Extraction and Characterization of Microcrystalline Cellulose from Walnut, Almond and Apricot Stone Shells

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Summary: Walnut, Almond and Apricot stone shells are abundantly available agro wastes worldwide and are sources of cellulose. In this study microcrystalline cellulose were isolated from these renewable biomasses through acid hydrolysis method. Isolation of microcrystalline was performed due to its potential significance in cosmetics, medicine and food industries. Acid hydrolysis is carried out at different concentrations of sulphuric acid. Surface morphology and elemental composition of microcrystalline cellulose was characterized with Scanning electron microscopy, energy dispersive x-ray spectroscopy and FT-IR spectroscopy. SEM clearly showed that microcrystalline cellulose obtained through high acid concentration has better structural similarities with commercial microcrystalline cellulose However microcrystalline cellulose obtained with low concentration of acid showed lower fibrillation. Elemental analysis revealed that amount of Sulphur impurity (1.17-1.18) is present in microcrystalline cellulose when hydrolyzed with high H_2SO_4 concentration while negligible (0.10-0.72) in microcrystalline cellulose treated with low concentration of H_2SO_4. It is also found that carbon and oxygen contents range in Walnut, almond and Apricot C; 50.89-58.73, 54.07-55.58, 54.19-55.62, O; 39.72-48.01, 43.54-43.71 and 41.75-44.34 respectively while FT-IR shows required functional groups in prepared MCC specifically representing beta 1-4 glycosidic linkage at 849 cm^{-1} that depicts improved cellulose content with in the sample. Thus, this work confirms that Walnut, Almond and Apricot stone are promising sources for microcrystalline cellulose.

Keywords: Valorization, Fibrillation, Renewable biomasses, Pre-treatment, Purification, Dewaxing.

Introduction

Cellulose is naturally occurring abundant biopolymer used in all kind of medical and industrial applications [1] it is a linear homopolymer of glucose. Chemical structure of cellulose depicts that it is a straight chain polymer that is made up of D-anhydrous glucose units which are joined together by beta 1-4 glucosidic bond [2]. Annual production of cellulose is calculated approximately 1011-1012 tons per year [1]. Since the tensile strength of cellulose is comparable to that of steel due to its distinct structure, it is therefore an essential source in the pulp and paper industries [3]. In addition, for clothing, men must use cellulose for 10,000 years [4]. Furthermore, it is used as raw material for the production of paper, rayon, cardboard and cellulose derivatives such as nanocrystals of cellulose, cellulose nanofibers and microcrystalline cellulose which are used as additives in the pharmaceutical, cosmetic and food industries [5]. Cellulose fibers were known widely over 5,000 years ago however its derivatives such nanocrystals of cellulose, powder cellulose and microcrystalline cellulose began to be used after 19th century [4]. Microcrystalline cellulose can be defined as "pure product of cellulose depolymerization, obtained by acid hydrolysis of native cellulose" it is an odourless, tasteless crystalline powder that can be obtained by hydrolysis of cellulose in acidic medium. The molecular formula of microcrystalline cellulose is $C_6H_{10}O_5$. Furthermore, due to fine particle size microcrystalline cellulose has great potential in several areas such as in food, cosmetics, pharmaceutical and polymer composite industries, it can be used as a binder in powder form while in the colloidal form it can be used as an emulsifier, a suspension stabilizer, water retainer in different pastes and creams [6]. Due to innumerable applications isolation of microcrystalline cellulose from different renewable agricultural biomasses drew much attention by employing different methods such as reactive extrusion, steam explosion, enzyme mediated and acid hydrolysis [7]. In literature it is found that various sources are being employed for the production of microcrystalline cellulose. For example it can be obtained from waste cotton [8], esparto grass [9], orange mesocarp [10], industrial agro waste such as fruits and vegeFig peels [11], corn husk [12] , mango kernel [13] , wheat straw [14] , peanut shells [15] , bamboo [16] , raw cotton linter [17], rice husk [18],

