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Preparation and Characterization of Cellulose and Nanocellulose From Pomelo (*Citrus grandis*) Albedo

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Abstract

Pomelo (*Citrus grandis*) peel is one of the under-utilized waste materials that have potential in the production of functional ingredients, due to its high fiber content. This study was conducted to isolate and characterize cellulose and nanocellulose from pomelo albedo. Cellulose was prepared via alkali treatment followed by bleaching process, while nanocellulose was produced via hydrolysis using sulfuric acid. The physicochemical and structural properties of the produced materials were characterized using proximate analysis, Fourier transform infrared spectroscopy (FTIR), and cellulose crystallinity index (CrI) by X-Ray Diffractometer (XRD) and water holding capacity (WHC). Proximate analysis showed that pomelo albedo contains 72.62% carbohydrate, 16.13% moisture, 6.27% protein, 3.41% ash and 1.56% fat. FTIR spectra for cellulose and nanocellulose confirms absorption bands characteristic of pure celluloses at 3334, 2902, 1630, 1427, 1030 and 896 cm^{-1}. The crystallinity index (CrI) of the isolated nanocellulose was found considerably higher than that of cellulose with the value of 60.27% and 57.47%, respectively. Water holding capacity (WHC) of nanocellulose was also higher ($p<0.05$) than cellulose with the value of 12.75 g water/g and 8.9 g water/g respectively. We conclude that pomelo albedo can be a principal source of natural cellulose and nanocellulose materials which can be further manipulated for food ingredient applications.

Keywords: Pomelo albedo; Cellulose; Nanocellulose; Morphology; Structural; Crystallinity

Introduction

Focuses on the need to find alternative fiber sources have been the subject of many researchers world-wide nowadays. Citrus, is one of the most consumed type of fruit over the world, due to low cost and bulk productivity as well as for their wholesome nutritional properties consisting of vitamin C, A and B, minerals (calcium, phosphorus, potassium), dietary fiber and many phytochemicalssuch as flavonoids, amino acids, triterpenes, phenolic acids and carotenoids [1,2]. However, the consumption of citrus fruits have led to the generating of residue (peel, pulp, seeds) which accounts approximately a 50% of the fruit weight and its moisture content [3-5]. This huge amount of waste can be considered as an agricultural waste, by the fact that it was discarded and contributes to the environmental pollution.

Due to its composition being rich in soluble and insoluble carbohydrates, citrus by-product shows great potential for the recovery of fibers which can be further used as functional food ingredient. Generally, citrus peel can be divided into two parts namely albedo (inner part or mesocarp) and flavedo (outer part or epicarp). Albedo, the white, spongy and cellulosic tissue is the principal citrus peel component and a potential fiber source due to its high fiber content. Additionally, it is also reported to possess good water and oil holding capacity as well good colonic fermentability and low caloric content [6].

Cellulose is a long chain polymer with repeating units of D-glucose, called pyranoses which are joined by single oxygen atoms (acetal linkages) between the C-1 of one pyranose ring and the C-4 of the next ring, called ß-1-4 linkages [7]. Each ß-1-4-glucopyranose bears three hydroxyl groups and is able to form intra and intermolecular hydrogen bonds that play a major role in determining the physical properties of cellulose [8]. Cellulose can also be used as starting materials for nanocellulose production via strong acid hydrolysis. This method introduced negative charges from the acidic substances to the structure of native cellulose and hydrolysed the amorphous part into nanosized fibers [9].

Citrus grandis, also known as pomelo is referred to the largest kind of citrus fruit native to southern Asia including Malaysia. The peels of pomelo contribute to 30% of the fruit weight and the consumption of this fruit has resulted in the production of a huge amount of peel. The albedo part of pomelo is very thick contributing to more than a kilogram fruit weight. Therefore, it is worth mentioning that the pomelo albedo is a great source for cellulose extraction compared to other types of citrus for its size and thickness of the albedo.

