Isolation and Characterization of Microcrystalline Cellulose from *Papaya* Stem

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ABSTRACT

Microcrystalline cellulose (MCC) was isolated from papaya stem, characterized and compared with a commercial brand MCC. The dried pulverized papaya stem was subjected to alkaline treatment using aqueous NaOH and NaOCl solutions. Alpha cellulose obtained was modified by partial hydrolysis using 2.5 M HCl at boiling temperature to produce microcrystalline cellulose. The Physicochemical properties such as bulk, tapped and true densities, degree of polymerization, particle sizes, moisture content, angle of repose etc were examined and compared with a commercial brand sample (C-MCC). Results have shown that the percentage yields of alpha cellulose and CU-MCC obtained from the starting dried stem material were 23.78 and 20.70, respectively. Fourier Transform Infrared (FT-IR) spectra of CU-MCC and C-MCC confirmed the presence of OH and (C=O) carbonyl functional group which are the main characteristics of cellulose. Scanning Electron Microscopy (SEM) showed individual rod-like and flat-shaped plank-like fiber structures with few bundled crystal packed forms for the CU-MCC and C-MCC respectively. The C-MCC has a high thermal stability at 408°C when compared to 367°C of the hydrolysed CU-MCC. This may be resulting from the morphological characteristics of the fiber structures. CU-MCC had poor powder flow properties and an appreciable thermal resistance.

Keywords: Cellulose, Characterization, Microcrystalline cellulose, SEM, FT-IR.

INTRODUCTION

The amount of agricultural wastes generated in the African region annually demonstrates the inadequacies of various African countries to follow global trend of recycling [1]. Adeyi reported that large quantities of wastes produced in Nigeria are underutilized yearly [2]. The burning of agro-wastes and the inappropriate disposal of wastes (such as open dumping) are some of the regular practices of waste disposal and management in Nigeria. Previous studies have shown that burning of agro-wastes reduce crop yield [3], causes climate change and health complications mainly respiratory diseases such as bronchitis, eye irritation, asthma etc. [4]. The indiscriminate dumping of such waste also creates an unsightly and unaesthetic environment.

Papaya scientifically recognized as *Carica papaya* L, in the family Caricaceae [5], is a fast growing herbaceous, semi-succulent plant with a self-supporting stem which branches only when injured [6,7]. It is a big tree-like plant, having spirally arranged leaves confined at the uppermost part of the trunk and mostly a single stem of about 5-15 meters tall. Papaya leaves are 50-70 cm large in diameter and palmately lobed with 7 lobes. The large fruits maturing into 15 to 45 cm long and 10 to 30 cm diameter appear on the axils of the leaves [8]. Previous study has shown that the lignin, cellulose and hemicellulose content of papaya bast fiber (plantation and green house) are approximately 20%, 53% and 29%, respectively [9]. Papaya stem can be an interesting source of fiber for the production of cellulose if processed further instead of its disposal as waste, since the plants are normally replaced after 3-5 yrs when the fruit yield is said to be at maximum in the prime third year. At that point, the fiber

material is said to be available in large scale and further processing into microcrystalline cellulose can be a great source of income to the producer.

Microcrystalline cellulose (MCC) is one of the natural polymers that have different functionalities. It is obtained from a specialized grade of alpha cellulose in its purified form by the process of acid hydrolysis or action of enzymes. Many of its vast applications include; pharmaceutical, cosmetics, food, cosmetics etc. Furthermore, the usefulness has also been extended in the making of paint, paper and nonwoven textiles, oil field services, medicine and composites due its properties such as high strength, aspect ratio and flexibility [10]. MCC is one of the few materials that has the capacity to perform dual functions of producing adequately hard and rapidly disintegrating tablets mainly as a result of the swelling of its particles and consequent weakening of the bond forces holding them together [11]. Wood and cotton linters have remained the main commercial sources of MCC. However, alternative low cost sources such as oil palm biomass [12], water gourd [13], Alfa grass [14], soy-bean hulls [15], Orange peel [16], Water Hyacinth [17], Oil palm frond [18], pod husk and stalk of fluted pumpkin [19] etc. have been reported.