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rice straw [19], corncob [20],banana rachis [21] and agricultural residues including bagasse and rice cotton stalks [22] . The focus of this study is valorization of Almond, walnut and apricot stone shells into microcrystalline cellulose. Our country generates a huge amount of renewable biomass such as Walnut shells, Almond and Apricot stone shells in addition to wheat, rice, maize and other renewable biomass every year. These shells are a suiFig source for the generation of microcrystalline cellulose because of the large number of production and presence of cellulose in them. Conventionally it is used for power generation by burning or incinerated which exploits the environment. For environmentally friendly use, we can valorize it into value-added product microcrystalline cellulose through hydrolysis. Moreover; renewable agricultural biomass is a cheaper source to produce microcrystalline cellulose. Indeed, several researchers have isolated microcrystalline cellulose from agro waste via acid hydrolysis however the aim of present research is to utilize cellulose based agro waste Walnut, Almond and Apricot stone shells in order to prepare microcrystalline cellulose via acid hydrolysis. At the moment, to the best of my knowledge there is lack of research towards extraction of microcrystalline cellulose from these renewable agricultural biomasses.

Experimental

Materials

Walnut, Almond and apricot stone shells were purchased from local market of Gilgit Baltistan. Reagents and chemicals used in this method are sodium hydroxide (PocH SA≥ 98.8%) sulphuric acid (sigma Aldrich 95-98 %) acetic acid (Chem Lab ≥99.8%), chloroform (≥99%), absolute ethanol (≥99.9%) and Hydrogen peroxide.

Pre-treatment

Before pre-treatment Walnut, Almond and Apricot stone shells were washed and sun dried properly for 6-7 days, these dried shells were than ground into fine powder and sieved. Crushed fibers were than dewaxed with mixture of chloroform and ethanol 2:1 by volume for 24 hours under mechanical stirring than fibers were washed until the filtrate pH becomes neutral and dried properly in an open air at room temperature. The dried product was treated in 4wt% NaOH solutions at 80°C to 90°C for 2h to remove hemicelluloses with residual starch and pectin. Alkali treatment was conducted two times and after each treatment fibers were filtered and washed until the filtrate pH becomes neutral. Lignin in the fiber plant is removed by treating alkali treated sample with 100 ml diluted hydrogen peroxide solution under mechanical stirring for 1h at 70 °C and was repeated two times. The bleached fibers were subsequently filtered, washed with distilled water and air dried. Bleaching treatment is done to remove residual lignin content.

Purification of pre-treated Cellulose

Cellulose obtained after pre-treatment of almond, walnut and apricot stone shell powder was than purified by dispersing 10g dry weight basis of three samples in 1L of 0.5% aqueous NaOH separately for 6-7 days and filtered and washed continuously until the filtrate pH becomes neutral. Then the cellulose was redispersed for 1-4 days in 1L of 0.5% NaoH, again washing process is repeated until filtrate pH reached to neutrality. The cellulose was than oven dried at 70°C for 48h, prior to hydrolysis.

Sulfuric acid hydrolysis

Cellulose crystals were prepared by sulfuric acid hydrolysis. Isolation process followed in this study was adapted from maaloul et.al and Khili et. al with some modifications [23], [24].

Protocol 1

In this protocol extraction is carried out with a method of khili et al [24]. 5g of treated cellulose material of Walnut, Almond and apricot shell powder is dispersed in 50ml of distilled water separately. Then 54 ml of sulphuric acid is added drop wise for 60 minutes after that suspension is heated at 44 °C under magnetic stirring for 20 minutes, subsequently hydrolysis is stopped by adding cold ice water.

Centrifugation: Centrifugation is used to separate particles from a solution according to their size, shape and density, after hydrolysis the excess of H_2SO_4 was removed by centrifugation at 4000 rpm for 15 minutes. After Centrifugation obtained microcrystalline cellulose is washed with distilled water to bring pH to neutrality sun dried after that stored at room temperature for Analysis.