Thus, this study was conducted to evaluate the utilization potential of pomelo waste by isolating citrus-based cellulose and nanocellulose from its albedo. The quality of the resultant materials was then investigated to determine its physicochemical and structural properties.

Materials and Methods

Preparation of cellulose and nanocellulose from pomelo albedo

The pomelo was peeled and the albedo was separated and cut into

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small pieces and dried in a convection oven (Protech, Malaysia) at 50°C for 48 hours. Dried sample was grinded by using (dry blander, Panasonic) and the powder formed was sieved until certain sizes were achieved. Extraction of cellulose was performed by using alkaline treatment followed by bleaching.

A 50 g of dried albedo powder were weighed and transferred into a round bottom flask. Alkali solution (4 wt% NaOH) was added and the treatment was performed at reflux condition at 100-120°C for 2 h. The mixture was then filtered and washed with distilled water several times to remove lignin and hemicellulose that dissolved in the solution. The resultant fiber was dried before used for bleaching treatment.

Bleaching treatment was performed at reflux condition at 110-130°C for 4 hours after adding 60 g of fiber into 400 ml of each solution of 1.7% $NaClO_2$, acetic buffer and distilled water. The mixture was then allowed to cool before filtered and washed with distilled water until white cellulose was obtained. The cellulose obtained was dried by using freeze dryer (Labconco) at -39°C for 24 h.

Cellulose nanocrystal was prepared by using sulfuric acid hydrolysis. A 65 wt% H_2SO_4 was prepared before approximately 5% of cellulose fiber was added to the solution. The time and temperature was fixed at 45°C for 45 min in order to achieve the optimum yield. The hydrolyzed cellulose sample was washed five times by centrifugation (10,000 rpm, 10 min, and 10°C) to remove excess sulphuric acid. The suspension was then dialyzed against distilled water until a constant pH was achieved. The resultant cellulose nanocrystal suspension was stored at 4 ± °C until further used.

Proximate analysis

All analyses were performed in duplicate unless otherwise specified. Dried pomelo albedo and extracted cellulose were analysed for total nitrogen, fat, carbohydrate, and ash content using AOAC Official Methods [10]. The cellulose content of the dried pomelo albedo was determined using acetic/nitric acid method with slightly modification [11].

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectra were recorded on an attenuated total reflection Fourier transform infrared (ATR-FTIR) to analyze the chemical changes of the samples before and after each treatment, including alkali, bleaching and acid hydrolysis using a Perkin-Elmer FTIR spectrophotometer. FTIR spectral analysis was performed within the wave number range of 400-4000 cm^{-1}.

X-ray Diffraction (XRD)

The X-ray diffraction (XRD) patterns were obtained with an X-ray diffractometer (D8-Advance Bruker AXS GmbH) at room temperature (RT). Samples were scanned with a monochromatic Cu-Kα radiation source (λ= 0.1539 nm) in the step-scan mode with a 2θ angle ranging from 10° to 50° with a step of 0.04 and scanning time of 5.0 min. The crystallinity index (CrI) was calculated from the heights of the 200 peak (I_{002}, 2θ= 22.6°) and the intensity minimum between the 200 and 110 peaks (I_{am}, 2θ=18°) using the Segal method [12]. I_{002} represents crystalline material, while I_{am} represents the amorphous material.

$$\text{Crystallinity index (CrI\%)} = [(I_{002} - I_{am}) / I_{002}] \text{ x } 100$$

Water Holding Capacity (WHC)

Water holding capacities were measured using Traynham et al. [13]. 2 g samples were weighed and dissolved in 38 ml of distilled water in a centrifuge tube. The solution was then shaken for 10 minutes. After 10 minutes, the solution is placed in a centrifuge (3000 rpm, 30 minutes). Water in a centrifuge tube was removed. Precipitate and centrifuge tube were weighed. Water holding capacity is calculated using the equation shown below.

