CELLULOSE NANOCRYSTALS FROM UNDERUTILIZED *POLYTHIA LONGIFOLIA* SEED

NANOCRISTAIS DE CELULOSE DA SEMENTE SUBUTILIZADA *POLYALTHIA LONGIFOLIA*

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ABSTRACT

Polythia longifolia seed (PL), an underutilized seed in Nigeria was used as a starting material to prepare *Polythia longifolia* cellulose nanocrystals (PLN). *Polythia longifolia* cellulose (PLC) was first isolated and subjected to sulfuric acid hydrolysis followed by ultrasonication and homogenization. PL, PLC and PLN were characterized using Fourier transformed infrared (FTIR), x-ray diffraction (XRD), thermogravimetric analysis (TG) and scanning electron microscopy (SEM). PLC and PLN were evaluated for their heavy metal adsorption capacity, swelling capacity, water holding capacity and oil holding capacity. SEM images reveal elliptical granules of PLN while XRD shows a mixture of polymorphs of cellulose I and cellulose II. PLN displayed a better water holding, oil holding and swelling capacities. PLN also exhibited a higher adsorption capacity towards Cu^{2+} and Pb^{2+} ions than PLC. The results showed that *Polythia longifolia* seed can serve as a potential source of cellulose nanocrystals which might be useful in other applications.

Keywords: *Cellulose; Ethylenediamine; Hibiscus sabdariffa; Modified cellulose; Surface grafting*

RESUMO

Sementes de *Polythia longifolia* (PL), uma semente de subutilizada na Nigéria foi utilizado como um material de partida para preparar nanocristais de celulose *Polythia longifolia* (PLN). A celulose *Polythia longifolia* (PLC) foi inicialmente isolada e submetida a hidrólise com ácido sulfúrico seguida por homogeneização por ultrassonificação. As amostras de PL, PLC e PLN foram caracterizados por Transformada de Fourier Infravermelho (FTIR), difração de raios-x (DRX), análise termogravimétrica (TG) e microscopia eletrônica de varredura (MEV). As amostras PLC e PLN foram avaliados quanto à sua capacidade de adsorção de metais pesados, capacidade de inchamento, capacidade de retenção de água e capacidade de retenção de óleo. Imagens de MEV revelam grânulos elípticos de PLN, enquanto DRX mostram uma mistura polimorfica de celulose I e celulose II. A PLN demonstrou melhor retenção de água, melhor retenção de óleo e capacidade de inchamento. A PLN também exibiu uma capacidade de adsorção superior a PLC de íons Cu^{2+} e Pb^{2+} . Os resultados demonstraram que as sementes *Polythia longifolia* podem servir como uma fonte potencial de nanocristais de celulose que podem ser úteis em outras aplicações.

Palavras-chave: *Celulose; etilenodiamina; Hibiscus sabdariffa; Celulose modificada; enxertia de superfície.*

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INTRODUCTION

Cellulose which is the most abundant biopolymer on earth is considered one of the renewable resources for the production of chemicals and materials (Ragauskas *et al.*, 2006). Structurally, cellulose is highly ordered with the presence of reactive hydroxyl groups which can serve as means of improving on its properties for different applications.

There are several sources of cellulose; these sources include wood fiber, seed fiber, bast fiber, grasses, bacteria and algae (Nevell and Zeronian, 1985; Varshney and Naithani, 2011). The cellulose content of these sources varies and the process of obtaining the cellulose may not be that easy. Since there is increasing growth in the demand for cellulose, it is important to search for other sources which can meet this demand. Underutilized plant seeds such as *Polythia longifolia* seeds are potential resource that can be screened as alternative source of cellulose. *Polythia longifolia* belongs to the Annonaceae plant family. It is found in the tropics and known by different common names such as India fir tree, Buddha tree, false Ashoka, Glodogan tiang, Ashoka and Devdar (Singh and Karthikeyan, 2000). It is an evergreen plant which is famous for its use as an ornamental street tree (Farshori *et al.*, 2013). The plant is readily available in Nigeria but with little or no application which makes it underutilized.

