

Development of Biocomposites of MCC Extracted From Non-Wood Sources

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Abstract: The present work mainly focuses on the estimation of various components and the extraction of microcrystalline cellulose (MCC) from non-wood sources like Country Almond/Badam shell through acid hydrolysis. This hydrolyzed MCC was successfully used as reinforcement for development of biocomposites. Country Almond/Badam trees are found all over Kerala, India and they give nuts once in a year. Usually the nut shells are discarded and are becoming one of the sources of agricultural waste. During this investigation various components were isolated from the Country Almond shells and they were characterised using different spectral and analytical techniques thereby the composition of Country Almond shells was successfully determined for the first time. The properties of MCC especially the crystalline nature depends on the source from which is isolated. FT-IR spectra give evidence for the chemical structure of MCC. Morphology of MCC was evidenced from scanning electron microscopy. Transmission electron microscopy and atomic force microscopy reveal the agglomerated bundles of particles and rough surface of MCC. The extracted MCC was found to contain Cellulose I and Cellulose II polymorphs, and this was confirmed from the X-ray diffraction (XRD) studies. The MCC extracted from Country Almond shells has reasonably good thermal stability. Solution casting method was adopted for the development of green composite of Poly (vinyl alcohol) reinforced with MCC extracted from Country Almond shell. The mechanical property of developed composites has been enhanced by the addition of MCC. The dispersion of MCC in the PVA matrix and flocculation of MCC significantly influence the mechanical strength.

Keywords: Country almond/Badam shell; microcrystalline cellulose; bio composites

1 Introduction

Nowadays, waste especially polymeric waste management deserves prime concern. The thrust area of research in developing countries is mainly focused on this serious issue. Though, natural or agricultural waste management is not a severe problem, conversion of these agricultural wastes into value added products opens up new area of research. Lignocellulosic matter is the main part of agricultural waste. Lignocellulosic materials have been used and investigated for decades as raw materials in a number of applications. Lignocellulosic matter is obtained from wood and non-wood sources. It is having interesting utility in the broad areas as a functional material. The specific use of lignocellulosic materials mainly recognized in the areas of biomedical, optoelectronics, automobile, nanocomposite and other industrial applications [1-4]. Characteristically, natural fibres consist of cellulose, hemicelluloses, lignin, waxes and oils. Presence of these ingredients having significant relevance on the basic properties of the plant fibres such as mechanical strength, optical properties, thermal stability, rigidity, flexibility etc. make them suitable for functional materials [5,6].

The most important constituent of lignocellulosic material is cellulose. Hydrophilic nature, low density, biodegradability and above all its renewable nature are the characteristic features of cellulose [6]. Cellulose consists of both crystalline and amorphous regions and the amorphous region can be

removed by treating with mineral acids. Depending on the extent of removal of amorphous regions, microcrystalline cellulose (MCC) and other novel forms of cellulose like nanocellulose could be successfully prepared. Moreover, in order to meet the various demands it has often been subjected to both physical and chemical modifications. The extraction of cellulose from renewable source is becoming an important research area, therefore, our research team is mainly concentrated on the exploitation of various non-wood sources for the extraction of MCC, nanocellulose and development of their biocomposites [7-11]. MCC is a commercially important material used in a number of applications like pharmaceuticals, binder, texturizer, food additives, edible coatings, cosmetics, and also as reinforcement in polymer matrix etc. [12-14]. It is a white odorless, fine crystalline powder obtained from different sources and in fact both wood and non-wood sources have been successfully used for isolation of MCC. It has been reported that the crystalline nature and properties of MCC differ on the source of isolation. Country almond/ Badam, popularly known as Malabar almond is grown all over Kerala, India as well as in other tropical countries and gives seeds once in a year. These deciduous seeds are planted in gardens and even in road side as an ornamental shady tree. In India itself annual production of Country almond nuts has been estimated to be 20 lakh tonnes. The potential use of these nut shells has not been utilized well. Though, the extraction of MCC from different sources has been reported earlier, till date, no systematic investigations have been carried out on the exploitation of Country almond shells which are usually discarded as agricultural waste.

The development of polymer composite has been rapidly increasing because of its wide range of applications in different areas such as automobiles, super capacitors, optical devices and sensing. Moreover, in biomedical applications which include artificial blood vessels, artificial intestine, contact lenses and drug delivery system [15-17] where the role of MCC is very important. PVA has been found to be an excellent matrix for the development of biocomposites. The unique properties of PVA include its non-toxicity, semicrystalline nature, biodegradability, water solubility, film forming ability, good chemical and thermal stability and further it behaves as a polymeric stabilizer [18,19]. Depending upon the nature of polymer and additives the properties of PVA composite have remarkable changes. Due to the presence of hydroxyl group along the chain of PVA it could form hydrogen bond with the cellulose moiety because both the materials are hydrophilic in nature, eventually necessary interaction may occur at the interface of PVA and cellulose. Reinforcement of PVA with lignocellulosic materials can certainly enhance thermo mechanical properties and water adsorption [20,21]. In the present study green composite of PVA reinforced with MCC derived from a non-wood source Country almond shells has been developed by simple solution casting technique. Due to environmental advantages cellulose based biocomposites have been widely recognized as a potential material. Solution casting technique is a very simple cost effective method to develop film moreover this method can control the uniform thickness, dispersion of additives and maximum optical purity [22]. Therefore, the main objective of this investigation is to determine the composition of the Country almond shells, then to extract microcrystalline cellulose formed and characterize the prepared microcrystalline cellulose by different spectral and analytical techniques and finally development of their biocomposites.