Protocol 2

In this protocol extraction is carried out with a method of maaloul et.al [23].5g of Walnut, Almond and apricot stone shells were treated with 15%, 40%, and 30% H_2SO_4. Here the reaction mixture was maintained at 60 °C and hydrolysis time was fixed at 45 minutes subsequently a large amount of ice is added

to reaction beaker in order to stop hydrolysis. The resulting mixture was than washed with distilled water until filtrate pH becomes neutral and sundried and stored at room temperature for analysis.

Characterization of MCC

Prepared MCC were Characterized via SEM, EDS, and FTIR. SEM is done for the determination of morphological analysis of MCC while EDX is used for elemental analysis of microcrystalline cellulose, obtained from Walnut, Almond and Apricot stone shells and FT-IR is carried out for structural determination.

Results and Discussions

Energy dispersive x-ray spectroscopy (Edx)

Fig. 1, 2 and 3 given below demonstrate energy dispersive x-ray Spectra of cellulose microcrystal obtained from Walnut, Almond and Apricot stone shells. Walnut shells treated with 15% H_2SO_4 is identified as S1 and walnut shells treated with 98% H_2SO_4 is identified as S2. "Almond shells treated with 98% H_2SO_4 is identified as S3 and almond shells treated with 40% H_2SO_4 is identified as S4, Apricot stone shell treated with 30% H_2SO_4 is identified as S5 and Apricot stone shell treated with 98% H_2SO_4 is identified as S6. From observation given in Figure and Fig it is clearly demonstrated that a high concentration of sulphuric acid has a greater effect during cellulose hydrolysis, as S2, S3 and S6 has greater carbon content then S1, S4 and S5 it means that high concentration of acid releases more fibrils of cellulose. However, it is also found that oxygen weight % reduces with increase in % of carbon, it might be because of the removal of non-cellulosic materials, lignin and hemicelluloses. Furthermore, sample treated with less concentration of sulfuric acid contains less Sulphur content then sample which is treated with 98% H_2SO_4.

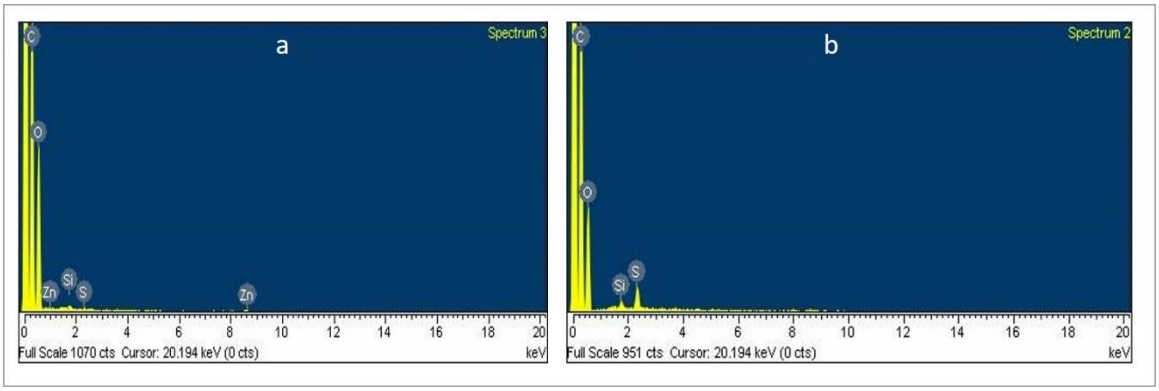

Fig. 1: EDX MCC obtained from Walnut Shells [a] S1 and [b] S2.

