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RESEARCH ARTICLE

STUDIES ON THE ISOLATION, STRUCTURAL AND FUNCTIONAL PROPERTIES OF ACETYLATED CELLULOSE FROM SAND BOX TREE (*Huracrepitans*).

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Abstract

Acetylation is one of the most significant reactions for the derivatization of cellulose and numerous works related to cellulose acetate have been reported in the literature. Information about the structural and spectroscopic properties with respect to the degree of derivatization of cellulose acetate is lacking. Cellulose was isolated from *Huracrepitans* (sand box tree) and chemically modified using acetic anhydride. The acetylation process of the cellulose has been investigated by means of Fourier transform infrared (FTIR) and X-ray diffraction techniques. Proximate analysis showed that the cellulose has low moisture content and have long shelf life. Acetylation was carried out at various temperatures, pH range, time range and concentration. The FTIR spectrograms showed new bands at 1730cm^{-1} which is responsible for acetyl group, X-ray pattern of native cellulose showed low intensity compared to acetylated cellulose showing high crystallinity in the acetylated cellulose. The thermal analyses (TGA and DTA) showed that the native cellulose was more thermally stable than the acetylated cellulose.

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Introduction:-

Cellulose is the most abundant renewable natural biopolymer on earth and is present in a wide variety of living species including plants, animals and some bacteria (Lima & Borsali, 2004). It is the main structural constituent of plants and regaining importance as a renewable chemical resource to replace petroleum-based materials (Beck et al., 2011; Maet al., 2011). The annual production of cellulose is estimated to be over 7.5×10^{10} tons (Habibi et al., 2010). Regardless of the sources, cellulose consists of a linear homopolysaccharide composed of D-glucopyranose units linked together by 1-4- β -linkages. The repeating unit is a dimer of glucose, known as cellobiose.

Cellulose, a linear polymer composed of glucose monomer, is predominantly located in the secondary wall. The three hydroxyl groups of the monomer and their ability to form hydrogen bonds play a major role in leading the crystalline packing which also governs the physical properties of cellulose (Maya et al., 2008). The degree of polymerization (DP) is up to 20,000, however, it varies widely and the value is around 10,000 in wood. Cellulose does not occur as an isolated individual molecule in nature, it is found as assemblies of individual cellulose chain-forming fiber cell wall.

Huracrepitans, commonly referred to as sand-box is an evergreen tree which belongs to the spurge family *Euphorbiaceae* which grows in the tropical regions of the world. The tree can be recognized by the presence of many dark conical spines that covers the bark and its large heart shaped leaves with prominent secondary veins. The fruits produced are pumpkin shaped seed pods which are usually green when fresh and brown when dry. The fruit is characterized by its tendency to break with an explosive sound when ripe and dry, splitting the seedpods into segments catapulting the seeds as far as 100 m.

In most parts of the world, the trees have been used as shade because of its large spreading branches. It is commonly planted in the cities and villages of the southwestern part of Nigeria. In Nigeria, it is known as “Odan Mecca” by the Kabba people of Kogi State, Nigeria and as “Aroyin” by the Ijesha people of Osun State, Nigeria, (Fowomola and Akindahunsi, 2007). The trees are about 9 m tall on the average, with the male and female flowers on the same tree. The flowers of the masculine are of dark red colour arranged laterally on the small branches.

In order to improve the functionality of cellulose based on its swelling capacity which is one of its advantages. So many types of modification can be carried out on cellulose which includes; carboxymethylation, oxidation, sulfonation, acetylation etc. One practical approach to make the surface of native fiber cellulose (NFC) more hydrophobic is the formation of ester groups (Ifuku et al., 2007). Acetylation of cellulosic fibers is an effective method in this regard. The principle of acetylation is the reaction of OH groups of cellulose with acetyl groups which cause plasticization of lignocellulosic fibers (Bledzki et al., 2008). In this contribution, we report the modification of cellulose from agricultural residue (non-food crop); sand-box seed (*Huracrepitans*) by simple acetylation method using acetic anhydride as a modifier. To the best of our knowledge, this work was the first time cellulose will be isolated from sand-box seed given information about the structural and spectroscopic properties with respect to the degree of modification.