2 Materials and Methods

2.1 Materials

Country almond shells were collected from Calicut University campus, Kerala, India during the month of January-February. Freshly fallen nuts/ dropped by using a long pole are collected from the ground by hand. The amount of extractives, Klason lignin, α -cellulose and ash content were estimated following the ASTM procedures. Acetic acid, toluene, sodium chlorite, sodium hydroxide, hydrochloric acid and sulphuric acid were obtained from Merck India.

2.2 Separation of Different Components

Removal of extractives: After removing the pith the seed shells were air dried, powdered well and meshed sample was kept in air oven at 100°C. 4g of the oven dried sample was extracted using

ethanol- toluene mixture (2:1) in a Soxhlet apparatus. The extracted residue was oven dried following the ASTM procedure. After evaporating the solvent from pre-weighed extraction flask the amount of extractives present in the Country almond shells was calculated.

Holocellulose: 2g of the extracted residue was agitated with 1.7% sodium chlorite in a water bath kept at 80°C by maintaining the pH 5 by adding acetic acid. The residue was filtered and thoroughly washed with distilled water till the washings were free from acid and finally oven dried at 100°C [23]. The amount of holocellulose was calculated.

Klason Lignin: 1g of the extracted residue was treated with cold 72% conc sulphuric acid. The mixture was stirred properly and subjected to stand for 2 h kept in a water bath. The concentrated solution was diluted to 3% concentrate sulphuric acid by adding distilled water. The diluted solution so obtained was boiled for 4 h. The mixture was filtered till the washings were free from acid and dried in air oven at 100°C.

α -Cellulose: 1g of obtained holocellulose was treated with 17.5% sodium hydroxide (25 mL), making three additions for about 45 minutes. The solution was kept for 1 h; sufficient quantity of distilled water was added to make the concentration of NaOH to 8.3%. The solution was filtered, washed with 10% acetic acid followed by washing with distilled water till the washings were neutral and dried at 100°C. The difference in weight, between holocellulose and α -cellulose gives the amount of hemicelluloses present in the Country almond shell.

Microcrystalline Cellulose: α -Cellulose (1g) was hydrolysed with 2.5N hydrochloric acid (17 mL) in a glass vessel at boiling temperature for about 15 min. The hot acid mixture was poured into 50 mL cold water followed by vigorous stirring and allowed to stand overnight. The MCC obtained was filtered, washed with distilled water till it became neutral and dried in a hot air oven at 60°C for half an hour.

Preparation of PVA-MCC Composite: Different weight percentage of MCC extracted from Country almond shells was dispersed in water containing PVA through ultrasonic treatment for 1 h at 30% amplitude. During this process, improper dispersion may result into agglomeration of MCC which affect the mechanical properties of the final composite. The PVA-MCC mixture was cooled in ice bath and the solution was transferred into a glass plate with controlled levelling. The air bubbles were removed by flaming and the solution was kept for 7 days at room temperature. The prepared films were labelled as P₀, P₁, P₂, P₃ and P₄ which contain 0.5, 1, 1.5 and 2 weight % of MCC, respectively.

2.3 Characterization

The different components extracted from Country almond shells and PVA reinforced MCC composite were characterized by various spectral and analytical techniques. FTIR spectra were recorded on a JASCO FTIR-4100 spectrometer using KBr pellet in the range of 4000-400 cm⁻¹. Thermogravimetry (TG) was performed using TA Q50 instrument. The decomposition was studied from room temperature to 650°C with a heating rate of 10 °/min, under nitrogen atmosphere to determine the thermal stability and decomposition temperature. X-ray diffraction studies were performed using the RIGAKU MINIFLEX-600 diffractometer with CuK_α ($\lambda = 1.54 \text{ \AA}$). The percentage of Degree of crystallinity (DC) was calculated by using the equation % of DC = $(I_{200} - I_{am}) / I_{200} * 100$, where I_{200} is the intensity of the 200 peak at $2\theta = 22.8$ and the I_{am} is the minimum intensity at the amorphous region of $2\theta = 18$. The morphology of MCC was studied using microscopic methods. The surface morphology was obtained using Hitachi SU 6600 variable pressure field emission scanning electron microscope. TEM observations were performed using JEOL model JEM 2100. AFM was taken on a WI Tec Alpha 300RA (Germany) in non-contact mode. Mechanical behaviour of MCC-PVA composite was evaluated by using SHIMADZU (AGX-PLUS-10 KN) having initial grip separation as 40 mm long, with a crosshead speed of 10 mm/min. Tensile strength measurement were performed at room temperature.