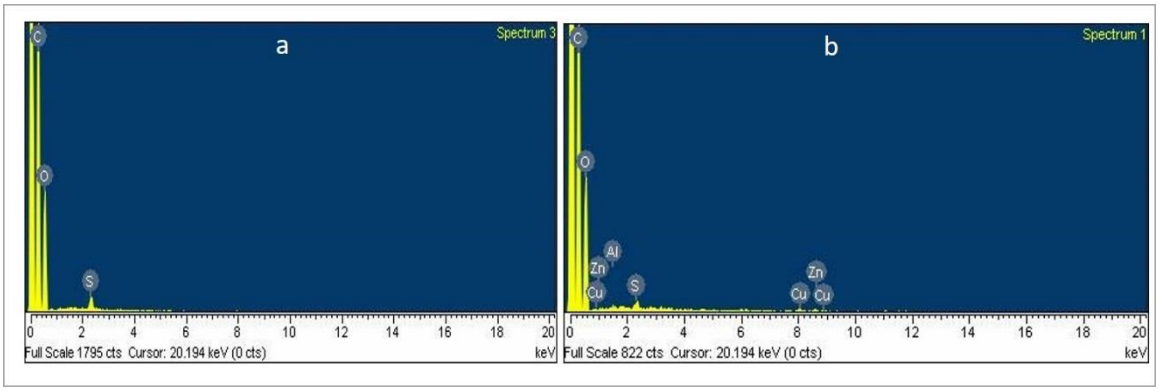

Fig. 2: EDX of MCC obtained from Almond Shells [a] S3 and [b] S4.

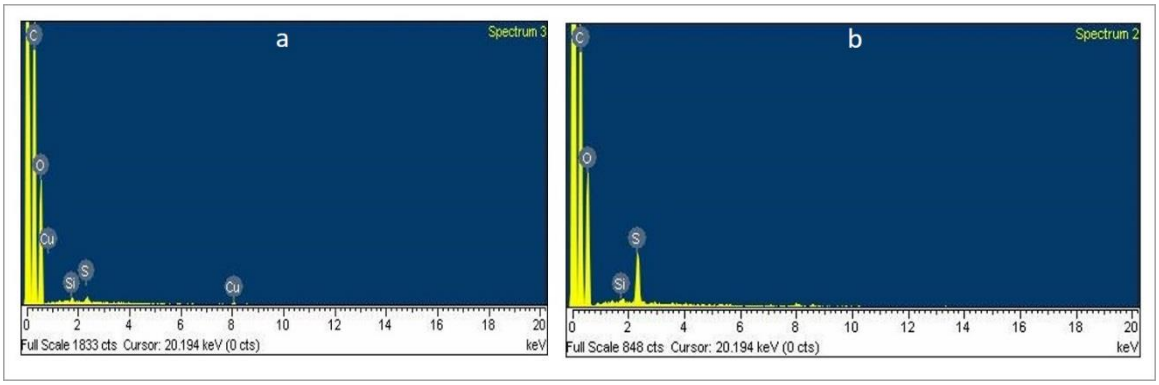

Fig. 3: EDX of MCC obtained from Apricot stone Shells [a] S5 and [b] S6.

Fig-1: Elemental weight %ages of walnut obtained through EDX.

Elemental weight percentages %	Walnut	
	S1%	S2%
C	50.89%	58.73%
O	48.01%	39.72%
S	0.10%	1.18%
Si	0.22%	0.38%
Zn	0.78%	00%
Total	100%	100%

Fig-2: Elemental weight percentages of almond shells obtained from EDX.

Elemental weight percentages %	Almond	
	S3%	S4%
C	55.58%	54.07%
O	43.71%	43.54%
Al	00%	0.16%
S	0.72%	0.40%
Cu	00%	1.10%
Zn	00%	0.73%
Total	100.00%	100.00%

Fig-3: Elemental weight percentages of apricot stones obtained through EDX.