Materials and methods:-

Huracrepitans seeds were collected from its tree around the mini campus of Olabisi Onabanjo University, Ago-iwoye, Ogun State, Nigeria. The seeds obtained were those dispersed by explosion of the matured and dry pods and reagents used were of analytical grade.

Isolation of cellulose:-

Cellulose was isolated using the method used by (Alemdar & Sain, 2008b; Wang & Sain, 2007c). This method involves delignification of the cellulosic material by alkaline hydrolysis in four stages as follows;

Delignification of the cellulosic material using 3.5% nitric acid and 0.01% sodium sulphite at 90°C for 2hr in a reaction vessel. The residue was washed severally with distilled water until filtrate became neutral to litmus papers. The washed cellulosic material was hydrolysed with 2% sodium hydroxide and 2% sodium sulphite for 1.5hr at 80°C. It was then washed until the filtrate became neutral to litmus paper. The cellulosic material was further delignified with 17.5% sodium hydroxide for 1hr at 70°C. The last stage involved the bleaching to obtain the cellulose with 3.5% sodium hypochlorite for 30mins at 40°C. This process is to ensure that colours that are not chemically bound to the cellulose were washed off.

Cellulose acetylation:-

A method used by Lawal et al., (2004) was used to acetylate the isolated cellulose. 15g of the dried cellulose were dispersed in 75ml of distilled water and stirred uniformly until a slurry was obtained. The pH of the slurry was adjusted to 9.0 using 1M NaOH. 10.02g of acetic anhydride was added and stirred with a magnetic stirrer at 30°C for 20mins while maintaining the pH at 9.0. The sample was washed 4 times with distilled water and air dried at 30±2°C for 48hr. This process was repeated for temperatures of 35, 40 and 45°C. This procedure was also repeated varying the time at 20, 30, 40 and 50mins, pH at 9.0, 10.0, 11.0 and 12.0 as well as the concentration of acetic anhydride from 10 to 25g.

Degree of acetylation:-

The degree of substitution of acetylation was determined according to Smith (1967). 5g of acetylated cellulose was placed in a 250 ml flask and 50 ml distilled water added and mixed. A few drops of phenolphthalein were added and the suspension was titrated with 0.1M sodium hydroxide to a permanent pink end point. 25ml of 0.45M sodium hydroxide solution was then added and the flask sealed tightly with a rubber stopper and shaken vigorously for 30mins. The stopper was removed carefully and washed together with the walls of the flask with distilled water. The saponified mixture containing excess alkali was then titrated against 0.2M HCl solution until disappearance of the phenolphthalein colour. The native cellulose was treated in the same manner to obtain blank value.

$$\text{Percentage acetyl (dry basis)} = \frac{(\text{blank titre} - \text{sample titre}) \text{ ml} \times \text{acid molarity} \times 0.043 \times 100}{\text{Sample weight in g (dry basis)}}$$

$$\text{Degree of Substitution(DS)} = \frac{162A}{4300 - 42A}$$

where A = percent acetyl (dry basis)

X-RAY DIFFRACTION (XRD):-

The X-ray diffraction pattern of native sand-box cellulose and its acetylated derivatives were recorded with XPert Pro X-ray diffractometer equipped with accelerator detector. The diffractograms were registered at Bragg angle (2θ) = 10- 80° at a scan rate of 5°/min. Multi-peak fitting was performed to get the integrated area of crystalline peaks and amorphous peak, and the degree of crystallinity [Xc (%)] was determined as follow

$$Xc (\%) = (A_c / A_t) - A_a \times 100\%.$$

Thermal analysis:-

TG and DTA were measured with Perkin Elmer Diamond TG/DTA instrument. The measurements were recorded in air atmosphere (flow rate; 50mL/min). The sample mass was 20mg and it was heated from room temperature to 600°C at the heating rate of 5°C/min.

