Trop J Nat Prod Res, February 2024; 8(2):6291-6299 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Effect of Coconut Water Storage Time and Inoculum Size of *Lentilactobacillus parafarraginis* on Dried Bacterial Cellulose Properties

Indah Y. Ningsih[1,2,3], Mochammad A. Hidayat[2,3], Bambang Kuswandi[3], Tristiana Erawati[4,5]*

[1]*Doctoral Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60115, East Java, Indonesia*
[2]*Biomaterials and Bioproducts Group, Faculty of Pharmacy, University of Jember, Jember 68121, East Java, Indonesia*
[3]*Chemo and Biosensor Group, Faculty of Pharmacy, University of Jember, Jember 68121, East Java, Indonesia*
[4]*Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60115, East Java, Indonesia*
[5]*Cosmetic Research Group, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60115, East Java, Indonesia.*

ARTICLE INFO

Article history:
Received 19 January 2024
Revised 23 February 2024
Accepted 26 February 2024
Published online 01 March 2024

ABSTRACT

Bacterial cellulose is a polysaccharide that possesses a chemical structure identical to cellulose derived from plants. Due to its high purity and distinct physicochemical properties, bacterial cellulose is extensively used in several industries, e.g., biomedical, food, and tissue engineering. The study aimed to evaluate the impact of the storage time of coconut water and the inoculum size of *Lentilactobacillus parafarraginis* on the properties of dried bacterial cellulose. In this study, coconut water was stored for 1, 2, and 3 days. The inoculum size used to produce bacterial cellulose was 4, 6, 8, and 10%. After fermentation for 9 days, bacterial cellulose was harvested and dried in an oven. The bacterial cellulose was evaluated for its organoleptic, weight, thickness, pH, swelling degree, moisture content, mechanical strength, and water vapour transmission (WVTR) properties. The FTIR spectrum and SEM image analysis were performed on the bacterial cellulose with optimum characteristics. The results showed that bacterial cellulose with coconut water stored for 2 days and inoculum size of 10% (BC210) exhibited optimum characteristics, indicating potential development as a new candidate biomaterial for broad applications.

Keywords: Bacterial cellulose, Coconut water, *Lentilactobacillus parafarraginis*, Storage time, Inoculum size

Introduction

Bacterial cellulose is an exopolymer constructed from units of *β*-1,4-D-glucopyranose and fermented using aerobic microorganisms. It has distinctive characteristics that enable it to be a valuable biomaterial for application in various industries. Bacterial cellulose is a fully biodegradable, environmentally friendly, non-toxic, chemically stable, and biocompatible substance. In contrast to plant cellulose, it is distinguished by its high degree of polymerization, crystallinity, and mechanical strength. Furthermore, bacterial cellulose exhibits higher hydrophilicity due to its smaller fiber diameter than plant cellulose.[1] The fiber also forms a three-dimensional network structure.[2,3] In its natural condition, the fiber network swells when exposed to water. The polymer properties, e.g., mechanical strength, crystallinity, degree of polymerization, and hygroscopicity, are significantly affected by many factors, including culture conditions (such as nutrients of growth medium and inoculum size), type of microorganisms, production method, fermentation time, and others.[4–9] The Hestrin-Schramm standard medium, despite its high cost, is widely utilized for bacterial cellulose synthesis. However, bacteria can be supplemented with alternative sources of nutrition. Carbon source alternatives include sucrose, glucose, fructose, mannitol, arabitol, and molasses/sugarcane molasses.

*Corresponding author. E mail: tristiana-e-m@ff.unair.ac.id; era_ffua@yahoo.co.id
Tel: +628121671607

Citation: Ningsih IY, Hidayat MA, Kuswandi B, Erawati T. Effect of Coconut Water Storage Time and Inoculum Size of *Lentilactobacillus parafarraginis* on Dried Bacterial Cellulose Properties. Trop J Nat Prod Res. 2024; 8(2):6291-6299. http://www.doi.org/10.26538/tjnpr/v8i2.29

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Meanwhile, alternative nitrogen sources are yeast extract, peptone, and corn steep liquor.[10,11] Recently, industrial and agricultural waste, e.g., coconut water, molasses, spoiled fruit cultures, fermentation liquid waste, and orange juice, have been used as nutrient sources in bacterial cellulose synthesis. [12–16] Substituting nutrients substantially decreases the production cost of bacterial cellulose and reduces environmental damage caused by improper handling of industrial waste.

In this study, coconut water was used as the main component of the fermentation medium due to its rich mineral content and high levels of fructose (32.52±0.227 – 39.04±0.824 mg/mL), glucose (29.96±0.243 – 35.43±0.510 mg/mL), and sucrose (6.36±0.06 – 0.85±0.010 mg/mL).[17] These sugars were considered the best carbon sources to produce bacterial cellulose.[18] The utilization of coconut water as a fermentation medium yielded a higher dry weight of bacterial cellulose than a combination of Hestrin-Schramm medium and coconut water.[19] In addition to its abundant and affordable availability, coconut water requires no pre-treatment before the fermentation process, making it efficient and cost-effective.[17] Unfortunately, coconut water quality was altered during storage due to the fermentation process by natural microorganisms, such as *L. paracasei*, *L. plantarum*, and *Pediococcus* sp.[20] This resulted in an increase of lactic acid bacteria and a reduction of the pH of coconut water.[21] Furthermore, the natural fermentation process altered the composition of coconut water, mainly sugar, the optimum carbon source for microbial growth.[22]

The first documentation of cellulose synthesis by microbes was released in 1886 when Brown identified *Acetobacter xylinum* as having the ability to synthesize cellulose.[23,24] It is now recognized that several microorganisms, e.g., Gram-positive bacteria, Gram-negative bacteria, and fungi, including yeast-like fungi, are capable of producing the polymer. Another bacterium potentially producing bacterial cellulose is *Lentilactobacillus parafarraginis*, previously known as *Lactobacillus parafarraginis*. The phenotypic characterization of *L. parafarraginis* strain A1 (KU495926) showed that it is a Gram-positive bacterium, a non-motile rod-shaped bacterium, with a length ranging from

approximately 0.75 to 2.75 μm and a width ranging from approximately 0.25 to 0.75 μm.[25] Other species from the *Lactobacillus* genus employed for bacterial cellulose production included *L. lactis*, *L. brevis*, and *L. plantarum*, as well as *L. acidophilus*.[26,27] Lactobacillus is a non-pathogenic bacteria and is classified as Generally Recognized as Safe (GRAS).[28] Previous studies reported that several *Lactobacillus* species were capable of producing bacterial cellulose with higher quality compared to *Gluconacetobacter xylinus*, *Gluconacetobacter* sp. gel_SEA623-2, and *Komagataeibacter xylinus*.[29–32]
In addition to bacterial species, inoculum size also affects the quality of bacterial cellulose. Therefore, the study optimized the production of bacterial cellulose due to differences in the duration of coconut water storage and investigated the effect of different inoculum sizes. Several characteristic measurements were performed to determine bacterial cellulose with optimum properties. Additionally, coconut water used in bacterial cellulose production was also characterized to comply with quality standards.