WHC (g water/g) = ((weight of centrifuge tube + precipitate) - weight of centrifuge tubes -sample weight) / (weight of sample)

Stastistical analysis

All the data for water holding capacity (WHC) between cellulose and nanocellulose were analysed by Independent Samples Test by using SPSS software version 22.0 for Windows (SPSS Inc., Chicago, USA). The results were expressed in mean and standard deviation.

Results and Discussion

Chemical composition of pomelo albedo

Proximate analysis was carried out to identify the chemical composition of pomelo albedo including its cellulosic content. The composition data as presented in Table 1, revealed that the dried pomelo albedo consisted mainly of carbohydrate (72%). This value was considerably higher than the carbohydrate content found in lemon pulp (70%) [14] and lemon albedo (59%) [15]. The moisture and protein content of pomelo albedo was also found at 16.13% and 6.27%, respectively. It was evident that these values are higher than those recorded in lemon albedo, orange peel and orange pulp [16,17].

Meanwhile, small amounts of ash (3.41%) and fat (1.56%) were observed. This is in agreement with Marin et al. [18] that outlined the amount of ash in citrus peel is in the range of 2.56-8.09%, while the amount of fat is at 1.51-4.00%. Meanwhile, the cellulose content in pomelo albedo was 21.29%, significantly greater than that reported in orange peel, at 14.4% [19]. These results suggest that pomelo albedo can be a better source for cellulose production compared to other types of citrus fruit peel.

FTIR spectroscopy analysis

The FT-IR technique was used to study the main functional groups present in pomelo albedo. Figure 1 shows the FT-IR spectra of the untreated pomelo albedo (a), NaOH treated pomelo albedo (b), bleached pomelo albedo (cellulose) (c) and acid hydrolysed pomelo albedo (nanocellulose). Generally, the cellular wall of most citrus peel contains insoluble polysaccharides that composed of pectin, cellulose, and hemicelluloses [20].

The most distinct absorption peak was observed on the untreated spectra (a), with the presence of a peak located at ~1734 cm^{-1} that indicates the presence of C=O stretching of the acetyl and uronic ester groups of polysaccharides, such as pectin, lignin and hemicellulose [21,22]. This peak was also related to the p-coumeric acids of lignin and/or hemicellulose [23]. The existence of this shoulder was reported by Ribeiro et al. [24] in untreated mandarin peels, where the presence of a peak located around ~1750 cm^{-1} indicates the presence of C=O

Composition	(% w/w, dry basis)
Carbohydrate	72.62 ± 0.42
Moisture	16.13 ± 0.16
Protein	6.27 ± 0.23
Ash	3.41 ± 0.05
Fat	1.56 ± 0.07
Cellulose	21.29 ± 1.90

Table 1: Chemical compositions of pomelo albedo

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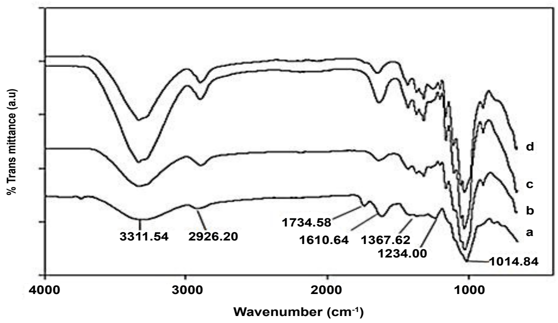

Figure 1: FTIR spectra of (a) ground pomelo albedo, (b) alkali-treated albedo, (c) bleached and, (d) acid hydrolysed pomelo albedo fibers.

streching which disappeared after treatment with a NaOH solution. Therefore, the absence of the peak from the spectrum (b) and (c) confirms the removal of the non-cellulosic materials from pomelo albedo.

It is also shown in Figure 1 that all of the samples have similar peak near 3400-3300 cm^{-1} region and 1610-1639 cm^{-1}, which indicates the presence of O-H stretching vibration and O-H bending of the absorbed water, respectively. The absorption peaks in the region of 1650-1610 cm^{-1} and around ~2900 cm^{-1} correspond to the O-H and C-H groups, respectively [25]. The vibration peak around ~1367 cm^{-1} can be clearly observed after alkali treatment and bleaching, which related to the bending vibration of the C-H and C-O bonds in polysaccharide aromatic rings [26].