FTIR:-

The IR spectra of celluloses were run as KBr pellets on IRPrestige-21 FTIR spectrometer in the frequency range 4000-500 cm⁻¹

Proximate analysis:-

Moisture content:-

This was determined by drying the seed flour to constant weight at 105°C in a clean, dry and weighed evaporating dishes. About 2g of the powdered sample was placed in the dish and placed in an oven to dry at 105°C. After 5hrs of drying, the sample was withdrawn and placed in a desiccator to cool down and weighed. The process of drying, cooling and weighing was repeated until a constant weight was obtained. The weight of water present was found by difference and expressed as a percentage as follows:

$$\text{Percentage moisture content} = \frac{(w_3 - w_2) \times 100}{w_3 - w_1}$$

w₁ = weight of dish

w₂ = weight of cellulose

w₃ = weight of cellulose and dish

Determination of Total Ash:-

5g of the sample was weighed into a previously dried, cooled and weighed silica crucible. The crucible and content were ignited, first gently over a low flame until charred, and then in a muffle furnace at 550°C until a white ash was obtained. The ash was moistened with distilled water, dried on a steam bath and then on hot-plate and re-ashed at 550°C to constant weight. The weight of ash was obtained by difference and expressed as percentage of the sample used following the method of Pearson (1981).

Determination of crude fiber:-

200ml of freshly prepared 1.25% H₂SO₄ (0.1275M) was added to 2g of seed flour which has been defatted by extraction with ether and brought to boil quickly. Boiling was hastened with plenty of warm water. The residue was then transferred quantitatively into a digestion flask. 200ml of 1.25% NaOH (0.313M) was added and boiled for 30mins. The mixture was then filtered and the residue washed free of alkali with water. The residue was then transferred dried into a weighed silica dish and dried to a constant weight at 105°C. The organic matter of the residue was burnt off by igniting for 30mins in a muffle furnace at 600°C. The ash left behind was cooled and weighed. The loss in weight on ignition was reported as crude fiber using the method of Pearson (1981).

Results and discussion:-

Table 1:- Proximate analysis of raw and isolated *Huracrepitans* fiber.

	Parameter Analysed	Huracrepitans fiber %	
		Raw	Isolated
1	Crude Fiber	1.16	1.37
2	Moisture content	13.38	13.69
3	Total Ash	2.28	2.37
4	Lignin	5.67	4.25
5	Hemi cellulose	20.11	18.67
6	Cellulose	18.65	22.30

Table 2:- Degree of substitution of acetylated cellulose.

Sample Temperature(°C)	% Acetyl	Degree of Substitution (DS)
30	3.68	0.02
35	2.94	0.15
40	2.57	0.21
45	2.21	0.27