Materials and Methods

Identification of plant material
The coconut water used in the study was obtained from mature coconut (*Cocos nucifera* L.) of the Kelapa Genjah variety grown in Kabuaran Village, Grujugan District, Bondowoso Regency, East Java, Indonesia, at an altitude of 425-447 meters above sea level in the coordinate of 7°58'31" S 113°46'17" E. The plant was collected in September 2022. The coconut plant was identified in the Botanical Laboratory of the Faculty of Mathematics and Natural Sciences, University of Jember at Jember, East Java, Indonesia, by Dr.rer.nat. Fuad Bahrul Ulum, S.Si., M.Sc. with voucher number 041/2022.

Identification of the bacterium
The *Lentilactobacillus parafarraginis* bacteria was identified through molecular analysis of the 16S rRNA gene conducted at the Professor Nidom Foundation by Dr. Reviany Vibrianita N., Apt., M.Farm. with a certificate of analysis no. 071122/PNF-XI/2022 (maximum score of 2811, total score of 2811, E-value of 0.0, query cover of 99%, and percent identity of 99.67%). The media for bacterial growth consisted of sucrose, acetic acid, ammonium sulphate, and coconut water, with the composition shown in Table 1.

Characterization of coconut water
Organoleptic test
The examination of the organoleptic appearance of coconut water was carried out using visualization of humans at room temperature, including colour, odour, and flavour.

pH determination
pH measurement of the coconut water was conducted utilizing the pH meter (Transinstrument WalkLAB HP9010, Singapore) at 25 °C. The electrodes were introduced to a double-filtered sample of coconut water, and the device showed a specific value representing the pH of the sample.

Ash content determination
The determination of ash content was conducted according to SNI 4268:2020.[33] The porcelain cup was heated with a low flame for 1 hour, then heated at 105 °C. The cup was placed in a desiccator and weighed (W). The procedure was repeated until a constant weight was obtained. A total of 5-10 g of coconut water, filtered twice, was placed into the cup and weighed (W1). The sample was heated at 100 °C, and a small amount of olive oil was introduced. Then, the sample was incinerated in a furnace (Carbolite ELF 11/14B, England) at 525 °C until it turned into white ash and cooled in a desiccator before being weighed. The cup was placed into the furnace at the same temperature for 1 hour, cooled in a desiccator, and weighed. The testing procedure was repeated until a constant weight was achieved (W2). The ash content of the sample was calculated using the following formula:

$$Ash\ content = \frac{W2-W}{W1-W}\ x\ 100\% \quad \text{.....(1)}$$

W = the empty cup weight
W1 = the empty cup and the sample weight
W2 = the empty cup and the ash weight

Potassium content determination
A 25 mL sample was destructed through dry ashing at 525 °C and then dissolved in a 50.0 mL volumetric flask containing 1 M HNO_3 solution. The sample was diluted to conform with the potassium standard curve (2, 4, 6, 8, and 10 ppm) to which 0.5% (w/v) CsCl solution was added. A blank solution was prepared using the same procedure. The absorbance of the standard solution, sample, and blank was determined using an atomic absorption spectrophotometer (Hitachi ZA-3000, Japan) at 766.5 nm.

Inoculum preparation
The formula used in the production of bacterial inoculum is shown in Table 1. The coconut water was stored at room temperature for 2 days, filtered twice, then heated at 90ºC for 5 minutes. To produce fermentation medium, sucrose, ammonium sulfate, and acetic acid were added to the prepared coconut water and thoroughly mixed. The mixture was sterilized using an autoclave (ALP CL-40L, Japan) at 121ºC for 15 minutes and cooled. Subsequently, the starter culture of *L. parafarraginis* was introduced to the medium. The inoculation was conducted for 9 days at 26-30ºC. The bacterial colony count was determined using the turbidity method.[34] The quantity of inoculum was determined using a UV-Vis spectrophotometer (ThermoFisher Scientific Genesys, USA) at 600 nm, resulting in an OD_{600} of 0.1, equivalent to $1x10^7$ CFU/mL.

Dried bacterial cellulose preparation
The process of bacterial cellulose preparation was similar to the bacterial inoculum preparation (Figure 1) based on the formula shown in Table 1. The preparation was performed using different coconut water storage times (1 day, 2 days, and 3 days) and inoculum size (4%, 6%, 8%, and 10%) at 25-30 °C. After a 9-day fermentation process, bacterial cellulose was harvested and purified. The wet bacterial cellulose washed using flowing water to remove impurities.[35,36] Subsequently, it was added to distilled water at 100 °C for 60 minutes. It was immersed in 0.5 M NaOH solution at 100 °C for 30 minutes, washed using running water, and repeatedly soaked in distilled water to achieve a pH of 7.0.[19,37] Then, it was dried at 60 °C for 48 hours to obtain the dried bacterial cellulose.

Evaluation of dried bacterial cellulose properties
Organoleptic test
The organoleptic examination of bacterial cellulose was visually performed at 25-30 °C. The determined organoleptic characteristics included colour, odour, and surface properties.

Weight and thickness determination
Weight and thickness were determined for both wet and dried bacterial cellulose. The wet weight was determined before drying, followed by drying at 60°C for 48 hours and weighing the dried bacterial cellulose.[38] The thickness was measured using a calliper (Inoki, Japan) with repeated measurements at 5 different positions of bacterial cellulose.

pH determination
The determination of pH was conducted using a pH meter. One percent of bacterial cellulose was introduced to 20 mL of distilled water.[39]

Table 1: Formula of bacterial inoculum preparation

Ingredients	Quantity (%)
Starter culture	10
Sucrose	2.5
Acetic acid	1
Ammonium sulphate	0.5
Coconut water	86

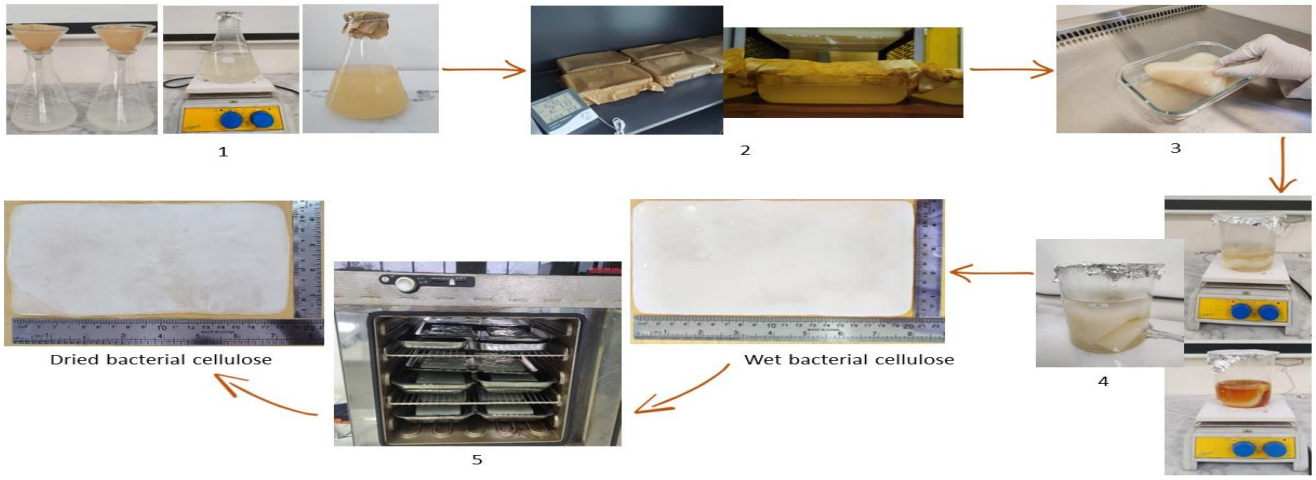

Figure 1: Production of dried bacterial cellulose
1: Fermentation medium production. 2: Fermentation process (after addition of starter culture). 3: Harvesting. 4: Purification. 5: Drying

