Antioxidant activity of biocellulose-based films incorporated with powder of soursop leaves (*Annona muricata* L)

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Abstract. Biocelluloses are natural materials that are produced from *Acetobacter* sp through fermentation in coconut water as the medium. In this study, biocellulose-based edible film was used as the carrier film for the antioxidant agents (the soursop leaf powder (*Annona muricata* L)). This leave is considered as a good source of natural antioxidant, while all parts of the fruit are used in traditional medicine. This work aimed to evaluate the antioxidant activity of biocellulose-based edible film added with soursop leave powder. The biocellulose was formed in slurry and leaves of fruit was crushed into powder. The edible films were prepared by mixing biocellulose slurry, carboxy methyl cellulose (CMC) as the homogenizer, and the leaves powder, casting, and drying in the oven at 40 °C for 24 h. The IC$_{50}$ of methanol extract of leaves powder and edible film containing 0.2 g leaves powder were 89.89 mg/mL and 363.26 mg/mL, respectively. All methanol extracts of edible films incorporated with soursop leaves powder inhibited DPPH about 71.10-86.80% and they remained above 70% after storage in a plastic bag at room temperature for one month. The edible films also had the thickness of 0.040-0.067 mm, the solubility of 80.30-90.20% and all compounds of edible films were still available based on SEM and FTIR results.

1. Introduction

Biocellulose is one of the natural and eco-friendly fibers which possess several unique properties including high tensile strength, high water absorption capacity, high crystallinity and an ultra-fine and highly pure fiber nanofibrillar structure [1,2]. Biocellulose is the result of *Acetobacter* sp fermentation in coconut water namely nata de coco. Biocellulose-based edible films have been known to contribute to reducing environmental contamination [3]. In addition, these films have been used as a carrier of food additives such as antioxidant or antimicrobial agent to improve their chemical characteristic [3]. Natural sources of antioxidants such as from plant or fruit have been attracted many people since a long time ago. It is because they have an effect to protect the human body or other materials against oxygen-derived from free radicals [4]. One of the antioxidant sources is *Annona muricata* L [5]. Several studies reported the medicinal purposes of leaves, seeds and roots of *A. muricata* plant that commonly called ‘Soursop’. Soursop leaf exhibits great antioxidant activity with the IC$_{50}$ value of 2.05 mg/mL and 0.91 mg/mL for ethanol and water extract, respectively [6]. The phytochemical screening demonstrated that the compounds which responsible for antioxidant activity were tannin, saponin, flavonoid, terpenoid, and phenolic with the percentage activity of 50-90% in the concentration of 20-200 µg/mL [7-9]. Based on 50% weight shrinkage and texture damage, Widiastuti *et al* reported that packaging using the starch-based edible film incorporated with soursop leaf extract could extend the storage time of red grapes [10]. Meanwhile, to the best of our knowledge, there is no study on incorporating the soursop leaves into the
biocellulose. Therefore, in this work, we evaluated the antioxidant activity of biocellulose-based films containing the powder of soursop leaves using DPPH method as well as the compound stability in the films for one-month storage. In addition, the morphological and physical properties of soursop leaf powder-incorporated biocellulose films were evaluated as well. The ability of biocellulose film to carry antioxidant agent can be an important novel material in the pharmaceutical field such as for capsule wrapper of multivitamins.

2. Experimental method

2.1. Materials
Biocellulose (BC) was supplied by a small enterprise in Cianjur, West Java, Indonesia. Each BC gel was washed with running tap water and boiled in distilled water to remove the remaining culture medium and bacteria cells until neutral pH was achieved. Carboxymethyl cellulose (CMC) was purchased from Himedia and used as it is. DPPH from Sigma Aldrich was used to check antioxidant activity.

2.2. Preparation of biocellulose pulp and soursop leaf powder
The neutralized BC gels with the addition of 40 w/w% of distilled water were crushed for about 15 min using a domestic blender (Philips, Japan). The obtained BC slurry was stored in a refrigerator until it was used. Fresh leaves of soursop were washed and dried in an oven at 50 °C for 24 h. The dried leaves were ground to powder using a home blender for 5 min. The powder was then stored in plastic sealed and placed in a desiccator.

