CHARACTERISATION OF ACETYLATED PULVERIZED JACK BEAN (*Canavalia ensiformis*) SEED COATS

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Abstract

The seed coats of jack bean (*Canavalia ensiformis*) were pulverized, modified via acetylation process and evaluated for their chemical properties, Gas Chromatography-Mass Spectroscopy (GC-MS) profile, Fourier Transform Infra-Red (FTIR) spectral profile, X-ray and morphological patterns. The unmodified sample possessed higher pH and ash content than the modified whereas an opposite trend was obtained for them in terms of moisture content and iodine absorption number. GC-MS indicated possible constituents as cyclononasiloxane, octadecamethyl, n-hexadecanoic acid and hexadecanoic acid, ethyl ether, which were in correlation with the observations obtained in the FTIR profile. The acetylated sample showed (O-H) spectral of normal polymeric hydroxyl group which had a characteristic signal of lignocellulosics. The XRD profile showed peaks of both the modified and unmodified as mixes of A- and B- polymorphs, that is, C-type, with high proportion of silicone. Though the pattern of the unmodified sample remained unchanged after modification, the crystallite size of the unmodified sample increased from 8.60 to 9.50. The micrographs of unmodified sample showed irregular shapes of the granules while those of the acetylated derivative were mixes of rod-like and irregular shapes. From the surface outlook, the acetylated sample appeared more crystalline and fibrous than the unmodified. This was carried out with the view to unveiling jack bean seed coats as potential filler.

Keywords: Acetylated, jack bean, pulverized, crystallite size

1. Introduction

Jack bean (*Canavalia ensiformis*), tropical climber producing long pendant green bean, belongs to the family of the *leguminasae*. It is a native of West Indies and Central America, but is now found scattered throughout the tropics and sub-tropics [1]. The jack bean seed, which is white in colour and nearly oblong in shape, is one of the neglected under-utilized legumes [1].

It can be grown relatively easily and produce high yields in the region of low altitude; high temperature and relative humidity. The environment of different locations plays an important role in the determination of quality and quantity of seed proteins. Location effect is relatively more important than that of cultivar of effect of protein content [2].

Presently, scanty reports on food and non-food applications of jack bean exist. Its nutritional values, anti-nutritional substances and suitability as food supplements for man, animals and fish have been reported [3-5]. Hydroxypropylation and ozone-oxidation of jack bean starch have been reported [5, 6].

The present work was borne out of curiosity to know the chemical constituents of jack bean seed coat, which, hitherto, is considered as a waste, and turn it to a useful raw material. As at the time of this research, the authors are not aware of any article on the characterization of seed coats of jack bean.

This paper is aimed at: (i) isolating and pulverizing seed coats of jack beans (*Canavalia ensiformis*), (ii) modifying the pulverized seed coats via acetylation, and (iii) characterizing both the unmodified and modified jack bean seed coat with a view to proposing its possible non-food application, especially as a potential filler.

2. Materials and Methods

2.1 *Materials*

Jack beans (*Canavalia ensiformis*) were freshly harvested from a farm in Auchi, Etsako-West Local Government Area, Edo State, Nigeria. All the reagents used were of analar grade.

2.2 *Preparation of Pulverized Jack Bean Seed Coat*

The seed coats of jack beans were manually separated from the embryo, dried in direct sunlight for 4-6 days, followed by thorough manual removal of notable foreign materials such as dirts, broken cotyledons and immature seeds. The dry seed coats were pulverised in a Willey Mill (Scientific Equipment), sieved into a fine particle (250 μm) and the sample packaged in a transparent polythene bag prior to analysis.