Swelling degree determination
The dried bacterial cellulose, sized 1.5 x 1.5 cm, was weighed and soaked in 25 mL of phosphate buffer solution (pH 7.4) at 25-30°C. The sample was carefully moved from the solution after 1, 4, and 6 hours. The remaining solution on the bacterial cellulose surface was removed using filter paper, and it was weighed. Calculation of swelling degree was carried out using the following formula:

$$Swelling\ degree = \frac{w2-w1}{w1}\ x\ 100\% \quad \text{.....(2)}$$

w1 is the weight before swelling, while w2 represents the weight after swelling.[39]

Moisture content determination
The dried bacterial cellulose, 2 x 2 cm, was put in a porcelain cup and heated at 90 °C for 24 hours. Calculation of moisture content was conducted based on the following formula:

$$Moisture\ content = \frac{m1-m2}{m2}\ x\ 100\% \quad \text{.....(3)}$$

m1 represents the initial weight of bacterial cellulose, and m2 is the final weight.[39]

Mechanical strength determination
The sample was cut into a size of 6 cm x 2 cm. Determination of mechanical strength was performed using a Universal Testing Machine (Hung Ta HT-2328, Taiwan) at 25 ± 2°C to measure tensile strength and elongation at break according to ASTM D1822 with a stretching rate of 1 mm/min and preload of 0.05 mPa.

Water vapor transmission (WVTR) determination
Five grams of anhydrous calcium chloride were added to a weighing bottle (Pyrex, China) as an adsorbent. After positioning the dried bacterial cellulose on top of the bottle and tying it up with thread, it was put in a desiccator (Pyrex, China) at 25 °C with a relative humidity of 75%. The following formula was applied to obtain the WVTR value:

$$WVTR = W/A \quad \text{.....(4)}$$

W represents the weight of bacterial cellulose after 24 hours (g), and A is the area of bacterial cellulose (m^2).[39]

FTIR spectrum and SEM analysis
Bacterial cellulose with optimum characteristics was analyzed for its structure using FTIR (Bruker Alpha, Germany) and SEM (Hitachi TM3000, Japan). The FTIR was run within a frequency range of 4,000-600 cm^{-1} and a resolution of 4 cm^{-1}. The spectrum was utilized to assess its functional groups. Furthermore, the SEM was operated at 15 kV and magnified up to 3,000 times.

Data analysis
The study was conducted in three measurements. The data was shown as the mean of all measures ± the standard deviation (SD). To assess significant differences between groups, One-way Analysis of Variance (ANOVA) and Least Significant Difference (LSD) tests were utilized using a significance level (α) of 5%.

Results and Discussion

The evaluation of coconut water as the primary constituent of the fermentation medium is presented in Table 2. The study revealed that fresh coconut water used in bacterial cellulose production fulfilled SNI 4268-2020 requirements regarding organoleptic properties, pH, ash content, and potassium content.[33]
The result of the evaluation of organoleptic properties is shown in Figure 2. It was observed that all samples had a similar organoleptic appearance. The bacterial cellulose showed a pale yellowish-white coloration, smooth surface, and odourless. Therefore, it was suggested that the storage time of coconut water and inoculum size had no impact on its organoleptic properties.
Also, the results of the weight and thickness determination of the dried bacterial cellulose are presented in Figures 3 and 4. From the results, it was evident that bacterial cellulose produced using coconut water with storage duration of 2 days and inoculum size of 10%, namely BC210, showed the highest wet weight, dry weight, wet thickness, and dry thickness with values of 156.5622±0.4327 g, 1.5910±0.0070 g, 13.7233±0.0205 mm, and 0.1067±0.0047 mm, respectively. Meanwhile, the lowest values of weight and thickness were obtained by bacterial cellulose with a 1-day storage period of coconut water and inoculum size of 4%, namely BC14, with values of 134.8662±0.0239 g, 1.4961±0.0001 g, 10.4833±0.0776 mm, and 0.0900±0.0000 mm, respectively.

Table 2: Characterization of fresh coconut water

Parameter	Test result	References[33]
Organoleptic test:		
Colour	Transparent, white	Normal
Odour	Normal	Normal
Flavour	Normal, slightly sweet	Normal
pH	5.08 ± 0.02	5.0-6.0
Ash content (%)	0.5299 ± 0.0047	≤ 0.6
Potassium content (mg/100 g)	179.2630 ± 1.7211	≥ 120

The duration of coconut water storage affected wet weight, dry weight, wet thickness, and dry thickness in the following order: 1 day < 3 days < 2 days. The longer the storage time of coconut water, the more acidic the pH. The previous study reported that *L. hilgardii* grew optimally in an acidic environment at a pH of 3.0, compared to pH 7.0 and 9.0.[25] In this study, storing coconut water for 2 days was indicated to produce the optimum pH for *L. parafarraginis* growth. Nevertheless, prolonged storage of coconut water led to an alteration in its composition due to natural fermentation processes. This decreased the essential contents of coconut water, particularly sugar, the best carbon source for microbial growth.[22] Consequently, the wet weight, dry weight, wet thickness, and dry thickness of bacterial cellulose produced by coconut water after a storage period of 3 days were lower than that stored for 2 days. In addition, inoculum size also affected the weight and thickness of bacterial cellulose. This finding aligned with earlier studies indicating that the higher the inoculum size, the higher the bacterial cellulose yield.[7,40]

The study proved that the pH of bacterial cellulose varied within the range of 4.57±0.02 - 6.43±0.02 (Figure 5). The result was consistent with the pH range of the skin, which was the application site for formulations using bacterial cellulose as a carrier of various active ingredients, namely 4.5 - 6.5.[41] The quality of coconut water changed during storage due to the fermentation process by natural microorganisms, such as *L. plantarum*, *L. paracasei*, and *Pediococcus* sp.[20] This contributed to an enhancement in the quantity of natural bacteria, mainly lactic acid bacteria and a reduction in the pH of coconut water.[21] Therefore, the longer the storage time of coconut water, the more acidic the pH of bacterial cellulose. Moreover, a larger inoculum size caused an increasing number of bacteria to produce an enhanced quantity of various acids, such as lactic acid, formic acid, acetic acid, propionic acid, and succinic acid, due to bacterial metabolism during fermentation. This caused the pH of bacterial cellulose to become more acidic. [42]