Cellulose and its nanocrystalline forms have received several applications, some of which cuts across pharmaceutical, food and several other house hold industries (Hon, 1996). Most modifications carried out on cellulose are aimed at adjusting its properties so as to make it suitable for certain requirements. Aside being a linear homopolymer, it is insoluble in water, biodegradable, nontoxic and stable over wide temperature and pH (Mathur and Mathu, 2001). In order to further increase the usefulness of cellulose, the properties can be improved by converting it to its nanocrystalline form. The aim of this study is to isolate cellulose from PL, convert the cellulose to its nanocrystalline form and characterized for possible use.

EXPERIMENTAL

2.1. Materials

The seeds of *Polythia longifolia* were obtained from a garden in Ibadan, Oyo state, Nigeria. This was later identified at the Department of Botany, University of Ibadan, Ibadan, Oyo state, Nigeria. The seeds were ground in an industrial mill, extracted with n-hexane as previously described by Adewuyi *et al.* (2012), air dried and stored in an airtight container. Sodium chlorite, sodium hydroxide, acetic acid, and all other chemicals used in this study were purchased from Sigma-Adrich (Brazil).

2.2. Isolation of cellulose from the seed of *Polythia longifolia*

Polythia longifolia seed cake (150 g) left after the extraction with n-hexane was weighed and transferred into a 2.5 L beaker. Alkali solution (2 wt% NaOH) was added and heated at 80°C for 5 h with continuous stirring using a Fisatom mechanical stirrer. The mixture was later filtered and washed with deionized water several times until alkali free and oven dried at 50°C. The treatment with 2 wt% NaOH was repeated twice. The brown product obtained was bleached with a mixture of solution which was made up of equal volumes (1:1) of acetate buffer (27 g NaOH and 75 mL glacial acetic acid, diluted to 1 L of deionized water) and aqueous sodium chlorite (1.7 wt% $NaClO_2$ in deionized water) as described by Flauzino-Neto *et al.* (2013). This was stirred at 80°C for 5 h. The resulting fibers were washed repeatedly in deionized water until the pH of the fibers became neutral. The bleaching step was repeated twice until the fiber became completely white and dried in an air-circulating oven at 50°C for 24 h to obtain the PLC.

2.3. Preparation of PLN

The PLC was hydrolyzed via sulfuric acid hydrolysis. Briefly, 15.0 g powder PLC was added to 150 mL 65 % sulfuric acid, maintained at 45°C and stirred vigorously using a Fisatom mechanical stirrer for 1 h. At the end of the acid hydrolysis, cooled deionized water was added to the resultant suspension and centrifuged at 7000 rpm for 10 min. This was dialyzed with deionized water to remove any non-reactive sulfate groups, salts and sugar until the solution reached pH 7.0. The purified suspension was ultrasonicated using a Cole Parmer ultrasonic processor (model CV 334, 20 kHz) for 30 min. Powdery PLN was finally obtained by freeze-drying.

2.4. Characterization

2.4.1. FTIR

The functional groups in PL, PLC and PLN were determined using FTIR (Perkin Elmer, spectrum RXI 83303). The samples were blended with KBr, pressed into pellets and analyzed in the range of 400 - 4500 cm^{-1}.

2.4.2. XRD

The X-ray diffraction pattern was obtained using X-ray diffractometer (XRD-7000X-Ray diffractometer, Shimadzu) with filtered Cu Kα radiation operated at 40 kV and 40 mA. The XRD pattern was recorded from 10 to 80° (2θ), with a scanning speed of 2.00° per minute.

2.4.3. TG

Thermal stability and fraction of volatile components of PL, PLC and PLN were monitored by TG. This was achieved using a simultaneous DTA-TG apparatus (SHIMADZU, C30574600245).

2.4.4. SEM

Surface morphology was studied using scanning electron microscope (SEM, JEOL JSM-6360LV, Japan and Quanta 200, FEG dua beam Bruker). Powdered PL, PLC and PLN were coated with gold using the sputtering technique in order to increase electrical conductivity and the quality of the micrographs.