Many studies have also been reported on the utilization of papaya stem fiber as a biosorbent to remove heavy metals [20-23], adsorbent to remove methylene blue (cationic dye) [24], reinforcement in green composite [25] and its wound healing property in albino rats [26]. No information exists on processing papaya stem fiber to microcrystalline cellulose and the evaluation of its physicochemical properties.

MATERIALS AND METHODS

Cellulose precursor

The Carica *papaya* stem (height- 10 m tall) was sourced from a felled tree of paw-paw that had stopped producing fruits in Azigbo, Anambra State, Nigeria. The stem was debarked, washed, cut into irregular chips and dried properly. The dried chips of the papaya stem were milled and allowed to cool to room temperature. The pulverized substrate was sieved through 2.mm Laboratory test sieve to obtain uniform size.

Preparation of microcrystalline cellulose

A slight modification of the procedure by Ohwoavworhua [27] was employed. 900 g of the sieved stem powder was macerated in 13 l of 2% w/v aqueous NaOH solution at 80°C for 3 h in a stainless steel immersed in a JP Selecta water bath [Precisdig (6001197)]. The resulting de-lignified mass was treated with 4.320 l of 3.5% w/v aqNaOCl solution at 80°C for 30 min. The bleached mass was subjected to treatment with 2.680 l of 17.5% w/v aqNaOH solution at 80°C for 1 h. The resultant α-cellulose undergoes the final stage of bleaching with 1.920 l of (1:1) aq solution of 3.5% w/v NaOCl for 1 h. The resultant mass obtained was repeatedly rinsed with distilled water until it is neutral to litmus paper. Excess moisture was squeezed off using a muslin cloth and the small mass obtained was then oven-dried in a JP Selecta Digiheat Oven at 65°C for 12 h. The alpha cellulose obtained was then hydrolyzed using HCl at a boiling temperature according to the procedure reported by Nwajiobi 19].

Physicochemical analysis

The identification, organoleptic characteristics, solubility tests, starch and dextrin were carried out according to British Pharmacopoeia (BP) specifications [28].

Moisture content

2 g of the MCC was placed in a petri dish and then oven-dried at 105°C for 3 h to a constant mass. The computation of the moisture content (%) was based on the initial air-dried weight.

Bulk density (B_d)

5 g of the MCC was placed in a 50 ml clean, dry measuring cylinder. The occupied volume by the sample without tapping was noted as the bulk volume while the bulk density was computed by dividing the mass of the sample by its bulk volume as expressed:

$$B_d = \frac{M}{V_B}$$

Where, M is the mass of the MCC, V_B is the bulk volume of the MCC.

Tapped density (T_d)

The tapping of the measuring cylinder containing the MCC on a wooden platform was carried out and after 500 taps; the occupied volume was noted as the tapped volume. The tapped density was then determined using the expression:

$$T_d = \frac{M}{V_T}$$

Where, M is the mass of the MCC, V_T is the tapped volume of the MCC.

True density (T_{td})

The liquid displacement method was adopted in determining the true density. This was carried out using the immersion fluid xylene and immersing the sample completely in a 26 ml pycnometer bottle capacity. The volume of the liquid displaced was measured and the density was computed using the expression;

$$T_{td} = [w/ \{(a + w) - b\} \times SG],$$

Where, w is the weight of MCC powder, **SG** is specific gravity of xylene, **a** represents sum of weights of bottle and solvent and **b** represents the sum of weights of bottle, solvent and the MCC powder.