The result of the degree of swelling is shown in Figure 6, which indicates that the storage time of coconut water affected the swelling capacity of bacterial cellulose in the following order: 1 day < 3 days < 2 days. Moreover, the higher the inoculum size, the higher the swelling degree. BC210 had the highest swelling degree of 301.8931±0.3177% within 24 hours. Meanwhile, BC14 exhibited the lowest swelling degree value, which was 142.2928±0.6267% within 24 hours. Previous studies reported that water absorption of native cellulose ranges from 90-350%.[43,44] This seemed to be related to the cellulose yield produced by bacteria. BC210 produced the highest yield of bacterial cellulose, equivalent to an enhancement in the amount of fiber formed in its morphological structure. The presence of more fibers led to an enhancement in the hydrogen bond formation between bacterial cellulose and water, causing a higher degree of swelling. The polar groups on the polymer chain of bacterial cellulose interact with water molecules by hydrogen bonding, increasing bacterial cellulose's swelling degree and volume. The unbound water that enters and exits the molecular structure of the material is responsible for maintaining the hydration level on the skin surface.[45] The high water absorption capacity of the bacterial cellulose leads to a plasticizing impact that affects its mechanical, permeability, and optical properties.[46–50] The ability to absorb and hold water allows the loading of liquid drugs and bioactive substances onto bacterial cellulose.[51] In addition, this characteristic enables bacterial cellulose to absorb exudate in wounds, promote wound healing, and enhance its role as a drug delivery system in wound dressing and cosmetics, such as sheet masks.[39]

The moisture content evaluation is depicted in Figure 7. The study demonstrated that the duration of coconut water storage affected the moisture content of bacterial cellulose in the following order: 1 day < 3 days < 2 days. Furthermore, an increase in inoculum size resulted in an enhancement in moisture content. The polysaccharide components of bacterial cellulose possess natural hydrophilic properties, allowing them to interact with the surrounding moisture. The polar groups of bacterial cellulose result in a high water-holding capacity.[52–55] Thus, BC210, with the highest number of fibers and the highest quantity of polar groups, produced the highest moisture content (9.0058±0.0414%), whereas BC14 had the lowest moisture content (6.1227±0.1668%). The moisture content value of the study meets the requirement of dried bacterial cellulose suitable for pharmaceutical formulations, 5 - 10%. The moisture content below 5% decreases hydrophilic properties and absorption capacity.[56] Furthermore, moisture in its structure ensures that bacterial cellulose remains consistently humid, thereby contributing to maintaining skin hydration and improving wound healing.[39]

Furthermore, the mechanical properties of bacterial cellulose are characterized mainly by tensile strength and elongation at break. Tensile strength refers to its maximum capacity to withstand stress or the stress level at which it fractures.

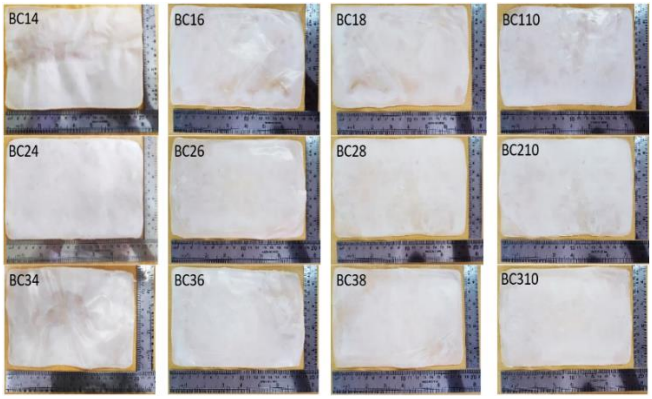

Figure 2: Dried bacterial cellulose with different coconut water storage times and inoculum size

BC14: Bacterial cellulose from 1-day storage of coconut water and inoculum size of 4%. BC16: Bacterial cellulose from 1-day storage of coconut water and inoculum size of 6%. BC18: Bacterial cellulose from 1-day storage of coconut water and inoculum size of 8%. BC110: Bacterial cellulose from 1-day storage of coconut water and inoculum size of 10%. BC24: Bacterial cellulose from 2-days storage of coconut water and inoculum size of 4%. BC26: Bacterial cellulose from 2-days storage of coconut water and inoculum size of 6%. BC28: Bacterial cellulose from 2-days storage of coconut water and inoculum size of 8%. BC210: Bacterial cellulose from 2-days storage of coconut water and inoculum size of 10%. BC34: Bacterial cellulose from 3-days storage of coconut water and inoculum size of 4%. BC36: Bacterial cellulose from 3-days storage of coconut water and inoculum size of 6%. BC38: Bacterial cellulose from 3-days storage of coconut water and inoculum size of 8%. BC310: Bacterial cellulose from 3-days storage of coconut water and inoculum size of 10%.

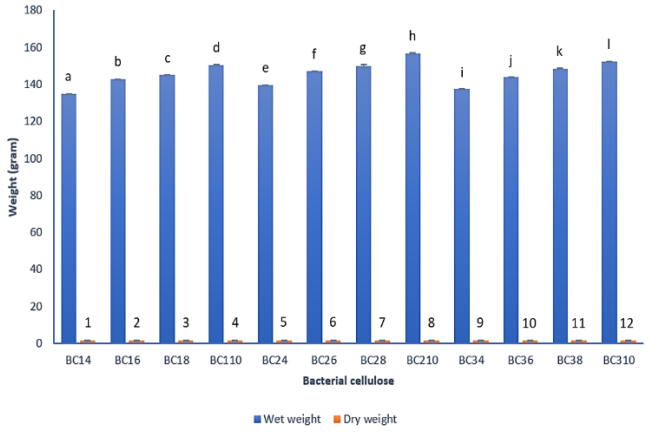

Figure 3: Weight evaluation of bacterial cellulose

Histograms represent the mean of 3 measurements ± SD. Different letters and numbers for histograms with the same colour denote significant differences based on the LSD test ($p < 0.05$).

Elongation at break, also known as ductility, is the material's capacity to deform under stress determined by calculating the area underneath the stress-strain curve of bacterial cellulose.[57] The mechanical strength evaluation is depicted in Figure 8. The study demonstrated that BC210 had the highest tensile strength and elongation at break, at

11.6313±0.0642 N/mm^2 and 63.6667±1.2472%, respectively. Meanwhile, BC14 exhibited the lowest tensile strength and elongation at break, at 4.0322±0.0073 N/mm^2 and 35.0000±0.8165%, respectively. Previous research showed that the mechanical strength of bacterial cellulose was mainly associated with the number of fibers, which was related to its weight and thickness.[39,58] Bacterial cellulose with the highest weight, BC210, indicated a greater number of fibers in its morphological structure, resulting in increased mechanical strength. Another study reported that mechanical strength for facial masks is greater than 0.1 kgf/cm^2 or 0.0098 N/mm^2.[59] The minimum elongation at break value for sheet masks is 30%. In addition, the minimum tensile strength for wound dressing is greater than 1 N/mm^2, and the minimum elongation at break is greater than 10%.[39]

Water vapor transmission (WVTR) is essential for pharmaceutical preparations, such as wound care and sheet mask formulations, which require optimum moisture conditions and efficient gas (oxygen) exchange.[39,45] As illustrated in Figure 9, the WVTR is enhanced in the following order according to the duration of coconut water storage: 2 days < 3 days < 1 day. Furthermore, an enhancement in inoculum size led to a reduction in WVTR value.

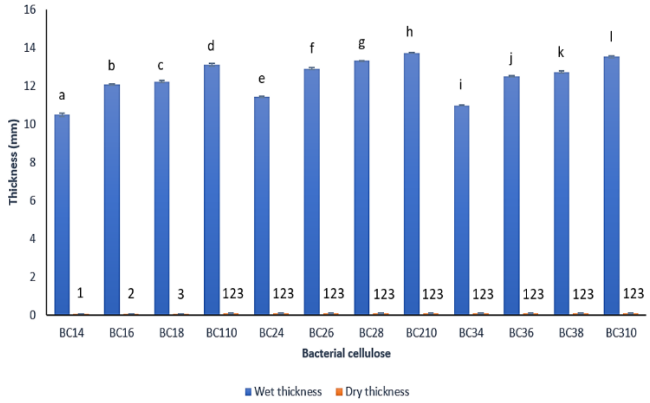

Figure 4: Thickness evaluation of bacterial cellulose
Histograms represent the mean of 3 measurements ± SD. Different letters and numbers for histograms with the same colour denote significant differences based on the LSD test ($p < 0.05$).

