Structural Characterization of Cellulose Obtained from Extraction Wastes of Graviola (*Annona muricata*) Leaf

Moonkyeung Choi¹, Yu-Ra Kang¹, In-Sook Lim², and Yoon Hyuk Chang¹

¹Department of Food and Nutrition, Kyung Hee University, Seoul 02447, Korea

²Chamhan Food, Inc., Gyeonggi 11018, Korea

ABSTRACT: Cellulose is one of the most common functional ingredients in food products and has been widely used as fat replacers and stabilizers. In the present study, the structural properties of cellulose obtained from extraction wastes of graviola (*Annona muricata*) leaf (CWG) were characterized via scanning electron microscope (SEM), X-ray diffraction (XRD), fourier transform-infrared (FTIR), and ¹³C nuclear magnetic resonance (NMR) analyses. Extraction and purification of CWG were accomplished by alkali treatment and bleaching processes. An elongated, fibrous structure of CWG was observed in SEM analysis. The XRD, FTIR, and ¹³C NMR spectra of CWG were compared with microcrystalline cellulose (control cellulose) and it was found that CWG exhibited similar structural characteristics to the control. XRD diffractogram of CWG showed typical peaks ($2\theta=15^\circ$ and 22.6°) of cellulose I. According to the specific peaks (898, 1,057, and 1,430 cm^{-1}) and chemical shifts (104.5, 88.5, 72~75, and 64.6 ppm) obtained by FTIR and NMR analyses, respectively, it was confirmed that cellulose was successfully extracted from CWG.

Keywords: graviola, graviola leaf, extraction wastes, cellulose, structural analysis

INTRODUCTION

Annona muricata, belonging to the family of Annonaceae, is distributed in tropical and subtropical areas such as Brazil, India, Philippines, and Central America. It is commonly known as 'graviola' and its leaves have been used for a variety of health problems by local communities because of its healing effects on headache, infections, diabetes, insomnia, and inflammation (1,2).

Cellulose is a type of insoluble dietary fiber and a major component of plant fiber composed of poly(1,4- β -D-anhydroglucopyranose) units. These linear glucan chain comprises highly regular intra- and intermolecular hydrogen bonds (3). Furthermore, it is one of the most common functional ingredients and has been used as fat replacer, fat reducing agent during frying, volume enhancer, binder, bulking agent, and stabilizer (4).

In general, graviola leaf has been consumed as infusion tea, concentrates, and concentrated powders in the food industry. In the production process of such products, extraction is essential. As a result of the extraction, a waste rich in dietary fiber is produced. A reuse of these extraction wastes can significantly reduce the amount of wastes that will be accumulated in the environment.

Thus, an efficient reuse of these wastes is of great importance, not only for minimizing the environmental impact, but also obtaining a higher benefit (5).

Although many researchers have reported the production of graviola leaf extracts using different solvents, including water and organic solvents, there are no studies covering the structural properties of cellulose obtained from extraction wastes of graviola leaf (CWG). Therefore, the objective of this study was to investigate the structural properties of CWG by means of scanning electron microscope (SEM), X-ray diffraction (XRD), fourier transform-infrared (FTIR), and nuclear magnetic resonance (NMR) analyses.

MATERIALS AND METHODS

Materials

Dried graviola leaf cultivated in Indonesia were purchased from DS International Co., Ltd. (Jeungpyeong, Korea) and stored in polyethylene bags at room temperature. Microcrystalline cellulose was purchased from Sigma Aldrich Co. (St. Louis, MO, USA) and used as control cellulose to compare structural properties with the cellulose obtained

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Correspondence to Yoon Hyuk Chang, Tel: +82-2-961-0552, E-mail: yhchang@khu.ac.kr

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from CWG. Chemicals and solvents were analytical grade.

Preparation of cellulose from CWG

Alkali treatment: The extraction wastes were used to produce cellulose from graviola leaf. The alkali treatment was performed to purify the cellulose by removing lignin and hemicellulose from CWG according to the method of Sheltami et al. (6) with some modifications. The ground CWG were treated with 2% (w/v) sodium hydroxide aqueous solution for 4 h at 100°C under mechanical stirring and washed several times with distilled water until the solvent was completely removed.

Bleaching process: Following alkali treatment, the material was bleached with a solution made up of equal parts (v:v) of acetate buffer and aqueous sodium chlorite (1.7% NaClO₂ in water, w/w) (6). The bleaching treatment was performed at 80°C for 4 h and the bleached material was washed repeatedly with distilled water until the pH of the material became neutral and subsequently dried at 50°C for 12 h in a dry oven.

