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Isolation, Percentage Yield Determination, Characterization, and Proximate Analysis of Cellulose from Bean Pods, Sandbox, and Wheat Offal

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Abstract
Cellulose is the most abundant, renewable, biodegradable and biomass substance in nature. It exhibits high flexibility, elasticity and bio-compatibility among its other properties. During pre-treatment in this study, the agricultural wastes from bean pod, wheat offal and sandbox were milled separately using an unbranded machine, sieved with a 250µm mesh to obtain fine-powdered materials which were then soaked inside water for 24 hours before boiling. Isolation by alkaline hydrolysis with sodium hydroxide was carried out inside a locally designed steel reaction vessel with a detachable electrically controlled mechanical stirrer at 90ºc for $2^1/_2$hrs. The cellulose obtained was bleached with sodium hypochlorite, $NaOCl_2$ to remove unbounded materials and then characterized using Fourier Transfer Infrared (FTIR) spectroscopy, to determine the functional groups present. Percentage yields of 47.4%, 36.3% and 34.6% were obtained for wheat offal, bean pod and sandbox respectively. The result obtained showed that obtaining cellulose from these agricultural wastes is economically viable and sustainable. The proximate analysis of the raw agricultural wastes and isolated cellulose was carried out according to the Association of Analytical Chemistry (AOAC) method, to determine the ash, moisture, lignin, hemicellulose, fat, crude fibre and protein values. The results showed a drastic reduction of ash, lignin and some other parameters in the isolated cellulose. This further revealed that the cellulose obtained was suitable for modification and wider applicability. Obtaining cellulose from agricultural wastes of bean pods, wheat offal and sandboxes for productive activities would reduce the volume of unwanted materials in our communities and eliminate or control the harmful effects of pollution in our environment.

Keywords: Agricultural waste, Cellulose, Renewable, Isolation, Proximate Analysis.

Introduction

Cellulose is the most abundant, renewable, biodegradable biomass in nature. Cellulose would be found in plant cell walls. It helps plants to become stiff and strong. Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$, that is, it is a polysaccharide compound consisting of a linear chain of several hundred to over ten thousand β(1 -4) linked D-glucose units (Romruen et al., 2022; Sher et al., 2021; Trilokesh & Uppuluri, 2019). As the most abundant renewable, biodegradable natural polymer, it is not too surprising that science and engineering continue to look to cellulose as a fundamental building block for a huge range of endeavors. They exhibit useful properties of thickening, thermal gelation, surfactancy, film formation, and adhesion. Furthermore, they are kinetically and thermodynamically more stable and appear easy to prepare and characterize. These characteristics earn them applications in areas such as pharmacy, cosmetics, food, oil drilling, paper, paint, textiles, construction, & adhesives (Kenawy et al., 2020; Davidson, 1980). A lot of Studies have revealed that obtaining cellulose from food items as feedstocks has led to scarcity, acute shortage and high cost of food in the society. It has also been observed from some studies that cellulose from inorganic–based materials exhibits poor flexibility, elasticity and biocompatibility. Hence their use as feedstocks has been discouraged. This work has explored the use of agricultural wastes from bean pod, sandboxes and wheat offal to obtain cellulose suitable for modification and subsequent applications in the industries. These agricultural wastes are readily available, easy to handle, low-toxic,

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biocompatible and of diverse sources (Romruen et al., 2022). Furthermore, the use of these agricultural wastes would help to remove them from the environment thereby combating pollutions in society.