2.5. Water holding capacity

Water holding capacity (WC) was determined as described by Zhang et al (2005). To achieve this, 0.5 g (W_1) of sample was dispersed in 10 mL of deionized water in a pre-weighed, clean centrifuge tubes (W) placed in a water bath at 37°C for 30 min. These were centrifuged for 15 min at 4000 rpm; the supernatant was removed and the centrifuge tubes with the distilled water soaked samples were weighed (W_2). Water holding capacity was estimated as:

$$WC\ \left(g\,g^{-1}\right)= \frac{\left(W_2-\left(W+W_1\right)\right)}{W_1} \qquad (1)$$

2.6. Oil holding capacity

The oil holding capacity (OC) was determined by weighing 0.2 g (W) of samples into a calibrated centrifuge tube containing 5 mL (V_1) of *Picralima nitida* seed oil. The mixture was properly stirred for 10 min after which it was centrifuged for 30 min at 5000 rpm. The supernatant oil (V_2) was gently removed while the absorbed oil was estimated as the difference between V_1 and V_2. The oil holding capacity was calculated as (Lu *et al*., 2013):

$$OC\ \left(mL\,g^{-1}\right)= \frac{V_1-V_2}{W} \qquad (2)$$

2.7. Swelling capacity

PLC and PLN were separately analyzed for their swelling capacity (SC) by accurately weighing 0.5 g (W) and placing it in a calibrated tube, measuring its initial bed volume (V_1), mixed with 10 mL of milliqui water and shaken vigorously. The tube with its content was place in water bath at 25°C for 24 h and the final volume (V_2) measured. The swelling capacity was calculated as (Lecumberri *et al*., 2007):

$$SC\ \left(mL\,g^{-1}\right)=\frac{V_2-\ V_1}{W} \qquad (3)$$

2.8. Heavy metal adsorption capacity

Lead nitrate ($Pb(NO_3)_2$) and copper sulphate ($Cu(SO_4).5H_2O$) salts were used in the preparation of the salt solutions in milliqui water. Metal adsorption study was carried out by separately shaking 0.1 g of PLC and PLN with 50 mL solutions (100 mg/L) of metal in different beakers at 25°C and 200 rpm for 3 h. This was later centrifuged for 10 min at 5,000 rpm and the metal concentrations before and after adsorption were determined using Atomic Absorption Spectrometer (Varian AA240FS). The metal ions adsorption capacity of PLC and PLN were calculated using equation:

$$q_e= \frac{\left(C_o-\ C_e\right)V}{M} \qquad (4)$$

Where q_e is the adsorption capacity in mg/g, C_o and C_e are initial and final concentrations (mg/L) of adsorbate (Pb^{2+} and Cu^{2+}) in solution respectively; while V and M are volumes (L) of metal ions solution and weight (g) of PLC and PLN used.

RESULTS AND DISCUSSION

3.1. Characterization

The yield of PLC obtained from *Polythia longifolia* was 51.20 %. The FTIR spectra of PL, PLC and PLN are presented in Fig. 1.

Large bands were found at 3250-3500 cm^{-1} in PL, PLC and PLN which were considered to be the stretching bands of -OH functional groups. Peaks at 1736 cm^{-1} was found in PL which was assigned to vibrational frequencies of C=O associated with acetyl and uronic ester groups of hemicellulose and ester linkage of the carboxyl group in lignin (Chen *et al*., 2012) while peaks seen at 1520 and 1448 cm^{-1} were assigned to C=C vibrational frequencies in lignin (Rosa *et al*., 2010; Chen *et al*., 2012). Band observed at 2924 cm^{-1} in all the samples was attributed to the C-H stretching of alkane. As previously reported by Chen *et al* (2012), peaks found in the region 800-1500 cm^{-1}in PL, PLC and PLN were considered as fingerprint region for cellulose which suggested that cellulose maintained its chemical identity irrespective of the treatment given to the sample. Within this range, peaks at 1040 cm^{-1} may be due to the C-O-C pyranose ring stretching vibration while absorption band at 904 cm^{-1} could be assigned to the stretching at β-(1→4) glycosidic linkages. Peaks at 815 and 1210 cm^{-1} were only seen pronounced in PLN which was attributed to the half-ester sulfate groups as a result of the sulfuric acid hydrolysis of PLC (Lu and Hsieh, 2010; Chen *et al*., 2012).

X-ray diffractogram of PL, PLC and PLN is presented in Fig. 2. The pattern is typical of semicrystalline materials having amorphous broad hump and crystalline peaks (Flauzino-Neto *et al*., 2013). The patterns as shown in the diffractogram display a mixture of polymorphs of cellulose I and cellulose II. The presence of cellulose II may be due to the 65 % sulfuric acid used which can also serve as a solvent for the cellulose apart from its capacity to hydrolyze the cellulose, this might have resulted in a re-precipitation of cellulose after hydrolysis (Borysiak and Garbarczyk, 2003; Flauzino-Neto *et al*., 2013).