2.3. Preparation of biocellulose-based edible film
Four formulas with different concentration of soursop leaf powder were prepared as shown in Table 1. Moreover, films with no addition of soursop leaf powder were also prepared as a control. The concentration of CMC was calculated based on the dry weight of 200 g of BC slurry. The film components (BS slurry, CMC, and soursop leaf powder) were mixed and homogenized at 60 °C. The mixtures were then degassed to remove the air bubble, cast onto a non-sticking tray, and dried in the air-circulating oven at 40 °C for 24 h. The films were cooled down to room temperature before peeling off from the tray.

| Sample | BC (g) | CMC (g) | Soursop leaf powder (g) |
|--------|--------|---------|-------------------------|
| A      | 200    | -       | -                       |
| B      | 200    | 0.14    | -                       |
| C      | 200    | 0.14    | 0.1                     |
| D      | 200    | 0.14    | 0.2                     |
| E      | 200    | 0.14    | 0.3                     |
| F      | 200    | 0.14    | 0.4                     |

2.4. Morphological and physical properties of BC-based films
The morphological analysis was performed using a scanning electron microscope (SEM) JEOL JSM IT-300 JAPAN at an operating voltage of 20kV. The BC films were mounted on the aluminum stubs using double-sided adhesive tape and then coated with a layer of gold (40–50 nm). They were then examined using a magnification of 10000 times. The structures of the films before and after incorporating soursop leaves were analyzed through Fourier-transform infrared (FTIR) spectra in the range of 4000–500 cm⁻¹ recorded with a Thermo Scientific Nicolet iS5 Spectrophotometer.

The thickness of the films was measured using digital micrometer (Mitutoyo) at five random points. The measurement results were then averaged. To determine the water solubility, the films were cut into a dimension of 2 cm x 2 cm and weighed (W₀). The samples were then immersed in distilled water for
30 min under a gentle stirring condition at room temperature. After that, any remaining pieces of films were taken out by filtering using nylon filter and dried in an oven at 105 °C for 24 h to obtain the final dry weight (W_1). Three replicates of each sample were measured. Water solubility of samples was calculated using the following equation.

\[
\text{Solubility (\%) } = \left( \frac{W_0 - W_1}{W_0} \right) \times 100 \%
\] (1)

Film color was measured in a Hunter L* a* b* color values by using a color difference meter (Murakami Color Research Lab, Japan). Measurements were performed at three distinct points of each film. L* represents the lightness, a* represents chromaticity on a green (-) to red (+) axis, and b* represents chromaticity on a blue (-) to yellow (+). During the measurement, films were placed on a white standard plate (L= 94.76, a = 0.795, b = 2.200). The total color difference (\(\Delta E^*\)) was obtained using the following equation [11]:

\[
\Delta E^* = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}
\] (2)

where \(\Delta L^*, \Delta a^*,\) and \(\Delta b^*\) are the differences between the color values of the samples and that of the white standard used as a background.

2.5. Antioxidant assay of BC-based films
The IC_{50} of soursop leaf powder was performed at the concentration of 500 to 50 ppm. A total of 0.1 g of film was extracted with 5 ml methanol for 1 x 24 h to obtain methanol extract. For the evaluation, 2.0 mL DPPH 0.1 mM solution was added with 1.0 mL of sample in a test tube, shaken and incubated in a dark room for 30 min. Then, the absorbance of the samples was determined using a spectrophotometer at 517 nm. The antioxidant activity was calculated as a percentage inhibition of DPPH, using the following equation:

\[
\% \text{ inhibition } = \left( \frac{A_0 - A_s}{A_0} \right) \times 100\%
\] (3)

where Ao is the absorbance of DPPH and As is the absorbance of the sample. IC_{50} values were determined using the linear equation of the graph of inhibition percentage versus sample concentration. To evaluate the storage life of the films, percentages of inhibition were also determined after one-month storage in a sealed plastic bag at room temperature.