2.3 *Preparation of Acetylated Pulverized Jack Bean Seed Coat*

40 g of unmodified pulverized sample was soaked in 2 % sodium hydroxide solution for 1 h at room temperature. This mixture was soaked in glacial acetic acid for 1 h at room temperature, followed by decantation. This was further soaked in 30% acetic anhydride, containing 1 drop of conc. tetraoxosulphate (VI) acid for 5 min. Thorough washing with distilled water was done, followed by drying at room temperature for 48 h before oven-drying for 2 h at 100 ^{o}C.

$$\text{Seed Coat–OH} + CH_3COOH \rightarrow \text{Seed Coat–O–}COCH_3 + H_2O$$

2.4 *Chemical Compositions*

Ash content, moisture content and pH values of the unmodified and acetylated samples were carried out by adopting the standard chemical method of AOAC [8]. Iodine absorption number was determined. 2 g of the sample was weighed into a glass vial containing 250 ml of standard iodine solution and stoppered immediately. The iodine filler mix were shaken vigorously for 1 min, centrifuged at 1600 rpm for 5 min and the iodine solution was decanted completely in one smooth motion into a 50 ml beaker. 20 ml of the decanted solution was pipetted into 250 ml Erlenmeyer flask and filtered with standard solution of sodium thiosulphate ($Na_2S_2O_3$) until a pale yellow colour was observed. Upon addition 5ml of starch indicator, titration continued until a drop of sodium thiosulphate changed blue colour to colourless. The volume of $Na_2S_2O_3$ was recorded as volume S while the volume of $Na_2S_2O_3$ solution, which was titrated against 20ml black sample to the neutral point, was recorded as volume B.

Iodine Absorption Number = $\frac{(B\text{-}S) \times V \times N \times 126.96}{B}$ where, S = ml of $Na_2S_2O_3$ required for the sample, B = ml of $Na_2S_2O_3$ required for the blank; V = volume of iodine solution used; N = molarity of sodium thiosulphate.

2.5 *GC-MS Analysis*

10 g of the sample was weighed and 99.9% n-hexane was used to extract the active ingredient with thorough mixing using an ultra sonicator for 5 h. The mixture was allowed to stand for 72 h, followed by filtration and consequent washing with n-hexane. The filtrate obtained was concentrated to 1 ml in the vial bottle and was taken for analysis on Gas Chromatography-Mass Spectrometer for chemical composition. The gas chromatographic analysis was performed on an Agilent Technologies (Model: 7890A) interfaced with Mass Selective Detector (Model: 55975C). The electron ionization was at a 70 V with an ion source temperature at 250 ^{o}C. Highly pure helium gas (99.9 % purity) was used as carrier gas while HP-5ms $(30\ mm \times 0.25\ mm \times 0.325 \mu m)$ was used as the stationary phase. The oven temperature was at 80 ^{o}C held for 4 min and ramped to 270 ^{o}C at the rate of 3.5 ^{o}C/min holding for 6 min. 1µ/l was auto injected.

2.6 *Fourier-Transform Infrared (FTIR) Spectroscopy*

Fourier Transform Infra-red (FTIR) spectra of the sample were obtained with a Nicolet AVATAR 360 Fourier Infra-red spectrometer using KBR disks. 1 mg of the sample was dispersed in a matrix of

KBR (100 mg) and pressed to form packet. The spectral was measured at a resolution of 4 cm^{-1} and 32 scan 3 was recorded per sample.

2.7 *X-Ray Diffractometry (XRD)*

The X-ray diffraction studies were carried out using a Siemens D5000 X-ray powder diffractometer (20 ^{o}C geometry, USA). The fine samples was filled into a sample holder and packed as a density as possible. Then, the sample was mounted into a X-ray diffractometer and copper ka, 2 λ (λ =1.540 μm and 1.544 A; 35 mA) will be generated to determine X-ray pattern. The scan was made from a distraction angle (2) of 1.5 to 70 at a 0.05 step size with a count time of 35. From the resulting X-ray pattern, peak positions were identified using the instrument's software and these positions were used to determine the crystalline nature of the filler samples [9].

Crystallite Size, $D_{(hkl)} = \frac{k\lambda}{B_{(hkl)} cos\,\theta}$

where, k = Scherer constant (0.84), λ = 1.54 μm, $B_{(hkl)}$ = FWHM (Full Width Half Maximum) and θ = Bragg's angle corresponding to FWHM

2.8 *Scanning Electron Microscopy (SEM)*

The samples were sprinkled onto the aluminum specimen stubs with double-sided adhesive tape and coated with a 30 nm layer of gold using a sputter coater [Polaron (Fisons) SC 515 VG Microtech, Sussex, UK]. The coated samples were observed using a Scanning Electron Microscope (FESEM Leo Supra 50 VP, Carl-Zeiss SMT, Oberkochen, Germany). Images were captured at different magnifications for morphological studies.