Elemental weight percentages %	Apricot stone shells	
	S5%	S6%
C	54.19%	55.62%
O	44.34%	41.75%
Cu	0.95%	00%
Si	0.25%	0.06%
S	0.28%	1.07%
Total	100.00%	100.00%

Fig 1,2 and 3 shows various percentage ratios of trace elements present in microcrystalline cellulose obtained from walnut, almond and apricot stone shells respectively and it is observed that mcc obtained from walnut shell by using low acid concentration contains trace number of elements such as Si, Zn and Mg while Zn is completely removed in cellulose crystals treated with high acid concentration. MCC obtained from almond shells by treating with low concentration of acid contains trace number of elements such as Al, Cu, and Zn which are removed to a greater extent in a sample which is treated with high acid concentration. Apricot stone shells show a similar pattern such as sample treated with high acid concentration removes or contains a negligible amount of trace elements while those treated with low acid concentration contains a trace number of elements such as Cu and Si.

Each spectrum and Fig show carbon and oxygen as major components of their composition hence it is observed that the prepared microcrystalline cellulose contains skeleton of carbon and oxygen with small number of impurities which disclosed that pure microcrystalline cellulose with small number of impurities is obtained, these impurities usually occur due to different chemical treatments, sulphur is detected as an impurity which came from sulphuric acid hydrolysis. Moreover, being agriculture residues small amount of trace elements were also found in MCC obtained from walnut almond and apricot stone shells [25-27]. Edx

elemental analysis of each sample correlates with characteristics of cellulose nanocrystals [28-31].

Scanning electron microscopy (SEM)

SEM is used to investigate the morphology of microcrystalline cellulose obtained from Walnut, Almond and apricot stone shells and presented in Fig. 4, 5 and 6 given below. Scanning electron micrograph shows that morphology of S1, S4, and S5 is smooth as compared to S2, S3 and S6. S2, S3 and S6 show rough surface with aggregates of irregular shapes of fibers, more cracks and damages are found in these samples it might be because of removal of cementing materials present around the fibers, furthermore roughness and irregularity of samples favours production of microcrystalline cellulose."

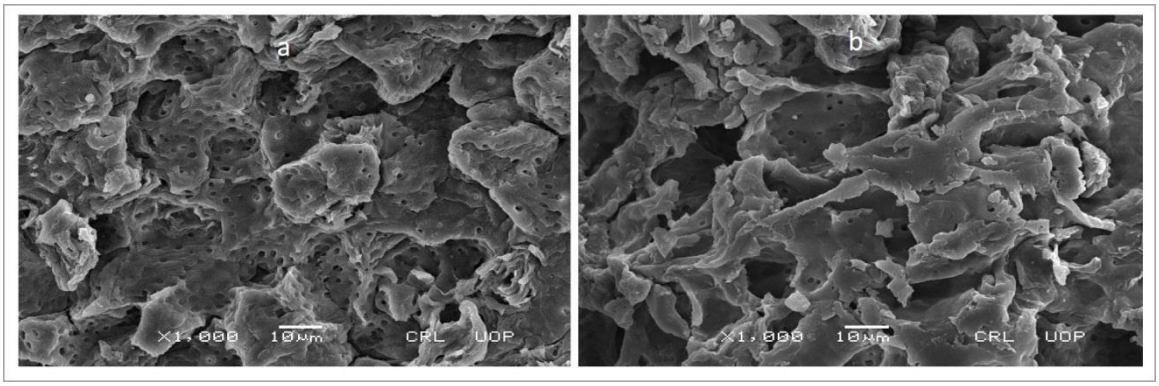

Fig. 4: SEM images of MCC obtained from walnut shell [a] S1 and [b] S2.

