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Extraction and Characterization of Microcrystalline Cellulose Obtained from *Cocos nucifera* Seed Pulp

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Abstract

The study aimed at investigating the physicochemical and powder properties of microcrystalline cellulose (MCC) extracted from coconut pulp in comparison with a commercial product. Alpha-cellulose was extracted from the pulp of coconut fruits and converted to MCC with dilute hydrochloric acid. The extracted MCC was subjected to physicochemical and powder characterizations as well as infrared spectrophotometric analysis and compared with Avicel®. Organoleptic properties and solubility of the extracted MCC were similar with those of Avicel®. The MCC moisture content, swelling and water sorption capacities were 5.40, 46.40 and 5.53% respectively. Extracted MCC exhibited powder properties; bulk density (0.30 g/mL), tapped density (0.38 g/mL), true density (1.25g/mL), Carr's index (20.51), Hausner's ratio (1.25), angle of repose (40.33°), porosity (80%) and particle size (100 µm). FTIR analysis revealed similarity in functional groups between the extracted MCC and Avicel®. Extracted MCC from Cocus nucifera pulp measured favourably with Avicel® in their physicochemical and powder properties and in their infra-red spectra. Hence, coconut seed is a viable local alternative source for the production of MCC.

Keywords: *Cocus nucifera*, microcrystalline cellulose, Avicel®, characterization

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INTRODUCTION

Microcrystalline cellulose (MCC) is a powder-like cellulose product that can be prepared from all forms of natural celluloses, alkali celluloses and regenerated celluloses (Vanhatalo and Dahl, 2014). It is produced from the chemical and mechanical degradation of cellulose as pure, partially depolymerized cellulose synthesized from α-cellulose precursor (Hindi, 2016). These common precursors used for MCC preparation such as wood and cotton might pose environmental problems and ecological imbalances; cottons are high-priced crop, while the use of wood pulp could reduce the availability of wood and can possibly contribute to deforestation activities. Therefore, finding alternative non-wood sources of MCC is necessary. Lignocellulosic waste materials are inexpensive, easily available and have been reported to consist of high cellulose of which include corn cob, pineapple leaf fibers, rice straw, sugarcane bagasse and coconut husk (Brinchi *et al,* 2013). Coconut can serve as an alternative to other sources of MCC since they are more abundant in the tropics. The husks and fiber are huge sources of lignocellulosic materials considered as agricultural wastes (Saini *et al*., 2015). Harnessing cellulose from these materials therefore solves the problem of deforestation, waste disposal and paves the way for more natural materials to be used as efficient pharmaceutical excipients. Coconuts also have a 33-44% cellulose content which is a good indication of the presence of microcrystalline cellulose (Muensri *et al.,* 2011).

The synthesis of MCC includes different processes such as reactive extrusion process, enzyme mediated process (Monschein *et al*., 2013), the steam explosion process and acid hydrolysis process (El-Sakhawy and Hassan, 2007; Chauhan *et al*., 2009). The acid hydrolysis process can be done using mineral acids such as H_2SO_4, HCl and HBr as well as ionic liquids. The role of these reagents is to destroy the amorphous regions while leaving the crystalline domains of the cellulose molecule (Udo *et al*., 2020). The acid hydrolysis method has preferred advantages over the other processes as it has a shorter reaction duration, it can be applied as a continuous process rather than a batch-type process and it consumes limited quantity of acid and produces fine particles of the MCC (Chauhan *et al*., 2009).

The importance of acid hydrolysis of lignocellulose fibres is derived from its ability to isolate micron-sized cellulose particles known as microcrystalline cellulose as well as nano-crystalline cellulose which can be used as an eco-friendly bio-filler for various applications in the medical, food cosmetics, and packaging industries (Abdul *et al.,* 2014). After coconut oil is extracted, the residue is usually disposed as waste, this residue is what this study sought to explore its usefulness. Thus, this study focused on isolating and characterizing the potential microcrystalline cellulose present in coconut seed pulp.