3. Results and Discussion

3.1 *Chemical Compositions*

The pH of the unmodified sample is 6.48, which indicates that the sample is less acidic (Table 1). That is, the acidity of the sample is very weak and this could lead to retardation of the cure rate of the sample. However, the inclusion of an anti-retardant will help overcome this set back. For the modified (acetylated) sample, the pH is 4.47, which indicate that the sample is very acidic. That is the acidity of the sample is very strong, and this could lead to the acceleration of the cure rate or making the sample susceptible and thereby giving rise to a proper cure rate.

The moisture content is used to indicate the sample properties to moisture absorption. From Table 1, the moisture content of the acetylated and the unmodified samples are 4.76% and 14.3% respectively, a low moisture content, which implies that the sample will have low resistance properties and agglomeration of the sample particles.

The ash content determines the carbon content that is present in the sample. From the results obtained, the ash content of both the acetylated and unmodified samples are 20.09 % and 12.50 % respectively. This indicates that the sample may possess low resistance to ageing.

The iodine absorption is used to confirm the reinforcing ability of the sample. The acetylated sample and the unmodified are 2.17 % and 1.98 % respectively. This simply indicates that the unmodified sample has lower absorption surface area than the acetylated derivative.

3.2 *GC-MS Profile*

The possible constituents of jack bean seed coats as revealed by GC-MS analysis are cyclononasiloxane octadecamethyl, n-hexadecanoic acid and hexadecanoic acid ethyl ester (Fig. 1). The presence of these constituents is confirmed by the FTIR patterns.

3.3 *FTIR Patterns*

The spectral characteristics of the unmodified and acetylated pulverized jack bean seed coats are presented in Fig. 2. Skeletal vibration, similar to silicon oxy-groups (of organic siloxane or silicone) and cyclohexane ring, is observed at absorption band, 1048 cm^{-1} in unmodified sample. Very strong N–O of nitro group is observed at 1540.26 cm^{-1}, which disappears upon modification. This may account for the

differences in the pH values of the samples. Nitro groups have high dipole moments, that is, greater polarity, resulting in low solubility in water. Hence, the disappearance of this group in the modified derivative of the sample may favour the relative solubilisation of the acetylated pulverized jack bean seed coat in water.

From the spectra, there are 3628.30, 3735.00 and 3821.64 cm^{-1} absorption bands observed for the acetylated sample, indicating free hydroxyl group (O–H) stretch. Hence, the modified derivative of the sample assumes a hydrophilic network for its inherent molecules. This, among other things, may portend the susceptibility of acetylated pulverized jack bean seed coat to moisture absorption and swelling.

The absorption bands of 3419.08 $cm^{-1}$and 3466.00 cm^{-1} observed for unmodified and acetylated samples respectively, are indications of (O–H) stretch of normal polymeric hydroxyl group. Broad peak of 3418 cm^{-1} signal as cellulose hydroxyl group to be a characteristic signal of lignocellulosics has been reported [10].

3.4 *X-ray Patterns*

Table 3 shows the characteristics of three major X-ray diffraction peaks of the unmodified and acetylated pulverized jack bean seed coats. X-ray diffraction peaks for the unmodified sample appear at 24.09^{o}, 18.31^{o} and 47.57^{o} 2θ, corresponding to interplanar d-spacing of 3.69Å, 4.84 Å and 1.19 Å (Figure 4.1). Likewise, the acetylated sample exhibits X-ray diffraction peaks, which appear at 24.19^{o}, 18.38^{o} and 28.85^{o} 2θ, corresponding to interplanar d-spacing of 3.67Å, 4.82 Å and 3.09 Å (Fig. 3). The modification process does not significantly alter the diffraction peaks and patterns of the unmodified samples. These peaks are indicative of mixes of A- and B- polymorphs. Hence, both the unmodified and acetylated samples are of C-type pattern. The elemental profile of the samples reveals high proportion of silicon. This is in agreement with the results of GC-MS analysis of the unmodified pulverized jack bean seed coats, showing high proportion of silicon in the sample. The proportion of silicon in the sample is unaltered by modification.