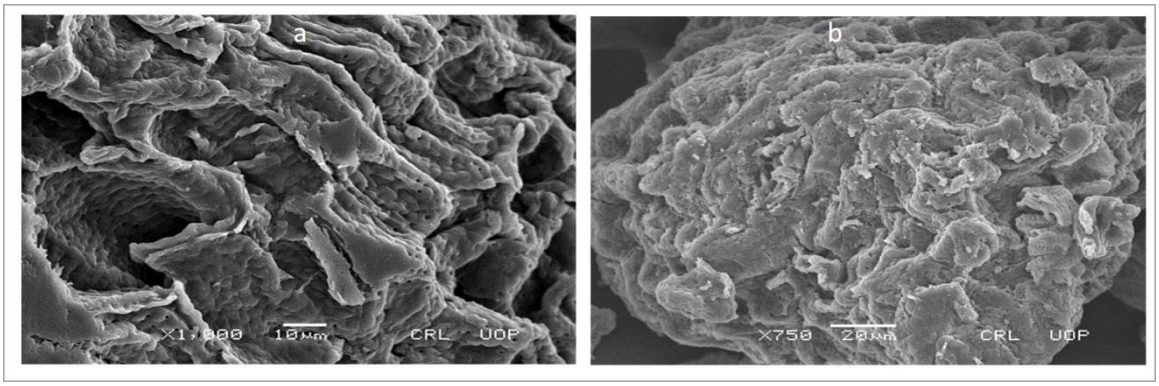

Fig. 5: SEM images of MCC obtained from Almond shells [a] S3 and [b] S4.

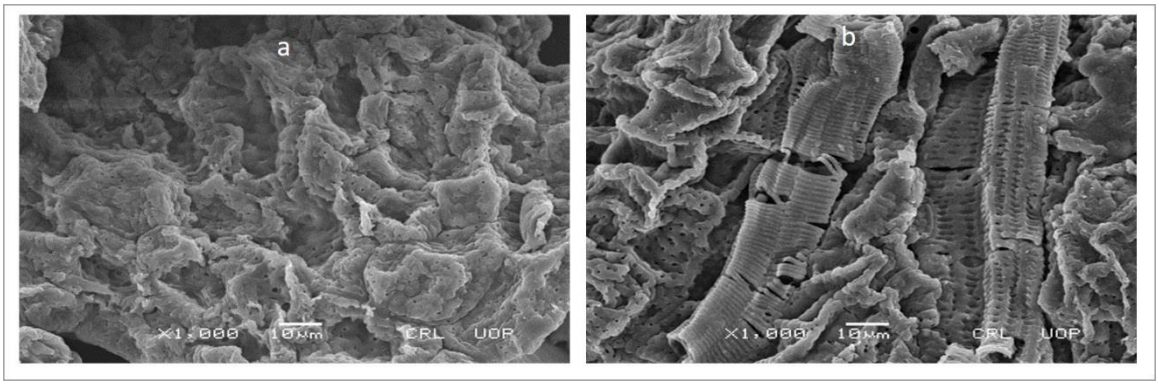

Fig. 6: SEM images of MCC obtained from Apricot stone shells [a] S4 and [b] S6.

From Figure it can be clearly observed that greater acid concentration is more favourable for the production of microcrystalline cellulose because sample treated with low acid concentration has unequal distribution of fibers and compact structure where fibers are not separated and formed clumps and bunches while those which are treated with high acid concentration shows cracks and damages which have much more structural similarities with Avicel 102 [31] For further better results temperature and time could be prolong.

Fourier Transform Infrared Spectroscopy (FT-IR)

Fig. 7, 8, 9 below presents the FT-IR spectra of fibers that shows similar patterns indicating that chemical functionality is not significantly influenced by varying concentration of acid, during acid hydrolysis. Various absorptions peaks were observed at 3558 cm-^{1}, 2925 cm-^{1}, 1639 cm^{-1}, 1424 cm-^{1}, 1367 cm-^{1}, 1000 cm-^{1},900-894 cm-^{1}. Here 3558 cm-^{1} represents OH vibration of cellulose, 2925 cm-^{1} is attributed as C-H stretching of cellulose, stretching between 1639 cm^{-1}-1745 is attributed as hemicellulose stretching that represents C=O vibration representing a reduced sharpness which determines the reduction of hemicellulose. However, decrement of peak sharpness at 1424 cm^{-1} is related to rearrangement of cellulose segments. The peak at 1000 cm-^{1} is probably attributed to ether group C-O-C representing cellulose order orientation and peak at 894 cm-^{1} represents beta 1,4-glycosidic linkage showing better content of cellulose in the sample.

