Bacterial cellulose-rambutan leaf extract (*Nephelium lappaceum L.*) composite: preparation and characterization

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Abstract. The purpose of this research is to produce bacterial cellulose-rambutan leaf extract (BC-RLE) composite. The BC-RLE composite was prepared by immersing BC gel into RLE under various immersion time of 7, 14 and 21 days with/without using UV light radiation, and then, its physical (water content, and swelling), mechanical (tensile and compressive strength) and structural (functional group and degree of crystallinity) properties were characterized. The results showed that the water content of BC-RLE composites was lower than that of pure BC gel and decreased as increased immersion time of BC gel in RLE. Further, the UV light radiation is given during immersion of BC gel in RLE also reduced the water content of BC-RLE composites. However, the swelling degree of BC-RLE composites increased as increased immersion time, and a similar tendency was also shown by applying the UV light radiation. Mechanical properties, tensile, and compressive strength of BC-RLE composites increased as increased immersion time and by applying UV light radiation. The FTIR results showed that there was no effect of UV light radiation on the structure of BC-RLE composites. The Degree of crystallinity of BC-RLE composite decreased by applying the UV light radiation during immersion time.

1. Introduction

Cellulose is a natural biopolymer that is abundant on earth. Cellulose can be synthesized by plants, algae, and some species of bacteria. Acetobacter xylinum (*A. Xylinum*) is one species of bacteria that can synthesize cellulose. Cellulose synthesized by bacteria is known as Bacterial Cellulose (BC) [1].

Cellulose produced by Acetobacter species has the same basic structure as cellulose in plants, but there are several advantages when compared to cellulose derived from plants. These advantages include high purity, high crystallinity, high mechanical strength, and uniformity [2]. Then, BC also has a high level of polymerization, non-toxic, non-allergenic, biocompatible, and can absorb water [3]. Also, the time needed for fermentation is short, and the degeneration is high. BC has a high-water content of 98-99% and can be safely sterilized without causing changes in characteristics [4] so that research on this BC is widely carried out.
Bacterial cellulose has been applied in various fields, one of them in the medical field such as pharmaceutical and prosthetic fields such as contact lenses, the field of drug delivery, and tissue change [5]. The use of BC as a network replacement is in the biomedical field, namely soft tissue replacement in the body, for example, bone growth material [6], bone connection with a bone (ligament), muscle connective with a bone (tendon) and cartilage (cartilage) [5].

Based on these facts, researchers are interested in researching BC. The obtained BC is expected to be applied in the biomedical field such as medicinal material and bone growth material. However, to apply BC in the medical world there are several obstacles including BC has a weakness that is easy to absorb liquid (hygroscopic) so that it is easily contaminated by microbes [4]. Then, BC has a compressive modulus or low compressive strength and strain ratio. This can be seen when BC is pressed from an angle perpendicular to the stack. Also, BC has low elasticity. If the BC is given a small pressure as it is pressed using a finger, the water contained in this BC sheet comes out, and the BC cannot return to its original form [5].

Based on the shortcomings of the BC, a study was carried out to improve the elasticity of BC. One way to obtain BC with high elasticity is by combining BC with other materials to form a new material in the form of composite. In previous studies conducted by Nakayama (2004) proved that the incorporation of BC with other materials such as gelatin and polyacrylamide can produce composites that have characteristics such as compressive strength, tensile strength, and higher elasticity than pure BC [7]. In this regard, researchers are interested in looking for other alternatives to improve the characteristics of BC elasticity through modification with the addition of another material. The material used was natural material, such as herbal plants which are widely available in Indonesia, one of which is the rambutan plant (*Nephelium lappaceum* L.) which will be combined with BC to form a composite. strong [8]. And it is very suitable when combined with bacterial cellulose matrix which is easily microgrid.

This study refers to research that had been done previously who made modifications to BC immersion media [9]. Media modification aimed to produce new composite materials that can be applied in the biomedical field [9]. Besides that, it is also expected that this new composite material is better, stronger, and high elasticity.

In this study, the immersion media used were rambutan leaf extract, and the same time variation was used which was 7.14 and 21 days without and using UV light. This treatment is expected to obtain cellulose composites of rambutan leaf extract (BC-RLE) which can be applied in the biomedical field.

