Preparation and characterization of bacterial cellulose-beeswax films

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Abstract. Bacterial cellulose (BC) based polymer films containing carboxymethyl cellulose (CMC) and beeswax as additives were produced. Control film was prepared by mixing of BC slurry and CMC, while treated films were produced by the addition of beeswax/Tween 80 solution in various concentrations. The objective of this work was to study the effect of beeswax content on properties of BC films. The films were prepared by solution casting technique and dried in convection oven at 40 °C overnight. Addition of beeswax dissolved in Tween 80 affected mechanical properties, significantly reducing the tensile strength but increasing elongation of the control film by 22 and 43%, respectively. Beeswax+Tween80 addition also improved water solubility by doubled from 36 to 73-78%. However, there was no significant difference of WVTR and hydrophobic properties among those films with the addition of beeswax in Tween 80 into the mixtures of BC slurry+CMC.

1. Introduction

Cellulose is one of the most abundant polysaccharide in nature. It is the main constituent in the cell wall of vascular plants, where it is in complex with hemicelluloses, pectin, the aromatic polymer lignin and numerous glycoproteins [1]. In addition to plants, numerous microorganisms, such as green algae and oomycetes and various bacteria are able to produce cellulose as well. Bacteria Acetobacter strain can produce bacterial cellulose (BC), which has similar molecular formula with plant cellulose. However, BC is free of hemicellulose, pectin, lignin, and other biogenetic products associated with plant cellulose [2]. BC also exhibits remarkable properties such as high degree of polymerization [3], high water holding capacity [4], high crystallinity [5], and high mechanical strength [6]. Due to its biocompatibility and biodegradability, BC has been proposed for use in numerous medical applications [7-9] or as suitable material for edible films [10,11].

The main interest in edible films and coatings is generally based on their potential to control some properties, such as moisture, oxygen, flavor and/or aroma transfer, either between food components or to/from the atmosphere surrounding the food, with a resulting increase in quality and shelf-life of the food [12]. For edible film to be used in food area, it should have appropriate water barrier properties, good mechanical strength, low cost of raw materials, and simple technology for production [13]. BC is considered to be able to fulfill those requirements except for its water barrier property and flexibility. To overcome this drawback, BC can be combined with hydrophobic compounds, such as neutral lipids, fatty acids or waxes. Among lipids, beeswax (BW) has been successfully used to reduce the water vapor permeability (WVP) of biodegradable films [14,15] due to its high hydrophobicity and solid state at
room temperature [16,17]. Moreover, it has been reported that BW could produce plasticizing effect to whey protein films [12]. BW is a mixture of many organic compounds, including wax esters, fatty acids, and hydrocarbons that exhibits viscoelastic behavior [18].

BC based films incorporated with BW have not been explored yet. This study will be another approach to improve biopolymer film properties for application in packaging field. Therefore, in the present study, effect of BW on the properties of BC-based films is investigated. Parameters such as thickness, solubility, water vapor transmission rate and water vapor permeability, and mechanical properties will be evaluated.

2. Experiment

2.1. Preparation of BC-based edible films

BC gels were purchased from local industry in Cianjur, West Java province, Indonesia. Washing, purification, and preparation of BC slurry were conducted as previously report [10]. A quantity of 200 g of BC slurry was casted on a non-sticky tray and dried overnight at 40 °C in a gear oven. The dried BC film was weighed and noted as the weight control for BC fiber. Another 200 g of BC slurry was stirred at 60 °C and added with carboxymethyl cellulose (CMC, Himedia). As the hydrophobic agent, BW (Cera Alba) with varied concentration was emulsified with 0.5 ml of Tween 80 (polyoxyl sorbitan monostearate, Merck). This solution was added into BC+CMC mixture and stirred until homogenous mixture was achieved. The blends were then degassed using a vacuum bell jar to remove the bubbles prior to cast onto the tray. Casting was conducted overnight at 40 °C in gear oven. BC based films were prepared according to formula in Table 1.

| Table 1. Formulation of BC based films incorporated with BW. |
|---------------------------------------------------------------|
| Sample | BC slurry (g) | CMC (g) | BW (g) | Tween 80 (mL) |
|--------|---------------|---------|--------|---------------|
| A      | 200           | 0.5     | 0      | 0.5           |
| B      | 200           | 0.5     | 0.005  | 0.5           |
| C      | 200           | 0.5     | 0.010  | 0.5           |
| D      | 200           | 0.5     | 0.015  | 0.5           |