A band around 1060 cm^{-1} that represents the C-O and C-H stretching vibration, confirms the structure of cellulose. Furthermore, the increase in the intensity of these groups indicates to the increase of the crystallinity of the samples [27,28]. Overall, the typical absorption band that appears in the extracted cellulose and nanocellulose from pomelo albedo spectra are similar to the characteristic of pure cellulose extracted from orange peel at 3426, 1631, 1434, 1031 and 895 cm^{-1} [19].

Crystallinity analysis

XRD pattern for all samples at different stages of treatments are shown in Figure 2. XRD analyses were done in order to determine the changes of the crystallinity and amorphous region of untreated and treated pomelo albedo. Alemdar and Sain [23] stated that crystallinity is expressed as the ratio of the diffraction from a crystalline region to the total diffraction of a sample. Therefore, the value of crystallinity index (CrI) was calculated using empirical Segal equation [29] and summarized in Table 2. Untreated pomelo albedo possesses the lowest percentage value of crystallinity (25.1%) since it contains a high amount of amorphous region.

However, upon chemical treatment (b) and (c), the crystallinity of the fibers slightly increased from 54.1% to 57.5% respectively, due to the removal of lignin and hemicellulose which exist in the amorphous region. The resultant cellulose was confirmed by the value from the diffactograms, where the common value of crystalline structure for cellulose I was shown by a major intensity peak located at a 2θ value of around 22°. Meanwhile, the amorphous region of cellulose I is characterized by the low diffracted intensity at a 2θ value of around 18° [30,31]. After sulfuric acid hydrolysis, the crystallinity of cellulose nanocrystals increased to 60.3%, implying that most of the amorphous regions were removed from the purified cellulose.

Overall, these data constitute good evidence to conclude that the obtained celluloses are partially crystalline celluloses. It was revealed that the amorphous portion quantitatively exceeds the crystalline portion. Additionally, the crystallinity indexes of these cellulose and nanocellulose are found to be lower than that of other common cellulose materials, at 70-80% [32]. This probably be due to the higher cellulose crystallinity which concurs a greater amorphous portion which consequently leads to greater permeability to water and other chemicals.

Water Holding Capacity (WHC)

The WHC of extracted cellulose and nanocellulose is as presented in Table 3. WHC is highly depending on several factors such as fiber processing the inherent chemical and physical structure of fiber [33] and its soluble dietary fiber content. The observed WHC for nanocellulose was higher than cellulose, with the value of 12.75 g water/g and 8.9 g water/g respectively. The size reduction from micron (µm) to nanometer (nm) produced smaller celluloses (nano) that are more uniform in size and has specific surface area that contributes to a better capacity of nanocellulose to hold large amount of water.

Moreover, the value of WHC of cellulose is lower than other types of citrus fiber, such as from grapefruit (9.77 g water/g) and orange (11 g water/g). This may be due to the removal of soluble components during the extraction to produce cellulose. These results suggest that nanocellulose isolated from pomelo albedo has great potential in food application as the new functional food ingredient, particularly as volume replacement, thickening or texturizing agent. The characteristics found in this citrus-based nanocellulose can be manipulated in the development of foods reduced in calories and that are rich in dietary fibre.

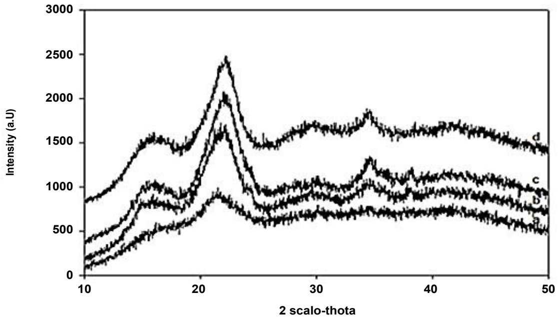

Figure 2: X-Ray diffraction patterns of (a) ground pomelo albedo (b) alkali-treated albedo, (c)bleached and (d) acid hydrolysed pomelo albedo fibers.