Thermal stability plays a key role in determining the use of nanocrystals as reinforcing materials (Roman and Winter, 2004), the TG is shown in Fig. 3. The curves in PL, PLC and PLN reveal a loss at around 48 -135°C which was attributed to loss of water molecules. PL exhibited loss in mass at around 160-240°C which was considered as being loss of hemicellulose and some other volatile matters while mass loss around 245-340°C was attributed to loss of lignocelluloses (Carrier *et al*., 2011). In PLC and PLN, the first step noticed in the degradation was loss of mass around 185-240°C which was accounted for as being degradation leading to 1,4 and 1,6 anhydroglucopyranoside while the second step at around 245-335°C was considered as being depolymerization at 1,4 glycosidic bond as previously reported (Chauhan *et al*., 2000; Sharma, 2012). The SEM images are presented in Fig. 4. The surface morphology of PL showed that it has an irregular shape and size. PLN appeared as an aggregation of rod-like assemblies of nanocrystals while PLC appeared as stacked particles as aggregates of macromolecules.

3.2. Water holding, Oil holding and swelling capacities of PLC and PLN

Water holding capacity estimates the ability of any material to hold water over a period of time most especially when undergoing certain processes. The water holding capacity of PLC (3.71 g/g) is lower than that of PLN (8.93 g/g). The higher water holding capacity of PLN over PLC suggests the possible use of PLN in applications requiring moisture retention (Lu *et al*. 2013). This value has been found higher than a few reported data in literature as shown in Table 1. The oil holding capacity was found to be 8.11 mL/g for PLN and 3.55 mL/g for PLC. This capacity has been related to the ability of material surfaces to hold or adsorb organic compounds or molecules which also bothers mainly on the porosity of the structure of such material (Yalegama *et al*. 2013). This *parameter is important in food industry* (Tizazu and Emire 2010) and it is related to texture, hydrophobic character and other food quality properties (Shad *et al*., 2013; Rafieian and Keramat 2015). The swelling capacity also plays an important role in finding application for a cellulosic material. It is a show of the amount of water that such material can absorb. The swelling capacity of PLC was 2.88 mL/g while that of PLN was 7.06 mL/g. The capacities of PLC and PLN were compared with some reported values as shown in Table 1.

3.3. Heavy metal adsorption capacity

PLC and PLN were evaluated for their ability to adsorb Cu^{2+} and Pb^{2+} ions from aqueous solution. The presence of these heavy metals (Cu^{2+} and Pb^{2+}) have been reported in food, water and soil as a result of rapid population growth, domestic activities, agricultural practices and industrialization (Su *et al.*, 2013). It is important that these heavy metals are removed from their sources before they come in contact with human, plant or animal.

The adsorption capacities of PLC and PLN are expressed in Fig. 5. Both PLC (35.99 mg/g) and PLN (42.05 mg/g) adsorbed Cu^{2+} ions; these values are higher than what was reported by Jiang *et al*. (2010) and Putra *et al*. (2014). The affinity of PLC and PLN for Cu^{2+} ions was higher than what was observed for Pb^{2+} ions. The adsorption capacity of PLC towards Pb^{2+} was 2.65 mg/g while that of PLN was 15.54 mg/g. For both metal ions, PLN demonstrated a better adsorption capacity than PLC.

CONCLUSION

This study evaluated the isolation of cellulose from *Polythia longifolia* seed and its hydrolysis to nanocrystalline cellulose using 65 % sulfuric acid. The PL, PLC and PLN were characterized, analyzed for their ability to adsorb heavy metals, water holding, oil holding and swelling capacities. The present work has revealed that cellulose nanocrystals can be prepared from PL suggesting the use of PL as a renewable source for the production of cellulose nanocrystals.