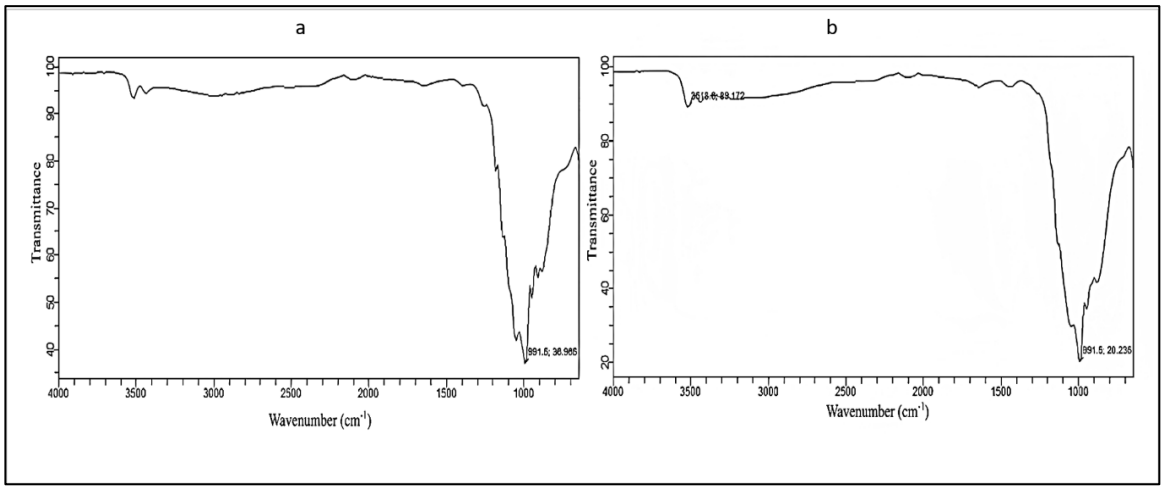

Fig. 7: FTIR spectrum of MCC obtained from walnut shells [a] S1 and [b] S2.

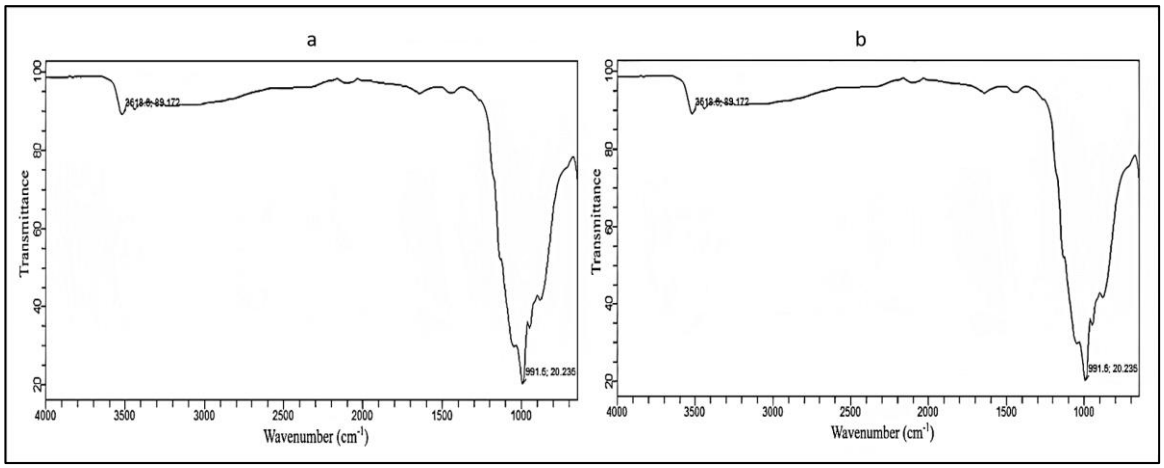

Fig. 8: FTIR spectrum of MCC obtained from Almond shells [a] S3 and [b] S4.