Materials and Methods

Sandbox was collected from Olabisi Onabanjo University in Ago-Iwoye, Ogun State, Nigeria. Bean pod and Wheat offal were obtained from Alaba market, Ojo, Lagos state. All chemicals used were of high analytical grade. The agricultural wastes were sorted and cut into chips. They were milled separately using an unbranded machine and sieved through a 250µm mesh aperture to obtain finely powdered texture materials.800g of the powdered agricultural wastes were weighed into the steel reaction vessel and soaked in water for 24 hours. The materials were then filtered, and washed thoroughly with distilled water until filtrate became clear. The materials were boiled in water for 1hr, filtered, and washed with distilled water. Isolation of cellulose in this study was carried out by methods similar to that of Trilokesh and Uppuluri (2019), Ibikunle et al. (2016), and Azubike et al. (2012). For the isolation and delignification reaction, 3.0L of 3.5% HNO_3 and 2.0L Na_2SO_3, which were previously prepared, were poured inside the vessel containing the material and the reaction proceeded for $2\frac{1}{2}h$ at 90°c with constant stirring. This was followed by filtration and washing with distilled water until the filtrate became clear and neutral to both blue and red litmus papers. Further delignification process continued with the addition of 2.0L of 2% NaOH and 2.0L of Na_2SO_3 at 80^{o}C for 2hr under constant stirring. This was followed by filtration and washing with distilled water until the filtrate became neutral to both blue and red litmus papers. Final delignification by alkali hydrolysis was carried out with 3.0L of 17.5% NaOH under constant stirring for 2hrs 30mins at 70^{o}C. Then, filtration and thorough washing were made with distilled water until the filtrate became neutral to blue and red litmus papers. The Material obtained was bleached with 3.5% $NaOCl_2$ for 45 minutes under constant stirring at $50°c$. This was followed by washing until the filtrate became clear and neutral to red and blue litmus papers. The final product obtained was air-dried, milled into fine powder material and kept in a dried container. The above process was repeated for Sandbox and wheat offal respectively.

The percentage yield of each sample was determined thus;

% Yield = $\frac{W_2}{W_1} X\ 100\ \%$

Where: W_1= initial weight of raw (powdered) material.

W_2= final weight of dried isolated cellulose

The functional groups of cellulose isolated were determined using Perkin Elmer Spectrum RXI Fourier Transform Infrared Spectrophotometer. The infrared spectra of these samples were measured in the wavelength regions of 4000 and 400cm^{-1} respectively. The standards recommended by the - Association of Official Analytical Chemistry - AOAC (2005), were adopted in the methods used for estimating Ash, Moisture, Crude fibres, Lignin, Hemicellulose, Protein and Fat contents of the agricultural wastes and the isolated cellulose.

Results

Pre-Treatment and Isolation

The pretreatment method enhanced the re-localization of lignin, ash and hemicellulose to the surface which made them to be more accessible to delignification process by alkaline hydrolysis that resulted in higher yields of cellulose. (Fig. 1). Also, the pretreatment process allowed for the removal of more lignin and ash contents, as was earlier observed in the works of Mathura and Maharaj (2024), Yu et al. (2021), and Qasim et al. (2020).

Percentage (%) Yield of Isolated Cellulose

Wheat offal had the highest yield of 47.4%. The percentage yield for the bean pod was 36.3% and that of the sandbox was 34.6% (Table 4.1). The results were similar to those obtained from the works of Nuruddin et al. (2011), Bono et al. (2009) and Atanu et al. (2006). Alkaline hydrolysis of the lingo-cellulosic materials with $HNO_{3(aq)}$ made the hemicelluloses solubilise while further treatment with NaOH $_{(aq)}$ disrupted the lignin structure and linkages between cellulose and lignin that led to their ultimate destruction.

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Table 1: Percentage (%) yield of cellulose from sample

Agricultural Waste	% Yield
Bean pod	36.30 (290.4g)
Sandbox	34.60 (276.8g)
Wheat offal	47.40 (379.2g)