3.5 *Scanning Electron Microscopy*

Table 4 shows the particle properties of unmodified and acetylated samples with the aid of SEM (Scanning Electron Microscope). From the table, the unmodified sample possesses higher particle properties than its acetylated derivative in terms of major axis, circumference, convex hull, circumscribed circle diameter, pixel count and elongation.

The micrographs of the unmodified and acetylated samples observed with scanning electron microscope are displayed in Fig. 4. The granules of the unmodified sample do not assume a conventional shape. Hence, they are irregular, non-uniform granules. The granules of the acetylated sample are mixes of rod-like and irregular shapes. The surface outlook of the acetylated sample appears more crystalline and fibrous than the unmodified.

4. Conclusion

The pulverized jack bean seed coat has been studied to ascertain its physical behaviour and possible constituent. The unmodified pulverized jack bean seed coat is weakly acidic with low moisture content, high susceptibility to oxidative degradation and low surface area compared to its acetylated derivative. Absorption bands resembling the characteristic signal of lignocellulosics is observed in the acetylated derivative of the pulverized jack bean seed coat. The XRD profiles show that the crystallite size is more favoured by modification. Both the unmodified and the modified derivative exhibit the same XRD pattern of C-type, which is a mix of A- and B- polymorphs. The SEM analysis shows irregular shapes of the unmodified granules and mixed shapes of the acetylated granules. Both unmodified and acetylated can possibly serve as fillers in rubber compounding.

5. References

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Table 1: Chemical composition of unmodified and acetylated pulverized jack bean seed coats

Sample	pH	Moisture Content (%)	Ash Content (%)	Iodine Absorption Number
Unmodified	6.48±0.10	4.76±0.01	20.49±0.10	1.98±0.01
Acetylated	4.47±0.10	14.30±0.02	12.50±0.11	2.17±0.01

Table 2: Major peak characteristics of unmodified and acetylated samples

Sample	Peak I				Peak II				Peak III			
	I	2θ	D	RI	I	2θ	d	RI	I	2θ	d	RI
Unmodified	1954	24.09	3.69	100	574	18.31	4.84	27	328	47.57	1.19	22
Acetylated	1224	24.19	3.67	100	291	18.38	4.82	22	340	28.85	3.09	19

I = Intensity (counts); 2θ = Bragg's angle; d = d-spacing; RI = relative intensity

Table 3: Crystallite sizes of unmodified and acetylated samples

Sample	B(hkl)	θ ($2\theta^o$)	Crystallite Size, D(hkl)
Unmodified	0.17	24.09	8.60
Acetylated	0.15	24.19	9.50

B(hkl)= FWHM (Full Width Half Maximum), θ(corresponding Bragg's angle to FWHM); D(hkl) = $\frac{k\lambda}{B_{(hkl)}cos\theta}$

Table 4: Particle properties of unmodified and acetylated pulverized jack bean seed coats

Property	Sample	
	Unmodified	Acetylated
Circle equivalent diameter (µm)	90.20	92.60
Major axis (µm)	118.00	115.00
Minor axis (µm)	70.00	75.40
Circumference (µm)	518.00	408.00
Convex hull (µm)	375.00	337.00
Circumscribed circle diameter (µm)	147.00	130.00
Area (µm²)	7.34×10^3	7.91×10^3
Volume by area (µm³)	5.46×10^5	6.28×10^5
Pixel count	9309.00	6925.00
Elongation	0.375	0.343

