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Sicklepod (*Senna obtusifolia*) Seed Processing and Potential Utilization

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Sicklepod (*Senna obtusifolia*) is a leguminous plant that infests soybean fields in the southeastern United States. Its seeds contain a variety of toxic, highly colored compounds, mainly anthraquinones together with a small amount of fat. These compounds contaminate and lower the quality of soybean oil when inadequately cleaned soybean seed from this area is processed. The sorting of sicklepod seed from a soybean harvest is an additional economic burden on the farmer beyond the cost of proper disposal of the weed seed to avoid worsening field infestation. Fortunately, sicklepod seed also contains substantial amounts of carbohydrates and proteins. These edible components when freed from anthraquinones have a market in pet food as well as potential in human foods because of the high galactomannan ratio of the polysaccharides. Sicklepod seed was dehulled, and the ground endosperm was defatted, followed by sequential solvent extraction of the defatted seed meal to isolate the anthraquinones, carbohydrates, and protein components into their respective classes. Each class of isolate was spectroscopically identified.

KEYWORDS: Sicklepod; *Senna obtusifolia*; polysaccharides; galactomannan; anthraquinones; proteins

INTRODUCTION

Sicklepod [(*Senna obtusifolia* L,) H.S. Irwin & Barneby] is a leguminous weed species in soybean and other field crops in the southeastern United States. Although the seeds of this plant have long been used medicinally in the Orient, only recently has its polysaccharide component attracted industrial interest. Sicklepod seed has been reported to contain 14−19% protein, 5−6% fat, and 66−69% carbohydrate (*1*). Among other components of the seed are anthraquinones, which are highly colored and toxic. Several anthraquinones have earlier been isolated and identified from the seeds of *S. obtusifolia* (*2a*,*b*). Studies estimating the anthraquinone content of the seeds of *S. obtusifolia* at various stages of maturity and aging have also been reported (*3*). Emodin, an anthraquinone isolated from *S. obtusifolia*, has been implicated as a mosquito larvicidal agent (*4*). In a recent study, an extract of *Senna tora* (L.) Roxb., a related species containing emodin, physcion, and rhein (also present in *S. obtusifolia*), has been shown to exhibit antifungal activity against phytopathogenic fungi (*5*).

A previous study of actual processing of the seed for gums has shown that 41% of sicklepod seed weight could be extracted with a sodium hydroxide solution and that the gums accounted for a large part of the extract (*6*). The seed gum can be used in the food, feed, paper, textile, petroleum recovery, and pharmaceutical industries. Sicklepod gum is sold commercially and is composed of polysaccharide chains consisting mainly of a 5:1 to 6:1 ratio of mannopyranosyl to galactopyranosyl units. There is considerable interest for growing sicklepod as a new crop, because of the valuable gum. Additionally, it is economically desirable to be able to utilize all parts of the sicklepod seed. In this paper, we report an integrated process for dehulling the seed by dry milling and sieving and subseqently separating anthraquinones, gum, and protein fractions in the endosperm fraction by wet processing.

MATERIALS AND METHODS

Materials and Reagents. Sicklepod seeds were obtained from the Wilder Farm, Raleigh, NC. Acetone, absolute alcohol, anhydrous diethyl ether, potassium hydroxide, and petroleum ether were purchased from Fisher Scientific (Chicago, IL). Activated carbon was obtained from Aldrich Chemical Co. (St. Louis, MO). Infrared spectra were recorded on a Bomem MB series FT-IR spectrometer (Bomem, Quebec, Canada) with a DTGS detector. Sieving was done on an RO-TAP sieve shaker (Mentor, OH). Protein analysis was performed on a Leco carbon/hydrogen/nitrogen determinator, model CHN-2000 series (Leco Corp., St. Joseph, MI).

