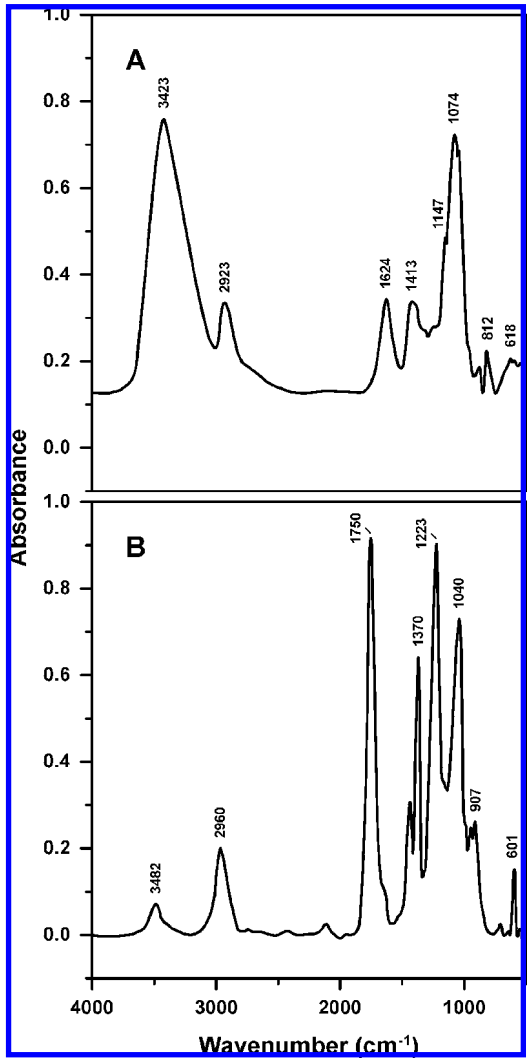

Figure 6. FTIR spectra of (**A**) extracted sicklepod polysaccharide; (**B**) its acetylated derivative.

1370 cm^{-1} band corresponding to the -CH_3 deformation mode of the acetyl ester is observed relative to the much weaker CH_2 bending mode at 1434 cm^{-1}. A very strong 1223 cm^{-1} stretching absorption band is also evident for the C-(OC-O) mode of the ester and a strong 1040 cm^{-1} for the C-CH_2-O band of the substituted primary alcohol moiety. These spectral features are characteristics expected in an acetylated carbohydrate. Additionally, the ^{13}C NMR spectrum was also consistent with what would be expected of an acetylated polysaccharide. The spectrum contained resonance lines for the ester carbonyls in the range of 168.5−170.5 ppm; the anomeric carbons of the polysaccharide are represented by resonances between 100.2−88.72 ppm, whereas the C-4, C-2, C-3, and C-5 resonances are seen between 70.07 and 67.64 ppm; the C-6 resonances are observed between 63.38 and 59.93 ppm; and the -CH_3 of the acyl groups is observed between 19.99 and 20.54 ppm. Further analysis of the freeze-dried polysaccharide by the HPLC-GPC technique using a Synchropak GPC 100 column eluted with water at 0.5 mL/min with refractive index detection indicated a molecular mass range of 25−27 kD, using dextrans as standards. This seed is unique in containing not only carbohydrates, oil, and proteins but also a potential gold mine of medicinally important phytochemicals that could be domestically produced by careful fractionation of the seed constituents.

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