Hausner's Index (Ha_r)

This was calculated from the tapped density and bulk density values of the MCC powder.

$$Ha_r = T_d/B$$

Powder porosity

This was determined from the true and bulk densities values when fitted into the equation below;

$$P_p = 1 - B_d/T_{td} \times 100$$

Where B_d is the bulk density, T_{td} is the true density and P_p is the porosity

Compressibility index C_I

This was computed from the bulk and tapped densities values when fitted into the equation:

$$\text{Compressibility index } (C\%) = [(T_d - B_d)/ T_d] \times 100$$

Hydration capacity (H_c)

1 g of the MCC sample was introduced into each of the four 15 ml plastic centrifuge tubes and 10 ml distilled water was added, shaken and then mixed for 2 min. After 10 min of standing, the mixture was immediately centrifuged at 1000 rpm for 10 min on a Sorvall bench centrifuge. The weighed sediment obtained after decanting the supernatant was used to compute the hydration capacity as the ratio of sediment weight to the dry sample weight [29].

$$H_C = [(\text{sediment weight} - \text{tube weight})/ \text{dry sample weight}]$$

Swelling capacity (Swell ability)

This was determined alongside with the hydration capacity according to the study reported by Ohwoavworhua [27] and was calculated as follows:

$$\text{Swelling capacity } (S_c) = [(V_2 - V_1)/V_1]$$

V_1 = Occupied tapped volume prior to hydration
V_2 = Volume occupied after hydration

Angle of repose (a)

The method as reported by Achor [13] was used in determining the static angle of repose. The tangent of the angle of repose was computed using the equation.

$$\text{Tan } a = 2h/D$$

Where, h is the height of the heap of powder and D is the diameter of the base of the heap of powder

Particle size analysis

This was determined by microscopic method using a trinocular microscope (SXY-m50) and s-viewer application was used in taking accurate readings. The average diameters of the magnified particles were determined for the sample size of 100 particles.

Degree of polymerization and Molecular weight determination

The procedure as established in a study reported by Nwajiobi [19] was adopted in determining the degree of polymerization and Molecular weight.

Scanning Electron Microscopy (SEM)

SEM was carried out on the MCC powder to study the surface morphology. The MCC powders were sputter-coated with gold for 3-4 min and then observed at 10 KV accelerated voltage in a SEM, JSM 5400 (JEOL ltd., Japan)

Fourier Transform (FTIR) Spectroscopy

The MCC powders were characterized using a Shimadzu FTIR-8400S by mixing about 5µg with a little amount of KBr and compressed into its disc. It was then scanned between 4000-500 cm^{-1} and the spectra was recorded and studied.

Thermo-gravimetric analysis (TGA)

Perkin-Elmer Thermal analysis controller was used to determine the thermal stability of the MCC powder at a heating rate of 10°C/min in nitrogen atmosphere.

RESULTS AND DISCUSSION

Table 1: Identification and organoleptic properties

Test	CU-MCC	C-MCC
Organoleptic characteristics	White, odorless, tasteless and granular powder	White, odourless, tasteless, granular
Identification with iodinated zinc chloride	Violet-blue	Violet blue
Starch and Dextrin	Negative	Negative
Solubility (n-hexane, water, acetone, ethanol)	Insoluble	Insoluble

The organoleptic properties (Table 1) have shown that the cellulose samples were white, odourless, tasteless and granular powder. The violet-blue coloration observed from identification test indicates the presence of cellulose; starch and dextrin were also considered absent. The cellulose samples were insoluble in these solvents; ethanol, n-hexane, acetone but soluble in Cuprammonium hydroxide. Alpha cellulose has a percentage yield of 23.75 while the % yield of MCC obtained from the alpha cellulose was 87.71%. Thus, the CU-MCC yield resulting from the initial dry substrate was 20.70% (w/w). Accordingly, CU- MCC % yield was expected to reduce due to the elimination of some semi-crystalline regions during the stages of acid hydrolysis and washing steps.

