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Comparative Study of Bacterial Cellulose Film Dried Using Microwave and Air Convection Heating

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Abstract. An investigation was conducted to analyze and compare the properties of bacterial cellulose (BC) films dried using microwave and air convection heating. Prior to the drying process, 90% of the water content inside the BC pellicles was removed through vacuum filtration. After that, the required drying time was only 3-5 min using microwave heating, 95% shorter than that observed for air convection heating. The properties of the BC sheets were characterized by Fourier transform infrared (FTIR), X-ray diffractometer (XRD), color difference meter, and tensile tester machine. The results showed that the structure of the BC films was the same for the BC films dried by microwave and air convection heating, i.e. cellulose I, as observed from FTIR spectra and XRD diagrams. Moreover, their color (L*, a*, and b* values) and mechanical properties were also almost identical. However, a slightly lower crystallinity and a higher swelling degree were observed for the BC film dried using microwave heating. These results suggest that microwave heating could be an alternative method of drying BC pellicles in order to shorten the processing time and reduce energy consumption when compared to air convection heating.

Keywords: *bacterial cellulose; convection oven; edible film; microwave; swelling; vacuum filtration.*

1 Introduction

Bacterial cellulose (BC) has gained considerable attention as an in-depth research topic because of its remarkable properties, including high crystallinity, high tensile strength, high water holding capacity, ultrafine fiber network and simple production [1-3]. Because of its biocompatibility, BC has been applied in wet form in numerous medical applications, for example for blood vessels and scaffolds in tissue engineering of cartilages [4,5] as well as in desserts, fruit cocktails, or fruit jellies. BC is also used in dry form (film, powder) in applications such as acoustic membranes, optic films and edible films [6,7].

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Drying is an important factor in preparing BC films because the applied drying conditions and methods can greatly affect the films' functionalities and properties. Several researchers have reported methods to prepare BC films, namely vacuum drying, supercritical drying, or freeze drying [8-10]. Zhang *et al.* employed vacuum drying to obtain BC films, resulting in some defects, such as cranny and cavity configuration as a result of speeding up the moisture evaporation, which led to a decline in mechanical properties [10]. The supercritical drying process produced very light films with more than 96% porosity but it involves complex procedures, while the freeze drying method required more than 10 h to get dry films [8]. Among these methods, drying at room temperature or hot air flow drying are the methods most commonly used to obtain dried BC film because of their simplicity. However, it should be noted that they require a long time to remove the water from the BC pellicles, whereas for industrial applications generally a fairly rapid drying process is needed.

Recently, the idea of using microwave heating for drying products, such as cotton cellulose or garlic powder as well as edible films, is on the rise because of its fast heating rate, low cost and simple process [11-14]. Microwaves are high-frequency electromagnetic waves with wavelengths in the range of 1 mm to 1 m and the frequency range of 300 MHz to 300 GHz [15]. This process involves the ability of materials to absorb microwave energy and then transform it into heat. In cellulose, the energy is expected to be absorbed selectively by the highly polar water molecules inside the materials. Therefore, microwaves transfer energy more rapidly and efficiently than convection heating, which relies on thermal conductivity. In the scientific literature, no studies on using microwave heating to dry BC can be found.

The objective of this study was to dry BC pellicles using microwave heating and study their properties. For comparison purposes, BC pellicles were also dried by air convection heating and characterized. For this purpose, 90% of the water content inside the BC pellicles was removed prior to the drying process. It is important to establish that the properties of the dried BC pellicles were not affected by the microwave treatment and resulted in similar or better properties than with conventional drying methods. Therefore, the structural, physical and mechanical properties of the BC films obtained from both drying methods were investigated and compared.

2 Methodology

2.1 Materials

Coconut water and sugar were bought at a traditional market in Bandung, Indonesia. Bacterial inoculum was obtained from a local industry in Cianjur, Province of West Java, Indonesia. Chemicals such as acetic acid glacial and sodium hydroxide were purchased from Merck.