3. Results and discussion

3.1. Morphological and physical properties of BC-based films
Photos obtained by a digital camera for all films are shown in Figure 1. Visual examination of the BC films without soursop leaves powder (Figure 1A and 1B) showed that the films were opaque, paper-like film, and white. In general, transparent films are preferred as packaging because they affect consumer choice of the products. However, opaque films are also an important feature when films are used as a barrier to light. Addition of soursop leaf powder (green colour) into the BC-based films changed the appearance of films, in which the films turned to green as a function of soursop leaf content as shown in Figure 1C-1F. Some green spots were observed on the surface of the films indicating the powder were not dispersed homogeneously and not small enough to penetrate between the BC fibers.

Quantitatively, the total color difference of the films is presented in Table 2. BC-only and BC-CMC films have a total color difference of 10.7 and 11.6, respectively. Incorporation of a small amount of soursop leaf powder into the BC-based films significantly increased total color different for about three times, where the lightness and a* values decreased, while b* values increased. The reduction trend of L* values showing a darkening tendency in film color, probably due to the darkening of soursop leaf powder as an effect of drying temperature. Lightness reduction due to the presence of antioxidant agent was also reported by other [12]. Declining a* values of the films containing soursop leaf powder suggested a trend to greenish, while b* values indicated a yellower appearance. This is expected due to the green color of the leaves.
Figure 1. Photos of BC-based films: (A) BC only, (B) BC+CMC, (C) BC+CMC+0.1g SL powder, (D) BC+CMC+0.2g SL powder, (E) BC+CMC+0.3g SL powder, and F) BC+CMC+0.4g SL powder.

Table 2. Physical characteristics of BC-based films.

| Film | Mixture | Thickness (mm) | Colour Test | Solubility (%) |
|------|---------|----------------|-------------|----------------|
| A    | BC      | 0.027±0.001    | L*: 90.4 a*: -1.8 b*: 11.3 ΔE: 10.7 | 89.5          |
| B    | BC+CMC  | 0.027±0.003    | L*: 90.2 a*: -1.6 b*: 12.3 ΔE: 11.6 | 91.5          |
| C    | BC+CMC+0.1g SL powder | 0.040±0.005 | L*: 78.3 a*: -4.9 b*: 32.9 ΔE: 35.5 | 80.3          |
| D    | BC+CMC+0.2g SL powder | 0.046±0.002 | L*: 72.1 a*: -4.9 b*: 36.5 ΔE: 41.7 | 81.2          |
| E    | BC+CMC+0.3g SL powder | 0.061±0.007 | L*: 61.1 a*: -3.1 b*: 38.1 ΔE: 49.3 | 84.3          |
| F    | BC+CMC+0.4g SL powder | 0.067±0.006 | L*: 57.3 a*: -2.0 b*: 34.9 ΔE: 49.7 | 90.2          |

Moreover, Table 2 presented the thickness of the films. This parameter is important in determining the feasibility of the films to be applied as packaging and it was also influenced by the formulations added with antioxidant extract [13]. As can be seen in Table 2, the more the components were added, the more the thickness of the obtained films. The thickness of the films incorporated with soursop leaves was in the range of 0.04 – 0.07 mm. This range of thickness is quite effective to stop the migration of oxygen and contaminants that can lead to product spoilage.

Images obtained by SEM for all films are presented in Figure 2. According to SEM images, the surface morphology of BC-based films showed smooth and homogenous with bundled of BC fiber network. The BC containing CMC film (Film B) presented denser morphology and more homogenous fibers than BC-only film (Film A) suggesting good incorporation of CMC in BC matrix. CMC filled the cavities of the BC ultra fine network to form a solid structure. CMC is commonly used as a stabilizing and suspending agent in pharmaceutical formulation [14]. With increasing soursop leaf powder content from 0.1 to 0.3 g, the surfaces of BC-based films (Film C-F) became slightly rough but still homogenous, indicating an even dispersion of the powder in BC matrix. However, further increasing the soursop leaf powder to 0.4 g (Film F) resulted in higher surface roughness of BC-based film, which could be attributed to the large particle size of the powder.