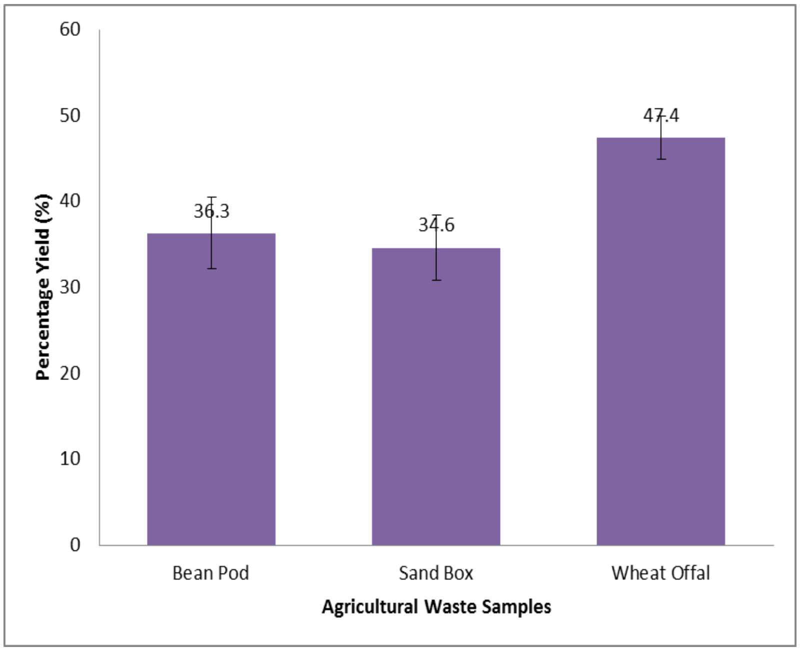

Fig. 1: Percentage (%) yield of isolated Cellulose

Functional groups present in the cellulose.
The Infrared spectra of the isolated cellulose from bean pod, sandbox and wheat offal are shown in Table 2.

Table 2: Functional Groups Present in Isolated Cellulose

S/N	*Rice Husk	Bean Pod	Sandbox	Wheat Offal	Functional groups Interpretation
1.	3322.5	3336.35	3360.00	3439.00	stretching vibration of –OH
2.	2901.97	2902.28	2900.22	2902.00	C-H stretching of CH_2
3.	1632.10	1626.42	16.38.79	1628.27	-OH bending (of absorbed water)
4.	1419.62	1420.22	1429.90	1429.70	C-C bending
5.	1384.80	1374.09	1371.00	1328.00	-OH in plane bending
6.	1122.70	1062.97	1045.00	1059.42	-C-O- stretching
7.	771.50	841.98	892.92	897.95	β-glycosidic linkage

*Archana et al. (2014)

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Proximate analysis

The moisture content in the raw agricultural wastes was found to be highest (15%) in wheat offal and lowest (5.2%) in sandbox. For the isolated cellulose, the percentage of moisture observed was, 44.6%, 11.98% and 7.77% for bean pod, sandbox and wheat offal respectively. Therefore, the moisture content in the raw agricultural wastes showed a significant ($p < 0.05$) reduction compared with the isolated cellulose. The results were similar to the findings in the earlier works of some researchers (Lawal, 2004; Francisca et al., 2023). The results obtained from the ash content were between 5.2% and 16.8% in the raw agricultural wastes and 2.12% to 4.1% in the isolated cellulose. Ash contents were observed to be higher in raw agricultural wastes. The highest (16.85%) was found in the sandbox and the lowest (5.24%) in the bean pod. In the isolated cellulose, the sandbox had the highest (4.1%) ash content value, and the bean pod yielded the lowest value of 2.2%. The crude fibre value obtained was between 2.0% and 6.82% in the raw agricultural wastes and between 3.45% - 5.45% in the isolated cellulose. Hence, the raw agricultural wastes contained higher crude fibre than the isolated cellulose. Wheat offal had the highest (6.4%) crude fibre from agricultural waste, and bean pod had the lowest (2.0%). Cellulose isolated from the sandbox contained the highest (5.45%) value of crude fibre, while bean pod had the least with 3.46%. Lignin content reduced drastically in the isolated cellulose compared with the raw agricultural wastes. This confirmed that delignification occurred. The lignin content range was between 15.6% - 26.5% in the raw agricultural wastes. Sandbox had the highest value of 26.5%, while wheat offal gave the least per cent of 15.6%.

In the isolated cellulose, the lignin content range was between 4.84% - 17.78%. The highest percentage yield value of 17.78% was obtained from the sandbox. The least was from wheat offal (4.84%). Hemicellulose content was found to be lower in the isolated cellulose than in the raw agricultural wastes. The range for Hemi-cellulose content was obtained between 61.5% - 39% for raw agricultural wastes. In the isolated cellulose, it was between 31.6% and 36.0%. Hemicellulose content was highest (61.55%) in wheat offal and lowest (39.0%) in bean pod. For the raw agricultural wastes, the highest (36.6%) was produced by the sandbox, while the bean pod gave the lowest per cent of 31.6% in the isolated cellulose. Protein content was higher in the raw agricultural wastes compared with the isolated cellulose. The protein value was between 25.6% - 7.0% in the raw agricultural wastes. Bean pod had the highest (25.57%) and sandbox the lowest (6.95%). In the isolated cellulose, the sandbox has the highest (6.52%) protein content and the bean pod the lowest (3.47%). Fat did not show any significant difference in both isolated cellulose and raw agricultural wastes. However, the values were between 2.5% - 0.080% in the raw agricultural wastes and 1.47% to 0.14% in the isolated cellulose. Bean pod (1.47%) produced the highest value of fat and wheat offal had the least per cent fat content of 0.14%. In the raw agricultural wastes, bean pod contained the highest (2.50%) value of fat and wheat offal the lowest (0.80%). The moisture contents in the isolated cellulose was found to be between 5.0% - 7.0%, which was similar to previous works by (Azubuike & Okhamafe, 2012; Azubuike et al., 2011; Oderinde et al., 2009).