Methods. *Dry Milling of Sicklepod Seeds.* Sicklepod seeds (24.38 kg) were milled following the scheme shown in **Figure 1**. The seeds

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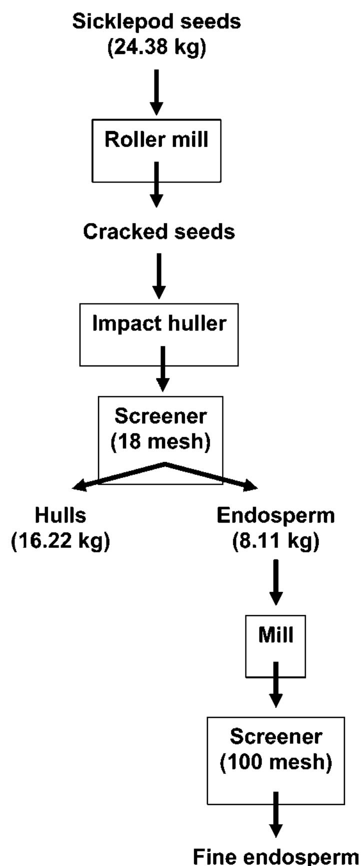

Figure 1. Flowchart of dry milling of sicklepod seeds.

were cracked by using a roller mill (Roskamp model SP900-12, (Roskamp Champion, Waterloo, IA) with a roller gap set at 2.0 mm. The cracked seeds were then passed through an impact mill (Forsberg model 15-D impact huller, Forsbergs Inc., Thief River Falls, MN) running at 1500 rpm to shake the cotyledons (meats) from the cracked seeds. The loose meats were then separated from the hulls using a Rotex model 12A screener (Rotex Inc., Cincinnati, OH) fitted with an 18 mesh (1.03 mm opening) screen. The milling of 24.38 kg of seed resulted in two streams of separated materials: meats (8.11 kg) and hulls with some unseparated meats (16.22 kg).

Fourier Transform Infrared (FT-IR) Spectrometry. A dried sample of the extracted oil was pressed between two NaCl disks (25 mm × 5 mm) to give a thin transparent oil film for analysis by FT-IR spectrometry. For solid samples (carbohydrates, proteins, and anthraquinones) typically 1.0 mg of dried sample was pulverized with spectrometric grade dry KBr (300 mg) in a stainless steel vial. The powdered sample was then placed in an IR die and compressed in a Carver press at 24000 lb/in.^{2} to give a transparent disk. To ascertain the detection limit for the anthraquinones, a smaller sample size, 0.10 mg in 600 mg of KBr, was used to produce the FT-IR spectrum in **Figure 4**. 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.

Subsequent Milling and Extraction. The above process only partially separated the endosperm from the hulls, giving a meats/hulls fraction (**Figure 1**). The meats/hulls endosperm mixture was further milled using a Retsch mill and sieved through a 100 mesh screen. This cleaned endosperm fraction (120 g) was defatted with petroleum ether in a Soxhlet extractor for 20 h. The petroleum ether extract was concentrated under reduced pressure to remove the solvent, giving rise to a dark red oil; yield of this fraction was 6.00 g (5.0%). The FT-IR spectrum of this oil is shown in **Figure 2**: ν_{NaCl} cm^{-1}, 3009 (H−C= stretch), 2954 (CH_3− asym stretch), 2925 vs (CH_2− asym stretch), 2854 s (CH_2−, CH_3− sym stretch), 1746 s (C=O), 1654 w (C=C breathing mode), 1465 m (CH_2− deformation), 1378 w−m (CH_3 deformation), 1238 m (CCO_2− stretch), 1164 medium to strong, 1099 m (−CHOCH−), 722 weak to medium (CH_2− wag).