Structural analysis of CWG

Field emission-SEM (FE-SEM): The morphological features of microcrystalline cellulose (control cellulose) and CWG were examined using a FE-SEM (SUPRA55, Carl Zeiss Meditec AG, Oberkochen, Germany). Before testing, the samples were fixed onto a specimen holder with the help of double sided carbon tape and then sputtered with platinum powder using an ion sputter coater. Each sample was observed at an accelerating potential of 10 kV under high vacuum condition of image magnifications of 200, 800, and 5,000 were used.

XRD: XRD patterns were obtained by a D8 Advance (Bruker AXS Advanced X-ray Solutions GmbH, Karlsruhe, Germany). X-ray generator tension and current were 40 kV and 40 Ma, respectively. Microcrystalline cellulose (control cellulose) and CWG were scanned through the range of 2 θ from 5° to 50°.

FTIR: FTIR spectra were measured using a FTIR spectrometer (Spectrum One Syste, PerkinElmer, Inc., Waltham, MA, USA). Microcrystalline cellulose (control cellulose) and CWG were ground with potassium bromide (KBr) and pressed into a disc. Spectral scanning range was 4,000 to 500 cm⁻¹.

¹³C solid-state NMR spectroscopy: Cross polarization (CP)/MAS ¹³C solid-state NMR experiments were performed on a Bruker Advance II 500 MHz spectrometer (Bruker Corp., Billerica, MA, USA) operating at 25°C. The spectrometer was equipped with a 4 mm ¹H/BB magic angle spinning probe. The operating frequency of carbon was 125 MHz. The spinning rate was 5 kHz, acquisition time was 50 milliseconds (ms), contact time was 2 ms, and delay time was 5 s.

RESULTS AND DISCUSSION

Extraction and purification of CWG

In the food industry, graviola leaf has been mainly used as food materials in the forms of infusion tea, extract concentrate or concentrated dry powder. Thus, it has been reported that there is a large amount of extraction wastes after producing them. Generally speaking, CWG have no economic values, although they are rich in cellulose, which can be utilized as a food additive in many food products. In most cases, a huge amount of wastes is burned or disposed of. Therefore, this study was conducted to increase the utilization of CWG and to search the new cellulose source.

The cellulose from various plant materials, such as cassava bagasse (7), mulberry bark (8), and pineapple leaf (5), was usually obtained by alkali treatment and bleaching process. Thus, in the present study, alkali treatment using NaOH and bleaching process were used to produce CWG. Fig. 1 shows the photographs of CWG after each stage of treatment. After alkaline treatment, the color of

Fig. 1. Photographs of (A) extraction wastes of graviola leaf (CWG), (B) alkali-treated CWG, and (C) alkali-treated and bleached CWG.

Fig. 2. Field emission scanning electron microphotograph of microcrystalline cellulose (control cellulose) and cellulose obtained from extraction wastes of graviola leaf (CWG). (A) control cellulose 200 $\times$, (B) control cellulose 800 $\times$, (C) control cellulose 5,000 $\times$, (D) CWG 200 $\times$, (E) CWG 800 $\times$, and (F) CWG 5,000 $\times$.

CWG was changed from greenish brown to brownish orange. After bleaching treatment, the color of CWG became yellowish white. The color changes of CWG were due to the removal of non-cellulosic materials and other impurities such as lignin, hemicelluloses, pectin, and wax from CWG after chemical treatments (9).

SEM analysis

Fig. 2 indicates SEM photographs of microcrystalline cellulose (control cellulose) and CWG. All CWG had larger particle sizes than the control cellulose. According to the manufacture's guidelines, the control cellulose was obtained by the acidic hydrolysis of cellulose fiber. On the other hand, CWG was produced without acidic hydrolysis. Therefore, the lower particle size of the control cellulose can be associated with the breakdown of the cellulose fiber during acidic hydrolysis.

XRD spectral analysis

Cellulose has a crystalline structure, differing from hemicellulose and lignin, which are amorphous in nature. According to Zhang and Lynd (10), cellulose has a crystalline structure due to hydrogen bonding and Van der Waals forces between adjacent molecules.

Fig. 3 shows the XRD patterns for the control cellulose and CWG. According to Klemm et al. (11) and Mahadeva et al. (12), the typical peaks of cellulose I are usually observed at 2θ values of around 15° and 22.6° . We found that both control cellulose and CWG had typical peaks at 2θ values of 15° and 22.6° , consequently indicating that CWG is type I cellulose.