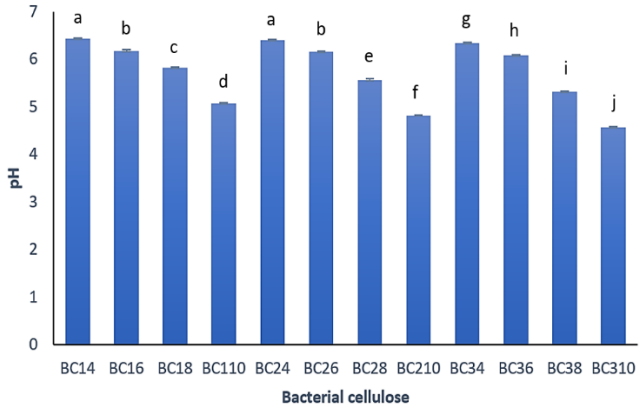

Figure 5: pH evaluation of dried bacterial cellulose
Histograms represent the mean of 3 measurements ± SD. Different letters denote significant differences based on the LSD test ($p < 0.05$)

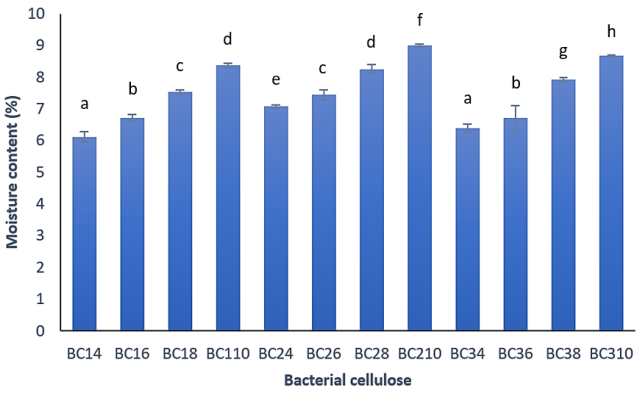

Figure 7: Moisture content evaluation of dried bacterial cellulose
Histograms represent the mean of 3 measurements ± SD. Different letters denote significant differences based on the LSD test ($p < 0.05$).

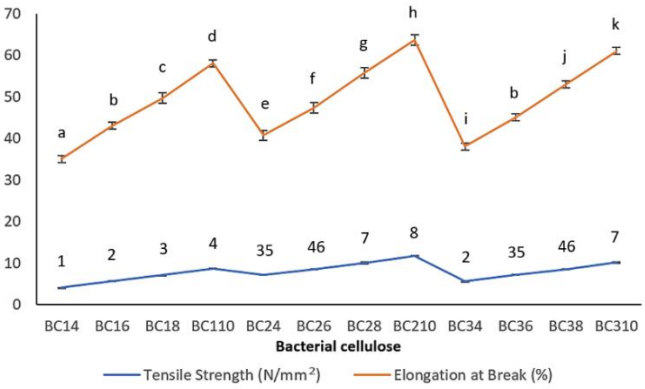

Figure 8: Mechanical strength evaluation of dried bacterial cellulose
Points represent the mean of 3 measurements ± SD. Different letters and numbers for the same line denote significant differences based on the LSD test ($p < 0.05$).

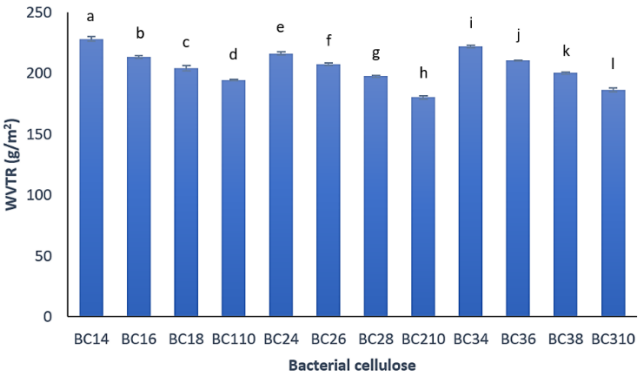

Figure 9: WVTR evaluation of dried bacterial cellulose
Histograms represent the mean of 3 measurements ± SD. Different letters denote significant differences based on the LSD test ($p < 0.05$).

Table 3: Identification of FTIR spectrum of BC210

No.	Wavenumbers of BC210 (cm^{-1})	Wavenumbers of reference (cm^{-1})	Identification of functional groups	Reference
1	3341.643	~3340	Stretching vibration of inter and intra O-H in cellulose	[30]
2	2908.179	~2900	CH_2 asymmetric and symmetric stretching of cellulose	[30]
3	2858.600	~2851	C-H stretching vibration of sugar rings	[61]
4	1644.616	~1650	H-O-H bending of absorbed water	[30]
5	1630.450	~1630	C-O stretching	[62]
6	1427.883	~1427	-OH bending	[61]
7	1413.718	~1420	CH_2 bending	[62]
8	1357.056	~1360	C-H bending	[30]
9	1332.974	~1335	C-H deformation or -OH in-plane bending	[63]
10	1311.726	~1314	CH_2 wagging at C-6	[61]
11	1280.562	~1280	C-H bending	[64]
12	1201.235	~1203	C-H bending	[64]
13	1158.739	~1160	C-O-C asymmetric stretching at β-glycosidic linkage	[61]
14	1104.910	~1108	C-C bonds of the monomer units from polysaccharide	[64]
15	1053.914	~1060	C-O-H bond of carbohydrate	[30]
16	1029.832	~1030	C-O-C ring skeletal vibration	[61]
17	667.195	~660	O-H out-of-phase bending vibration	[64]
18	889.594	~893	C-O-C stretching of pyranose ring and bending vibration of (1-4) β linkage	[30]

This has been suggested to be due to the increasing amount of fiber in the structure of bacterial cellulose, which led to a reduced interchain distance between polymer chains or fibers, which inhibited the exchange of water vapor. The study showed that WVTR values ranged from 180.0848±1.2992 to 228.1937±1.7605 g/m^2 in a 24-hour experimental period. A WVTR value below 840 g/m^2/24 hr indicates that bacterial cellulose is moisture retentive, while a value below 300 g/m^2/24 hr demonstrates an occlusive characteristic maintaining moisture on the skin.[60]

The FTIR spectrum analysis was conducted on bacterial cellulose with optimum characteristics, namely BC210. Figure 10 depicts the presence of 18 bands that represent the characteristic functional groups of bacterial cellulose identified in Table 3. The resulting bands undergo a maximum shift of 8 cm^{-1}, which was suggested to be due to fermentation medium and bacteria differences in bacterial cellulose production. However, the FTIR spectrum of bacterial cellulose using *L. parafarraginis* showed similarities with bacterial cellulose obtained from *L. hilgardii* IITRKH159.[30]