4 Results and Discussion

The observed chemical composition of Country almond shell powder is presented in Tab. 1. The distribution of lignin content in the cellulosic materials increases with age of the tree. The chemical composition is largely influenced by the growing condition of Country almond. As expected from the toughness of the shell the major component of country almond shell was found to be holocellulose followed by α -cellulose, hemicellulose and extractives. During extraction process all the soluble impurities (pectin, waxes, oil, gums, fats, chlorophyll etc.) present in the Country almond shell powder was removed. The analyses show that the Country almond shell has reasonably good lignin and cellulose content which are comparable to peanut shell and sugar beet [24,25]. Therefore Country almond shells can be used as a potential source for the isolation of microcrystalline cellulose.

Table 1: Weight percentage of different components isolated from Country almond shells

Components	Weight %
Extractives	5 \pm .5
Klason Lignin	46 \pm 1.2
Holocellulose	47 \pm 1.6
α -Cellulose	30 \pm 0.6
Hemicellulose	17 \pm 0.8
Ash content	2 \pm .3

4.1 FT-IR Spectra

This technique is a useful tool to obtain information on various chemical changes involved during the extraction of different components from Country almond shell. Fig. 1 represents the FT-IR spectrum of (a) lignin and (b) holocellulose extracted from country almond shell powder. Lignin is a cross linked phenolic polymer having complex three dimensional structure. Cellulose along with hemicellulose constitutes holocellulose. From the FT-IR spectrum all structural and functional groups in the holocellulose and lignin are identified, which testifies the delignification process. The FT-IR spectra of holocellulose and lignin have common frequencies at 3400 cm^{-1} and 2920 cm^{-1} . These absorptions in both samples accredited to the O-H stretching and C-H stretching vibrations respectively [26]. The delignification process involved during the extraction process was confirmed from the absorbance at 1640 and 1516 cm^{-1} which can be attributed to aromatic skeletal vibrations of lignin. The frequency observed at 1740 cm^{-1} in Fig. 1(a) should be primarily due to carbonyl stretching in acetyl and uronic ester groups of hemicelluloses, which also confirms removal of lignin in holocellulose [27].

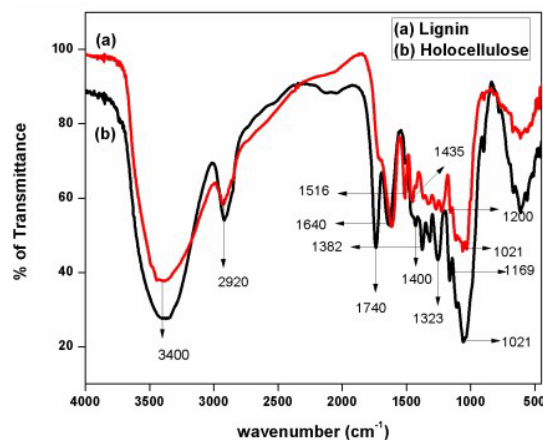


Figure 1: FTIR spectra of (a) lignin and (b) holocellulose

Fig. 2 represents the FT-IR spectrum of α -cellulose and MCC. The absorbance of all characteristic vibrations of α -cellulose was reduced compared to MCC. It corroborates the enhanced crystallinity of MCC than α -cellulose. The broad band obtained in the 3000-3600 cm^{-1} region is due to O-H stretching vibration which gives considerable information for the presence of inter- and intra- molecular hydrogen bonding. The observed red shift (10 cm^{-1}) of the O-H stretching band is explained as elongation of O-H bond. It shows that acid hydrolysis can reduce hydrogen bonding interaction between the individual chains. Frequency at 2900 cm^{-1} is due to C-H stretching of the methyl group. The frequency at 1643 cm^{-1} may be due to absorbed water present in the compound. Typical polysaccharide bending frequencies of CH, CH₂ and OH can be seen at 1437, 1373 and 1318 cm^{-1} respectively. The frequency at 1036 cm^{-1} is due to C-O stretching vibration [4,28].