Table 2:
Proximate analysis of raw agricultural wastes and isolated cellulose from Bean Pod, Sand Box, and wheat Offal.

		PARAMETERS						
S/N	Sample	Ash	Moisture	Crude fibre	Lignin	Hemicellulose	Protein	Fat
1.	RBP	5.24 ± 0.25	8.64 ± 0.58	2.00 ± 0.13	23.43± 1.53	38.96 ± 2.52	14.33± 0.25	1.47 ± 0.00
2.	CBP	2.18 ± 0.096	44.65± 1.15	3.46 ± 0.36	5.64 ± 1.00	31.63 ± 3.21	3.48 ± 0.10	2.51 ± 0.00
3.	RSB	16.85 ± 0.57	5.23 ± 0.051	6.82 ± 0.057	26.55 ± 1.53	36.00 ± 4.00	6.95 ± 0.15	2.40± 0.00
4.	CSB	4.0911 ±0.10	11.98 ± 0.77	5.46 ± 0.21	17.78 ± 0.57	39.84 ± 1.00	6.53 ± 0.20	0.92 ± 0.00
5.	RWO	8.30 ± 0.10	15.26 ± 0.00	6.42 ± 0.04	15.60s ± 1.52	33.90 ± 1.67	25.58 ±0.20	0.14 ± 0.00
6.	CWO	2.58 ± 0.12	7.77 ± 0.13	3.84 ± 0.025	4.84 ± 1.52	61.55 ± 1.73	3.56 ± 0.20	0.80 ±0.000

Abbreviations:
RBP: Raw agricultural waste of bean pod
CBP: Cellulose from bean pod
RSB: Raw agricultural waste of sand box
CSB: Cellulose from sand box
RWO: Raw agricultural waste of wheat offal
CWO: Cellulose from wheat offal

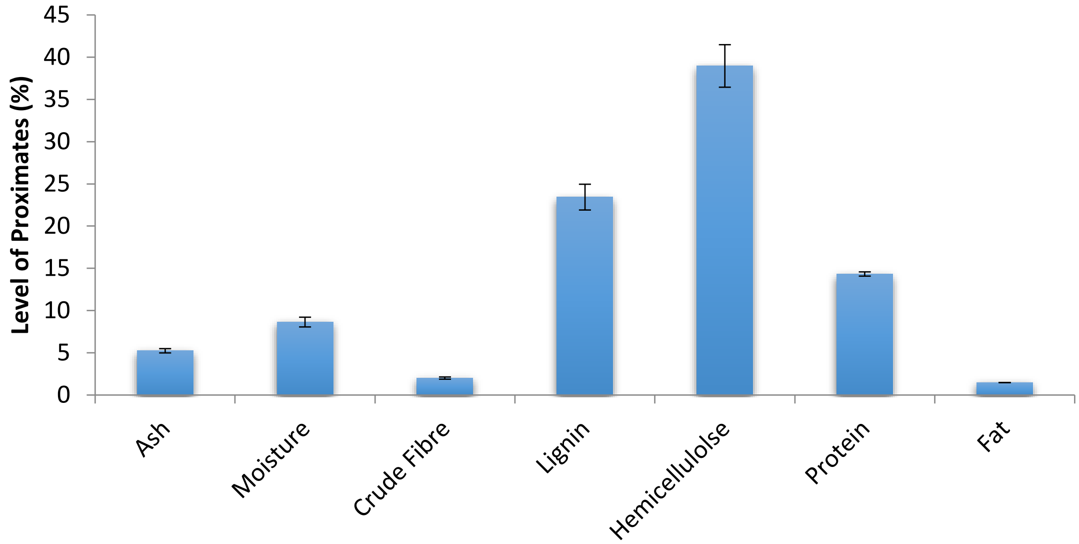

Fig: 2a. Proximate analysis of RawBean Pod. (RBP)