Samples	2θ (am)	2θ (002)	CrI (%)
Raw fibers	17.6	21.3	25.1
Alkali treated fibers	17.9	21.7	54.1
Bleached fibers	18.1	22.0	57.5
Acid hydrolysed fibers	18.3	22.2	60.3

Table 2: Crystallinity index (CrI) of pomelo albedo fibers at different stages of treatment

	Particle size	WHC (g water/g)
Cellulose	<500 µm	8.9^{b} ± 0.20
Nanocellulose	100-150 nm	12.75^{a} ± 0.63

(a) ground pomelo albedo (b) alkali-treated albedo

Table 3: Water holding capacity (WHC) of cellulose and nanocellulose from pomelo albedo

Conclusion

Cellulose and nanocellulose were successfully extracted from pomelo albedo. The physicochemical characterization data of these cellulose materials indicate good levels of purity, low crystallinities and good water holding capabilities. These findings proved that pamelo albedo can be utilized to produce valuable ingredients such as cellulose and nanocellulose which may be used in various food and industrial applications such as fillers in paper and other composites, water absorbents, or as raw materials for cellulose derivatives.

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References

1. Melèndez-Martínez AJ, Britton G, Vicario IM,Heredia FJ (2008) The complex carotenoid pattern of orange juices from concentrate. Food Chem 109: 546-553.
2. Roussos PA (2011) Phytochemicals and antioxidant capacity of orange (Citrus sinensis (l.) Osbeck cv. Salustiana) juice produced under organic and integrated farming system in Greece. Sci Hortic 129: 253-258.
3. Garcia-Castello EM, Mayor L, Chorques S, Argüelles A, Vidal-Brotons D, et al. (2011) Reverse osmosis concentration of press liquid from orange juice solid wastes: flux decline mechanisms. J Food Eng 106: 199-205.
4. Fernandez-Lopez J, Fernandez-Gines JM, Aleson-Carbonell L, Sendra E, Sayas-Barbera E, et al. (2004) Application of functional citrus by-products to meat products. Trends in Food Science & Technology 15: 176-185.
5. Pe rez-Alvarez JA, Fernandez-Lo pez J, Sayas-Barbera E, Navarro C, Sendra E (2001) Effect of citrus fiber (albedo) incorporation in cooked pork sausages. In Proceedings of International Food Technologists Annual Meeting.
6. Fernando F, María LH, Ana ME, Italo C, Fernando A (2005) Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. Food Chemistry 91: 395-401.
7. Kim J, Yun S, Ounaies Z (2006) Discovery of cellulose as a smart material. Macromolecules 39: 4202-4206.
8. John MJ, Thomas S (2008) Biofibres and biocomposites. Carbohydrate Polymers 71: 343-364.
9. Beck-Candanedo S, Roman M, Gray DG (2005) Effect of reaction conditions on the properties and behavior of wood cellulose nanocrystal suspensions. Biomolecules 6: 1048-1054.
10. AOAC (2000) Official methods of analysis of AOAC International (17thedn.). Gaithersburg, MD, USA: AOAC International.
11. Wright PJ, Wallis AFA (1998) Rapid determination of cellulose in plantation eucalypt woods to predict kraft pulp yield. TAPPI Journal 81: 126-130.
12. Segal L, Creely L, Martin AE, Conrad CM (1959) An empirical method for estimating the degree of crystallinity of native cellulose using X-ray diffractometer. Textile Research Journal 29: 786-794.
13. Traynham TL, Myers D, Carriquiry AL, Johnson LA (2007) Evaluation of Water-Holding Capacity for Wheat–Soy Flour Blends. Journal of the American Oil Chemists' Society 84: 151-155.
14. Brillouet JM, Rouau X, Hoebler C, Barry JL, Carre B, et al. (1988) A new method for determination of insoluble cell walls and soluble nonstarchy polysaccharides from plant materials. J Agri Food Chem 36: 969-979.