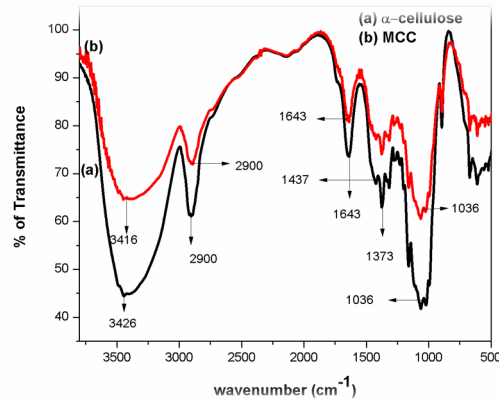


Figure 2: FTIR spectra of (a) α -cellulose (b) MCC

Fig. 3 shows the AT-IR spectrum of PVA and its MCC composites. The broad band of pure PVA at 3320 and the frequency at 2926 cm^{-1} attributed to O-H stretching in its side hydroxyl group and the CH₂ stretching vibrations respectively. The characteristic frequency of pure PVA is observed around 1144 and 1086 cm^{-1} . During the addition of MCC, O-H stretching frequency was blue shifted to 3307 cm^{-1} indicating the formation of hydrogen bonding between MCC and PVA. The characteristic frequency of pure PVA (1144 and 1086) shifted to low values which also suggest changes in the crystalline domains of PVA [29].

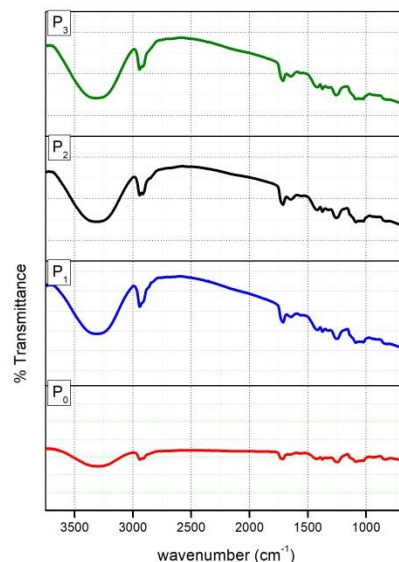


Figure 3: AT-IR spectrum of PVA and its composites, P₀, P₁, P₂, and P₃

4.2 Morphology

The morphology of different components extracted from Country almond shell was studied using SEM and TEM. Figs. 4(a)-4(c) shows the different morphological changes occurred during the extraction of MCC. Fig. 4(a) illustrates the agglomerated particles and rough surface of Country almond shell. This can be explained due to the presence of lignin, hemicellulose and other components. During the extraction process of MCC, hemicellulose and lignin are gradually removed. Fig. 4(b) represents the non-uniform rough domain of Klason lignin. The roughness of the isolated components was decreased due to successive chemical treatment. The SEM image of holocellulose (4c) revealed the agglomerated and amorphous domains. The SEM images of α -cellulose and MCC from Figs. 4(d) and 4(e) indicates the self-assembled and highly agglomerated structures like stacked flakes. The TEM and AFM results are shown in Fig. 5. The TEM image Fig. 5(a) confirms the particle morphology and aggregated state. The agglomerated particles having random orientation along with some non-agglomerated structures were found [5]. Similar observations were reported earlier. The morphology of MCC was studied further using AFM analysis. Fig. 5(b) shows the two dimensional AFM image and Fig. 5(c) shows the three dimensional height view of MCC. These images illustrate the surface roughness and aggregation of particles, which also reveals the micro structural behavior of MCC.

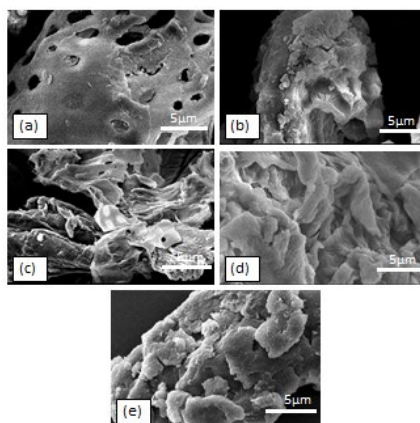


Figure 4: SEM images of (a) Country Almond shell powder, (b) lignin, (c) holocellulose, (d) α -cellulose and (e) MCC

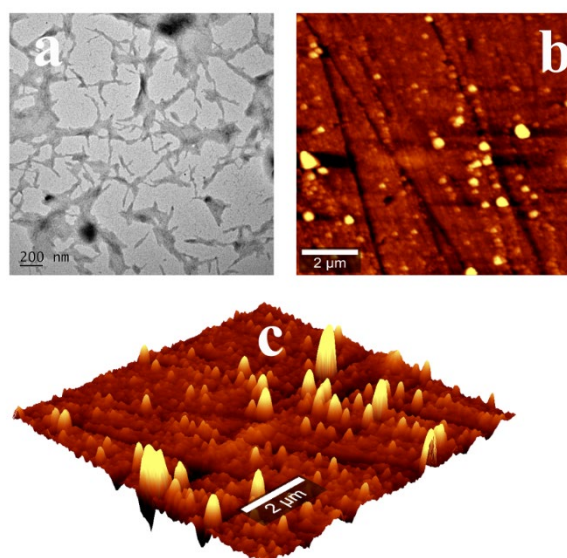


Figure 5: (a) TEM images of MCC, (b) AFM image of MCC (height view), (c) AFM image of MCC (3D view)