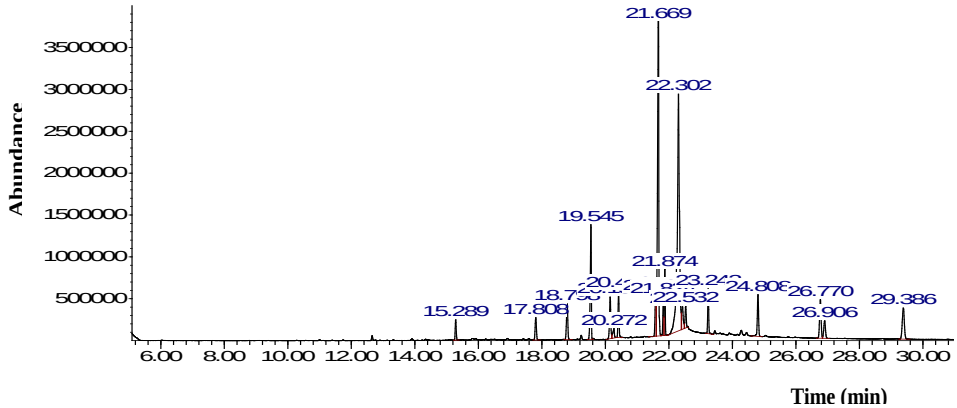
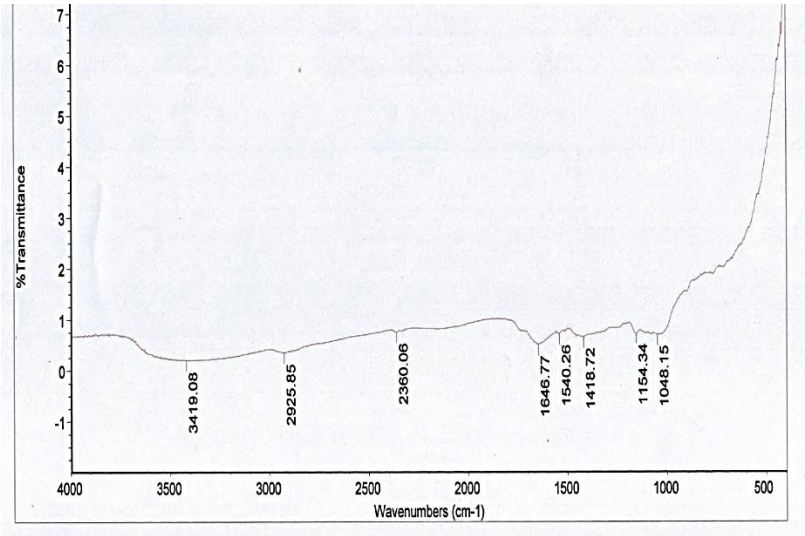
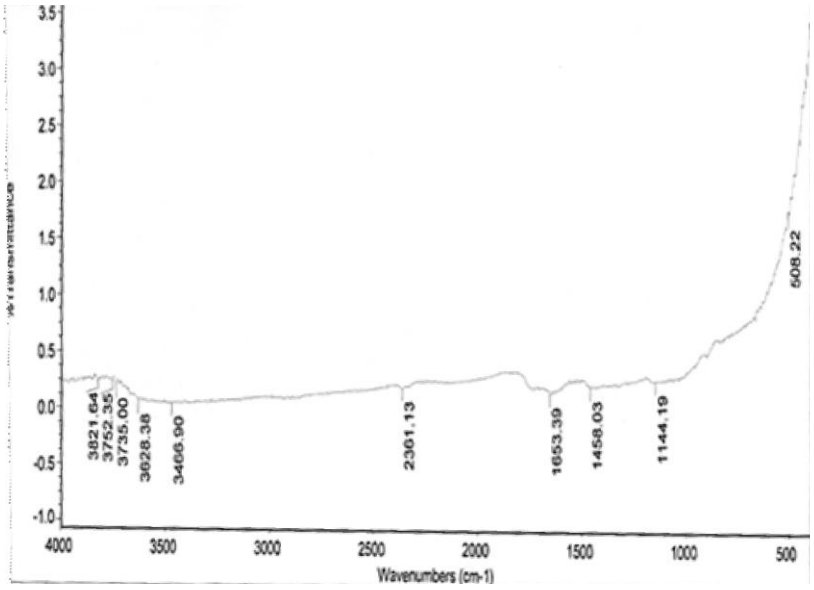