2.2 Production of Bacterial Cellulose

In the present study, coconut water was used as the main culture media for BC production. The coconut water was filtered and mixed with sugar (75 g/l), after which the pH was adjusted to 3.0 with acetic acid glacial. The liquid media was sterilized for 30 min and cooled down to room temperature. Protein denaturation due to the heating process is negligible since the growth of bacterial cellulose is not affected by different levels of nitrogen sources [16]. The medium was then put into several plastic containers, which had been placed in an oven at 50 °C for 24 hours prior to use for inoculation. Then, the bacterial inoculum was added to it with a ratio inoculum : medium of 1 : 5. Static incubations were performed at room temperature until the thickness of the BC pellicles was about 1 cm. The BC was harvested and washed repeatedly with running tap water until pH was neutral. Then, purification of the BC was carried out according to the previous method in [17] using 2% sodium hydroxide solution for 1 hour to remove any remaining organic materials embedded in the cellulose materials. The purified BC was then thoroughly washed with distilled water until pH was neutral.

2.3 Drying Process

About 18 x 12 cm of purified BC pellicles were cut and then vacuum filtrated to remove 90% of the water content in the BC pellicles. The water content was calculated by gravimetric method. The almost-dry BC sheet was then placed on the glass tray of a domestic microwave oven (MW) (Sharp model R-298H, 800 W, 2450 MHz) and heated at full power. The samples were dried until their moisture content was below 5% and the process times were noted. In the case of air convection heating, vacuum filtration was also performed to remove the same amount of water content. The almost-dry sheet was placed on the same glass tray used for the MW and dried in an air convection oven (Memmert) at 40 °C until the moisture content was below 5%. Up to 8 films for each drying method were prepared and the drying time was averaged. The dried BC films are referred to as BC-MW and BC-AC, indicating final drying with microwave and air convection heating, respectively.

2.4 Structural Analysis using Fourier Transform Infrared (FTIR) Spectroscopy and X-Ray Diffraction (XRD)

The film structure was investigated by Nicolet iS10 FT-IR spectrometer (Thermo Fisher Scientific) in attenuated total reflectance (ATR) mode. The spectra were collected by using air as a background from 4000 to 500 cm^{-1} at room temperature. A total of a 32 cumulative scans were made per sample with a resolution of 8 cm^{-1} in absorbance mode. Two samples were measured for each method.

An X-ray diffractometer (XRD), model D-2400 (Rigaku, Japan) was used to analyze the structure and crystallinity index of the resulted BC films. The films were placed in the X-ray holder and scans were performed over 5-40° at a speed of 4°/min. The wavelength of the CuKα radiation source used was 0.154 nm, generated at an accelerating voltage of 40 kV and a filament emission of 30 mA. The crystallinity index (CI) was estimated using the peak height method as shown in Eq. (1) [8,18].

$$\text{Crystallinity Index (\%)} = \frac{I_{200} - I_{min}}{I_{200}} x100\% \quad (1)$$

where I_{200} corresponds to the maximum intensity found between the scattering angles of 2θ = 22° and 23° and I_{min} corresponds to the intensity of the minimum peak, typically between 2θ = 18° and 19°, which accounts for the amorphous part of the cellulose. Two samples for each method were measured.

2.5 Physical Properties of Bacterial Cellulose Films

The percentage of swelling was determined according to the method reported by Wong, *et al.* [19] with some modifications. Three specimens for each sample were prepared with a size of 2 x 2 cm. These specimens were dried in an oven at 110 °C for 24 h prior to weighing using an analytical balance (Mettler Toledo AL204) to ensure that no water content remained in the samples. After that, the samples were immersed in 20 mL of distilled water at room temperature for 72 hours. The swelling degree was calculated with the following Eq. (2):

$$\text{Degree of swelling (\%)} = \frac{W_s - W_i}{W_i} x100\% \quad (2)$$

where Ws represents the mass of the film after 72 h soaking in water (mg) and Wi represents the mass of the samples in dried state (mg).

Color values of the films were determined by color difference meter (Murakami Color Research Lab). The film specimen was placed on the surface of a standard white plate (L = 93.8, a = -0.7, and b = 1.4). The values of L* (lightness), a* (redness/greenness), b* (yellowness/blueness) were evaluated by reflectance measurement. The analysis was conducted in duplicate and the mean values were computed.

