Incorporation of citrus essential oils into bacterial cellulose-based edible films and assessment of their physical properties

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Abstract. The use of edible films in food protection and preservation has recently gained more interest since they offer several advantages over synthetic packaging materials. Biocellulose (BC) offers great opportunity as edible film due to their unique physical and mechanical properties. In this study, biocellulose films were prepared by solution casting with addition of 30% carboxymethyl cellulose (CMC) and 30% glycerol as the homogenizer and plasticizer, respectively. Furthermore, various citrus essential oils (EOs) including lemon, lime, and sweet orange were added at 50% w/w of BC dried weight. The solutions were then cast on the tray and allowed to dry in the air convection oven at 40°C overnight. The films were characterized for water solubility, tensile strength (TS), elongation at break (EB), water vapour transmission rate (WVTR), and color. Those characteristics may influence consumer acceptability of the packaged products. Results revealed that addition of lemon and sweet orange EOs into BC-based edible film decreased water solubility and TS, but improved EB, as these oils acted as plasticizers in the film. However, different trend was observed for BC-based film incorporated with lime oil, which had higher solubility and TS, but lower EB and WVTR compared with that of control film. Addition of citrus EOs into BC-based films did not have much effect on color properties as stated in L*, a*, and b* values.

1. Introduction
Packaging has an important role in food protection and preservation. Research on edible films and coatings on food system has been conducted in order to improve food quality, add value to the edible polymer materials, and reduce synthetic packaging materials. An edible film or coating could be defined as a packaging made from edible materials [1]. Edible films are distinguished from edible coating by their manufacturing method and application in food. Edible films are dried forming thin material structures and are used to wrap the product or make a pouch or bags, while edible coatings are usually applied as a liquid to the outer surface of food product and allowed to dry to form thin layer structure.

Polysaccharides such as starch, cellulose derivatives, chitosan, and carrageenan, are most often used for edible films among others because of their film-forming properties. As one form of celluloses, biocellulose (BC) is produced by bacteria *Gluconacetobacter xylinus* (formerly known as *Acetobacter xylinum*) that secretes large quantities of cellulose as microfibrils and these microfibrils merge to form a large ribbon of cellulose in a medium containing carbon sources. Due to BC structure that consist only glucose monomer, it exhibits great properties such as unique nanostructure, high water holding capacity, high mechanical strength and high crystallinity [2-5]. BC has been identified as one of the suitable materials for edible film [6-7].

There are several requirements to be considered for edible films application in food area, such as appropriate water barrier properties, good mechanical strength, low cost of raw materials, and simple technology production [8]. Our previous study revealed that by simple production technology and with low cost of raw material, edible film from BC can be produced. Addition of glycerol to BC film gave higher efficiency in plasticizing BC-based films which was shown in increased elongation at break and in tensile strength [7]. Nowadays, one of the major challenges for edible film technologies is the design of antibacterial edible film using minimum amounts of active compounds, such as phenolic or...
essential oil (EO) compounds. Edible films can be used as a carrier of EO. EO interacts with polymer and plasticizer and forms part of chemical structure of the film [9]. EOs are “essential” in common sense that they carry a distinctive scent of the source plant and are generally extracted by steam distillation and other processes like maceration, cold pressing, or solvent extraction. Some studies have demonstrated the use of EO in edible film to inhibit the growth of microorganisms [10-11]. However, the use of essential oils also affects the physicochemical properties of edible films, particularly BC-based edible films, and these characteristics are relatively unknown. Therefore, it is important to evaluate the effect of EO addition on BC film characteristics. Thus, the aim of this work is focused on analyzing the effect of EO incorporation on the physicochemical behavior of BC-based edible films. The characteristics including solubility, mechanical properties, water vapour transmission rate (WVTR), and color at three different kinds of citrus essential oils was discussed. Those characteristics may influence consumer acceptability of the packaged products.

2. Experimental

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 [7]. BC slurry, CMC (Himedia) and glycerol (Merck) were mixed under magnetic stirring condition (named as control film). Lime, lemon and sweet orange essential oils purchased from Lansida were added at concentration of 50 %v/w over dried BC. The solutions were degassed under a vacuum bell jar to remove the bubbles prior to cast onto the tray. The trays were placed overnight at 40 °C in gear oven, and then cooled to room temperature before peeling the films off the trays.