MATERIALS AND METHODS

Materials

Avicel® PH101 (FMC Biopolymer, USA), sodium hydroxide and nitric acid (Guangdong Guanghua Sci-Tech, China), sodium hypochlorite (Reckitt and Colman Nig. Ltd., Lagos), hydrochloric acid, sodium nitrite and sodium sulphite (BDH Chemicals Ltd, Poole, England), phloroglucinol (Hopkin and Williams, UK) were employed in this study. All sieves were British Standard Sieves (Endecotts, UK) and water was double distilled. Matured coconut fruits were bought from a local market in Abraka, Delta State, Nigeria.

Extraction of α-cellulose from *Cocus nucifera* pulp

The hard backs of the matured coconut fruits were broken off and the edible pulp was washed, blended and soaked in distilled water. The coconut oil was extracted from the blended mixture by cold maceration and subsequently filtered. The residue was washed thoroughly

with distilled water and dried at 60 °C for 24 h in a hot air oven ((Kottermanns, Germany). The dried residue was pulverized into powder using a blender (Kenwood Ltd, UK).

Using the method of Azubuike and Okhamafe (2012), 300 g of the powdered residue was suspended in 3.0 L of 3.5% nitric acid in a steel container immersed in a water bath at 90 °C for 2 h to remove lignin in the form of soluble nitro-lignin. This mixture was then filtered and the residue was dispersed in 2.0 L solution containing 2.0% (w/v) each of sodium hydroxide and sodium sulphite held at 50 °C for 1.0 h. The mixture was stirred, washed with distilled water and filtered again. The sample material was then suspended in 750 mL of 17.5% sodium hydroxide solution for 30 min at 80 °C. The residue (α-cellulose) obtained was washed with distilled water, filtered and dried in a hot air oven at 60 °C for 16 h. The dried α-cellulose was bleached with 500 mL of 3.2% sodium hypochlorite in a beaker at 40 °C for 1.5 h. The bleached α-cellulose was thoroughly washed with distilled water, until it was neutral to litmus, filtered and then dried in an oven at 60 °C for 16 h. The resulting powder was passed through a 212 µm sieve, further dried at 60 °C for 1.0 h and weighed. The weight was used to calculate the percentage yield of the α-cellulose with respect to the weight of the coconut pulp material.

Hydrolysis of α-cellulose

The extracted α-cellulose powder was hydrolyzed with 2.0 N hydrochloric acid solution under reflux for 15 min with a solid-liquid ratio of 1:10. The mixture was poured into 1.0 L of distilled water and washed thoroughly by stirring and then allowed to settle and the supernatant decanted. Repeated washings were carried out until the supernatant was neutral to litmus. The sediment (microcrystalline cellulose) was air dried for 48 h. The dried MCC was stored in an airtight container until characterization.

Physicochemical characterization

Organoleptic properties

The organoleptic characteristics of the extracted MCC and Avicel® PH 101 were carried out in accordance with British Pharmacopoeia (2012) specification. Their colour, taste, odour and texture of both MCC powders were noted.

Tests for cellulose and lignin

The test for cellulose was carried out by dispersing about 10.0 mg of the microcrystalline cellulose powders in a test tube and adding few drops of iodinated zinc chloride solution for any colour change while the test for lignin content was done by wetting about 100 mg of the microcrystalline cellulose powders with few drops of concentrated hydrochloric acid on a glass slide. Two drops of phloroglucinol was then added to the wetted powders on the glass slide and heated over a Bunsen burner for complete evaporation of the powder's liquid content. Any colour change to the powder was noted with the slide examined under a light microscope (Eraga *et al.*, 2017).