In the XRD diffractogram, the peak at around $2\theta = 22.6^\circ$ of the control cellulose was sharper than that of the CWG. Alemдар and Sain (13) reported that a sharper diffraction peak was correlated with a higher degree of crystallinity in the cellulose structure. Accordingly, it was suggested in the present study that CWG had a lower degree of crystallinity compared to the control cellulose used in the food industry. Furthermore, Yoshida et al. (14) reported that a higher crystalline structure hindered an enzymatic hydrolysis of cellulose. In other words, an enzyme like cellulase can hydrolyze amorphous cellulose faster than crystalline cellulose. Therefore, in our study, CWG with a less crystalline structure can be used in various ways in food or biomass industries when compared to microcrystalline cellulose with a more crystalline structure.

Fig. 3. X-ray diffraction diffractograms of (A) microcrystalline cellulose (control cellulose) and (B) cellulose obtained from extraction wastes of graviola leaf (CWG).

ture because CWG can be hydrolyzed by enzymes more efficiently.

FTIR spectral analysis

FTIR analysis can be used to analyze compounds for their chemical structure and confirm their identities of organic and inorganic materials (15). The fingerprints of the functional groups obtained for CWG are labelled in Fig. 4 and were very similar to those of the control cellulose. In particular, the absorbance bands at around 898, 1,057, and 1,430 cm^{-1} were associated with the C-H rocking vibration, C-O stretching vibration, and symmetric CH_2 bending vibration, respectively, where these peaks are referred to cellulose form of the carbohydrates. Besides the above mentioned peaks, the peak at 1,640 cm^{-1} reflects the stretching O-H groups corresponds to the absorption of water and the peak at 2,900 cm^{-1} reflects the stretching vibration of the C-H groups. Additionally, the peak at 3,410 cm^{-1} is representative of the O-H groups and assigned to the adsorbed water (6,13,16). Consequently, based on the results, it was clearly confirmed

that the extraction of cellulose from CWG was successfully accomplished.

^{13}C solid-state NMR spectral analysis

^{13}C solid-state NMR spectra and chemical shifts of the control cellulose and CWG are shown in Fig. 5 and Table 1, respectively. Peaks have been assigned based on the previous literature (17-19) and are displayed on the spectra. Both the control cellulose and CWG spectra showed the corresponding signals for cellulose. All noticeable peaks of the carbon atoms corresponding to cellulose occurred between 60 and 110 ppm. The sharp peaks at 104.5 and 64.6 ppm are associated with ordered cellulose C-1 and C-6 carbons, respectively (17,18), while the peaks at 82.7 and 62.9 ppm were related to the disordered cellulose C-4 and C-6 carbons as well as to the less ordered cellulose chains of the crystallite surfaces (17, 19). Furthermore, the peaks around at 72~75 ppm corresponded to the C-2, C-3, and C-5 carbons of cellulose (18). Based on the results obtained from ^{13}C solid-NMR spectroscopy analysis, the existence of cellulose comprising CWG was clearly proved.

In conclusion, in the present study, the structure of CWG was elucidated by FTIR, XRD, and ^{13}C solid-NMR

Fig. 4. Fourier transform-infrared spectra of (A) microcrystalline cellulose (control cellulose) and (B) cellulose obtained from extraction wastes of graviola leaf (CWG).

Table 1. ^{13}C -chemical shifts of microcrystalline cellulose (control cellulose) and cellulose obtained from extraction wastes of graviola leaf (CWG)

IUPAC no. of carbon atom	^{13}C (ppm)	
	Control cellulose	CWG
1	104.47	104.54
2, 3, 5	72.27	71.98
	74.57	74.59
4	82.72	82.46
	88.47	88.47
6	62.94	62.28
	64.68	64.57

Fig. 5. ^{13}C solid-state nuclear magnetic resonance spectra of (A) microcrystalline cellulose (control cellulose) and (B) cellulose obtained from extraction wastes of graviola leaf (CWG).

spectroscopy analyses in comparison with microcrystalline cellulose as the control. The obtained results apparently indicated that non-cellulosic materials such as lignin and hemicellulose were removed from CWG during the alkali treatment and bleaching process, consequently leading to production of cellulose. We expect that CWG can be utilized in the food industry as cellulose-based additives like fat replacers and stabilizers or dietary supplements.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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