15. Ros JM, Schols HA, Voragen AGJ (1996) Extraction, characterisation, and enzymatic degradation of lemon peel pectins. Carbohydrate Research 282: 271-284.
16. Sariçoban C, Özalp B, Yilmaz MT, Özen G, Karakaya M, et al. (2008) Characteristics of meat emulsion systems as influenced by different levels of lemon albedo. Meat Sci 80: 599-606.
17. Nassar AG, AbdEl-Hamied AA, El-Naggar EA (2008) Effect of citrus by-products flour incorporation on chemical, rheological and organolepic characteristics of biscuits. World Journal of Agricultural Sciences 4: 612-616.
18. Marin FR, Soler-Rivas C, Benavente-Garcia O, Castillo J, Perez-Alvarez JA (2007) By-products from different citrus processes as a source of customized functional fibres. Food Chemistry 100: 736-741.
19. Bicu I, Mustata F (2011) Cellulose extraction from orange peel using sulfite digestion reagents Bioresource Technology 102: 10013-10019.
20. Grohmann K, Cameron RG, Buslig BS (1995) Fractionation and pretreatment of orange peel by dilute acid hydrolysis. Bioresource Technology 54: 129-141.
21. Sain M, Panthapulakkal S (2006) Bioprocess preparation of wheat straw fibres and their characterization. Industrial Crops and Products 23: 1-8.
22. Sgriccia N, Hawley M, Misra M (2008) Characterization of natural fibre surfaces and natural fibre composites. Composites Part A-Applied Science 39: 1632-1637.
23. Alemdar A, Sain M (2007) Isolation and characterization of nanofibres from agricultural residues – Wheat straw and soy hulls. Bioresource Technology 99: 1664-1671.
24. Ribeiro GC, Coelho LM, Oliviera E, Coelho MNN (2013) Removal of Cu (II) from ethanol fuel using mandarin peel as biosorbent. Bioresources 8: 3309-3321.
25. Khalil HPSA, Ismail H, Rozman HD, Ahmad MN (2001) The effect of acetylation on interfacial shear strength between plant fibre and various matrices. European Polymer Journal 37: 1037-1045.
26. Nacos M, Katapodis P, Pappas C, Daferera D, Tarantilis PA, et al. (2006) Kenaf xylan – A source of biologically active acidic oligosaccharides. Carbohydrate Polymers 66: 126-134.
27. Alemdar A, Sain M (2008) Biocomposites from wheat straw nanofibres: morphology, thermal and mechanical properties. Composites Science and Technology 68: 557-565.
28. Elanthikkal S, Gopalakrishnapanicker U, Varghese S, Guthrie JT (2010) Cellulose microfibres produced from banana plant wastes: Isolation and characterization. Carbohydrate polymers 80: 852-859.
29. Segal L, Creely L, Martin AE, Conrad CM (1959) An empirical method for estimating the degree of crystallinity of native cellulose using X-ray diffractometer. Textile Research Journal 29: 786-794.
30. Chen WS, Yu HP, Liu YX, Chen P, Zhang MX, et al. (2011) Individualization of cellulose nanofibres from wood using high-intensity ultrasonication combined with chemical pretreatments. Carbohydrate Polymers 83: 1804-1811.
31. Lu H, Gui Y, Zheng L, Liu X (2013) Morphological, crystalline, thermal and physicochemical properties of cellulose nanocrystals obtained from sweet potato residue. Food Research International 50: 121-128.
32. Reddy N, Yang Y (2009) Natural cellulose fibers from soybean straw. Bioresour Technol 100: 3593-3598.
33. Grigelmo-Miguel N, MartÍN-Belloso O (1999) Comparison of Dietary Fibre from by-Products of Processing Fruits and Greens and from Cereals. LWT - Food Science and Technology 32: 503-508.