### 2. Method

#### 2.1. Tools and Materials

The tools used to create and characterize BC-RLE are glassware (measuring flasks, chemical glasses, measuring cups), stirring rods, funnels, watch glass, analytical balance, universal pH paper, shakers, blenders, Compressive Strength and Tensile Strength, glass, Fourier Transform Infra-Red, X-ray Diffraction, UV lamp, micrometer screw, iron, plastic container, stainless steel pan, stove, knife, scissors, filter, cloth, plastic, washcloth, newspaper, rubber band, tissue, UV box and label paper. The materials used include pineapple, coconut water, urea fertilizer (CO(NH$_2$)$_2$), C$_6$H$_{12}$O$_6$, betel leaf, methanol, vinegar (CH$_3$COOH), starter A. xylinum, technical NaOH, HCl, aquades, and water

#### 2.2. BC Preparation

1.00 L of coconut water was put in a heated stainless-steel pan, 100.00 grams of sucrose, and 10.00 grams of urea (CO (NH$_2$)$_2$) were added. This solution was heated to boiling and acidified with 25% acetic acid (CH$_3$COOH 25%), in a hot condition the solution is transferred to a plastic container measuring 24 cm x 17 cm x 4 cm as much as 600.00 mL and covered with sterilized newsprint until the media reaches room temperature between 25-30°C. The media was then inoculated with 10% (v/v)
A. xylinum aseptically and fermented at room temperature until BC formed with a thickness of ± 1.00 cm, and then harvested [10,11].

2.3. Washing and purification of BC
The resulting BC in the fermentation process was washed using running water for ± 24 hours. Then, the BC was soaked in 2% (w/v) NaOH solution for ± 24 hours, and then, was washed again with running water until neutral condition. Then, BC can be stored in water. The water was replaced every 2 days [10].

2.4. Rambutan Leaf Extract Preparation (RLE)
The rambutan leaves used were old leaves, because they contain more secondary metabolites. Rambutan leaves were washed with clean water then soaked with HCl for ± 24 hours while stirring occasionally and washed again with clean water. Then it was heated at a temperature of 60 °C until a constant weight was reached. Then mashed and dissolved with methanol then filtered with filter paper.

2.5. BC-RLE Preparation
Soaked BC was cut to a certain size, then soaked in RLE for 7.14 and 21 days with and without UV light. During the immersion process, the sample was shaken using a shaker to produce BC-RLE.

2.6. Characterization of BC-RLE
2.6.1. Water content measurement. BC-RLE was determined by the water content utilizing the sample oven at a temperature of 105°C, then weighted. The percentage of water content can be calculated using the following equation.

\[ W_c(\%) = \frac{W_w - W_d}{W_w} \times 100\% \]  

Where: \( W_c \) is the percentage of water content, \( W_w \) is wet weight, and \( W_d \) is the dry weight

2.6.2. BC-RLE Swelling Degree measurement. BC-RLE dried gel was measured its thickness and weighed at its initial dry weight (\( W_d \)), then, soaked in distilled water for a certain time. Then, the surface of the sample was dried and immediately weighed. This weighing processed was carried out every 24 hours until a constant weight (\( W_w \)) was obtained. The percentage of the degree of swelling can be calculated using the following equation.

\[ DP(\%) = \frac{W_w - W_d}{W_d} \times 100\% \]  

2.6.3. Compressive strength measurement. Measurement of the compressive strength of BC-RLE was done using Compressive Strength equipment.

2.6.4. Tensile strength measurement. Measurement of the tensile strength of BC-RLE was done using tensile strength equipment.

2.6.5. Analysis of functional group using FTIR. The sample used for functional group analysis was an oven-dried sample using FTIR equipment.
2.6.6. Analysis of the degree of crystallinity using XRD. The sample used to calculate the degree of crystallinity is a sample that has been dried using an oven. The percentage degree of crystallinity can be calculated using the weighing method. The relationship between mass and crystallinity of cellulose is as follows.

\[
C(\%) = \frac{m_{\text{crystall}}} {m_{\text{crystall}} + m_{\text{amorf}}} \times 100\%
\]  

(3)

Where \( C(\%) \) is the percentage of crystallinity, \( m_{\text{crystall}} \) is mass of crystallin part and \( m_{\text{amorf}} \) is mass of amorf part.

3. Results and discussion

\textit{A. xylinum} bacteria produce cellulose fibers in the form of a layer of white pellicle which expands on the surface of the culture medium. The white layer is formed as bacterial cellulose (BC). Cellulose fibers start from the surface of the culture medium towards the bottom of the culture medium which forms the cellulose layer which was thicker until the solution that functions as a growing medium for bacteria continues to decrease.

BC formation process is a process that is susceptible to various disturbances, therefore to avoid the formation of imperfect BC (hollow and moldy) BC preparation must be carried out using sterile equipment, paying attention to the adequacy of nutrients used, oxygen adequacy, and acidity, temperature the room must be adjusted to the optimum temperature of the growth of \textit{A. xylinum} bacteria which is 28\(^\circ\)C-31\(^\circ\)C or at room temperature, then placed in a flat place and free from shock. The bacteria used must be active so that the formation of cellulose fibers can occur quickly.