In order to apply the films as edible packaging, one of the important properties is water solubility [10]. The components of the film should be able to soluble in water and easy to digest. Generally, cellulose is insoluble in water due to the strong intermolecular hydrogen bonding between cellulose molecules. However, in this study, BC film exhibited 89% water solubility, which was attributed from the fragmented BC used in the formula. The insoluble matter remains is the BC fibers. Furthermore, adding a water-soluble component such as CMC also contributed to the solubility of the resulted film,
thus increasing the water solubility. CMC is known to have good biocompatibility with hydrophilic matrices. It has many hydrophilic carboxyl groups and presents a hydrophobic polysaccharide backbone. Films contained soursop leaf powder also presented high water solubility, but they were slightly lower than those without soursop leaf powder. It might be due to the fiber content in soursop leaf powder.

![Figure 2. SEM micrographs of BC-based films surface with different concentration of soursop leaf powder.](image)

Insignificant changes also were not observed in FTIR spectra of the films, as can be seen in Figure 3. All films exhibited almost identical spectrum. Several common bands of cellulose are presented by all films, such as a broad peak around 3340 cm\(^{-1}\) for O-H stretching of hydroxyl groups, and the peak around 2880 cm\(^{-1}\) indicated the presence of C-H group. The COO\(^{-}\) of CMC was exhibited at 1590-1654 cm\(^{-1}\). The spectra region can be referred to as the intermolecular hydrogen bonds of cellulose [15,16]. From Figure 3, the addition of soursop leaves powder into BC-based film did not interfere with the main bonds of cellulose polymer. However, increasing the concentration of soursop leaf powder resulted in a broader band in the range of 3200-3500 cm\(^{-1}\) with the peak at 3340 cm\(^{-1}\) due to the presence of polyhydroxy groups (O-H stretching). In addition, BC-based films containing soursop leaf powder showed the peak at 1370 cm\(^{-1}\) representing C-H stretching which considered coming from alkaloid compounds.
3.2. Antioxidant activity of BC-based films
In this study, the ethanol extract of soursop leaf powder was about 85.48% and the IC$_{50}$ of soursop leaf powder was 89.89 mg/mL. These results indicate that soursop leaves perform an antioxidant activity. IC$_{50}$ is the concentration required to reduce 50% absorption intensity of DPPH solution. The previous studies also showed the free radical scavenging of soursop leaves was found to be effective 85.67% (ethanol extract) [7] and 79.95% (water extract) [17]. Another study reported that the IC$_{50}$ of leaves extract, powder extract, and nanoparticle powder of A. muricata L was 24.62, 52.08, and 46.88 µg/mL, respectively [18]. Flavonoids (phenolic compounds) are the most prevalent phenol coming from plants and proved as antioxidant agents.

The DPPH scavenging assay was used to determine the antioxidant activity of the film. Compared to other methods, the DPPH assay has many benefits, such as good stability, credible sensitivity, simplicity, and feasibility [19]. As can be seen in Table 3, methanol extracts of BC and BC-CMC films had insignificant antioxidant activities, with the value of 23.4 and 26.1%, respectively. After incorporating the soursop leaf powder into the formula, the inhibition percentage of DPPH increased to about 71.1–86.8%. The facts that mixing the components at 60 °C and drying the film at 40 °C did not affect the antioxidant activity of the films indicated that the active compounds in soursop leaves are quite resistant to medium heating.

In order to investigate the effect of shelf life on antioxidant activity, the percentage of inhibition was monitored again after one-month storage and it revealed that antioxidant activity in sample D, E and F still remained and effective against free radical DPPH > 50%. The decreasing antioxidant activity of the edible film might be due to the storage condition that was exposed to light and fluctuation of room temperature. However, it was reported that antioxidant properties of soursop leaves such as polyphenol and phenolic compounds are stable under different conditions (such as temperatures and lights) [20,21].