Table 2: Powder properties of the cellulose samples

Parameters	CU-MCC	C-MCC
Moisture content (%)	7.54	7.61
Bulk density (g/ml)	0.219	0.319
Tapped density (g/ml)	0.448	0.566
True density (g/ml)	1.464	1.5
Angle of repose (Φ^0)	41	40
Hausner's ratio	2.044	1.775
Compressibility index (%)	51.1	43.7
Hydration capacity	2.687	3.468
Swelling capacity (%)	13.3	20.8
Porosity (%)	85	86
Average particle size (µm)	6.796	5.865
Degree of polymerization	210	239
Molecular weight (g/mol)	33942	38718

The percent moisture contents of the cellulose samples (Table 2) were slightly above the acceptable limit of 7.5% as stated by the BP. Thus, both samples may require further drying before their usage in drugs formulation. The values of the bulk and tapped densities of the commercial MCC (C-MCC) is significantly higher than that of papaya stem microcrystalline cellulose (CU-MCC). A decrease in bulk density improves tablet ability; however, it often hinders flow-ability [30]. Again, lower bulk density makes for higher dilution potential which may better counteract the poor tableting properties of active pharmaceutical ingredients [31]. This implies that C-MCC may have better flow properties though it may require larger amount when compressing tablet due to lower loading volume while CU-MCC may have better tableting properties. Both densities
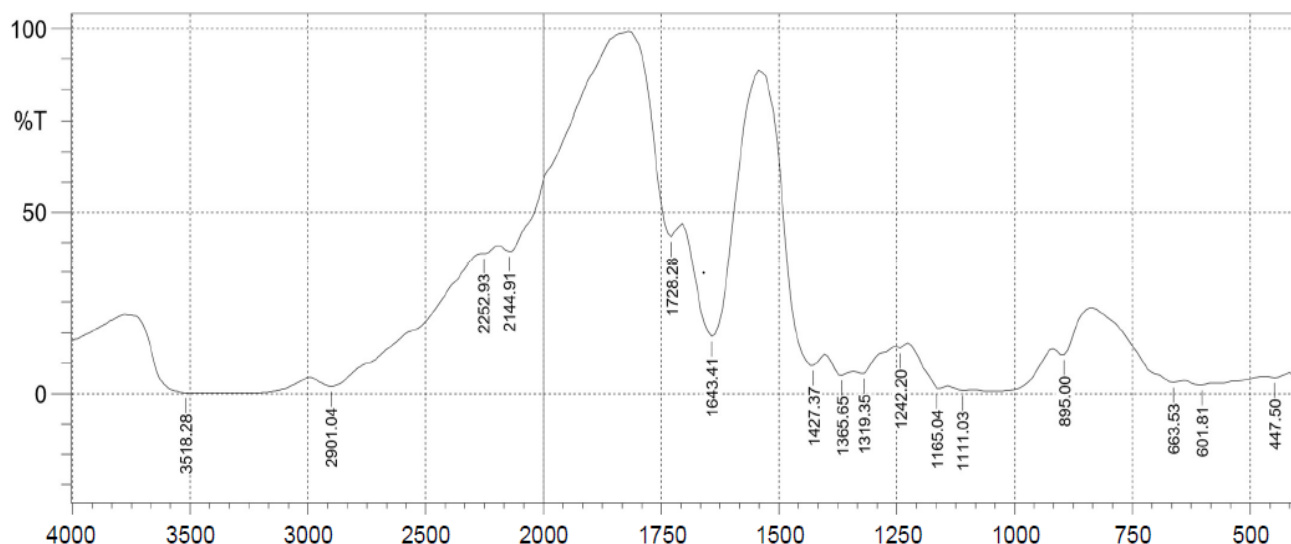
depend on the packing behavior of the cellulose samples in a confined space. Particle sizes and shapes, particle size distribution, moisture content etc. are some of the factors affecting the bulk and tapped densities.

The true density of commercial MCC (C-MCC) is slightly higher than the papaya stem microcrystalline cellulose (CU-MCC). Higher true density enhances compressibility. Furthermore, higher true density is an indication of a high degree of crystallinity which is in accordance with the report by Stamm [32].