4.3 XRD Pattern

Fig. 6 (a) shows the X-ray diffraction pattern of Country almond shell, (b) that of α -cellulose and (c) for MCC. X-ray diffraction pattern of α -cellulose reveals the existence of mixture of polymorphs of cellulose I and cellulose II. The existence of cellulose II can be evidenced by characteristic peaks at $2\theta = 12$ (110), 20 (210) and 22 (200) [30,31] and cellulose I by the presence of peaks at $2\theta = 22$ (200) and 34.6 (004) [32,33]. The X-ray diffraction pattern showing the doublet indicates the transformation of native cellulose from cellulose I to cellulose II [34]. It has been well established that cellulose exists as different polymorphs I, II, III and IV where cellulose I is abundant in natural source. As already been reported, polymorph I can be converted to cellulose II or III. Polymorph II, the most thermodynamically stable form of cellulose is obtained by solubilisation and recrystallization. This can be explained that in the lattice structure of cellulose, -ONa groups replaces hydroxyl groups and in addition during washing with water, sodium ions linked are removed and ultimately leads to the formation of cellulose II [35,36].

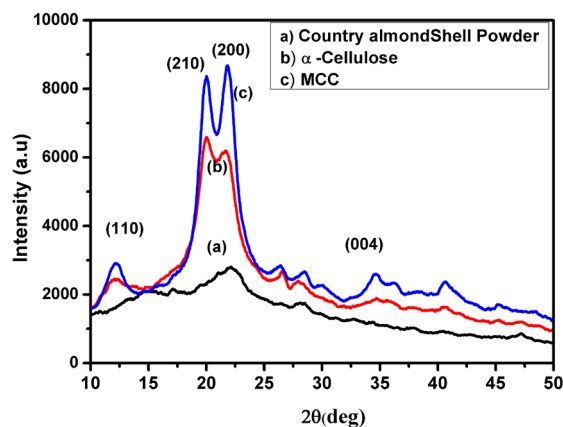


Figure 6: XRD pattern of (a) Country almond shell powder, (b) α -cellulose, (c) MCC

The intermolecular bonds are gradually weakened by increasing the alkaline concentration due to swelling and by penetration inside the cellulose fibre matrix. Since 17.5% sodium hydroxide solution was used during the extraction of cellulose, which is high enough concentration to convert cellulose I into cellulose II. Crystalline transformation in natural fibres generally occurs with high alkali concentrations [37,38]. The main difference between these two forms of cellulose lies in the packing of crystalline structure, cellulose II has an antiparallel packing but cellulose I has a parallel orientation. From the XRD patterns (Fig. 6) it can be clearly understood that there is increased crystallinity while going from Country almond shell to MCC because distinct peaks have been observed for α -cellulose and MCC but that of Country almond shell the XRD pattern is not well defined. The degree of crystallinity was enhanced from 54% (α -cellulose) to 67% (MCC). This degree of crystallinity matches with that of the already reported values [29].

4.4 Thermal Analysis

Figs. 7(a)-7(d) show the thermal degradation curves of Country almond shell, holocellulose, α -cellulose and MCC respectively. The first decomposition in each of the curve observed around 100°C is naturally due to the evaporation of water. From Fig. 7(a), it can be seen that Country almond shell shows a wide decomposition in the range 230-600°C, can be attributed to the presence of lignin, holocellulose and other components present. The main decomposition of holocellulose from Fig. 8(b) is observed around 230-350°C. Similar decomposition behaviour has already been reported for MCC obtained from different sources [39]. The presence of hemicellulose is confirmed by the appearance of shoulder around 250°C from the DTG curve of Fig. 8. The higher stability is observed for MCC than α -cellulose and holocellulose. From Figs. 8(c) and 8(d) it can be seen that α -cellulose and MCC have almost same decomposition temperature in the range of 250-380°C, which is due to degradation process

of cellulose such as the decarboxylation, depolymerisation and decomposition of glycosyl units [40]. DTG results of Country almond shell, holocellulose, α -cellulose and MCC are presented in Tab. 2.