2.2. Characterization of BC-based edible films

Film thickness was measured before the WVP test using a digital micrometer (Mitutoyo, Japan), taking measurements at 3 random positions on the film. Averaged values of the thickness were used in all the WVP calculations. Solubility was calculated as the percentage of film dry matter dissolved during immersion in distilled water for 24 h. Pieces of film (2 cm x 2 cm) were cut from each film and dried at 105 °C in an oven for 5 h. Samples were weighed to the nearest 0.0001 g and noted as initial dry weight (w₀). Samples were then immersed in 50 ml distiller water for 24 h under constant stirring at 100 rpm. Undissolved film materials were filtered through nylon filter, followed by oven drying at 105 °C for 24 h. The samples were then weighed and noted as wᵢ. Samples were measured in triplicate and the percentage of total soluble matter (% solubility) was calculated as follow.

\[
\% \text{ solubility} = \left( \frac{w₀ - wᵢ}{w₀} \right) \times 100\%
\]

(1)

The mechanical properties were characterized by Orientec UCT-5T universal testing with 100 kgf load cell according to ISO 527- 1993E standard method. The samples were cut by dumbbell cutter according to ISO 527-2 type 5A. Prior to measurement, the thickness of each specimen along the narrow area was measured using digital micrometer (Mitutoyo) and averaged. At least five specimens of each sample were measured and computerized calculated to obtain the average value. The specimens were mounted in the film-extension grips of the testing machine and stretched at a rate of 10 mm/min until breaking. The measurement was conducted at 23 °C and RH 50%.
Water vapor transmission rate (WVTR) of films was determined gravimetrically according to ASTM E96, modified by Gontard et al [19]. The test film was sealed to a glass cup having 16 cm² mouth areas and containing 10 g of dried silica gel (0% RH; 0 mmHg water vapor pressure). The cups were placed in a desiccator maintained at 100% RH (32.23 mmHg water vapor pressure) with distilled water. The water vapor transferred through the film and absorbed by the desiccant was determined from the weight gain of the silica gel recorded at various times. The cups were weighed using analytical balance at 24, 38, 72, and 96 h intervals. Changes in weight of the permeation cell were recorded to the nearest 0.0001 g. All tests were conducted in triplicate at room temperature. WVTR and WVP were determined using the following equations (Equation 1, 2):

\[
WVTR = \frac{\Delta m}{\Delta t A} 
\]

\[
WVP = WVTR \left( \frac{L}{\Delta p} \right) 
\]

where \(\Delta m/\Delta t\) is the weight gain per time (g/day), \(A\) is the exposed surface area of the film (m²), \(L\) is the thickness of the film (mm), and \(\Delta p\) is the difference of partial pressure (32.23 mmHg) [19].

3. Result and discussion

3.1. Film formation

Films were slightly white in color with opaque appearance as a function of BW concentration (Figure 1). They were homogenous that indicates good compatibility and the film’s forming capability among those materials. BW is hydrophobic, therefore surfactant is needed for water based formulation. In this work, Tween 80 served as non-ionic surfactant and used as an emulsifier for improving miscibility of BW in aqueous BC slurry due to its high hydrophilic lipophilic (HLB) value i.e. 15 [20]. Moreover, no air bubbles or holes were observed throughout the films since all bubbles have been removed by degassing techniques prior to casting the films. These air bubbles were a concern for film production, because they could produce a reduction in the tensile strength and other film properties [21]. Control film (sample A) made with BC and CMC was strong enough and slightly rigid when stretched manually by hand. Modified films with BW and Tween 80 (sample B, C, and D) were more opaque in appearance and flexible enough to be handled. The addition of BW affected the films thicknesses (Table 2). When the wax content increased, the higher molecular contact between cellulose (BC and CMC) and wax compound might weaken the aggregation forces of the polymer chain, making the matrix more open [22].