The compressibility index and Hausners' ratio are indirect measurements that determine the strength and interparticulate friction built up by the cellulose samples in their arch in hopper and the ease with which such arch collapse. Angle of repose assesses the internal and cohesive friction between the powder samples. They provide indirect information relating to flow rate, cohesiveness and particle sizes for which a small value indicates better flowability [33]. The result of angle of repose, compressibility index and Hausners ratio of CU-MCC are significantly higher than the C-MCC. This suggests that the commercial MCC (C-MCC) may have better flowability when compared to CU-MCC. Cohesion and friction are two fundamental forces affecting powder flow [34]. Moisture content of a powder also affects its flow rate as an increase in the moisture content decreases the ability to flow smoothly [35]. However, both cellulose samples have poor powder flow properties.

Hydration capacity and swelling capacity assess the sensitivity of moisture of the MCC powders. The values of hydration capacity and swelling capacity obtained for commercial MCC (C-MCC) is significantly higher than the papaya stem microcrystalline cellulose (CU-MCC).This may be due to increased surface area arising from the small particle sizes of the C-MCC powder as well as the porosity. However, since MCC is sensitive to atmospheric humidity, the storage should be in a tightly closed container. The degree of polymerization is an identity test which confirmed that the prepared sample (CU-MCC) and the commercial MCC (C-MCC) have their degrees of polymerization within the official limit $DP \leq 350$ [28]. The high porosity values of the samples will help facilitate their compressibility as the degree of material densification (tapped density) correlates to its porosity which is a function of the void volume [36].

Figure 1: Scanning electron micrograph images of a) papaya stem MCC (CU-MCC) (b) Commercial MCC (C-MCC)

Figures 1a and 1b are the scanning electron micrograph images of CU-MCC and C-MCC at 500 and 1000 magnifications. CU-MCC images showed long individual rod-like shaped fibre structures with a few bundled crystal packed forms while the C-MCC has shorter fibers than long fibers which exist as irregular individual flat-shaped plank-like fibers. The unevenness of their surfaces may be due to the processing conditions that led to the elimination of some binding materials around their fibre bundles [37]. The surface coarseness assists nanocrystals production through hydrolysis [38].The shapes of the powder particles also revealed their effect in the packing behavior and flow properties. These are reflected in the physical properties of the MCC samples.

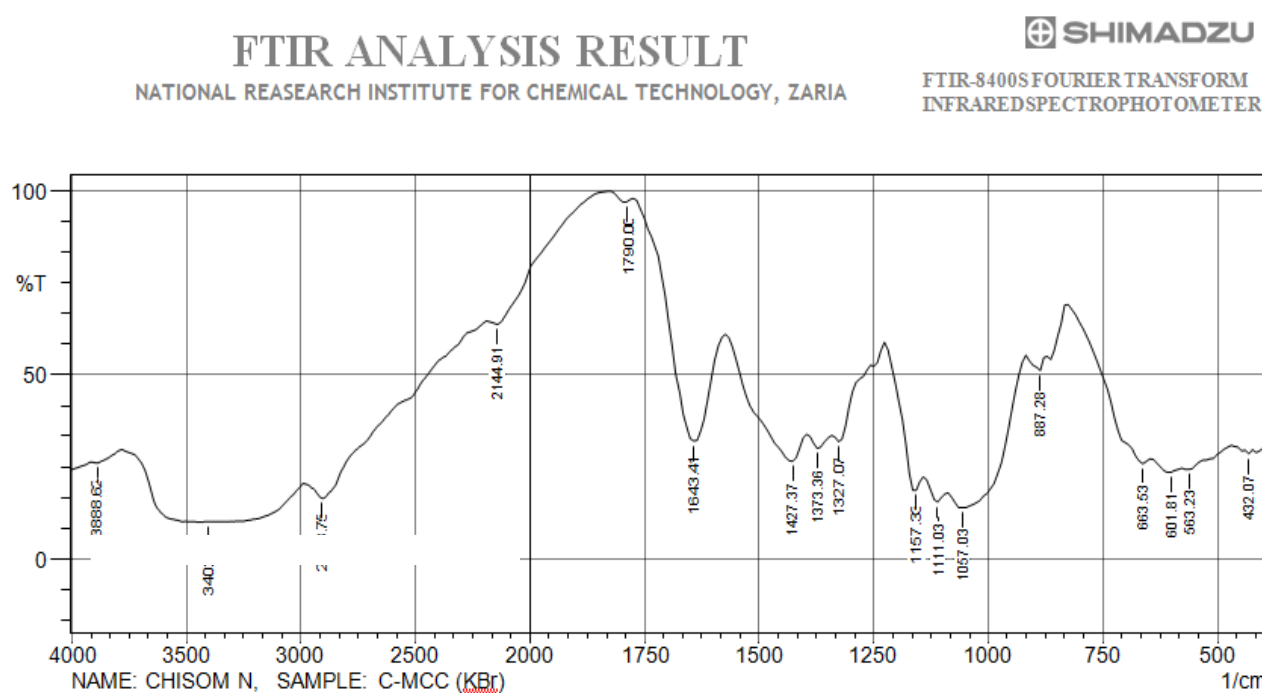**Figure 2: Fourier transform infrared (FT-IR) of CU-MCC**