2.6 Mechanical Properties of Bacterial Cellulose Films

After pre-conditioning at 23 °C and 50% for at least 48 hours, the films were cut using a dumbell punch according to ISO 527-2 type 5A. The thicknesses of the specimens were measured at five points along a narrow area with ±0.001 mm precision using a digital micrometer (Mitutoyo, Japan) and averaged. The specimens were placed vertically onto a tensile grip clamp with a 50-mm initial grip separation and the stress-strain curves were recorded with a tensile test machine (Orientec UCT-5T) according to the ISO 527-1993E standard method. The cross-head speed was set at 10 mm/min. At least 5 specimens were tested for each sample.

3 Result and Discussion

The microwave heating process was designed to reduce the drying time while maintaining the unique characteristics of BC films. BC pellicles contain a huge amount of water (more than 95%) so that reducing the water content to below 20% is important to shorten the total drying time. In this work, vacuum filtration was used to squeeze the BC pellicles and force the water out of the pellicles. A significant amount of water reduction could be observed by the decline of the BC film's thickness, which was proportional to the decrease of the water content in the BC.

Based on visual assessment, the physical appearances of the samples were slightly different. After microwave heating, the surface of the BC films was quite smooth; elucidating rapid drying using the microwave oven did not influence the physical appearance of the BC film. This could be because in the microwave drying method, the energy being absorbed by the water molecules inside the BC pellicles and the vibration of the water molecules lead to distribution of the heat throughout the BC gel. Therefore, heating by microwaves occurs in the bulk of the material and not only on the surface, as it occurs in conventional heating [20]. Moreover, rapid water evaporation can retain the tension inside the BC fibers so that a smooth surface can be obtained. Zhang, *et al.* revealed that microwave drying can be the most effective technique to dry products of moisture content below 20%, as also performed in this research [21]. In air convection, wrinkled films are observed as a result of the heat transfer mechanism. This is because the speed of water evaporation is too fast for the BC fibers to be able to retain their integrity and dimensions as a result of the heat transfer mechanism in air convection heating.

More than five samples were dried by microwave and air convection heating. Because of the volumetric and spatial heating of microwave radiation, the drying time decreased significantly compared to air convection heating, in agreement with a previous study [22]. It was recorded that the required drying time was 3-5 min with microwave heating, while it took 3 to 5 hours with air convection heating. For further analysis to ensure that the microwave heating did not influence the structure and properties of the BC films, the films from the 3-min microwave and 5-hour air convection heating were selected due to the best physical appearance of these films.

3.1 Structural Analysis of Bacterial Cellulose Films

FT-IR spectroscopy has been shown to be a rapid, simple and non-destructive tool for studying polysaccharide structures. Cellulose can consist of cellulose I (native cellulose), cellulose II (regenerated cellulose), and/or amorphous cellulose, which can be distinguished from the absorption bands of their FTIR spectra. Figure 1 shows a comparison of the FTIR spectra for both drying methods, confirming the presence of common functionalities of cellulose in BC. The figure also shows that the FTIR spectrum of BC-MW was the same as that of BC-AC, which means the structures of both films were the same. This phenomenon reveals that the rapid water evaporation in microwave heating process did not cause changes in the molecular structure.

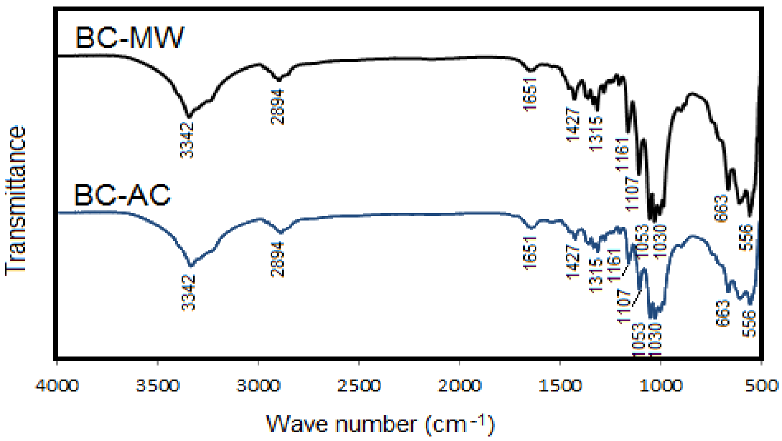

Figure 1 FTIR spectra of BC films final dried by microwave (BC-MW) and air convection heating (BC-AC).