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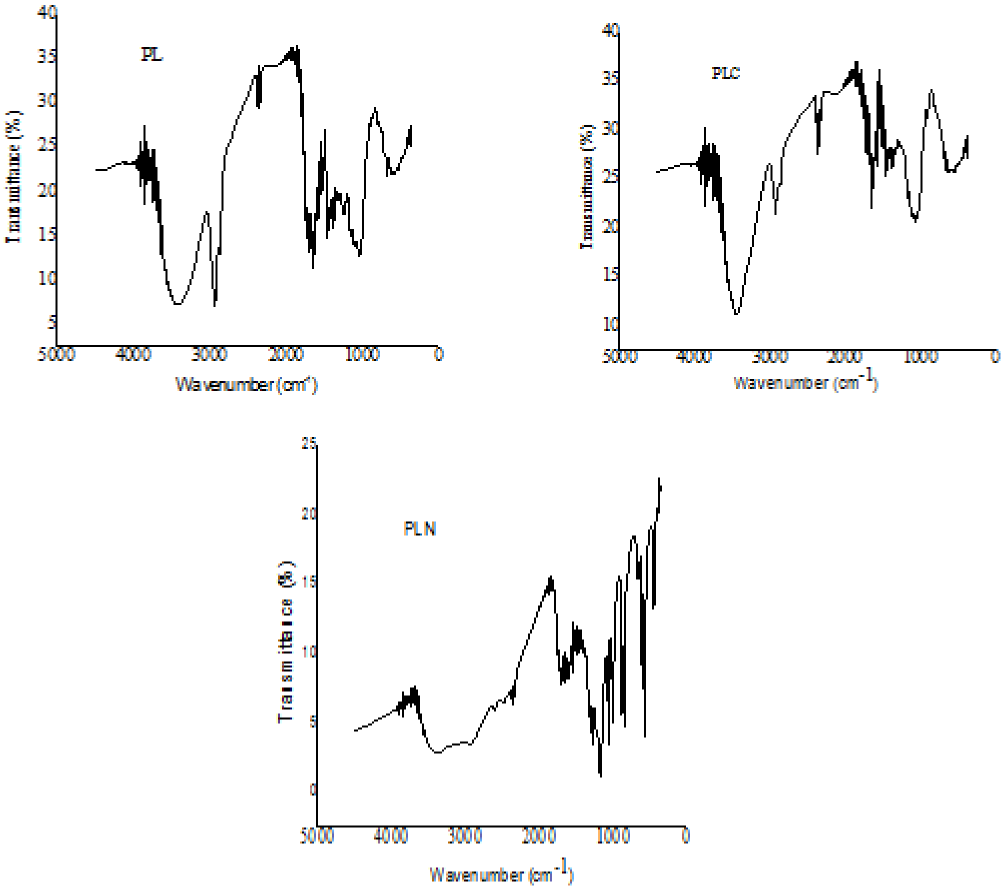