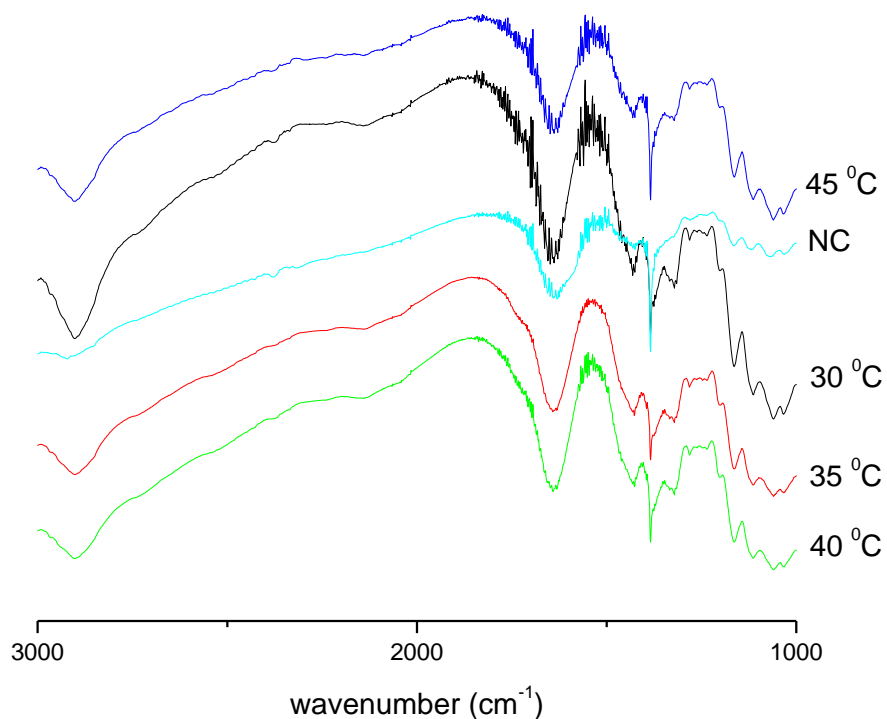


Fig 1:- FTIR showing the effect of temperature on native cellulose (NC) and acetylated cellulose.

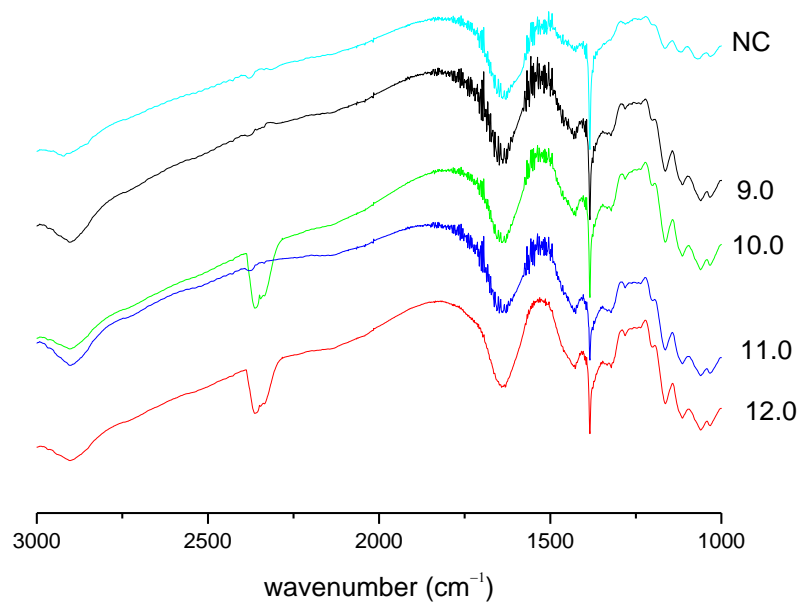


Fig 2:- FTIR showing the effect of pH on acetylated cellulose and native cellulose (NC).