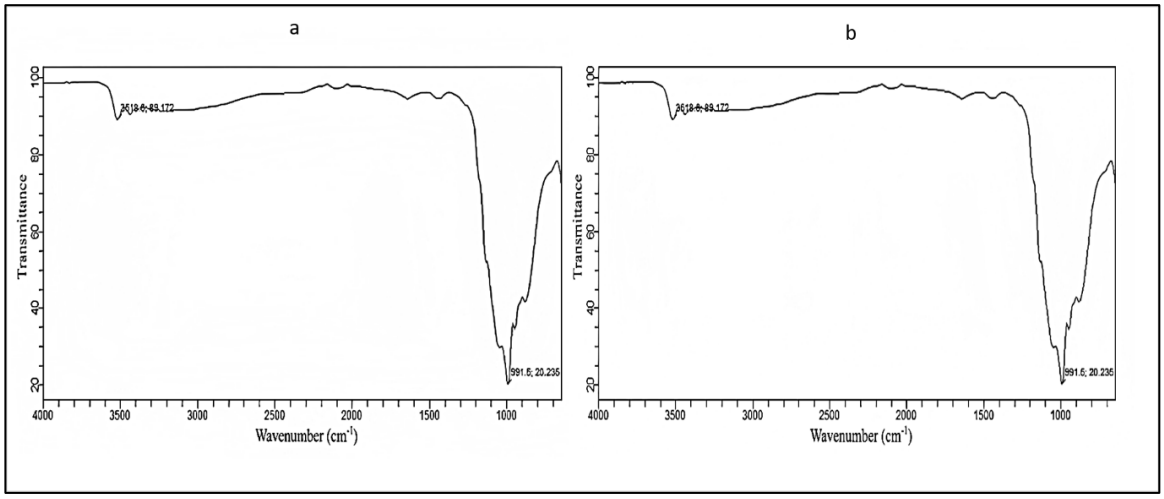

Fig. 9: FTIR spectrum of MCC obtained from Apricot stone shells [a] S5 and [b] S6.

Conclusion

Findings of present study have revealed that renewable agricultural biomasses Walnut, Almond and Apricot stone shells can be used as potential raw material for production of microcrystalline cellulose, by using simple methods like pre-treatment, alkali treatment, bleaching and acid hydrolysis. The prepared microcrystalline cellulose was characterized by energy dispersive x-ray spectroscopy scanning electron microscopy and FT-IR spectroscopy. EDX analysis revealed cellulose crystals obtained by treating with 98% acid contained sulphur that came from sulphuric acid hydrolysis. However, microcrystalline cellulose treated with low acid concentration contain less sulphur and contained other trace elements Zn, Si, Cu etc. Morphological analysis showed that microcrystalline cellulose obtained by treating with less acid concentration in hydrolysis is not much similar to commercial microcrystalline cellulose. FT-IR spectra shows required functional groups in prepared MCC. It is also found that microcrystalline cellulose which is treated with high acid concentration has much structural similarities with Avicel. Thus, it illustrates that valorization of microcrystalline cellulose from these potential substrates can be optimize under different factors such as time, temperature and acid concentration during hydrolysis.

To conclude, present study revealed that Walnut, Almond and Apricot stone shells were successfully valorised into microcrystalline cellulose thus these agricultural residues could be used in environmentally friendly way.

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