Figure 1: FTIR OF PL, PLC AND PLN

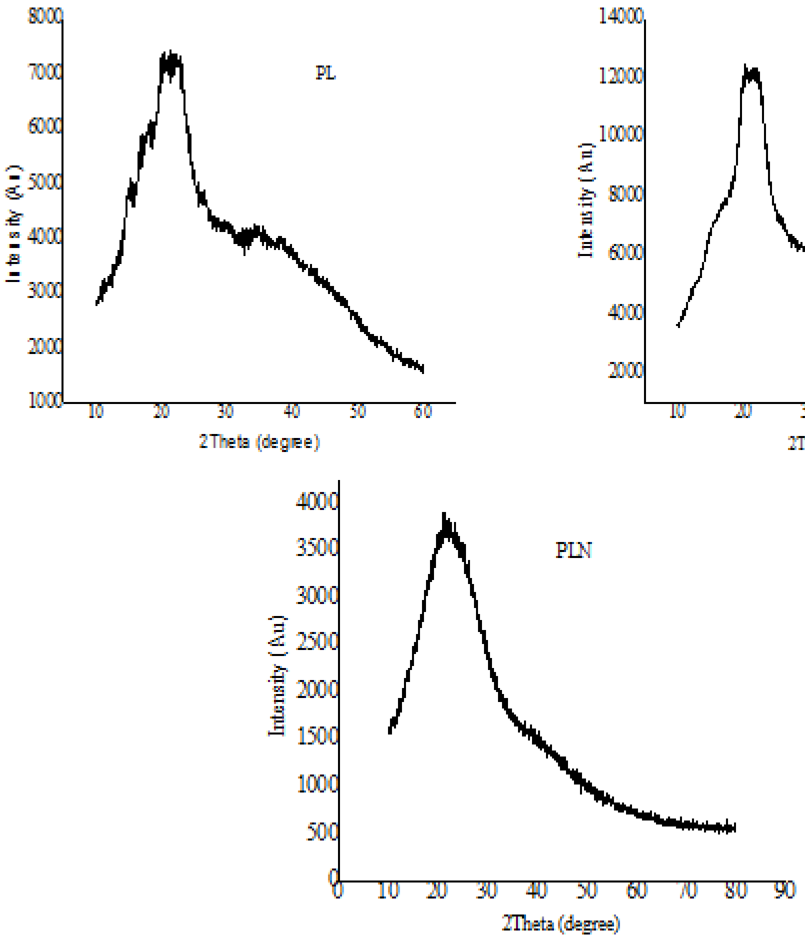

Figure 2: XRD OF PL, PLC AND PLN

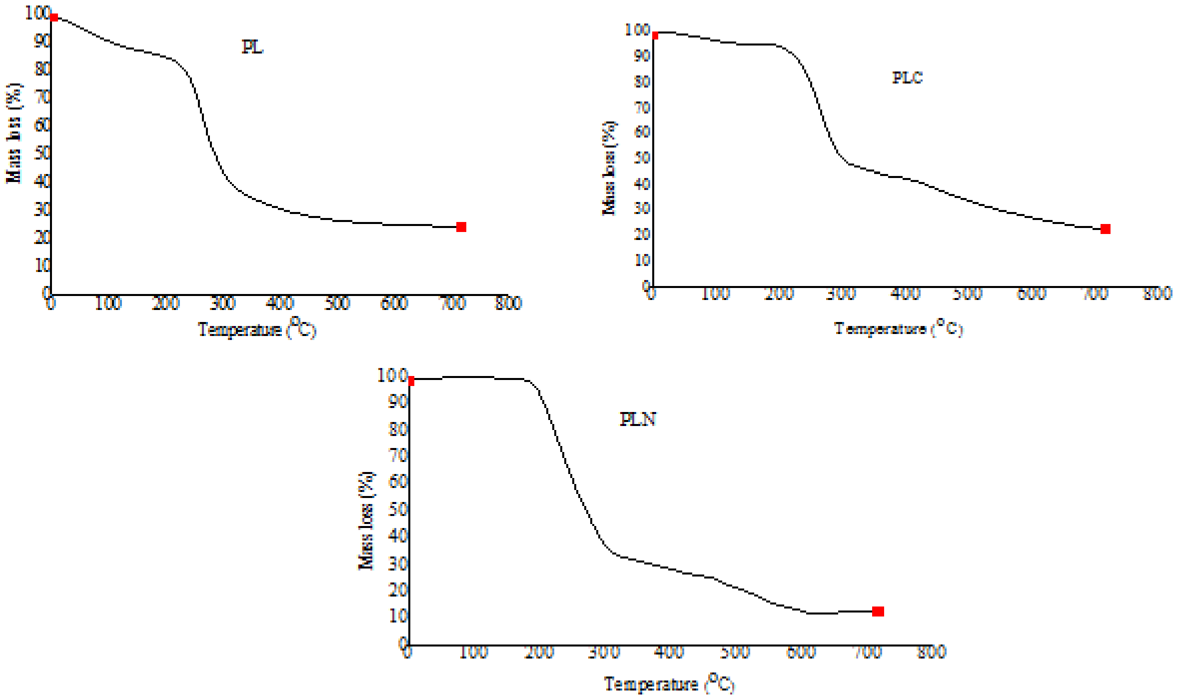

Figure 3: TG OF PL, PLC AND PLN

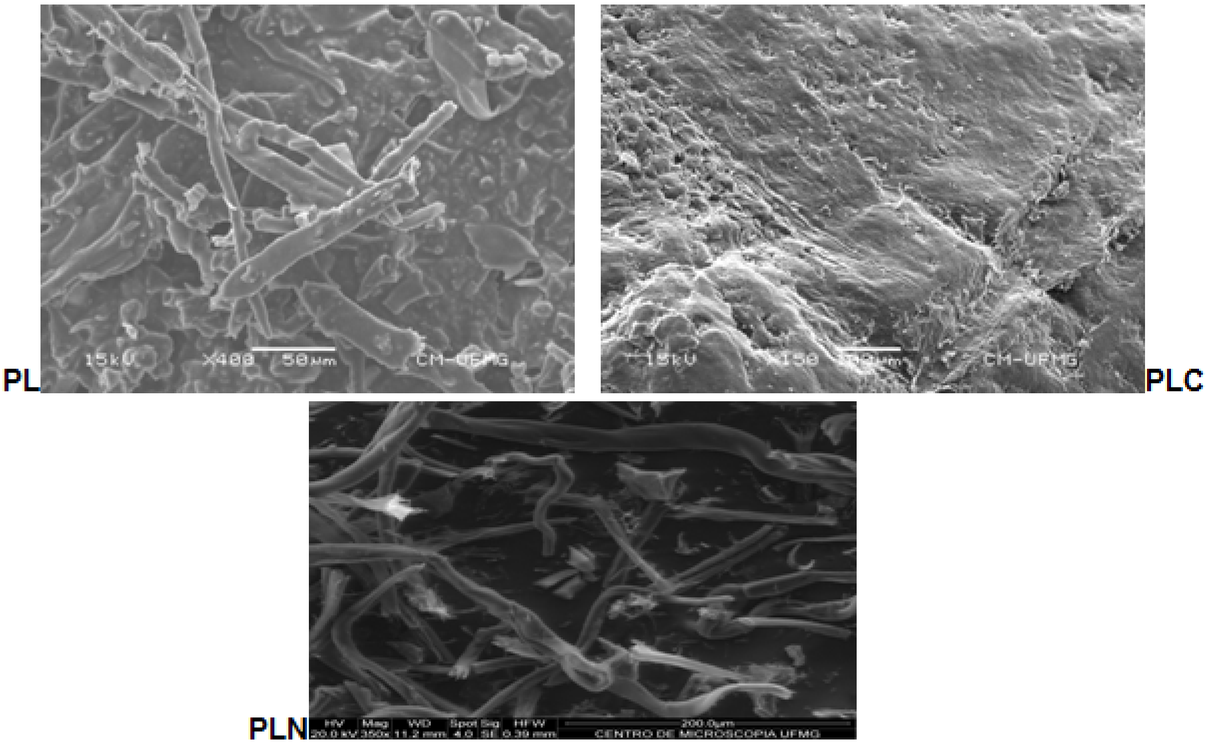

Figure 4: SEM OF PL, PLC AND PLN

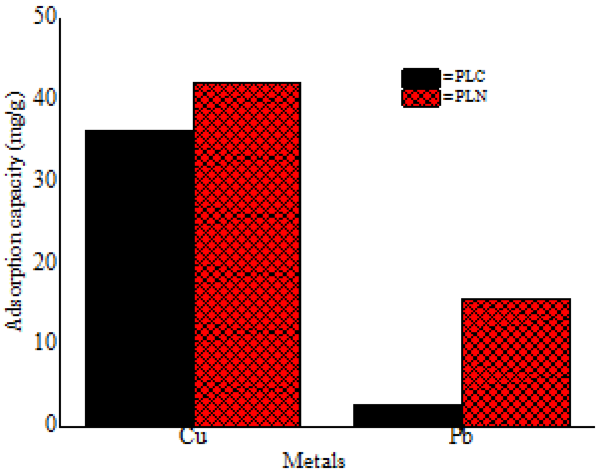

Figure 5: ADSORPTION CAPACITY OF PLC AND PLN

Table 1: Comparison of the water holding capacity, oil holding capacity and swelling capacity of PLC and PLN with other reported values in literature

Sample	**Water holding capacity (g/g)**	**Oil holding Capacity (mL/g)**	**Swelling Capacity (mL/g)**
Red bean flour Ashraf *et al.*, (2012)	4.419	4.553	-
Standard flour Menon *et al.*, (2014)	1.02	2.50	4.00
White lupin Tizazu and Emire (2010)	2.65	1.82	-
Gelatin Rafieian and Keramat (2015)	8.59	0.67	-
Coconut kernel Yalegama *et al.*, (2013)	8.13	4.57	5.50
Cowpea Benítez *et al.*, (2013)	2.00	0.80	5.50
PLC (Present study)	3.71	3.55	2.88
PLN (Present study)	8.93	8.11	7.06

- = No result found