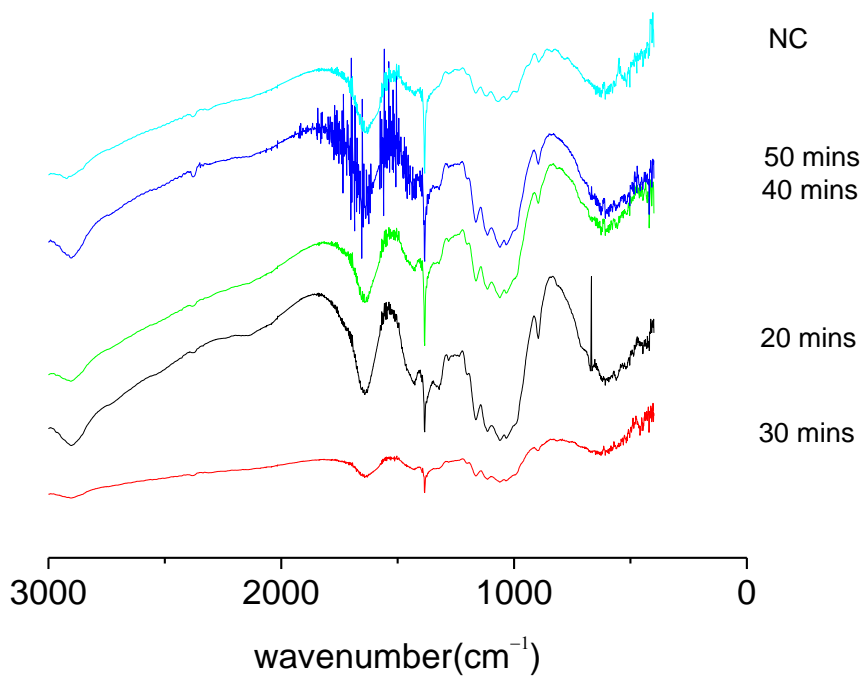


Fig 3:- FTIR showing the effect of time on acetylated cellulose.

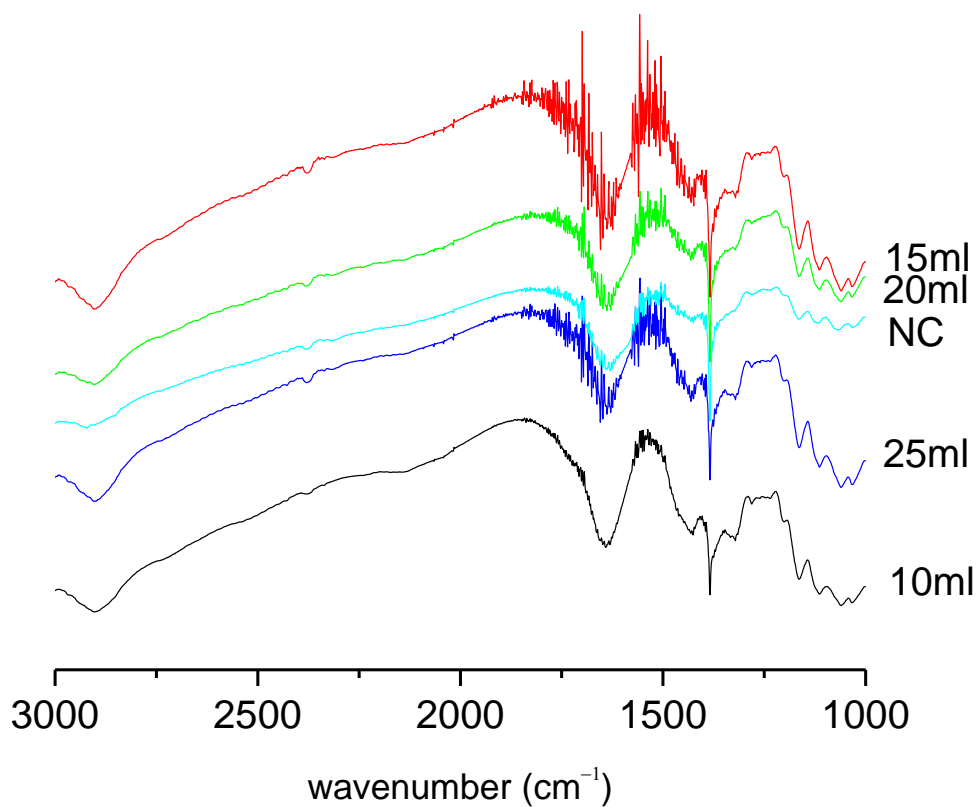


Fig. 4:- FTIR showing the effect of different quantities of acetic anhydride on the cellulose.