**Table 3. Antioxidant activity of edible film incorporated with soursop leaf (SL) powder.**

| Sample code | Mixture                  | Inhibition DPPH (%) |
|-------------|--------------------------|---------------------|
| A           | BC                       | 23.4                |
| B           | BC+CMC                   | 26.1                |
| C           | BC+CMC+0.1g SL powder    | 71.2                |
| D           | BC+CMC+0.2g SL powder    | **86.8**            |
| E           | BC+CMC+0.3g SL powder    | 83.9                |
| F           | BC+CMC+0.4g SL powder    | 81.8                |

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In this study, the best antioxidant activity among samples was edible film D whereas containing 0.2 g powder leaves. The IC\textsubscript{50} value of that film was 363.26 μg/mL which confirmed that BC-based film incorporated with soursop leaf powder had antioxidant activity.

4. Conclusion
Biocellulose-based edible film incorporated with 0.1-0.4 g of soursop leaf powder (Annona muricata L) showed antioxidant activity and stability after one-month storage in a sealed plastic bag at room temperature. The highest inhibition percentage was demonstrated by BC-based film contained 0.2 g of soursop leaf powder with an IC\textsubscript{50} value of 363.26 μg/mL. The addition of soursop leaf powder did not influence the morphological characteristic, structure, and solubility of BC-based film. They only affect the total color difference of the films.

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References
[1] Keshk S M 2014 J Bioproces. Biotechniq. 4 150
[2] Mohammad S M, Rahman N A, Khalil M S and Abdullah S R S 2014 Adv. Biol. Res. 8 307–13
[3] Aldana D S, Andrade-Ochoa S, Aguilar C N, Contreras-Esquivel J C and Nevárez-Moorillón G V 2015 Food Control 50 907–12
[4] Almeida M M B, De Sousa P H M, Arriaga A M C, Do Prado G M and Magalhaes C E C 2011 Food Res. Int. 44 2155–9
[5] Akomolafe S F and Ajayi O B 2015 Int. Food Res. J. 22 238–8
[6] Gavamukulya Y, Abou-Elella F, Wamunyokoli F and El-Shemy H A 2014 Asian Pac. J. Trop. Med. 7 S355–63
[7] Muthu S and Durairaj B 2015 Eur. J. Experimental Biol. 5 39–45
[8] Agu KC and Okolie P N 2017 Food Sci. Nutr. 5 1029–36
[9] Bryan-Thomas J 2016 Int. J. Sci. Res. Publications 6 490–5
[10] Widyastuti E, Sedyadi E and Prabawati S Y 2016 Biol. Med. Nat. Prod. Chem. 5 55–9
[11] García F T and Sobral P J d A 2005 LWT–Food Sci. Technol. 38 289–96
[12] Gaofeng Y, Hua L, Bingjie Y, Xiaoe C and Haiyan S 2015 Molecule 20 11034–45
[13] Torres-León C, Vicente A A, Flores-López M L, Rojas R, Serna-Cock L, Alvarez-Pérez O B and Aguilar C N 2018 LWT–Food Sci. Technol. 97 624–31
[14] Tongdeesoontorn W, Mauer L J, Wongruong S, Sriburi P and Rachtanapun P 2011 Chem. Central J. 5 1–8
[15] Indrarti L and Indriyati 2017 IOP.Conf. Series: Earth and Environmental Sci. 60 012018
[16] Martelli S M, Motta C, Caon T, Alberton J, Bellettiini I C, do Prado A C P, Barreto P L M and Soldi V 2017 LWT–Food Sci. Technol. 77 21–9
[17] Yesi D, Deni R and Affiah H 2017 Res. J. Pharm. Biol. Chem. Sci. 8(1S) 275–80
[18] Volf I, Ignat I, Neamtu M and Popa V I 2014 Chem. Papers 68 121–9
[19] Deng J, Cheng W and Yang G 2011 Food Chem. 125 1430–5
[20] Boakye A A, Wireko-Manu F D, Agbenorhevi J K and Oduro I 2015 Int. Food Res. J. 22 262–8
[21] Wei Z L, Sui K C, Hock E K, Chiauw M S and Hip S Y 2016 Acta Sci. Pol. Technol. Aliment. 15 419–28