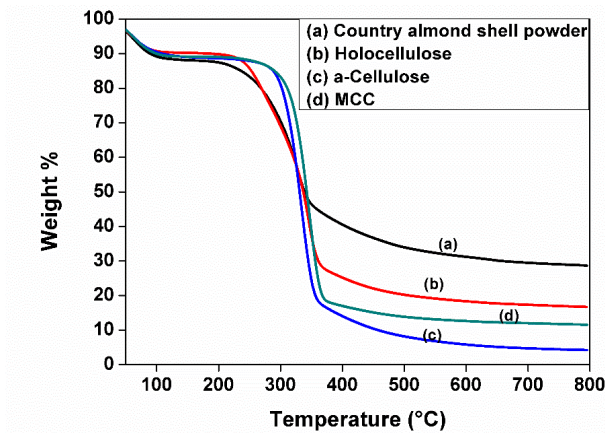


Figure 7: TG curves of (a) Country Almond shell powder (b) Holocellulose (c) α -cellulose (d) MCC

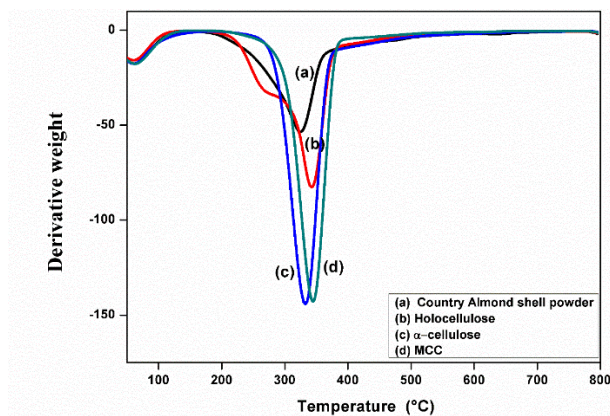


Figure 8: DTA curves of (a) country almond shell powder (b) holocellulose (c) α -cellulose (d) MCC

Table 2: DTG data of country almond shell, holocellulose, α -cellulose and MCC

Sample	T _{onset} (°C)	T _{max} (°C)
Country almond shells	217	323
Holocellulose	223	341
α -Cellulose	263	331
MCC	267	343

4.5 Mechanical Studies

The mechanical properties of PVA and PVA reinforced MCC composite were studied using tensile tests. The hydrophilic nature of PVA and MCC enhance the hydrogen bonding between hydroxyl groups of neighboring molecules. Tab. 3 shows Young's modulus, tensile strength and elongation at break of PVA-MCC composites. The MCC content significantly changes the mechanical properties of the developed composites. The Young's modulus of pure PVA film was observed to be 36 MPa. The mechanical properties of PVA was enhanced by the addition of 1 weight % of MCC (Young's modulus = 44.6958 MPa, % elongation = 223.470%). The increased mechanical properties suggest strong

interfacial interaction and good dispersion between MCC and PVA [41]. When compared to the previous work in the literature [42,43] it was observed that the small addition of MCC can significantly enhance the mechanical strength of PVA film. The decreasing mechanical properties of P₃ and P₄ observed may be due to the aggregation of MCC at higher filler content. Negative effect on the mechanical properties of natural fibre reinforced polymer composites has also been reported.

Table 3: Mechanical properties of PVA-MCC composites

Samples	%Elongation (%)	Young's Modulus (MPa)	Tensile Strength (MPa)
P ₀	185.295	36.1761	0.20127
P ₁	190.987	34.4770	0.18546
P ₂	223.470	44.6958	0.20232
P ₃	182.973	30.8593	0.17145
P ₄	132.586	26.8307	0.20200

5 Conclusions

The composition of agricultural wastes of Country almond/Badam shells has been established by standard methods of analysis. It has been found that Country almond shells contain Klason lignin, holocellulose, α -cellulose and hemicellulose. The separated components were characterized by using different analytical and spectral tools. The analysis reveals that Country almond shell has reasonable lignin content and also rich in cellulose level. Though we usually discard almond shell as an agricultural waste, it can be successfully utilized as a potential source for MCC. It can also be utilized as a potential additive to enhance the property of polymers as composites. The isolated α -cellulose has been successfully converted in to MCC by the removal of the amorphous regions by acid hydrolysis. The extracted MCC has high crystallinity than that of α -cellulose. The micro structural behavior of extracted MCC from almond shells has been proclaimed by SEM, TEM and AFM results. The mechanical property of the developed PVA-MCC biocomposite was investigated and it was found that the low concentration of MCC can significantly enhance the mechanical properties. Due to agglomeration of MCC the mechanical strength of the composite was decreased at higher concentration of MCC.

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References

1. Chao, L., Bin, L., Haishun, D., Dong, L., Yuedong, Z. et al. (2016). Properties of nanocellulose isolated from corncob residue using sulphuric acid, formic acid, oxidative and mechanical methods. *Carbohydrate Polymers*, 151, 716-724.
2. Iwamoto, S., Nakagaito, A. N., Yano, H., Nogi, M. (2005). Optically transparent composites reinforced with plant fiber-based nanofibers. *Applied Physics A*, 81, 1109-1112.
3. Benoit, B., Julien, B., Seema, S., Claude, D., Eric, L. (2016). Mechanical and antibacterial properties of a nanocellulose-polypyrrole multilayer composite. *Material Science and Engineering*, 69, 977-984.
4. Trache, D., Hazwan Hussin, M., Hui Chuin, C. T., Sabar, S., Nurul Fazita, N. et al. (2016). Microcrystalline cellulose: Isolation, characterization and bio-composites application-A review. *International Journal of Biological Macromolecules*, 789-804.