Figure 3: Fourier transform infrared (FT-IR) of C-MCC

From Figures 2 and 3, the samples displayed broad absorption within the band region of 3400-3600 cm^{-1} which depicts O-H stretching vibration due to intramolecular and intermolecular hydrogen bonding. The band at 2901 and 2909 cm^{-1} is attributed to the C-H stretching of CH_2 groups in C-6 present in MCC [12]. The spectra band at 1643 cm^{-1} is due to water molecules absorbed and the occurrence of this band is attributed to the strong interaction between cellulose and the water molecules [39]. A peak was observed at 1728 cm^{-1} which indicates a trace of hemicellulose for the CU-MCC sample. Previous studies have shown that the band at 1700-1740 cm^{-1} corresponds to either the acetyl or uronic ester groups of hemicelluloses or ester linkage of the carboxylic group of ferulic and p-coumaric acids of lignin and/or hemicellulose [39,40]. The band at 1427 cm^{-1} is as a result of CH_2 − bending (i.e., CH_2 of pyran ring symmetric scissoring). The CH deformation vibration was at 1365 and 1373 cm^{-1} while CH_2 rocking vibration was denoted by these bands 1319 and 1327cm^{-1}. The peaks associated with the C-O-C stretching at the β (1-4) glycosidic linkage was at 1165 and 1157 cm^{-1}. The band at 1111 cm^{-1} is a symmetric in-phase ring stretching, C-C and C-O stretching. The regions assigned to 895 and 887 cm^{-1} is due to the C-H out of plane asymmetric ring stretching in cellulose due to β- linkage and also to the semi-crystalline form in the cellulose.