Solubility

The gravimentric method was used in determining the solubility of the MCC powders. Exactly 100 mg quantity of the microcrystalline cellulose powder was dispersed at 30 °C (room temperature) in 10 mL of water in a test-tube and shaken. The mixture was filtered with a pre-weighed filter paper. The residue on the filter paper was air dried and weighed**.** The difference in weights of the residue and the MCC powders was used as a measure of solubility of the powder.

pH

Exactly 1.0 g of the MCC powders was shaken with 50 mL of distilled water and centrifuged for 5 min. Thereafter, the pH of the supernatant layer was determined using a pH meter (3510 model, Jenway, England)

Moisture content

About 2.0 g of the MCC powders was transferred into a Petri dish and dried in an oven at 60 °C until a constant weight was obtained. The moisture content was expressed as a percentage of the difference in weight loss of the initial and final weights of the MCC powders after drying.

Swelling capacity

About 10 g of the MCC powder was poured into a 100 mL measuring cylinder and tapped several times to give a tapped volume (V_1). Some quantity of distilled water was added to the powder to form a dispersion and finally made up to the 100 mL mark. The dispersion was allowed to stand for 24 h and the volume of the sediment (V_2) noted. The swelling capacity was computed with Equation 1 (Okhamafe and Azubuike, 1994).

$$Swelling\ capacity = \frac{V_2 - V_1}{V_1} \times 100 \quad \ldots\ldots\ (1)$$

Moisture sorption capacity

Moisture sorption capacity was carried out by pouring 2.0 g of the MCC powder into a pre-weighed tarred Petri dish. Even distribution of the powder over the surface of dish was ensured by gentle taps to the dish. The samples were then placed in a desiccator containing a glass of distilled water (RH = 100%) at room temperature. The Petri dish was brought out of the desiccator and weighed every 24 h until a constant weight was obtained. The amount of water sorbed by the powder was calculated from the weight difference between the initial and final weights of the Petri dish with powder (Audu-Peter *et al*., 2004).

Characterization of powder properties

Flow rate and angle of repose

Using the funnel method, about 25 g of the MCC powder was poured into a plugged glass funnel clamped to a retort stand and hanging about 5.0 cm above a flat surface. The plug was removed from the tip of the funnel and the time taken for the MCC powder to completely flow through the orifice of the funnel was recorded. Also, the height of the heap formed by the granules and the diameter of the heap base was measured and recorded. The process was carried out three times and their average values were computed and recorded. The angle of repose (θ) was calculated using the Equation 2.

$$\theta = \tan^{-1}(h/r) \quad \ldots\ (2)$$

Where h is the height of the powder heap and r is the radius of the circular base.

Bulk and tapped densities

A 25 g of the MCC powder was weighed into a 100 mL graduated measuring cylinder and the volume occupied by the granules was recorded. The graduated cylinder still containing the powder was then tapped 100 times and the volume occupied after tapping was recorded. The bulk and tapped densities were calculated using Equations 3 and 4.

$$\text{Bulk density} = \frac{\text{Weight of powder}}{\text{Volume of powder}} \quad \ldots\ldots\ (3)$$

$$\text{Tapped density} = \frac{\text{Weight of powder}}{\text{Tapped volume of powder}} \quad \ldots\ldots\ (4)$$

Carr's index and Hausner's ratio

Using Equation 5, the difference between the tapped and bulk density of the MCC powders divided by the tapped density and the ratio expressed as a percentage to give the Carr's index. While the ratio of the tapped density to the bulk density of the MCC powders was calculated as the Hausner's ratio or quotient with Equation 6.