Moreover, strong hydrogen bonding between cellulose chains was presented by broad peaks centered at 3342 cm^{-1} for both drying methods, as shown in Figure 1. A sharp peak appeared at 2894 cm^{-1} and 1427 cm^{-1}, showing the CH stretching arising from symmetric deformation of CH [23]. The observed peak at 1161 cm^{-1} indicates C-O-C stretching and the peaks at 1030 cm^{-1} to 1053 cm^{-1} are associated with C-O stretching [24]. These peaks confirm that the BC films obtained in this work was cellulose I, which is composed of monoclinic Iβ and triclinic Iα. According to Sugiyama, *et al.*, the band near 750 and 3240 cm^{-1} can be designated as triclinic Iα crystalline cellulose, whereas those near 710 and 3270 cm^{-1} are indicative of monoclinic Iβ cellulose [25]. Unfortunately, these peaks were obscured in the spectra obtained here because of the broad peak at about 3200 cm^{-1}.

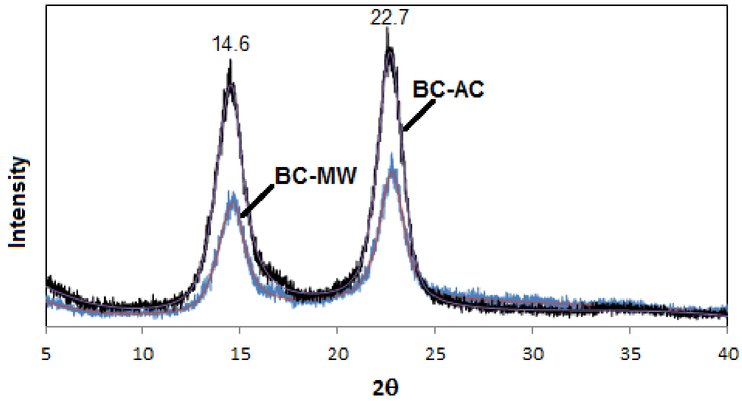

Figure 2 XRD patterns of BC films final dried by microwave (BC-MW) and air convection heating (BC-AC).

Figure 2 shows XRD patterns in the range of 2θ = 5-40°. Two strong diffraction peaks at ~14° and ~22° are the common XRD spectra of BC, which are sometimes accompanied by another relatively weak peak at ~16° [26]. Herein both XRD patterns showed two distinct peaks at 2θ = 14.6° and 22.7°, supporting the evidence from the FTIR spectra that both drying methods resulted in BC films with identical structures. These peaks are also the typical profile of cellulose I. The peak at 2θ = 14.6° represents the projection of the (110) plane of cellulose Iα and the $(1\bar{1}0)$ plane of cellulose Iβ in crystalline form, while the peak at 2θ = 22.7° represents the projection of (110) cellulose Iα and (200) cellulose Iβ [8,27,28]. The peaks as shown in Figure 2 belong to cellulose Iβ, as also reported in other studies [10,29]. The structure of cellulose Iβ, appearing in the BC films because of the alkali treatment prior to the drying process. Alkali treatment or hydrothermal annealing can convert Iα, which preponderates in cellulose from bacterial origin into cellulose Iβ [8,26]. Moreover, BC is a semi-crystalline material, hence broad diffraction peaks are usually observed [8].

According to the peak height method, the crystallinity index for BC-AC and BC-MW was 88.5 ± 0.7% and 84.0 ± 2.4%, respectively. The higher crystallinity index of the BC-AC film compared to that of the BC-MW film suggests a higher degree of intra- and intermolecular hydrogen bonding between the cellulose chains in the BC-AC film [30]. The energy from the microwaves may disturb the hydrogen bonding between the BC's microcrystalline chains, resulting in the lower crystallinity of BC-MW. It is also possible that the longer time of air convection drying allowed more time for cellulose molecules to move and better orient themselves, resulting in higher crystallinity. The values of crystallinity in this work are comparable with the values reported by Pa'e, *et al.*, who found a crystallinity index of about 87-89% when drying BC using oven, tray and freeze drying [9].