Room Temperature Solvent Extraction. The remaining endosperm meal was dried in a vacuum oven at 20 °C to remove residual petroleum ether. The dry meal (100.0 g) was then placed in a 3.0 L Erlenmeyer flask equipped with a magnetic stirrer. In modifications of the procedures of Bayerlein et al. (*7*), Varshney et al. (*8*), and Khanna and Gupta (*9*), 700 mL of an acetone/water mixture (4:3) was added at room temperature and stirred vigorously for 20 min. Stirring was stopped at the end of this time, and the solids were allowed to settle. The supernatant extract was filtered and saved. The solids were resuspended and stirred with a fresh 700 mL of acetone/water mixture. This process was repeated until ∼6.0 L of extract had been collected. The extracts progressively darkened with each succeeding acetone/water addition while the solids became lighter from the original golden yellow endosperm. After the third trituration, further addition of acetone/H_2O to the solids and stirring resulted in suspended solids that would not settle on cessation of agitation. This flocculent material was filtered, washed, and freeze-dried separately from the bulk solids. Yield of freeze-dried flocculent material was 6.60 g (5.5%) of solids. The bulk of the insolubles was resuspended in dilute alkali (0.1 M ammonium hydroxide) and separated into two fractions. The minor portion was a flocculent suspension that was separated and freeze-dried to yield 14.0 g (11.7%). Protein analysis of samples of this material and the earlier flocculent solids indicated the samples were 76.3% dry protein. Its infrared spectral absorption bands were consistent with those expected for proteins (3405−3200 cm^{-1} s, N−H stretch; 1653 cm^{-1} vs, amide I; and 1546−1530 cm^{-1} s, amide II band) (**Figure 3**). The freeze-dried major insoluble component gave a mass of 46.0 g and was analyzed to be 88.5% dry protein. This final component retained a tinge of color that resisted removal. It, however, gave a normal infrared spectrum displaying strong absorption bands characteristic of proteins, namely, bands centered at 3401 cm^{-1} s, N−H stretch; 1655 cm^{-1} vs (amide I); and 1530 cm^{-1} s (amide II).

Isolation of Anthraquinones. Aliquots of the combined aqueous extract (1500 mL) were transferred into a separatory funnel containing 300 mL of anhydrous diethyl ether. The mixture was shaken and allowed to separate, and the reddish ether phase was transferred and saved, whereas the aqueous layer was re-extracted with fresh ether until the ether layer became very pale yellow or colorless. The combined ether extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to yield 5.60 g (4.6%) of anthraquinone component based on the amount of starting endosperm. Thin-layer chromatography (TLC) of this isolate in hexanes/ethyl acetate/glacial acetic acid (10:5:2) as solvent gave yellow spots with R_f values of 0.58, 0.42, 0.36, 0.23, 0.10, and 0.03, with a brown spot at the origin (**Figure 4**). The IR spectrum of the isolate at 1.33 ppm in KBr (**Figure 5**) showed the following bands: 3412 cm^{-1} broad medium (OH), 3006 cm^{-1} w (HC=), 2925 medium to strong (HC), 2854 m (CH sym stretch), 1738 cm^{-1} weak to medium, 1679 cm^{-1} m (C=O), 1655 cm^{-1} vs (C=C), 1635 cm^{-1} vs, 1620 cm^{-1} vs, 1601 cm^{-1} m (HC=C), 1575 cm^{-1} weak to medium (C=C), 1470 cm^{-1} m (−CH−), 1281 cm^{-1} vs (O=CC), 1210 cm^{-1} m (OC=C), 745 cm^{-1} (HC out-of-plane wag).

Recovery of Carbohydrate Component. The combined aqueous phase after ether extraction was then treated with activated carbon at 45 °C and filtered to remove residual colorants. The resulting colorless aqueous filtrate was then freeze-dried to yield the polysaccharide fraction (16.60 g, 13.8%): mp 227−230 °C dec; $[\alpha]_D^{20}$ +36.7° (*c*, 27.4 mg, 0.1 N NaOH). The FT-IR spectrum (**Figure 6**) of this isolate in KBr pellet showed bands at 3401 cm^{-1} vs; 2930 cm^{-1} medium to weak, 2859 cm^{-1} medium to weak, 1631 cm^{-1} m, and 1140 cm^{-1} vs.