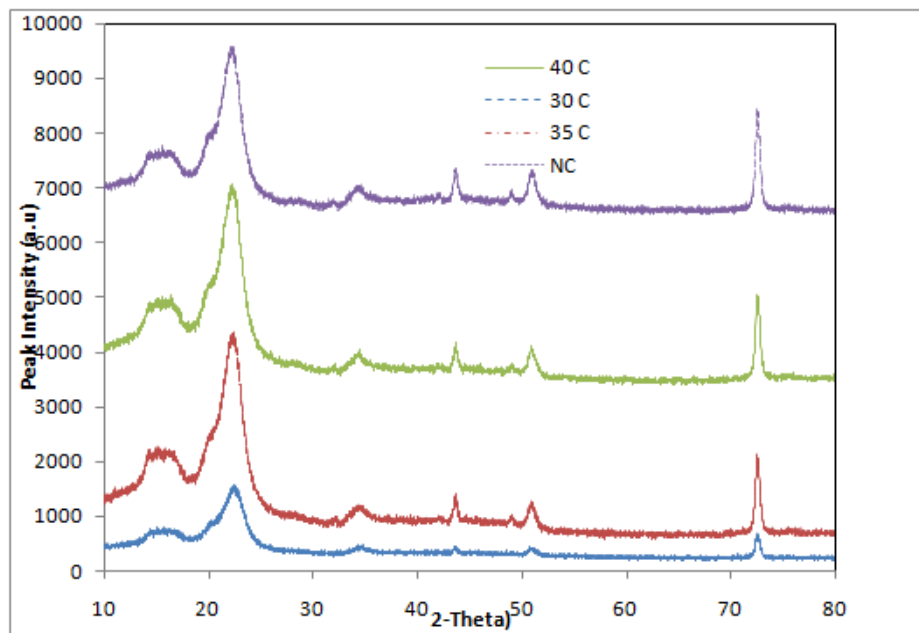


Fig 5: XRD showing the effect of temperature on the acetylated cellulose.

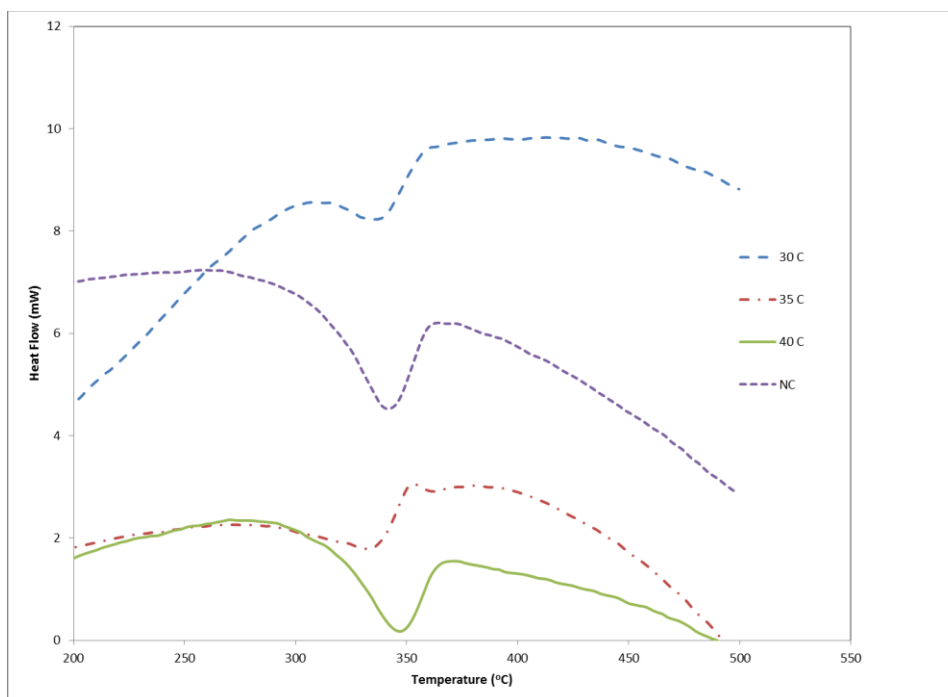


Fig 6:- DTA curve of native cellulose (NC) and its acetylated derivatives (AC) showing the effect of temperature on acetylated cellulose.