3.2 Physical Properties of Bacterial Cellulose Films

The swelling ability of biopolymer film is one of the most important properties for application as a food packaging or edible film. The drying process may influence the swelling properties of the films due to a reduction in the plasticizing effect of the water molecules and promotion of the formation of additional hydrogen bonds [31]. In this work, the films from microwave heating showed a higher swelling degree compared to the air convection films, as shown in Table 2. This is consistent with the lower crystallinity of the BC-MW films. The swelling degrees for both samples were in the range of 210-280%, which is comparable to the values observed by Pa'e *et al.* [9]. Based on our observations, the films still maintained their integrity in the swelling study, even after more than 72 h. This means that the dried BC films did not dissolve in water.

The film's color can be a factor in consumer acceptance. The L*, a*, and b* values for both drying methods are presented in Table 1. It shows that both drying methods gave similar color values. The value of L* (> 90) indicates that BC films tend to be white and this agrees with our visual observation, which showed that the BC films were white and opaque. A browning reaction because of the higher temperature in microwave heating compared to air convection heating did not happen in the BC films. This observation disagrees with that reported by Mayachiew & Devahastin [32]. The negative values of a* and positive values of b* indicate that the BC films tended to be green and yellow, respectively.

Table 1 Swelling degrees and color properties of bacterial cellulose films.

Drying method	**Swelling degree (%)**	**Color**		
		L*	**a***	**b***
Air convection oven	242 ± 34	94.3	-4.4	5.0
Microwave	264 ± 26	93.7	-3.1	4.9

3.3 Mechanical Properties of Bacterial Cellulose Films

Figures 3(a) and 3(b) show the tensile strength and elongation of the dried BC films. The tensile strengths of BC-MW and BC-AC were almost the same, both within the range of the standard deviation. On the other hand, BC-MW had lower elongation at break than BC-AC. The higher elongation of the BC-AC sample may have been caused by the extension of wrinkles found in this film, as previously mentioned. Furthermore, microwave heating may slightly compress the fibril arrangement so that it becomes difficult to extend with the applied force, thus resulting in lower elongation. The mechanical behavior of the BC-dried films obtained in this work were much higher than in a previous report [9]. This may be due to differences in treatment after harvesting. In this study, the BC gels were subjected to an alkali treatment prior to drying, which could have improved the mechanical properties of the BC [6,33].

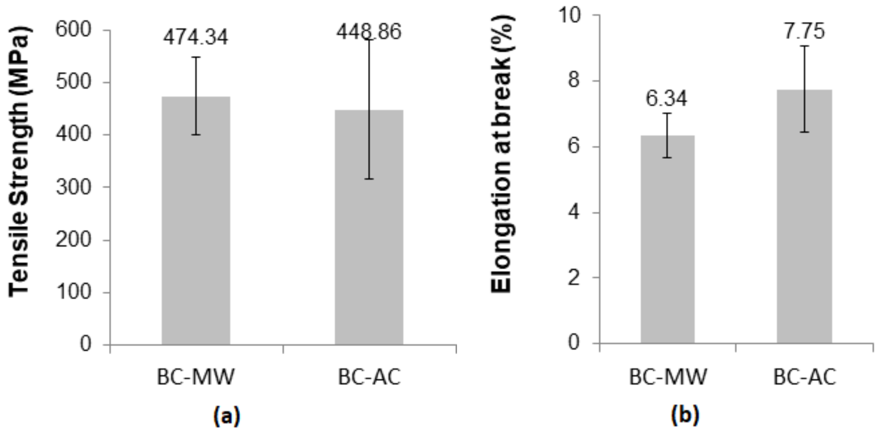

Figure 3 Mechanical properties of BC films final dried by microwave (BC-MW) and air convection heating (BC-AC): (a) tensile strength, and (b) elongation at break.

4 Conclusion

BC films were successfully obtained by microwave heating, offering a great advantage in terms of treatment time and effectiveness. Film drying using microwave heating was 95% faster compared to using air convection heating. The results showed that there were no significant structural or physicochemical behavior differences in their properties between microwave and air convection heating as confirmed through analysis of FTIR, XRD, color and mechanical properties. The structure of cellulose I was observed in the FT-IR spectra and XRD diagrams of the BC films. However, slightly lower crystallinity and higher swelling degree were observed for the BC film dried by microwave heating. This phenomenon opens up the possibility to use microwave heating to dry BC.

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