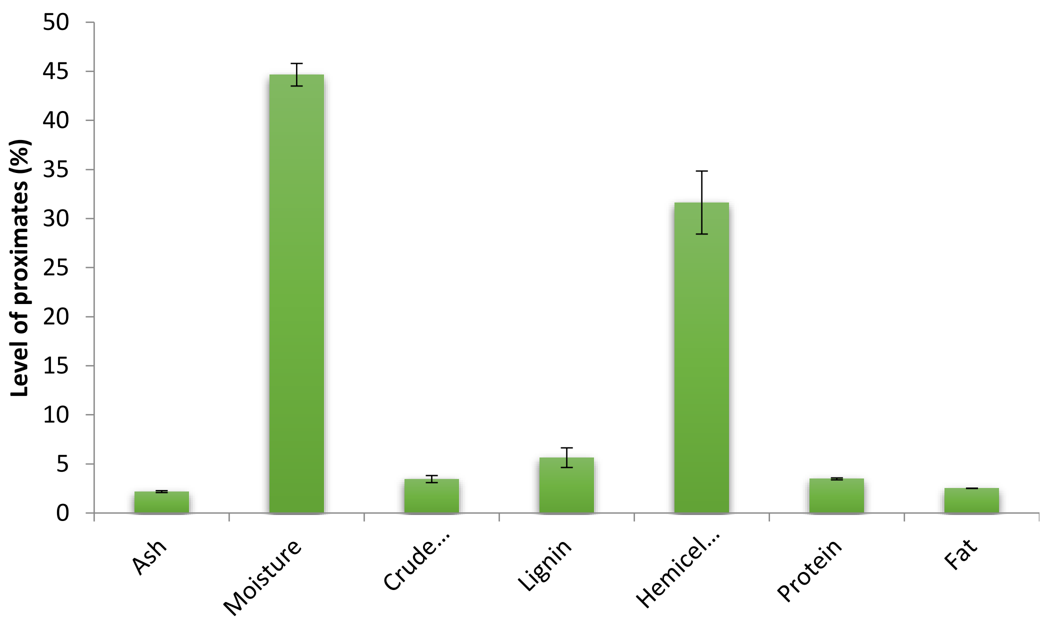

Fig 2b: Proximate analyses of isolated cellulose from Bean Pod (CBP)

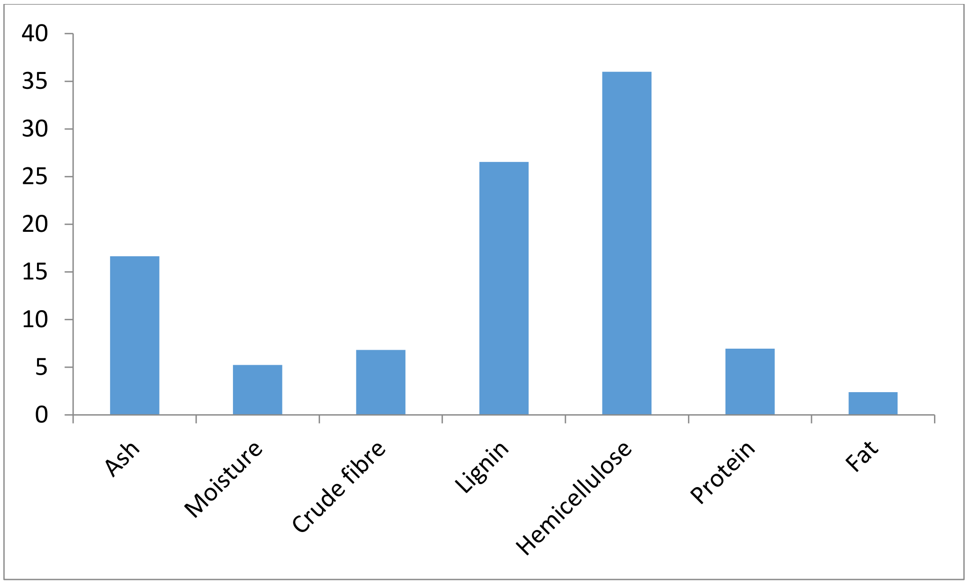

Fig: 3a. Proximate analyses of Sand Box (RSB)