The analysis of morphological structure using SEM was performed on BC210 as bacterial cellulose with optimum characteristics, as illustrated in Figure 11. The SEM image of BC210 is similar to the fiber structure of bacterial cellulose using *L. hilgardii* IITRKH159. It is known that the cellulose of *L. hilgardii* IITRKH159 bacteria yielded thinner and finer fibers than those produced by *K. xylinus*.[30] The fiber arrangement of BC210 was a complex structure composed of tightly arranged cellulose fibers that were intricately woven together in a three-dimensional network. Compact fibers and a decreased volume of pores also characterized the structure of bacterial cellulose, which was related to a reduction in WVTR value.[65] The fiber structure reduced the distance and quantity of trapping sites for water molecules. In contrast, a dense bacterial cellulose structure was associated with the enhancement of moisture content. The density enhancement of microfibers led to high water holding in the system, mainly because of hydrogen bond formation. This contributed to a decrease in the amount of free bulk water and thus reduced water evaporation.[66]

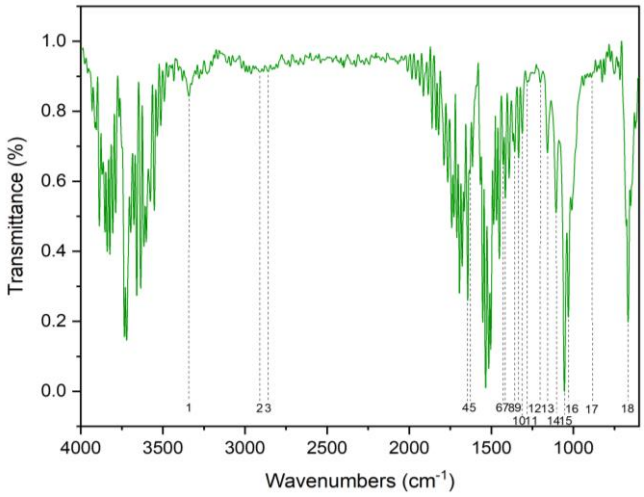

Figure 10: FTIR spectrum of BC210

Conclusion

The study utilized coconut water as the main ingredient for preparing *L. parafarraginis* growth medium for bacterial cellulose production. According to the evaluation of bacterial cellulose characteristics, BC210 exhibited optimum properties, including organoleptic test, weight, thickness, pH, swelling degree, moisture content, mechanical strength, and WVTR. The FTIR spectrum analysis showed that BC210 had several characteristic functional groups of bacterial cellulose. Moreover, the analysis of the SEM image revealed that BC210 exhibited a morphological structure similar to bacterial cellulose using bacteria of the same genus, namely *L. hilgardii* IITRKH159.

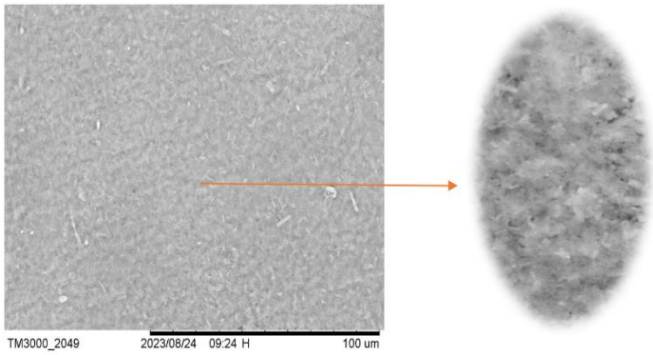

Figure 11: Scanning electron micrograph of BC210 in 1000 and 3000x magnification

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors thank the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia through Dissertation Completion Scholarship-Indonesian Education Scholarship (Beasiswa Bantuan Penyelesaian Disertasi-Beasiswa Pendidikan Indonesia) 2023 Batch II.

References

1. Ye S, Jiang L, Su C, Zhu Z, Wen Y, Shao W. Development of gelatin/bacterial cellulose composite sponges as potential natural wound dressings. Int J Biol Macromol. 2019; 133:148-155. doi:10.1016/j.ijbiomac.2019.04.095
2. Illa MP, Sharma CS, Khandelwal M. Tuning the physiochemical properties of bacterial cellulose: effect of drying conditions. J Mater Sci. 2019; 54(18):12024-12035. doi:10.1007/s10853-019-03737-9
3. Sederavičiūtė F, Bekampienė P, Domskienė J. Effect of pre-treatment procedure on properties of Kombucha fermented bacterial cellulose membrane. Polym Test. 2019; 78:105941. doi:10.1016/j.polymertesting.2019.105941
4. Hu W, Chen S, Yang J, Li Z, Wang H. Functionalized bacterial cellulose derivatives and nanocomposites. Carbohydr Polym. 2014; 101:1043-1060. doi:10.1016/j.carbpol.2013.09.102
5. Hodel KVS, Fonseca LM dos S, Santos IM da SS, Cerqueira JC, dos Santos-Junior RE, Nunes SB, Barbosa JDV, Machado BAS. Evaluation of different methods for cultivating *Gluconacetobacter hansenii* for bacterial cellulose and montmorillonite biocomposite production: Wound-dressing applications. Polymers. 2020; 12(2):267. doi:10.3390/polym12020267
6. Revin VV, Liyas'kina EV, Sapunova NB, Bogatyreva AO. Isolation and characterization of the strains producing bacterial cellulose. Microbiology. 2020; 89(1):86-95. doi:10.1134/S0026261720010130
7. Yanti NA, Ahmad SW, Muhiddin NH. Evaluation of inoculum size and fermentation period for bacterial cellulose production from sago liquid waste. J Phys Conf Ser. 2018; 1116(2018):1-7. doi:10.1088/1742-6596/1116/5/052076
8. Kim JA, Jung WS, Chun SC, Yu CY, Ma KH, Gwag JG, Chung IM. A correlation between the level of phenolic compounds and the antioxidant capacity in cooked-with-rice and vegetable soybean (*Glycine max* L.) varieties. Eur Food Res Technol. 2006; 224(2):259-270. doi:10.1007/s00217-006-0377-y
9. Devanthi PVP, Pratama F, Kho K, Taherzadeh MJ, Aslanzadeh S. The effect of *Dekkera bruxellensis* concentration and inoculation time on biochemical changes and cellulose biosynthesis by *Komagataeibacter intermedius*. J Fungi. 2022; 8(1206):1-13. doi:10.3390/jof8111206
10. Keshk S, Sameshima K. Evaluation of different carbon sources for bacterial cellulose production. Afr J Biotechnol. 2005; 4(6):478-482.
11. Buldum G, Bismarck A, Mantalaris A. Recombinant biosynthesis of bacterial cellulose in genetically modified *Escherichia coli*. Bioprocess Biosyst Eng. 2018; 41(2):265-279. doi:10.1007/s00449-017-1864-1
12. Velasquez MT, Bhathena SJ. Role of dietary soy protein in obesity. Int J Med Sci. 2007; 4:72-82. doi:10.7150/ijms.4.72
13. Kongruang S. Bacterial cellulose production by *Acetobacter xylinum* strains from agricultural waste products. Appl Biochem Biotechnol. 2008; 148(1-3):245-256. doi:10.1007/s12010-007-8119-6
14. Wu JM, Liu RH. Cost-effective production of bacterial cellulose in static cultures using distillery wastewater. J Biosci Bioeng. 2013; 115(3):284-290. doi:10.1016/j.jbiosc.2012.09.014
15. Cao Y, Lu S, Yang Y. Production of bacterial cellulose from byproduct of citrus juice processing (citrus pulp) by *Gluconacetobacter hansenii*. Cellulose. 2018; 25(12):6977-6988. doi:10.1007/s10570-018-2056-0
16. Amorima J, Costab A, Galdino Jr. C, Vinhasa G, Santos E, Sarubb L. Bacterial cellulose production using fruit residues as substract to industrial application. Chem Eng Trans. 2019; 74:1165-1170.
17. Tan TC, Cheng LH, Bhat R, Rusul G, Easa AM. Composition, physicochemical properties and thermal inactivation kinetics of polyphenol oxidase and peroxidase from coconut (*Cocos nucifera*) water obtained from immature, mature and overly-mature coconut. Food Chem. 2014; 142:121-128. doi:10.1016/j.foodchem.2013.07.040
18. Rani MU, Udayasankar K, Appaiah KAA. Properties of bacterial cellulose produced in grape medium by native isolate *Gluconacetobacter* sp. J Appl Polym Sci. 2011; 120(5):2835-2841. doi:10.1002/app.33307
19. Kamarudin S, Sahaid K, Sobri T, Mohtar W, Radiah A, Norhaliza H. Different media formulation on biocellulose production by *Acetobacter xylinum* (0416). Pertanika J Sci Technol. 2013; 21(1):29-36.
20. Suryani S, Purnawati Y, Gemaeka Putri S, Rahmawati R, Akbar Y, Yusra Y. Novel probiotic isolation of coconut water's helpful lactic acid bacteria cure Covid-19 patients. ARRUS J Eng Tech. 2022; 2(1):1-11. doi:10.35877/jetech724
21. Pramana WA, Septinova D, Riyanti R, Husni A. Pengaruh air kelapa hasil fermentasi terhadap kualitas fisik daging broiler. Jurnal Riset dan Inovasi Peternakan. 2018; 2(2):7-13.
22. Santosa B, Wignyanto W, Hidayat N, Sucipto S. The quality of nata de coco from sawarna and mapanget coconut varieties to the time of storing coconut water. Food Res. 2020; 4(4):957-963. doi:10.26656/fr.2017.4(4).372
23. Brown AJ. The chemical action of pure cultivations of *Bacterium aceti*. J Chem Soc T. 1886; 49(0):172-187. doi:10.1039/CT8864900172
24. Skočaj M. Bacterial nanocellulose in papermaking. Cellulose. 2019; 26(11):6477-6488. doi:10.1007/s10570-019-02566-y
25. Allen-McFarlane R, Allen AD, Bansal G, Eribo B. Isolation and characterization of *L. parafarraginis* (KU495926) inhibiting multidrug-resistant and extended spectrum β-lactamase gram-negative bacteria. J Microbiol Biotechnol