5. Habibi, Y., Lucia, L. A., Rojas, O. J. (2010). Cellulose nanocrystals: chemistry, self-assembly, and applications. *Chemical Reviews*, 110, 3479-3500.
6. Kvien, I., Tanem, B. S., Oksman, K. (2005). Characterization of cellulose whiskers and their nanocomposites by atomic force and electron microscopy. *Biomacromolecules*, 6, 3160-3165.
7. Kargarzadeh, H., Mariano, M., Gopakumar, D., Ahmad, V., Thomas, S. et al. (2018). Advances in cellulose nanomaterials. *Cellulose*, 1-39.
8. Achor, M., Oyeniyi, Y., Yahaya, A. (2014). Extraction and characterization of microcrystalline cellulose obtained from the back of the fruit of *Lageriana siceraria* (water gourd). *Journal of Applied Pharmaceutical Science*, 4, 57.
9. Kalita, R. D., Nath, Y., Ochubiojo, M. E., Buragohain, A. K. (2013). Extraction and characterization of microcrystalline cellulose from fodder grass; *Setaria glauca* (L) P. Beauv, and its potential as a drug delivery vehicle for isoniazid, a first line antituberculosis drug. *Colloids and Surfaces B: Biointerfaces*, 108, 85-89.
10. Shanmugam, N., Nagarkar, R., Kurhade, M. (2015). Advances in cellulose nanomaterials. *Indian Journal of Natural Products and Resources*, 6, 42-50.
11. Suryadi, H., Sari, H. R. (2017). Preparation of microcrystalline cellulose from water hyacinth powder by enzymatic hydrolysis using cellulose of local isolate. *Journal of Young Pharmacists*, 9, S19.
12. Uesu, N. Y., Pineda, E. A. G., Hechenleitner, A. A. W. (2000). Microcrystalline cellulose from soybean husk: effects of solvent treatments on its properties as acetylsalicylic acid carrier. *International Journal of Pharmaceutics*, 206, 85-96.
13. Bhatnagar, A., Sain, M. (2005). Processing of cellulose nanofiber-reinforced composites. *Journal of Reinforced Plastics and Composites*, 24, 1259-1268.
14. Oksman, K., Mathew, A. P., Bondeson, D., Kvien, I. (2006). Manufacturing process of cellulose whiskers/polylactic acid nanocomposites. *Composites Science and Technology*, 66, 2776-2784.
15. Paralikar, S. A., Simonsen, J., Lombardi, J. (2008). Poly (vinyl alcohol)/cellulose nanocrystal barrier membranes. *Journal of Membrane Science*, 320, 248-258.
16. Frackowiak, E., Khomenko, V., Jurewicz, K., Lota, K., Béguin, F. (2006). Supercapacitors based on conducting polymers/nanotubes composites. *Journal of Power Sources*, 153, 413-418.
17. Holbery, J., Houston, D. (2006). Natural-fiber-reinforced polymer composites in automotive applications. *Jom*, 58, 80-86.
18. Ramakrishna, S., Mayer, J., Wintermantel, E., Leong, K. W. (2001). Biomedical applications of polymer-composite materials: a review. *Composites Science and Technology*, 61, 1189-1224.
19. Schmedlen, R. H., Masters, K. S., West, J. L. (2002). Photo cross linkable polyvinyl alcohol hydrogels that can be modified with cell adhesion peptides for use in tissue engineering. *Biomaterials*, 23, 4325-4332.
20. Wan, W., Campbell, G., Zhang, Z., Hui, A., Boughner, D. (2002). Optimizing the tensile properties of polyvinyl alcohol hydrogel for the construction of a bioprosthetic heart valve stent. *Journal of Biomedical Materials Research: An Official Journal of the Society for Biomaterials, the Japanese Society for Biomaterials, and the Australian Society for Biomaterials and the Korean Society for Biomaterials*, 63, 854-861.
21. Lu, J., Wang, T., Drzal, L. T. (2008). Preparation and properties of microfibrillated cellulose polyvinyl alcohol composite materials. *Composites Part A: Applied Science and Manufacturing*, 39, 738-746.
22. Roohani, M., Habibi, Y., Belgacem, N. M., Ebrahim, G., Karimi, A. N. et al. (2008). Cellulose whiskers reinforced polyvinyl alcohol copolymers nanocomposites. *European Polymer Journal*, 44, 2489-2498.
23. Ibrahim, M. M., El-Zawawy, W. K., Nassar, M. A. (2010). Synthesis and characterization of polyvinyl alcohol/nanospherical cellulose particle films. *Carbohydrate Polymers*, 79, 694-699.
24. Wise, L., Murphy, M., Addiecs, A. (1946). Chlorite holocellulose, its fractionation and bearing on summative wood analysis and studies on the hemicelluloses. *Paper Trade Journal*, 122(2), 35-43.
25. Boonmee, A. (2012). Hydrolysis of various Thai agricultural biomasses using the crude enzyme from *aspergillus aculeatus* iizuka fr60 isolated from soil. *Brazilian Journal of Microbiology*, 43, 456-466.
26. Xiong, R., Zhang, X. X., Tian, D., Zhou, Z. H., Lu, C. H. (2012). Comparing microcrystalline with spherical nanocrystalline cellulose from waste cotton fabrics. *Cellulose*, 19, 1189-1198.