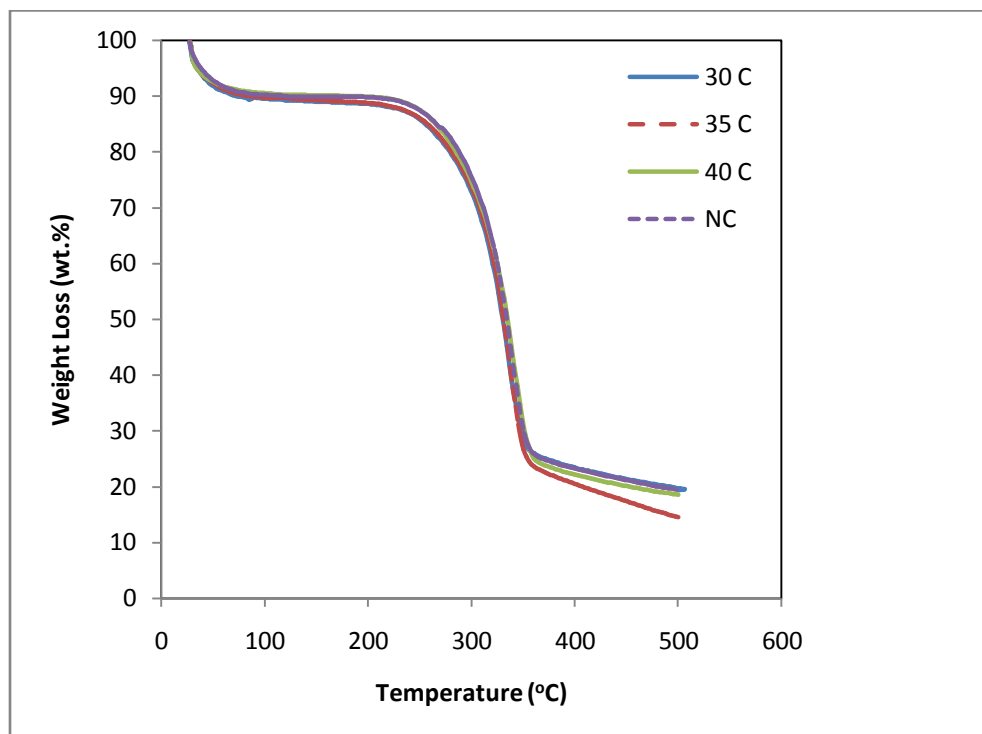


Fig.7:TGA curve showing the effect of temperature on native cellulose (NC) and acetylated cellulose (AC).

Proximate analysis:-

From the result obtained in table 2, Fibre content of fine blend *Huracrepitans* (1.16%) was observed to be slightly lower than that for the isolated powder (1.37%). The percentage ash content (2.28%) of isolated *Huracrepitans* was also lower than that of raw (2.37%). Percentage Lignin and hemi celluloses (5.67 and 20.11) was also lower in the isolated *Huracrepitans* sample (4.25 and 18.67) and the cellulose content (22.30) was also observed to be higher in the isolated sample indicating that cellulose isolation was successful. Moisture content of *Huracrepitans* (13.38%) was slightly lower than the isolated sample (13.69%) indicating that the seed will have a long shelf life. The result agreed with those reported by Oderinde et al., (2009).

Degree of substitution:-

Table 3. Shows the degree of substitution DS of acetyl group. The result showed that increase in temperature increased DS and this favors the substitution of acetyl group.

FTIR:-

Fig.1 shows the effect of temperature on the FTIR spectra of the native cellulose (NC) and its acetylated derivatives. The broad band in the region $3400-3500\text{ cm}^{-1}$ is due to OH-stretching vibration and it gives considerable information of the hydrogen bonds. It was noticed that the OH-stretching band in the native cellulose is sharper than its acetylated derivatives. Another band at 2900 cm^{-1} shows CH_2 symmetric stretching vibration. Also, the sharp band at 1730 cm^{-1} shows C=O stretch of an acetyl group which confirms that acetylation has actually occurred in the cellulose and a peak at 1635 cm^{-1} indicates adsorption of water, this is in accordance with the report of Lawal et al., (2008) and Heinze et al., (2013).