Figure 1: Chemical constituents of jack bean seed coat

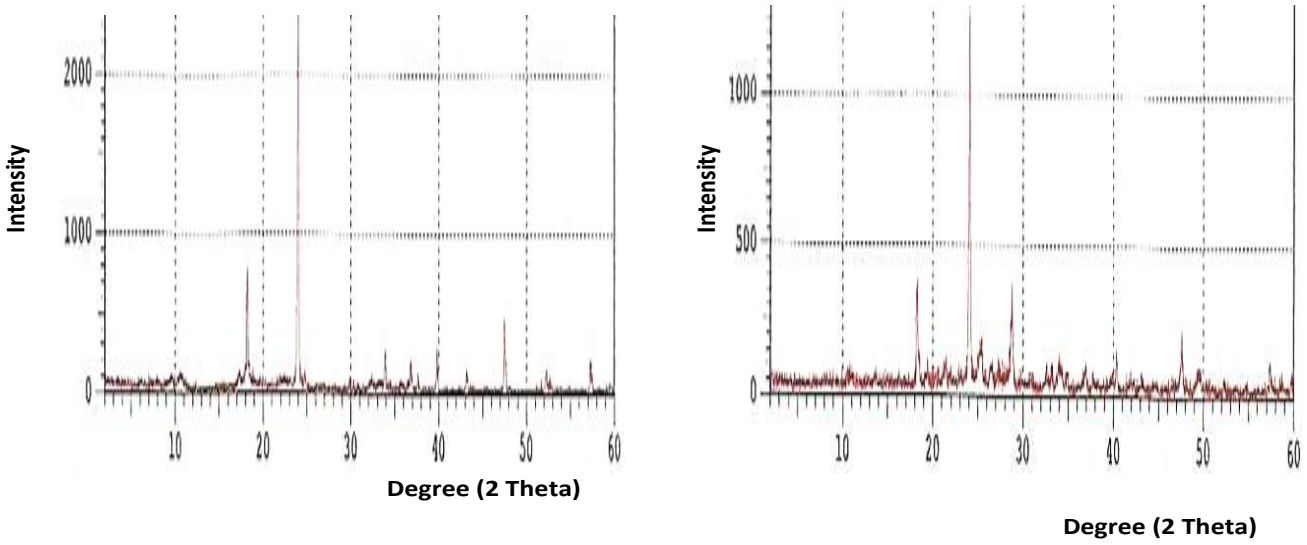


(a)



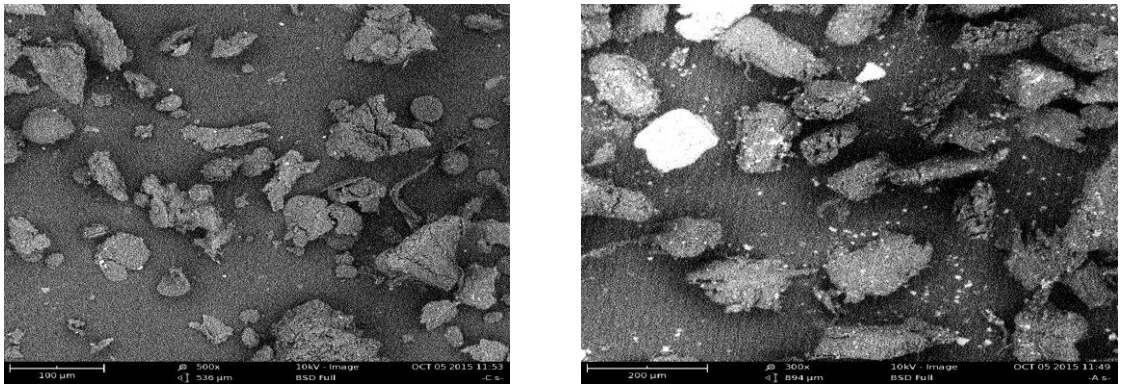
(b)

Figure 2: FTIR spectra of: (a) unmodified and (b) pulverized jack bean seed coat



(a) (b)

Figure 3: X-ray patterns of: (a) unmodified, (b) acetylated pulverized jack bean seed coat



(a) (b)

Figure 4: Scanning electron micrographs of: (a) unmodified and (b) acetylated samples