RESULTS AND DISCUSSION

In this preliminary dry-milling process, sicklepod seeds were milled at room temperature as shown in **Figure 1**. Because the seeds were not preclassified into the appropriate grades, repetition was necessary to achieve a particle size amenable to efficient solvent extraction of seed components from the milled endosperm. Consequently, finer cleaning of the endosperm

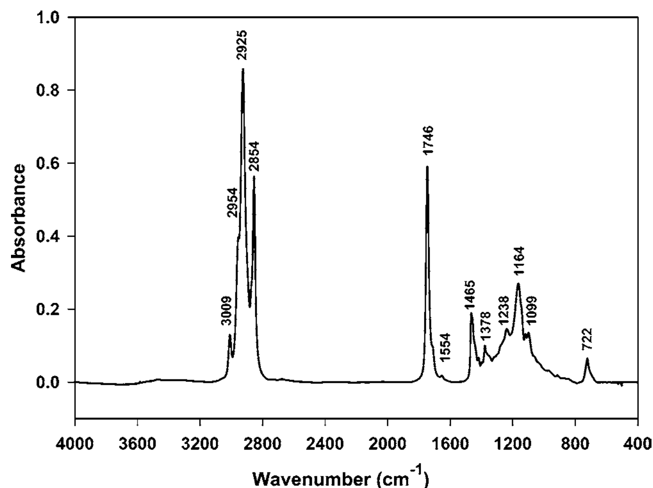

Figure 2. FT-IR spectrum of sicklepod oil.

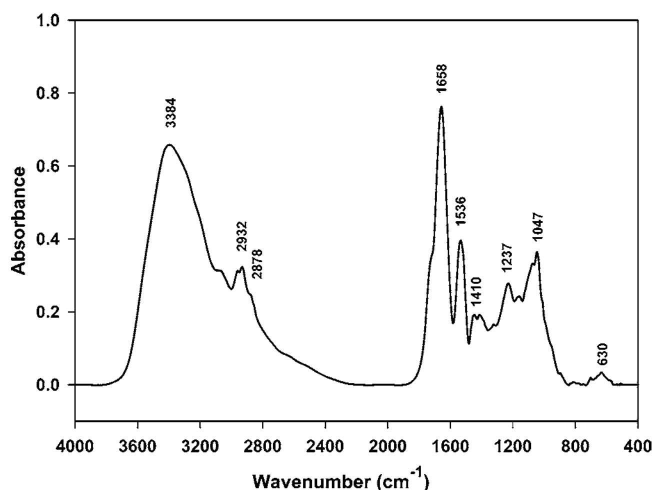

Figure 3. FT-IR of the protein component.

required secondary grinding and sifting of the prior milled sample through 80 and 100 mesh sieves. The cleaned, powdered endosperm was then defatted in petroleum ether using a Soxhlet to give a dark red oil after solvent removal. The oil yield was 6.00 g (5.0%) based on the starting mass of endosperm. Its infrared spectrum (**Figure 2**) included no absorbances characteristic of anthraquinones. Most of the latter show IR (major) bands at 1701−1679 cm^{-1} sh, 1629−1625 cm^{-1} vs, 1571 cm^{-1} m, 1457 cm^{-1}s, 1285 cm^{-1} vs, 755 cm^{-1} s, and a strong broad band for rhein and emodin centered at ~3400 cm^{-1} (**Figure 5**).

Exhaustive extraction of the defatted endosperm in aqueous acetone gave insoluble residues that totaled 66.60 g, or 55.5% (w/w), based on the starting endosperm. Infrared spectroscopy (**Figure 3**) and protein analyses of this material showed it was essentially 76−88% protein.