Food Sci. 2019; 8(4):1041-1053. doi:10.15414/jmbfs.2019.8.4.1041-1053
26. Adetunji V, Adegoke G. Bacteriocin and cellulose production by lactic acid bacteria isolated from West African soft cheese. Afr J Biotechnol. 2007; 6(22):2616-2619. doi:10.5897/AJB2007.000-2418
27. Sumardee JNS, Mohd-Hairul AR, Mortan SH. Effect of inoculum size and glucose concentration for bacterial cellulose production by *Lactobacillus acidophilus*. In: IOP Conf Ser: Mater Sci Eng. Vol 991. IOP Publishing Ltd; 2020:1-5. doi:10.1088/1757-899X/991/1/012054
28. Elbanna K, El Hadad S, Assaeedi A, Aldahlawi A, Khider M, Alhebshi A. In vitro and in vivo evidences for innate immune stimulators lactic acid bacterial starters isolated from fermented camel dairy products. Sci Rep. 2018; 8(12553):1-15. doi:10.1038/s41598-018-31006-3
29. Seto A, Saito Y, Matsushige M, Kobayashi H, Sasaki Y, Tonouchi N, Tsuchida T, Yoshinaga F, Ueda K, Beppu T. Effective cellulose production by a coculture of *Gluconacetobacter xylinus* and *Lactobacillus mali*. Appl Microbiol Biotechnol. 2006; 73(4):915-921. doi:10.1007/s00253-006-0515-2
30. Khan H, Kadam A, Dutt D. Studies on bacterial cellulose produced by a novel strain of *Lactobacillus* genus. Carbohydr Polym. 2020; 229:115513. doi:10.1016/j.carbpol.2019.115513
31. Gunduz G, Erbas Kiziltas E, Kiziltas A, Gencer A, Aydemir D, Asik N. Production of bacterial cellulose fibers in the presence of effective microorganism. J Nat Fibers. 2019; 16(4):567-575. doi:10.1080/15440478.2018.1428847
32. Kim KM, Kim J, Yang KW. Effect of acetic acid concentration and mixed culture of lactic acid bacteria on producing bacterial cellulose using *Gluconacetobacter* sp. gel_SEA623-2. Korean J Microbiol. 2014; 50(3):227-232. doi:10.7845/kjm.2014.4062
33. Badan Standarisasi Nasional. Standar Nasional Indonesia SNI 4268-2020 Air Kelapa Olahan. Published online 2020:1-20.
34. Thongwai N, Futui W, Ladpala N, Sirichai B, Weechan A, Kanklai J, Rungsirivanich P. Characterization of bacterial cellulose produced by *Komagataeibacter maltaceti* P285 isolated from contaminated honey wine. Microorganisms. 2022; 10(3):1-18. doi:10.3390/microorganisms10030528
35. Indrianingsih AW, Rosyida VT, Apriyana W, Hayati SN, Darsih C, Nisa K, Ratih D. Antioxidant and antibacterial properties of bacterial cellulose-Indonesian plant extract composites for mask sheet. J Appl Pharm Sci. 2020; 10(7):37-42. doi:10.7324/JAPS.2020.10705
36. Indrianingsih AW, Rosyida VT, Jatmiko TH, Prasetya DJ, Poeloengasih CD, Apriyana W, Nisa K, Nurhayati S, Hernawan, Darsih C, Pratiwi D, Suwanto A, Ratih D. Preliminary study on biosynthesis and characterization of bacteria cellulose films from coconut water. In: IOP Conf Ser: Earth Environ Sci. Vol 101. Institute of Physics Publishing; 2017. doi:10.1088/1755-1315/101/1/012010
37. Kuswandi B, Jayus, Oktaviana R, Abdullah A, Heng LY. A novel on-package sticker sensor based on methyl red for real-time monitoring of broiler chicken cut freshness. Packag Technol Sci. 2014; 27(1):69-81. doi:10.1002/pts.2016
38. Fitri R, Reveny J, Harahap U, Syahputra HD, Nasri N. Anti-acne activity from biocellulose mask formula containing (*Aloe vera* (L.) Burm. F) essence combined with vitamin E. Indonesian J Pharm Clin Res. 2021; 04(01):2021-2022.
39. Sulastri E, Zubair MS, Lesmana R, Mohammed AFA, Wathoni N. Development and characterization of ulvan polysaccharides-based hydrogel films for potential wound dressing applications. Drug Des Devel Ther. 2021; 15:4213-4226. doi:10.2147/DDDT.S331120
40. Aswini K, Gopal NO, Uthandi S. Optimized culture conditions for bacterial cellulose production by *Acetobacter senegalensis* MA1. BMC Biotechnol. 2020; 20(1). doi:10.1186/s12896-020-00639-6
41. Tranggono RI, Latifah F. Buku Pegangan Dasar Kosmetologi. 1st ed. (Djajadisastra J, Adimukti P, eds.). Sagung Seto; 2014.
42. Gänzle MG. Lactic metabolism revisited: Metabolism of lactic acid bacteria in food fermentations and food spoilage. Curr Opin Food Sci. 2015; 2:106-117. doi:10.1016/j.cofs.2015.03.001
43. Mohammadkazemi F, Khademibarangenani R, Koosha M. The effect of oxidation time and concentration on physicochemical, structural, and thermal properties of bacterial nano-cellulose. Polym Sci Ser A. 2019; 61(3):265-273. doi:10.1134/S0965545X19030088
44. de Amorim JDP, de Souza KC, Duarte CR, Duarte I da S, Ribeiro F de AS, Silva GS, de Farias PMA, Stingl A, Costa AFS, Vinhas GM, Sarubbo LA. Plant and bacterial nanocellulose: production, properties and applications in medicine, food, cosmetics, electronics and engineering. A review. Environ Chem Lett. 2020; 18(3):851-869. doi:10.1007/s10311-020-00989-9
45. Portela R, Leal CR, Almeida PL, Sobral RG. Bacterial cellulose: A versatile biopolymer for wound dressing applications. Microb Biotechnol. 2019; 12(4):586-610. doi:10.1111/1751-7915.13392
46. Nisar T, Wang ZC, Alim A, Iqbal M, Yang X, S Lijun, Guo Y. Citrus pectin films enriched with thinned young apple polyphenols for potential use as bio-based active packaging. CyTA J Food. 2019; 17(1):695-705. doi:10.1080/19476337.2019.1640798
47. Cazón P, Vázquez M, Velazquez G. Environmentally friendly films combining bacterial cellulose, chitosan, and Polyvinyl Alcohol: Effect of water activity on barrier, mechanical, and optical properties. Biomacromolecules. 2020; 21(2):753-760. doi:10.1021/acs.biomac.9b01457
48. Cazón P, Velazquez G, Vázquez M. Characterization of mechanical and barrier properties of bacterial cellulose, glycerol and polyvinyl alcohol (PVOH) composite films with eco-friendly UV-protective properties. Food Hydrocoll. 2020; 99:105323. doi:10.1016/j.foodhyd.2019.105323
49. Ludwiczak S, Mucha M. Modeling of water sorption isotherms of chitosan blends. Carbohydr Polym. 2010; 79(1):34-39. doi:10.1016/j.carbpol.2009.07.014
50. López-Palestina CU, Aguirre-Mancilla CL, Raya-Pérez JC, Ramirez-Pimentel JG, Vargas-Torres A, Hernández-Fuentes AD. Physicochemical and antioxidant properties of gelatin-based films containing oily tomato extract (*Solanum lycopersicum* L.). CyTA J Food. 2019; 17(1):142-150. doi:10.1080/19476337.2018.1564793
51. Shah N, Ul-Islam M, Khattak WA, Park JK. Overview of bacterial cellulose composites: A multipurpose advanced material. Carbohydr Polym. 2013; 98(2):1585-1598. doi:10.1016/j.carbpol.2013.08.018
52. Azeredo HMC, Barud H, Farinas CS, Vasconcellos VM, Claro AM. Bacterial cellulose as a raw material for food and food packaging applications. Front Sustain Food Syst. 2019; 3. doi:10.3389/fsufs.2019.00007
53. Tsouko E, Kourmentza C, Ladakis D, Kopsahelis N, Mandala I, Papanikolaou S, Paloukis F, Alves V, Koutinas A. Bacterial cellulose production from industrial waste and by-product streams. Int J Mol Sci. 2015; 16(12):14832-14849. doi:10.3390/ijms160714832
54. Keshk SM. Bacterial cellulose production and its industrial applications. J Bioprocess Biotech. 2014; 04(02):1-10. doi:10.4172/2155-9821.1000150
55. Jozala AF, de Lencastre-Novaes LC, Lopes AM, Santos-Ebinuma V de C, Mazzola PG, Pessoa Jr A, Grotto D, Gerenutti M, Chaud MV. Bacterial nanocellulose production and application: a 10-year overview. Appl Microbiol Biotechnol. 2016; 100(5):2063-2072. doi:10.1007/s00253-015-7243-4