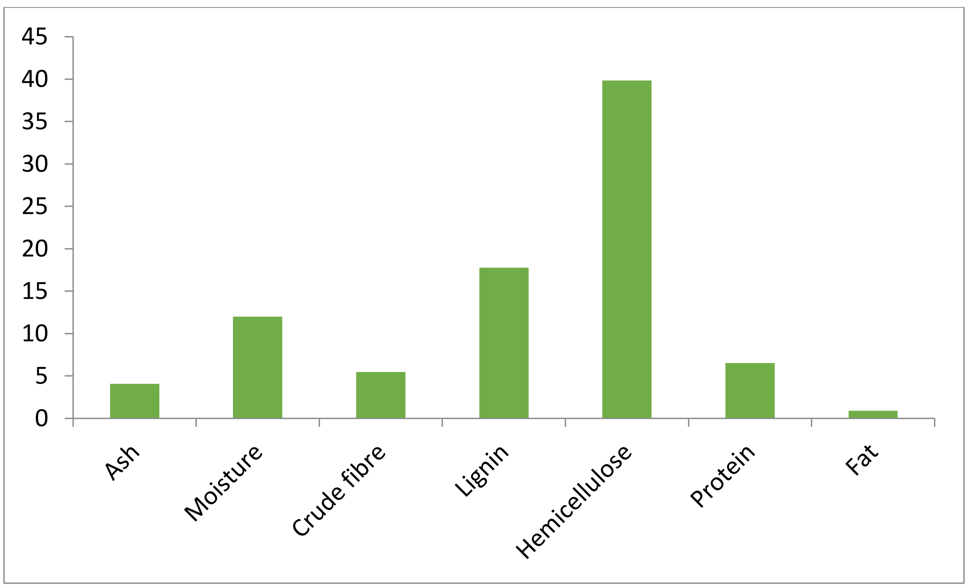

Figure 3b: Proximate analyses of cellulose from Sand Box. (CSB)

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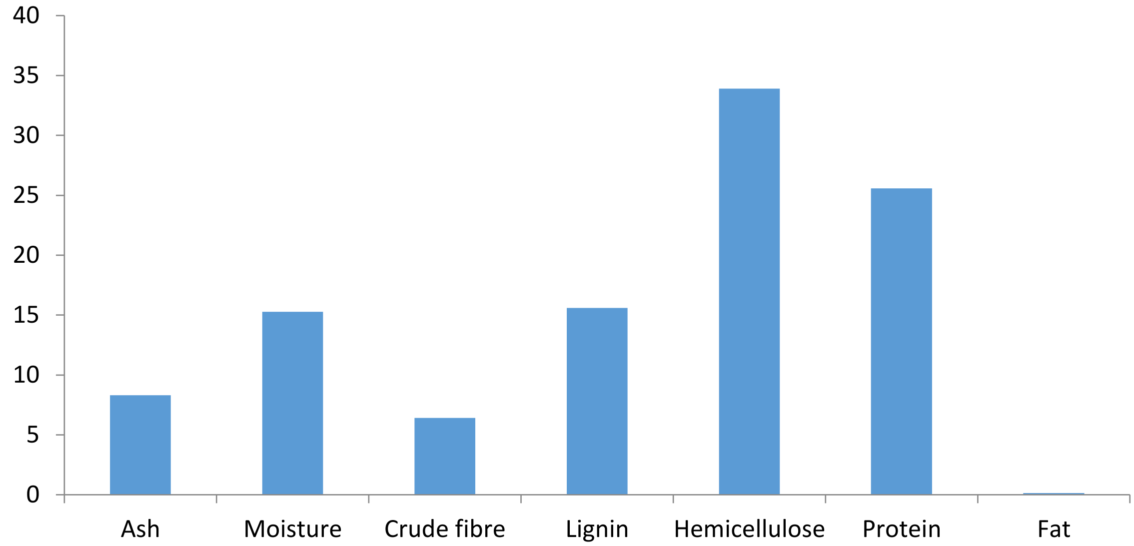