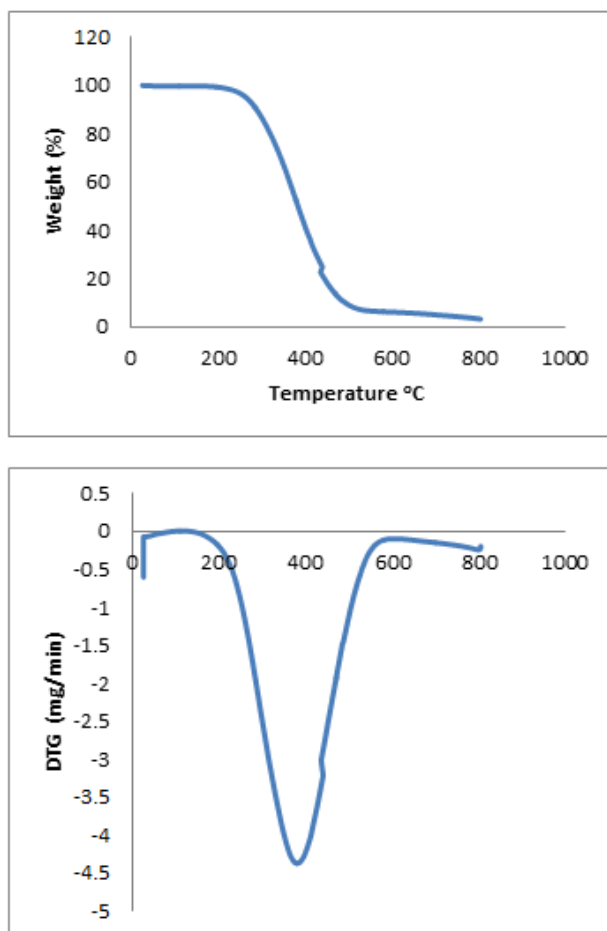
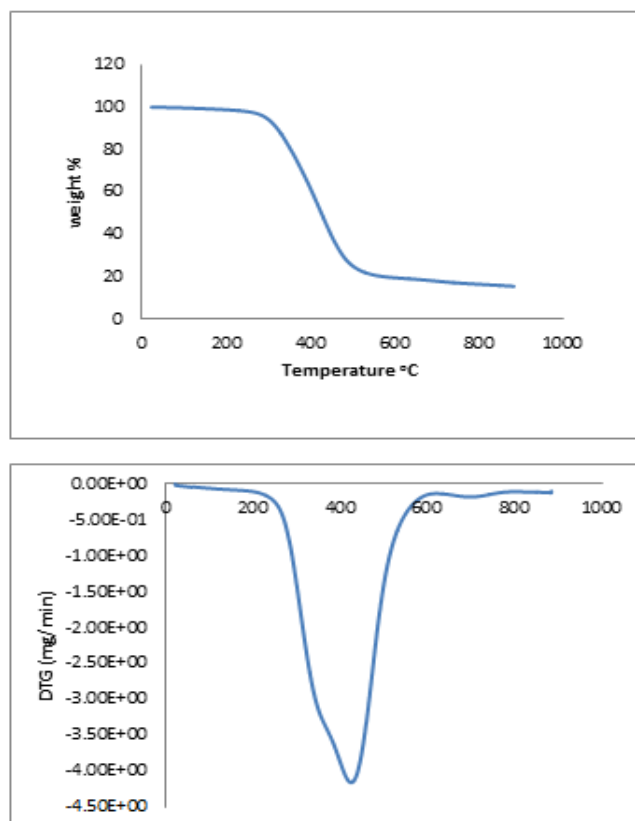**Figure 4: TGA/DTG curve for CU-MCC**

Figure 5: TGA/DTG curve for C-MCC

From Figures 4 and 5, the thermogram showed two-step degradation with the first thermal event occurring below 150°C. This corresponds to the removal and evaporation of water molecules in the cellulose samples. The onset decomposition temperatures are 224°C and 272°C while the derivative weight loss curve which determines the major decomposition peak (T_{max}) occurs at 367°C and 408°C for CU-MCC and C-MCC respectively. At this point cellulose degradation takes place which involves the decomposition of the glycosyl units resulting to the evolution of gases. The decomposition peak of CU-MCC is greater than the decomposition of MCC from jute (°C) and oil palm biomass (326°C) as reported by M.S. Jahan [12,41] respectively. CU-MCC sample has an appreciable thermal stability.

CONCLUSION

The characterization of CU-MCC obtained from paw-paw stem shows that the sample has poor powder and flow properties. From the thermal analysis, the sample has fair thermal resistance when compared to other established reports. The SEM revealed long strands of rod-like shaped fiber particles and FT-IR identified the main functional groups associated with cellulose. CU-MCC may be used for other high-temperature related applications since it has an appreciable thermal stability.

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