2.2. Characterization of BC-based edible films
Water solubility of the films was determined according to the method of Ojagh et al. [12]. Pieces of film (2 cm x 2 cm) were cut from each film and dried at 110 °C in an oven. Samples were weighted to the nearest 0.0001 g and noted as initial dry weight (w₀). Samples were then placed into beaker with 20 mL aquades and heated at 60°C for 3 hr. The remaining pieces of film after soaking were filtered through nylon filter, followed by oven drying at 110 °C until its weight get constant and noted as final dry weight (wᵢ). Samples were measured in 2 replicates and the percentage of total soluble matter (% solubility) was calculated as follow.

\[
\% \text{ solubility} = \left(1 - \frac{wᵢ}{w₀}\right) \times 100\%
\]  

Mechanical properties were characterized by Orientec UCT-5T universal testing with 100 kgf load cell according to ISO 527-1993E standard method. Dumbbell-shaped specimens were obtained from each film according to ISO 527-2 type 5A. The measurement was conducted at 23°C. At least five specimens of each sample were measured and computerized calculated to obtain the average value.

Water vapor transmission rate (WVTR) was measured using gravimetric modified cup method based on ASTM E96 [13-14]. The thickness of each sample was determined in five random positions. The edible films were firmly fixed onto the opening of glass cups containing of silica gel and then, these cups were placed in a dessicator with distilled water. These cups were weighed using an analytical balance (±0.0001 g) at interval of 24 hr during 5 days. The samples were measured in 3 replicates. WVTR was calculated from the slope of the regression analysis of weight gain versus time divided by the exposed film area.

Color values of films were measured using color difference meter (Murakami Color Research Lab) and expressed in L*, a* and b* color space. L* represents the lightness, a* represents chromaticity on a green (-) to red (+) axis, and b* represents chromaticity on a blue (-) to yellow (+). For measurement, films were placed on a white standard plate (L* = 93.8, a* = -0.7 and b* = 1.4). Whiteness index was estimated using the following equation [15].
\[ \text{WI} = 100 - ((100 - L)^2 + a^2 + b^2)^{1/2} \]  
(2)

The color was expressed as difference of color (\(\Delta E^*\)), accordingly to the equation [16].  
\[ \Delta E^* = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{1/2} \]  
(3)

where \(\Delta L\), \(\Delta a\), and \(\Delta b\) correspond to the variation between the color parameter of film and that of white standard used as background. Film opacity was calculated with reflectance measurements of each film (n=2) with black and white backing plates, using the following equation [17].

\[ \text{Opacity} = \frac{Y_{\text{black backing}}}{Y_{\text{white backing}}} \times 100\% \]  
(4)

where \(Y\) is the color values of the film with the black or white backing plates.

3. Result and discussion

3.1. Film appearance

The BC-based edible films formed using variations of citrus essential oils were visually homogeneous, no crack or brittle areas, and no visible pores or bubbles. They were easy to handle and easy to peel from the casting plates.

3.2. Water solubility

Film solubility is an important factor for edible applications. Film solubility at temperature above room temperature is advantageous if the films are consumed with a pre-heated product prior to consumption and it may also be an important factor to determine biodegradability of film when used as packaging wrap. The water solubility of BC-based edible films with and without EOs is shown in Table 1. It would be expected that hydrophilic compounds should increase a film’s solubility, whereas hydrophobic compounds should decrease it [18]. In this study, incorporating lemon and sweet orange EOs into BC-based film decreased water solubility of edible films, which is in accordance with the expectation. Lower solubility of film contained sweet orange EO compared to film contained lemon EO may be explained by an increase in interaction between the hydroxyl group of BC chains and sweet orange EO components, leading to a decrease in availability of hydroxyl group, and thus reducing polysaccharide-water interactions. On the other hand, incorporating lime EO into BC-based edible film increased water solubility, probably due to less lime EO components that interact with hydroxyl group of BC chain, leading to an increase in availability of hydroxyl group, and thus enhancing BC-water interactions.

| Formulation | Solubility (%) |
|-------------|----------------|
| BC+CMC+glycerol (control) | 26.21±0.56 |
| BC+CMC+glycerol+lemon EO | 25.79±0.48 |
| BC+CMC+glycerol+lime EO | 28.24±2.61 |
| BC+CMC+glycerol+sweet orange EO | 19.71±1.96 |

Cellulose is a hydrogen bond cross-linked polymer. Despite being hydrophilic, cellulose is insoluble in common solvents, because it has rigid long chains and strong intra and inters hydrogen bonds [19]. Usually, BC-based film is insoluble in water. However, in this study, water solubility of BC-based films was in the range of 20-28%. It can be explained that water went inside the cellulose’s structures and broke apart the intermolecular attractions between CMC and BC. Therefore, the low percentage of
solubility of BC-based edible films elucidates only CMC was dissolved in water while the remaining solid was fragmented BC fibers.

3.3. Mechanical properties of BC-based edible films
Mechanical properties of films were characterized by measuring the tensile strength (TS) and elongation at break (EB), which are key indicators of a film’s strength and flexibility. TS exhibits the maximum stress during tensile testing before failure, while EB means film stretchability prior to breakage.