Fig: 4a. Proximate analyses of wheat Offal. (RWO)

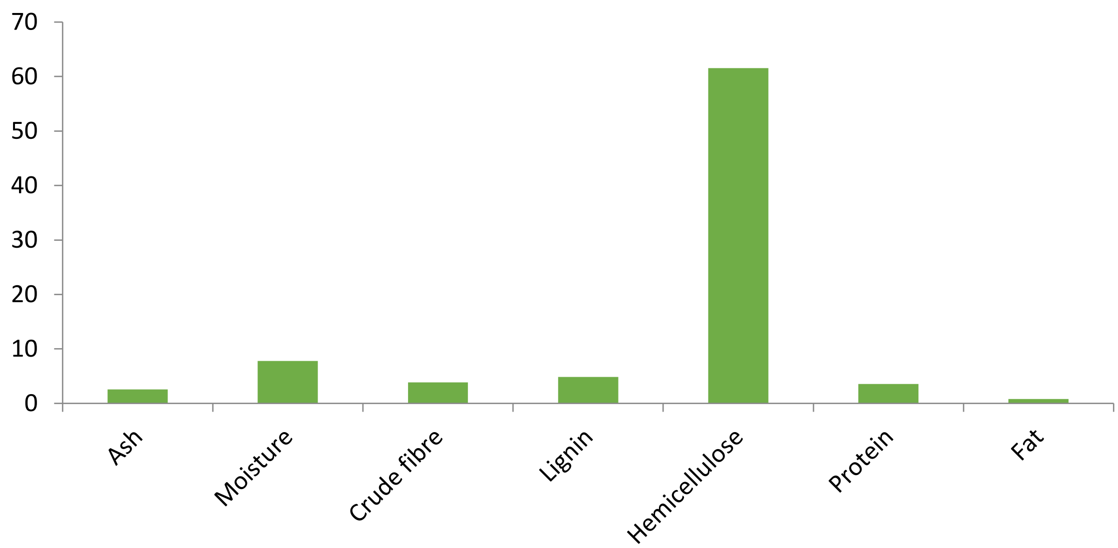

Figure 4b: Proximate analyses of Wheat Offal. (CWO)

Conclusion

Isolation of cellulose from food items has resulted in scarcity and high cost of food in society. Consequently, the use of agricultural wastes as feedstocks for obtaining cellulose is currently receiving greater attention. This research showed that the pretreatment method increased accessibility of the surface area and size of pores leading to a decrease in crystallinity and polymerization degree, which enhanced the yield of cellulose. The findings were strictly comparable to those of Badejo et al. (2018) and Lawal (2004) further, the proximate analysis showed a reduction in organic cations and lignin in the cellulose. Characterisation by Fourier Transfer Infrared Spectroscopy revealed the presence of various functional groups in the structure of cellulose. This study espoused the practise of obtaining cellulose from bean pod, sandboxs and wheat offals, and narrated the opportunities in utilising of isolated cellulose from agricultural wastes, as potent feedstocks for industrial applications. The Alkali treatment for the removal of lignin, ash and hemicellulose among other materials, made the isolation process promising (Qasim et

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al., 2020). The results of cellulose yields in this study were comparably similar to earlier works that were reported in literature.

The imperatives for this study had provided that the use of agricultural wastes of bean pod, sandbox and wheat offal for obtaining cellulose would be of immense opportunities in removing wastes from the environment, thereby reducing pollution.

Recommendations

1. The incessant practice of obtaining cellulose from food items should be discouraged. Greater efforts at getting cellulose should be geared towards the utilization of agricultural wastes such as bean pod, sandboxes and wheat offal. The pretreatment method used in this research was recommended for enhanced yield of cellulose from agricultural wastes and for efficiency in the isolation and conversion processes of cellulose. Thus, it would be recommended that further related studies be made to improve the efficiency of isolation and conversion processes of cellulose for wider application.
2. The major intermediate by-products obtained such as lignin and hemicellulose could be formulated into livestock feeds as food supplements. The availability of livestock feed supplements from waste products would restrict grazing activities and reduce farmer/herdsmen clashes in Nigerian local communities.
3. Agricultural wastes from bean pod, sandboxes and wheat offal were under-utilised and should be properly harnessed with view to converting them into useful products of more economic value such as cellulose-derivatives.

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