![Figure 1. Physical appearance of BC based films without (A) and with (B, C, D) BW.](image)

| Sample | Thickness (µm) | Solubility (%) |
|--------|----------------|----------------|
| A      | 32.7 ± 6.8     | 33.5 ± 4.0     |
| B      | 35.0 ± 4.0     | 78.8 ± 8.1     |
| C      | 41.3 ± 2.3     | 79.5 ± 1.9     |
| D      | 49.7 ± 8.1     | 66.6 ± 14.0    |
3.2. Water solubility

Film solubility in water is an important factor for edible applications. For example, films for water soluble pouches or edible strips must be readily soluble, while films on high-moisture foods must be insoluble. Table 2 shows water solubility of the films. BC films without BW were partially soluble in water of which its solubility 33.5±4.0% maintaining their integrity during immersion because of its rigid long chains and strong intra and inters hydrogen bonds [23]. Low percentage of solubility of BC-based edible films elucidates that only CMC was dissolved in water while the remaining solid were fragmented BC fibers. On the other hand, films with BW were highly soluble with its solubility ranging of 67-79% and broken apart in water after 24 h of immersion. This trend was in contrast to what have been reported previously [14] in which increasing the amount of BW decreased solubility. This indicates that the addition of BW into BC films weakened intra-molecular interactions in the aqueous condition compared to those of films without BW. The relatively high solubility of BC-BW films in water could possibly make the films appropriate for soluble pouches, like cellulose ether-based soluble pouch which is currently available commercially [24].

3.3. Mechanical properties of BC-based edible films

![Figure 2. Mechanical properties of BC-based edible films with variation of beeswax.](image)

Tensile properties are essential for edible films and coatings. It relates to durability as well as coating ability to increase mechanical integrity of foods [12]. High tensile strength (TS) is necessary for edible film in order to withstand the normal stress encountered during the application, shipping, and
food handling. However, flexibility of edible films, i.e. elongation at break (EB) is also required for application of edible films. Elastic modulus (EM) is a measure of the intrinsic stiffness, durability or resilience of a film.

Comparison of mechanical properties of BC based films, with and without BW is depicted in Figure 2. TS of control film (BC+CMC) was 118.4±28.2 MPa. This value is comparable to our previous report [25]. It was observed that TS and EM of films were significantly decreased by the incorporation of BW. TS decreased by 22%, while EM decreased by 46%. Observed decrease with addition of BW might be due to the increase of free volume between polymer chains as reported earlier for starch films by Brandelero et al [26]. On the other hand, addition of BW remarkably increased the flexibility of films as shown by their EB values. Thus, it appears that BW had a plasticizing effect on BC based film. The presence of a significant amount of free fatty acids in BW may increase chain mobility and contribute to the flexibility of films. This result is in agreement with previous study, which demonstrated the plasticizing effect of BW in whey protein films [12]. These findings also support those of Tanaka et al [27] who studied the effect of different types of lipid materials on fish water soluble proteins (FWSP) composites films. They reported that the incorporation of BW remarkably increased the flexibility of films. Increasing concentration of BW at the same content of Tween 80 did not change the mechanical properties of BC based films as shown in B, C, and D films.

3.4. Water vapor transmission rate (WVTR) and water vapor permeability (WVP)

WVTR is the rate of water vapor permeating through the film, which is determined from the slope of the regression line of sample weight versus time graph and then divided by the area of the film being exposed to the transmission (Equation 2). Table 3 tabulates the WVTR values as well as WVP values calculated from the WVTR values (Equation 3). Table 3 shows that WVTR increased with an increase in the level of beeswax concentration. The similar trend was also observed for WVP values. These results were unexpected, because the addition of BW to the BC based film was supposed to reduce the transmission of water due to the hydrophobicity of BW. Study published by Auras et al [21] with cassava starch films containing glycerol and BW also documented this effect. They explained the phenomenon might be due to the lack of interaction of the lipid with the matrix, in our case with BC. This incompatible mix increased the chance for water molecules to migrate through the films through the BC/CMC/BW interface.

Table 3. The slope of the linear regression line of the sample weight vs time graph, the WVTR, and WVP of the BC based films with and without beeswax.

| Sample | Slope, ΔW/Δt (g/day) | WVTR (g/day)/m² | WVP (g.mm/(m².day.mmHg)) |
|--------|----------------------|-----------------|-------------------------|
| A      | 0.0072               | 4.43            | 0.00433                 |
| B      | 0.0073               | 4.46            | 0.00484                 |
| C      | 0.0088               | 5.36            | 0.00687                 |
| D      | 0.0093               | 5.75            | 0.00886                 |

4. Conclusion

BC based films with incorporating BW were successfully produced with a stable film structure. The preparation technique was very simple through casting method. The addition of beeswax improved the water solubility of films from 50 to 63-83%. It also influenced mechanical properties of films, significantly reducing the tensile strength and elastic modulus as well as increasing elongation. However, different concentration of BW did not affect much on mechanical properties of films. Addition of 0.005 g of BW showed the lowest WVTR of BC-BW film.
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