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \quad \ldots\ldots. \quad (5)$$

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \quad \ldots\ldots. \quad (6)$$

True density

A 25 mL specific gravity bottle (glass pycnometer) was filled with liquid paraffin, cleaned of any residual liquid paraffin and weighed (a). The bottle was emptied, rinsed with acetone and dried. About 1.0 g (b) of the MCC powder was poured into the bottle and then filled with liquid paraffin. It was weighed (c) after cleaning off the residual paraffin from the bottle. The various weights recorded were used to calculate the true density of the powder using Equation 7 (Irwin *et al*., 2002; Ohwoavworhua *et al*., 2007).

$$\rho = \frac{b}{[(a+b)-c]} \times S \quad \ldots. \quad (7)$$

Where ρ is the particle density of the powder and S is the specific gravity of liquid paraffin

Powder porosity

The porosity of the MCC powders was calculated by subtracting the ratio of the bulk density to the true density from one and expressed as a percentage using Equation 8.

$$\text{Powder porosity} = 1 - \frac{\text{Bulk density}}{\text{True density}} \quad \ldots\ldots. \quad (8)$$

Particle size analysis

Sieves of different aperture sizes were arranged in decreasing order of sizes with a receiver placed at the base. The nest of sieves was clamped on a standard mechanical sieve shaker (Endecott's Ltd, UK). A 10 g amount of the MCC powder was weighed and placed on the topmost sieve and the mechanical shaker was operated for 30 min. Thereafter, the particles obtained in the different sieves were weighed and the average diameter was calculated using Equation 9 (Ansel *et al.,* 2005).

$$Average\ diameter = \frac{[\sum (\%\ retained) \times (mean\ aperture)]}{100} \quad \ldots\ldots. \quad (9)$$

Infrared spectroscopy

The MCC powders were analyzed using Fourier transform infrared spectroscopy (FTIR). Potassium bromide (KBr) pellets containing 1.0% of the samples were prepared and placed in the sample compartment. Spectra were recorded on a Perkin-Elmer FT-IR spectrometer. The scanned range was 4000 - 400 cm^{-1}, with a resolution of 4 cm^{-1}. Three samples were subjected to this analysis; the extracted α-cellulose, MCC from the α-cellulose and Avicel® powders.

Statistical analysis

Experiments were carried out in triplicates and mean values reported with standard deviation. Differences between means obtained from the evaluation of the MCC powders were subjected to student's T-test at 5.0% level of significance using GraphPad InStat 3.10.

RESULTS

The quantity of α-cellulose (35.10 g) derived from the extraction process gave a percentage yield of 11.60%. Results from the characterization of the extracted MCC and a commercial Avicel® product are shown in Table 1. The organoleptic properties of the extracted MCC and Avicel showed white, tasteless and odourless powders with gritty texture and both powders were insoluble in water at room temperature. Both powders showed a slight violet-blue colour on reaction with iodinated zinc chloride solution to confirm the presence of cellulose and negative for the phloroglucinol test, showing absence of lignin. The pH of the extracted MCC was 5.6 and that of Avicel® was 6.1. Their moisture contents were within the BP specification and ranges form 5.0 - 7.0%. However, the swelling capacity and water sorption capacity for the extracted MCC were 46.40 and 5.53% respectively and those for Avicel® were 45.25 and 5.52% respectively.

The Hausner's ratios for the extracted MCC and Avicel® powders were 1.22 and 1.20, respectively while their Carr's indices were 18.42 and 17.50%, respectively. The extracted MCC powder exhibited a flow rate of 2.90 g/sec with an angle of repose of 40.33°, while those for Avicel® powder were 2.96 g/sec and 39.43° respectively. The powder porosity and mean particle size obtained for extracted MCC were 80% and 100 µm respectively while of Avicel® were 75% and 50 µm respectively.

FTIR analysis

The FT-IR spectra of the α-cellulose, extracted MCC and Avicel® powders are shown in Figure 1. On comparison of the spectra pattern of the extracted MCC and Avicel®, a general characteristic spectra of cellulose was observed with pronounced changes in intensities at band 740.80 and 1072.7 cm^{-1} (conforming to ß-glycosidic linkages), 1093 cm^{-1} (ring vibration and C-OH bonding), at 2500 to 3450 cm^{-1} (C-H asymmetric and symmetric tensile vibrations) and at 3000 to 4000 cm^{-1} (OH stretching bond).