Isolation of the anthraquinone content was achieved by partitioning the aqueous extract with diethyl ether. Concentration of the combined and dried organic layers under reduced pressure yielded 5.60 g (4.6% w/w), containing a dark red solid mixture of anthraquinones. This value is apparently much higher than that previously reported (~1−2%) in whole seed (*3*). Because this is a known endosperm component, the observed higher value in contrast to whole seed content is not unexpected. In an attempt to identify the extracted anthraquinones, the TLC procedure of Crawford et al. (*1*) was modified to achieve better spot resolution (**Figure 4**). From a comparison of the migration values of the components in our extract (lane 4) to those of the

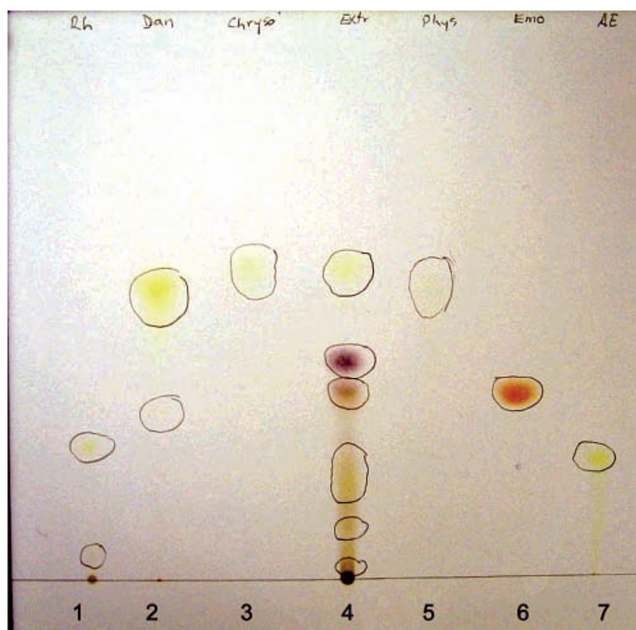

Figure 4. TLC of anthraquinone extract and standards (from left to right: rhein, danthron, chrysophanic acid, extract, physcion, emodin, aloe-emodin).

available standards in our solvent system (hexanes/EtOAc/AcOH; 10:5:2), the $R_f = 0.58$ for the fastest component closely matched that of chrysophanic acid (1, 8-dihydroxy-3-methyl-anthracen-9,10-dione), lane 3. All of the visible spots on the developed chromatogram were yellow, but after spraying with 5% KOH/methanol and heating, some of the spots changed color. The spot with $R_f = 0.42$ became purple following KOH spraying and heating. This component did not correspond to any of the standards available to us. A purplish- orange spot of $R_f = 0.36$ matched the R_f value of emodin (lane 6). In addition to the brown spot at the origin, there were three smeared spots between $R_f = 0.36$ and the origin; the fastest of these has a top segment matching the R_f of rhein (4,5-dihydroxyanthraquinon-2-carboxylic acid), lane 1, whereas its middle portion matched the R_f value of aloe-emodin (1,8-dihydroxy-3-hydroxymethyl-anthraquinone), lane 7. The standards danthron (1,8-dihydroxy-anthracen-9,10-dione), lane 2, and physcion (1,8-dihydroxy-6-methoxy-3-methylanthracen-9,10-dione), lane 5, were unrepresented in the components of the extract. The IR spectral characteristics of the available standards of these compounds showed weak to medium absorption bands in the 3500−2700 cm^{-1} region except 4,5-dihydroxyanthraquinone-2-carboxylic acid (rhein) and 1,3,8-trihydroxy-6-methylanthracen-9,10-dione (emodin), which have strong to moderate band intensities, respectively, in this region. Sharp absorption bands are observed for all anthraquinones in the 1740−1000 cm^{-1} spectral region. To ascertain the detectability of low levels of anthraquinones in the IR spectra, the isolated anthraquinone mixture at 1.33 ppm in KBr solid solution gave a broad band of moderately strong intensity centered at 3412 cm^{-1} for the O−Hs and moderate alkyl C−H stretch frequencies at 2925 and 2854 cm^{-1}. The lower frequency region of the spectrum consisted of a weak 1738 cm^{-1} band, a slightly broadened intense absorption band encompassing 1679, 1655, 1635, and 1620 cm^{-1} for the conjugated carbonyls, and the HC=and C=C puckering modes with shoulders at 1601 and 1586 cm^{-1}. The remaining spectral region showed bands at 1486 cm^{-1}, 1456 cm^{-1} m (−CH− deformation), 1281 cm^{-1} vs (O=CC), 1210 cm^{-1} m (OC=C), and 745 cm^{-1} (HC out-of-plane wag) (**Figure 5**). The additional