27. Kline, L. M., Hayes, D. G., Womac, A. R., Labbe, N. (2010). A simplified determination of lignin content in soft and hard wood via UV-spectrophotometric analysis of biomass dissolved in ionic liquids. *BioResources*, 5, 1366-1383.
28. Fang, J., Sun, R., Tomkinson, J. (2007). Isolation and characterization of hemicelluloses and cellulose from rye straw by alkaline peroxide extraction *Cellulose*, 7, 87-107.
29. Subair, N., Purushothaman, E. (2016). Sago seed shell: determination of the composition and isolation of microcrystalline cellulose (MCC). *Cellulose*, 23, 1803-1812.
30. Borysiak, S., Garbarczyk, J. (2003). Applying the WAXS method to estimate the supermolecular structure of cellulose fibers after mercerization. *Fibres and textiles in eastern Europe*, 11, 44.
31. Soyekwo, F., Zhang, Q. G., Lin, X. C., Wu, X. M., Zhu, A. M. et al. (2016). Facile preparation and separation performances of cellulose nanofibrous membranes. *Journal of Applied Polymer Science*, 20, 2379-2392
32. Peng, Y., Gardner, D. J., Han, Y., Kiziltas, A., Cai, Z. et al. (2013). Influence of drying method on the material properties of nanocellulose I: thermostability and crystallinity. *Cellulose*, 20, 2379-2392.
33. Shahabi-Ghahafarrokh, I., Khodaiyan, F., Mousavi, M., Yousefi, H. (2015). Preparation and characterization of nanocellulose from beer industrial residues using acid hydrolysis/ultrasound. *Fibers and Polymers*, 16, 529-536.
34. Lani, N., Ngadi, N., Johari, A., Jusoh, M. (2014). Isolation, characterization, and application of nanocellulose from oil palm empty fruit bunch fiber as nanocomposites *Journal of Nanomaterials*, 13.
35. John, M. J., Anandjiwala, R. D. (2008). Recent developments in chemical modification and characterization of natural fiber-reinforced composites. *Polymer Composites*, 29, 187-207.
36. O'sullivan, A. C. (1997). Cellulose: the structure slowly unravels. *Cellulose*, 4, 173-207.
37. Abraham, E., Deepa, B., Pothan, L., Jacob, M., Thomas, S. et al. (2011). Extraction of nanocellulose fibrils from lignocellulosic fibres: a novel approach. *Carbohydrate Polymers*, 86, 1468-1475.
38. Fortunati, E., Puglia, D., Monti, M., Peponi, L., Santulli, C. et al. (2013). Extraction of cellulose nanocrystals from Phormium tenax. fibres. *Journal of Polymers and the Environment*, 21, 319-328.
39. Djalal, T., André, D., Kamel, K., Riad, B., Nicolas, B. (2014). Physico-chemical properties and thermal stability of microcrystalline cellulose isolated from Alfa fibres. *Carbohydrate Polymers*, 104, 223-230.
40. Sebio-Puñal, T., Naya, S., López-Beceiro, J., Tarrío-Saavedra, J., Artiaga, R. (2012). Thermogravimetric analysis of wood, holocellulose, and lignin from five wood species. *Journal of Thermal Analysis and Calorimetry*, 109, 1163-1167.
41. Peresin, M. S., Habibi, Y., Vesterinen, A. H., Rojas, O. J., Pawlak, J. J. et al. (2010). Effect of moisture on electrospun nanofiber composites of poly (vinyl alcohol) and cellulose nanocrystals. *Biomacromolecules*, 11, 2471-2477.
42. Subair, N., Sreejith, M. P., Jinita, T. V., Shaniba, V., Aparna, K. B. et al. (2017). Development of green composites of poly (vinyl alcohol) reinforced with microcrystalline cellulose derived from sago seed shells. *Polymer Composite*, 1-7.
43. Sun, X., Lu, C., Liu, Y., Zhang, W., Zhang, X. (2013). Melt-processed poly(vinyl alcohol) composites filled with microcrystalline cellulose from waste cotton fabrics. *Carbohydrate Polymers*, 101, 642-649.