The formation of BC starts from the hydrolysis stage of sucrose to glucose and fructose by the enzyme sucrase. Sucrose at this stage comes from granulated sugar as an additional nutrient and coconut water as a culture medium. The content of sucrose in coconut water was around 9.18 mg/mL, besides that coconut water also contains other sugars such as 7.25 mg/mL glucose and fructose 5.25 mg/mL [12].

The process of washing and purifying BC of fermented products is important. The fermentation process for ± 7 days produces a yellowish-white BC. Pure BC should be white, but the resulting BC was yellowish due to the trapping of the medium between the BC fibers formed. To remove the remnants of the medium, BC was washed by immersing the BC in water and also drained with water for ± 24 hours, then purification followed by soaking using 2% NaOH (w/v) for ± 24 hours at room temperature. Thus, soaking with 2% NaOH can increase the purity of cellulose produced so that the relationship between the chains in cellulose gets stronger through hydrogen bonds between chains and the cellulose structure becomes tighter [13]. Then, it was washed again with water, this treatment aims to neutralize the pH of the BC.

RLE preparation aims to make filler components in composites. Rambutan leaves used are old rambutan leaves, because the older rambutan leaves contain more secondary metabolites which are expected to be composite fillers. Rambutan leaves were extracted by maceration method, namely extra-cation using methanol solvent. Dry rambutan leaves were soaked with a ratio of sample weight and solvent volume of 1: 10 for 24 hours. The extract solution obtained was filtered using filter paper. The results obtained from this process were dark green rambutan leaf extract.

BC-RLE preparation aims to produce a new material (composite) that has better physical, mechanical and structural properties than BC (matrix) and RLE (filler). BC-RLE preparation was done by soaking the BC in RLE with a variation of the soaking time of 7.14 and 21 days with and without using UV light and stirring using a shaker. Irradiation using UV light was expected to increase bond strength in BC-RLE, UV light used in this study has a wavelength of 380-315 nm. The use of shakers was intended to make RLE (filler) enter the BC cavity. If the shaker was not used then the RLE will
settle on the bottom of the immersion container, so there was no adsorption or absorption into the BC (matrix).

![Figure 1](image1.png)

**Figure 1.** (a) BC which is 2x2 cm in size and 15x2x1 cm before being soaked with RLE (b) BC which is 2x2 cm in size and 15x2x1 cm after being soaked with RLE

Based on the research that has been done it can be seen that the BC which was soaked in RLE for 7.14 and 21 days was brown compared to BC without soaking. Comparison of BC without immersion and BC which has been soaked in this RLE can be seen in Figure 1.

Water content measurement was done to determine the amount of water content contained in BC, RLE, and BC-RLE. Determination of water content is one of the important parameters to determine the physical properties and quality of the BC produced [14]. Based on the results obtained from the water content of BC, RLE, and BC-RLE, it can be seen in Figure 2 that it can be seen that the percentage of water content from BC was obtained at 99.340%. These results indicated that the water content of BC was very high and following the research conducted by Pa'e (2014) which states that the normal BC water content is more than 90% [15]. Whereas, the percentage of water content from RLE obtained is 99.08%.

![Figure 2](image2.png)

**Figure 2.** Effect of immersion time on the percentage of water content

Based on the data obtained it can be seen that in general the longer the immersion time, the percentage of water content decreases. Based on the results obtained above, it can be concluded that the longer the immersion time, the more fillers absorbed by the matrix and replace the water contained in the matrix. The binding that occurs was crossing between matrix and filler. in the BC (matrix) immersion process in RLE extract (filler) a physical absorption process occurs because the filler in a
place replacing the water in the matrix is characterized by a decrease in the water content of BC-RLE from BC, and did not result in the formation of new substances. Soaking BC in RLE in a UV light environment has a lower water content compared to soaking it without a UV light environment. The effect of UV light, RLE extract will enter more water in the BC.

Measurement of the degree of swelling of BC in water is one of the important basic properties of cellulose fibers. Swelling shows how much the polymer chain can expand when interacting with a solvent (water) at a certain time. The percentage of the degree of swelling can be seen in Figure 3. The results obtained from the measurement of the degree of swelling showed that the immersion time affects the percentage of the degree of swelling produced, the longer the immersion of BC in the RLE, the more the filler entering the matrix so that the degree of swelling of the BC-RLE increase. This is because the pores of BC-RLE with the longest immersion will be smaller with regular interconnections, with the filler (RLE) entering. BC interconnects with high interconnections make this area easier for the diffusion of water molecules so that the swelling capacity is higher [16].