Fig .2 shows the FTIR spectra of the AC at various pH. The broad band observed in the $3400-3500\text{ cm}^{-1}$ region is due to OH-stretching and a band at 2900 cm^{-1} region is also due to CH_2 symmetric stretching vibration. A sharp peak at 1730 cm^{-1} shows C=O stretch of an acetyl group and also the peak found at 1635 cm^{-1} is due to adsorption of water. This indicates that alkaline medium favors acetylation of cellulose.

Fig.3 shows the FTIR spectra of the effect of time on acetylated cellulose (AC). On the spectra, at 20 minutes acetylation has been completed. Fig.4 shows the FTIR spectra of the effect of different quantities of the acetic anhydride on the cellulose. The prominent peaks are those of OH-stretching vibration at $3400-3500\text{ cm}^{-1}$, CH_2 symmetric stretching at 2900 cm^{-1} and the adsorption of water at 1635 cm^{-1} . The peaks observed using 10 ml of acetic anhydride are sharper than other peaks with other quantities, this implies that using 10 ml of acetic anhydride enhances acetylation and could be the optimum quantity.

Thermal stability:-

Fig. 7 shows the TGA of the NC and AC. This analysis was done in order to compare the thermal stability of the native cellulose with the acetylated cellulose. The initial weight loss of the cellulose started from 50°C to 145°C with 93% and 89% weight loss respectively which is due to the evaporation of moisture. The temperature range between 160°C to 240°C shows the depolymerisation of non-cellulose such as hemicellulose and the sharp weight loss from 360°C indicates the degradation of the crystallinity of the cellulose. At 380°C , there is a slight difference in the amount of native cellulose (20%) and that of the acetylated cellulose (15%). This reveals that the structures and crystallinity of the cellulose are related to the degradation of the cellulose sample and it indicates that the crystalline cellulose have higher thermal stability. This is in line with Ouajai and Shanks (2005).

Fig. 6 shows the differential thermal analysis (DTA) of the NC and AC. The DTA carried out on the native cellulose showed a peak at 320°C while at 340°C the acetylated cellulose also showed degradation. This indicates that native cellulose is more thermally stable than the acetylated derivatives.

X-ray diffractometry:-

XRD analyses of NC and AC were done in order to determine the structural and chemical change of the acetylated cellulose. Cellulosic fibers consist of 3 components namely Lignin, Hemi celluloses and α - celluloses. Cellulose shows crystalline nature while lignins are amorphous in nature. As a result, the crystallinity of the fibers should increase after treatment. A typical cellulose diffractogram shows peak at $2\theta = 22^\circ$, a shoulder peak in the region 11.4° , 15.3° (Bonds et al., 2006) which shows the presence of celluloses. The acetylated cellulose shows strong crystalline nature due to its higher diffraction intensity at $2\theta = 22^\circ$.

The diffractograms of NC from *Huracrepitans* showed the diffraction intensity at $2\theta = 21.0^\circ$ and a shoulder in the region $2\theta = 11.4^\circ, 17.6^\circ$ while the acetylated cellulose exhibit diffraction intensity at 22° and lower intensities of $2\theta = 13.8^\circ$ and 18.6° . The broad peaks of the NC are due to the amorphous nature of the lignin. The higher peak intensity of the AC from fig.5 indicates the complete removal of non-cellulose due to alkaline hydrolysis during steam explosion. Thus, the inter-fibrillar regions are likely to be less dense and less rigid and thereby make the fibrils more capable of rearranging themselves. This result is similar to that observed by Gassan and Bledzki (1999).

Conclusion:-

Cellulose was isolated from *Huracrepitans* and was subjected to alkaline hydrolysis. The structural and spectroscopic changes in cellulose acetates observed by the XRD and FTIR revealed different mechanisms of acetylation. The chemical composition confirmed that the hydrolysis results in higher percentage of cellulose and lower percentage of lignin. FTIR also confirms that acetylation actually occurred on the native cellulose. Thermal analysis showed that the native cellulose degrades faster than its acetylated derivatives. XRD analysis also showed higher crystallinity in the native cellulose.

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