The effect of citrus essential oils and control on the mechanical properties of BC film samples is shown in Table 2. Compared with the control film, incorporation of lemon and sweet orange EOs into BC-based films showed a decreased in TS and increased in EB of the BC-based films compared to the control due to the effect of plasticized behavior of these oils. This is common result when EOs were introduced into the films and also in agreement with the literatures [15,20]. On the other hand, different trend was observed for BC-based film enriched with lime oil, which had higher TS and lower EB than that of control film, presumably because lime oil EO might consist mostly of terpene-like compound rather than lipids. It might interfere the interaction between polymer chains and reduce flexibility of BC-based film [13].

Table 2. Mechanical properties of BC-based edible films with and without essential oils.

| Formulation                      | TS (MPa)    | EB (%)   |
|----------------------------------|-------------|----------|
| BC+CMC+glycerol (control)        | 106.6±6.6   | 22.9±1.4 |
| BC+CMC+glycerol+lemon EO         | 101.8±4.2   | 27.4±1.1 |
| BC+CMC+glycerol+lime EO          | 133.1±22.5  | 20.6±3.6 |
| BC+CMC+glycerol+sweet orange EO  | 92.8±22.5   | 26.6±2.7 |

3.4. Water vapor transmission rate (WVTR)
WVTR is an important property for films used as edible food coatings, because most natural biopolymers are very sustain to water. WVTR of BC films containing citrus essential oils is shown in Table 3.

Table 3. Water vapor transmission rate (WVTR) of BC-based edible films with and without essential oils.

| Formulation                      | WVTR (gr/cm²/day) |
|----------------------------------|-------------------|
| BC+CMC+glycerol (control)        | 1.69±0.19         |
| BC+CMC+glycerol+lemon EO         | 1.61±0.17         |
| BC+CMC+glycerol+lime EO          | 1.41±0.08         |
| BC+CMC+glycerol+sweet orange EO  | 1.78±0.11         |

Incorporating lemon and lime EOs caused a slight decreased of WVTR compared to that of control film. In contrast, addition of sweet orange EO slightly increased the WVTR of the film. Water vapor transfer generally occurs through the hydrophilic portion of the film. Essential oil’s chemical nature also plays an important role in the barrier properties of edible films. The composition of the citric fruits is generally composed of 90% terpenes, 5% oxygenated compounds, and less than 1% non-volatile compounds such as waxes and pigments [21]. Different amounts of monoterpenes could influence the hydrophobicity. In other words, monoterpenes is the governing factor in determining the hydrophobicity [13]. The presence of a hydrophobic disperse phase, even at small ratio, limits water
vapour transfer since it introduces the interfere in hydrophilic phase and increases the tortuosity factor of mass transfer [15].

3.5. Color
Color attributes are of prime importance because they directly influence consumer acceptability [22]. Addition of EOs in BC-based films may change the native color of BC-based films. However, in this study, there was no significant change in L* value for all films, whereas incorporation of citrus EOs on BC slightly influenced a* and b* value as shown in Table 4. As a result, ΔE values changed upon of citrus EOs on BC-based films. The interaction of citrus EOs did not significantly influence the white index the BC film. Furthermore, the opacity of films increased with addition of citrus EOs. Sweet orange EO gave the highest opaque percentage, followed by lime EO. This property is important in various film applications, particularly if the film is used as a surface food coating or for improving product appearance.

Table 4. Color parameters of BC-based edible films with and without essential oils.

| Film sample          | L*  | a*  | b*  | Color difference (ΔE) | White index (WI) | Opacity (%) |
|----------------------|-----|-----|-----|-----------------------|------------------|-------------|
| Control              | 92.7| 0.4 | 10.1| 8.8                   | 87.53            | 46.39       |
| Lemon                | 92.4| 0.3 | 8.6 | 7.4                   | 88.52            | 48.38       |
| Lime                 | 92.4| 0.2 | 10.6| 9.3                   | 86.96            | 53.68       |
| Sweet orange         | 92.4| -0.4| 10.4| 9.2                   | 87.13            | 74.89       |

4. Conclusion
Some physicochemical characterizations of BC-based films blended with citrus essential oils (EO) have been carried out. Addition of various citrus oils into BC-based films showed an effect on solubility, mechanical properties, WVTR and opacity of BC films. The film that contained sweet orange EO presented the most opaque color compared to others. Tensile analyses showed that addition of sweet orange and lemon oils could enhance films’ flexibility. WVTR decreased when lemon and lime EOs were incorporated. However, whiteness index were not significantly affected by addition of citrus EOs-BC films.

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