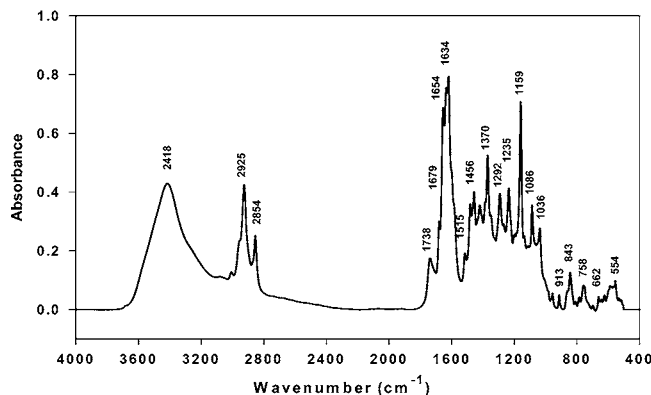

Figure 5. FT-IR of the anthraquinone extract.

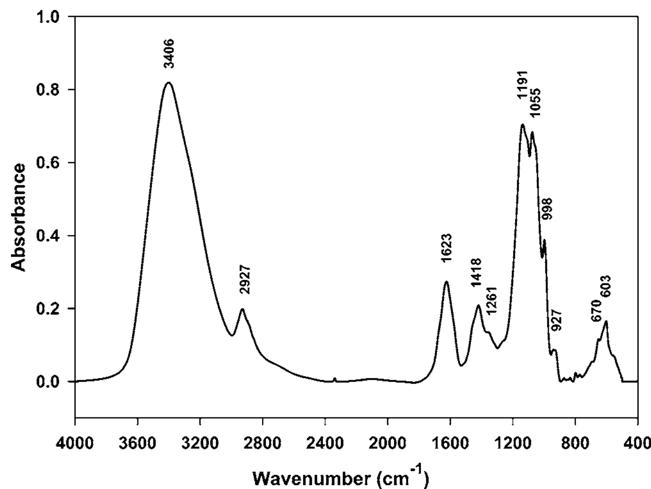

Figure 6. FT-IR of the carbohydrate component.

1655 cm^{-1} band does not seem to be a characteristic anthraquinone frequency and may have originated from slight protein contamination.

The polysaccharide component was recovered from the decolorized, freeze-dried aqueous phase. The total amount of carbohydrate obtained was 16.60 g, 13.8% w/w of the starting endosperm, and is colorless. This value is much lower than that earlier reported for total carbohydrates in whole sicklepod seed (*1*), but it is much more in accord with the percentage of extractables as observed by Abbott et al. (*6*) and Varshney et al. (*8*). The FT-IR spectrum of this isolate (**Figure 6**) is that typical of carbohydrates: a very strong and broad OH stretch centered at 3401 cm^{-1} due to hydrogen bonding, a weak −CH alkyl stretch at 2930 and 2859 cm^{-1}, and a broad moderate band centered at 1631 cm^{-1} for the OH bending mode of water, usually observed at 1640 cm^{-1} in polysaccharides. The characteristically strong −CCO− stretching modes of the secondary and primary alcohol functional groups are not resolved in this sample, but are centered at 1140 cm^{-1}.

The processes described here have allowed for the dehulling of sicklepod seed, grinding of the resulting endosperm, and separation of the component fat, anthraquinone, polysaccharide, and protein fractions via solvent extraction of the meal. A scale-up of the wet chemistry to kilogram quantities is in progress.

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