Table 1: Some properties of the microcrystalline cellulose powders

Properties	MCC powders	
	Extracted MCC	**Avicel®**
Appearance	White	White
Taste	Tasteless	Tasteless
Odour	Odourless	Odourless
Texture	Gritty	Gritty
Solubility (Ambient temperature)	Insoluble	Insoluble
Presence of cellulose	Positive	Positive
Presence of lignin	Negative	Negative
pH	5.6	6.1
Moisture content (%)	5.40	5.35
Swelling capacity (%)	46.40	45.25
Water sorption capacity (%)	5.53	5.52
Bulk density (g/cm^3)	0.30	0.33
Tapped density (g/cm^3)	0.38	0.41
Carr's index (%)	18.42	17.50
Hausner's ratio	1.22	1.20
True density (g/cm^3)	1.55	1.58
Flow rate (g/sec)	2.90	2.96
Angle of repose (°)	40.33	39.43
Porosity (%)	80	75
Mean particle size (µm)	100	50

DISCUSSION

The percentage yield of α-cellulose reported in this work showed an acceptable yield, even though there is a difference in the percentage yield reported by other researchers. Using the nitric acid method of extraction, Ohwoavworhua *et al.* (2009) reported a 15.0% yield for groundnut husk and Azubuike *et al.* (2012) had a 26% yield for corn stalks while Eraga *et al.* (2017) reported a 30.22% yield for cassava stems.

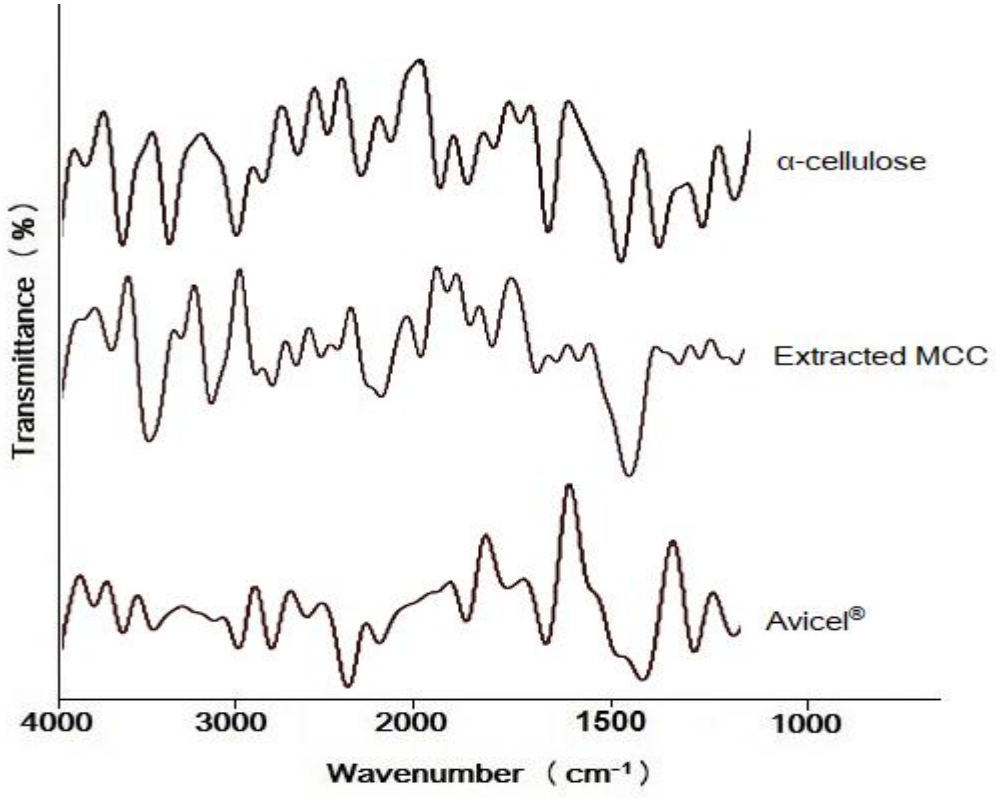

Figure 1: FTIR spectra of the extracted α-cellulose, microcrystalline cellulose (MCC) and Avicel® powders

The organoleptic properties and solubility of the MCC powders were similar between the extracted MCC and Avicel®. The chemical test carried out on MCC powders showed a slight violet-blue colour on reaction with iodinated zinc chloride solution to confirm the presence of cellulose and negative for the phloroglucinol test, showing absence of lignin. This indicates that the process of extraction did not significantly alter the properties of extracted MCC powders.