![Image of Figure 3](image.png)

**Figure 3.** Graph of the effect of immersion time on% degree of swelling

Compressive strength is a test of the capacity of a material or structure to determine the resistance of a material to a load or pressure that tends to change its shape and size. The compressive strength of a sample identifies the quality of a sample structure. The higher the strength of the structure, the higher the quality of a sample produced. Compressive strength testing is an important parameter in designing or making a material structure because this test can determine the quality of BC produced [17].

In this study, the compressive strength was obtained as shown in Figure 4. Based on Figure 4, it can be explained that the BC sample soaked in RLE its compressive strength increases with increasing immersion time. BC-RLE-UV and BC-RLE-TUV did not significantly affect the compressive strength of the sample. BC has a compressive strength of 0.953 MPa.
Figure 4. Effect of immersion time on Compressive Strength BC-RLE

BC-RLE without and using UV light has increased compressive strength along with the increase in immersion time. The longer the immersion time, the higher the compressive strength of BC-RLE. Increased compressive strength occurs due to an increase in RLE content in the matrix.

The tensile strength test is the maximum tensile test that can be retained by BC-RLE material during testing until it is cut off. Tensile strength testing is an important parameter to determine the quality of BC-RLE produced. The higher the tensile strength, the better the quality of the BC produced. Based on the results obtained, it can be concluded that the tensile and strain values affect the elasticity value produced. The elasticity value is proportional to the tensile value and inversely proportional to the strain value produced.
**Figure 5.** Graph of the effect of immersion time on a) tensile, b) strain and c) elasticity.

The results of the functional group analysis using FTIR can be seen in Figure 6. Based on Figure 6 the functional groups and wave numbers generated from the spectra can be seen in Table 1.

| Sample          | Culmination (cm\(^{-1}\)) |
|-----------------|-----------------------------|
|                 | O-H | C-O-C | C-H | C=C (aromatic) |
| BC              | 3340.95 | 1039.10 | 2919.50 | - |
| Rambutan Leaf   | 3301.03 | 1043.31 | 2923.60 | 1622.61 |
| Rambutan Leaf   | 3340.90 | 1008.30 | 2925.57 | 1630.57 |
| BC-RLE TUV      | 3329.63 | 1032.87 | 2917.29 | 1634.73 |
| BC-RLE UV       | 3334.24 | 1034.62 | 2922.14 | 1608.92 |

Based on the results obtained, BC-RLE did not produce new peaks but only experienced a monochromic and hypochromic shift. The red or bathochromic shift is the maximum absorption shift to a higher wavenumber and a blue or hypochromic shift is the maximum absorption shift to a lower wavenumber.
The results of the analysis of the degree of crystallinity using XRD can be seen in Figure 7. Figure 7 shows that the diffractogram of BC formed does not have a significant difference when compared to the diffractogram of BC-RLE TUV and BC-RLE UV. Based on the peaks formed it can be concluded that BC and BC-RLE are cellulose type I. This is following Liu and Hu (2008) that cellulose peaks of type I appear to the range at 2\(\Theta\) = 15\(^o\), 16\(^o\), 3\(^o\) and 22.7\(^o\) [18].
The determination of the crystallinity of BC-RLE can be determined from the diffractogram formed in Figure 7. In this study, the crystallinity of BC-RLE was determined using the Hermans- Weidingermethod method [19]. Based on the calculation of this method, the resulting degree of crystallinity can be seen in Table 2.

Table 2 shows that the crystallinity of BC is higher than that of BC-RLE TUV and the crystallinity of BC-RLE TUV is higher than BC-RLE UV. The difference in the crystalline intensity of BC, BC-RLE TUV, and BC-RLE UV shows that BC is more crystalline than BC-RLE TUV and BC-RLE UV which causes differences in mechanical properties of BC, BC-RLE TUV, and BC-RLE UV.

| Sample           | $M_{\text{total}}$ (gram) | $M_{\text{amorphous}}$ (gram) | $M_{\text{crystal}}$ (gram) | % crystallinity |
|------------------|---------------------------|-------------------------------|-----------------------------|----------------|
| Pure BC          | 0.2196                    | 0.0623                        | 0.1573                      | 71.630         |
| BC-RLE TUV       | 0.2186                    | 0.0689                        | 0.1497                      | 68.481         |
| BC-RLE UV        | 0.2106                    | 0.0774                        | 0.1332                      | 60.380         |

4. Conclusion

Based on the results of the research that has been done, it can be concluded the water content and the degree of mixing of the BC-RLE UV are lower than the BC-RLE TUV and BC. The longer the BC immersion time in RLE decreases the percentage of water content and degree of swelling produced. The longer the BC immersion time in RLE will increase the mechanical properties (tensile strength and compressive strength) produced. The degree of crystallinity of BC-RLE UV is lower than that of BC-RLE TUV and BC. UV light aid affects the physical and mechanical properties of the BC-RLE.

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