56. Clasen C, Sultanova B, Wilhelms T, Heisig P, Kulicke WM. Effects of different drying processes on the material properties of bacterial cellulose membranes. Macromol Symp. 2006; 244(1):48-58. doi:10.1002/masy.200651204
57. Wang Y, Zhao X, Li D, Wu Y, Wahid F, Xie Y, Zhong C. Review on the strategies for enhancing mechanical properties of bacterial cellulose. Published online 2022. doi:10.21203/rs.3.rs-1783762/v1
58. Cazón P, Vázquez M, Velazquez G. Novel composite films based on cellulose reinforced with chitosan and polyvinyl alcohol: Effect on mechanical properties and water vapour permeability. Polym Test. 2018; 69:536-544. doi:10.1016/j.polymertesting.2018.06.016
59. Surini S, Auliyya A. Formulation of an anti-wrinkle hydrogel face mask containing ethanol extract of noni fruit (*Morinda citrifolia* L.) for use as a nutracosmeceutical product. Int J Appl Pharm. 2017; 9:74-76. doi:10.22159/ijap.2017.v9s1.41_47
60. Seaman S. Dressing selection in chronic wound management. J Am Podiatr Med Assoc. 2002; 92(1):24-33.
61. Akintunde MO, Adebayo-Tayo BC, Ishola MM, Zamani A, Horváth IS. Bacterial cellulose production from agricultural residues by two *Komagataeibacter* sp. strains. Bioengineered. 2022; 13(4):10010-10025. doi:10.1080/21655979.2022.2062970
62. Rusdi RAA, Halim NA, Norizan MN, et al. Pre-treatment effect on the structure of bacterial cellulose from Nata de Coco (*Acetobacter xylinum*). Polimery. 2022; 67(3):110-118. doi:10.14314/polimery.2022.3.3
63. Castro C, Zuluaga R, Putaux JL, Caro G, Mondragon I, Gañán P. Structural characterization of bacterial cellulose produced by *Gluconacetobacter swingsii* sp. from Colombian agroindustrial wastes. Carbohydr Polym. 2011; 84(1):96-102. doi:10.1016/j.carbpol.2010.10.072
64. Wang S, Han YH Chen J, Zhang D, Shi X, Ye Y, Chen D, Li M. Insights into bacterial cellulose biosynthesis from different carbon sources and the associated biochemical transformation pathways in *Komagataeibacter* sp. W1. Polymers. 2018; 10(9):963. doi:10.3390/polym10090963
65. Wang J, Wan YZ, Luo HL, Gao C, Huang Y. Immobilization of gelatin on bacterial cellulose nanofibers surface via crosslinking technique. Mater Sci Eng C. 2012; 32(3):536-541. doi:10.1016/j.msec.2011.12.006
66. Gelin K, Bodin A, Gatenholm P, Mihranyan A, Edwards K, Strømme M. Characterization of water in bacterial cellulose using dielectric spectroscopy and electron microscopy. Polymer. 2007; 48(26):7623-7631. doi:10.1016/j.polymer.2007.10.039