Powder flowability is indirectly measured by the angle of repose, Hausner's ratio, and Carr's compressibility index. Hausner's ratio indicates the inter-particle friction between particles, a ratio greater than 1.25 indicates poor flow, hence, the Hausner's ratio for the extracted MCC and Avicel® indicated a fair flow (Ohwoavworhua and Adelakun, 2010). Carr's index shows the aptitude of a material to diminish in volume and Carr's index below 16% indicates good compressibility, while values above 35% indicate cohesiveness (Staniforth, 1996). The Carr's indices for the extracted MCC and Avicel® indicates a fair to good compressibility, however, Avicel® showed superior flow property compared to the extracted MCC powders. Furthermore, there was no significant difference in the angles of repose for the extracted MCC powders and Avicel® and both MCC powders showed poor flowability.

The porosity of a powder is due to the voids between the particles as well as pore within the particles (Nwadiogb *et al.*, 2015). This indicates the level of perforation in the powder bed, which connotes that the particles are spaced from each other and this effect is related to the particle size distribution as well as the densities of the powders. The results obtained for both the extracted MCC and Avicel® were similar and revealed a high porosity in the powders.

The pH of the extracted MCC and Avicel® were within the BP specification and showed that the extracted MCC was weakly acidic. The moisture content indicates the amount of water in the MCC powders. Their moisture contents were within the BP specification and indicates that the powders contained acceptable amount of moisture. Swelling is the most accepted mechanism for tablet disintegration (Alebiowu *et al.,* 2003). It reflects the increase in volume of cellulose following water uptake (Ohwoavworhua *et al.,* 2009). Microcrystalline cellulose obtained from *Cocus nucifera* had a slightly higher swelling index than the commercial cellulose which indicates that the extracted MCC powders absorbed only little portion of water. Furthermore, the porosity of the tablet is important to the performance of disintegrant with swelling as its mode of action as a porous matrix having large void spaces could suppress the swelling action of the tablet disintegrant (Markl and Zeitler, 2017). The high porosity of these cellulose powders may have affected their swellability by suppressing the swelling action of the cellulose. The water sorption capacity indicates the sensitivity of the cellulose to adsorb moisture and the physical stability of the cellulose when stored in a humid environment. The water sorption capacity of the MCC powders and commercial Avicel® was similar.

FTIR spectroscopy can give direct information about changes in chemical functionality and structural analysis of the extracted material after delignification and after acid hydrolysis. Though minor differences were observed on comparing the spectra of the extracted α-cellulose, MCC powder and Avicel®, these differences were related to the intensities of the bands. Also, there were some extra peaks seen in the spectral pattern of the MCC in relation to Avicel®. These extra peaks might have been as a result of extraneous compounds that might have been contained in the extracted α-cellulose. Similar finding has been reported in a study involving the extraction of MCC from cassava fermentation waste (Eraga *et al*., 2015). Hence the need for the optimization of the α-cellulose extraction process to ensure the extraction of α-cellulose of high purity.

CONCLUSION

Microcrystalline cellulose (MCC) powder was successfully extracted from coconut seed pulp. The MCC powder obtained competed favourably with the commercial brand in their physicochemical and powder properties with little or no differences in their infra-red spectra. Owing to the findings in this study, *Cocus nucifera* may therefore be considered as a suitable alternative source for commercial production of microcrystalline cellulose.

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