Food Research 8 (4) : 291 - 300 (August 2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Quality improvement of nata bioedible film using *Acetobacter xylinum* and kombucha symbiotic culture of bacteria and yeast as starter

[1,*]Nurhayati, N., [2]Narulita, E. and [3]Pujiati, P.

[1]*Department of Agricultural Products Technology, Faculty of Agricultural Technology, University of Jember, Jember 68121, Indonesia*
[2]*Department of Biology Education, Faculty of Teacher Training and Education, Universitas of Jember 68121, Indonesia*
[3]*Graduate School of Biotechnology, University of Jember, Jember 68121, Indonesia*

Article history:
Received: 1 May 2022
Received in revised form: 27 June 2022
Accepted: 5 April 2023
Available Online: 7 August 2024

Keywords:
Acetobacter xylinum,
Cellulose,
Food,
Nata,
Kombucha,
SCOBY

DOI:
https://doi.org/10.26656/fr.2017.8(4).225

Abstract

The trend towards bio-edible films is being promoted to reduce the use of plastic packaging, specifically as primary packaging for food. Therefore, this study aimed to improve the quality of nata as bioedible film by using a starter during fermentation to produce microbial metabolites such as the cellulose matrix, "nata". The fermentation of nata was carried out using coconut water as substrate, while the starter used single culture (*Acetobacter xylinum*) and multiculture (kombucha symbiotic culture of bacteria and yeast (SCOBY)). The starter (15% v/v) was inoculated on the coconut water substrate which had been formulated and adjusted at pH 3-4, then incubated for 14, 18 and 22 days. Fermentation using the kombucha SCOBY for 22 days of fermentation resulted in good quality nata biofilm. The results showed that the yield of nata reached 35.44% w/v with 14.76 mm of thickness, 5 cm of diameter, 0.86 N/nm^2 of elasticity, 34.02-52.01 MPa of tensile strength, and 2.3-2.6% of elongation. The functional groups profile of nata edible film was OH, CH, C=C, and aromatic rings. The results of this study showed that kombucha SCOBY has good potential as a starter for producing bioedible films.

1. Introduction

Several studies were carried out on bioedible packaging to develop an environmentally friendly film as well as to reduce the use of plastic packaging, which is difficult to decompose by soil and causes environmental pollution both in the soil and water. Plastic consists of millions of monomers that cannot be broken down by microorganisms (Salhofer *et al*., 2021; Kremer *et al.,* 2021). Therefore, it is necessary to find alternative packaging, which is environmentally friendly, without synthetic chemicals, and easily degraded.

Several studies reported that biodegradable film is a good product, which can be used for developing environmentally friendly packaging that is designed to advance and develop packaging technology and public awareness. Furthermore, environmentally friendly packaging is usually made of starch, cellulose, collagen, protein, or lipids that are easily broken down by microbial decomposers (Rusianto *et al*., 2020).

Wang *et al*. (2022) developed an edible bioactive film. Previously, Saputra *et al*. (2021) developed a bioactive and functional film from chitosan, but the process involves the role of synthetic chemical compounds. Therefore, it relates to existing efforts to produce bioedible film that does not include the addition of synthetic chemical compounds, such as biodegradable film, through the fermentation process to produce a cellulose matrix.

Cellulose as edible film can be used from cellulolytic microbial metabolites such as *A. xilynum*, *Gluconacetobacter* (De Filippis *et al*., 2018; Blanco *et al*., 2020). Additionally, synergistic growth between bacteria and yeast known as SCOBY (Symbiotic Culture of Bacteria and Yeast) can also produce a cellulose matrix such as nata (Urbahillah *et al*., 2021; Nguyen *et al*., 2021).

The synergy of microbial growth between bacteria and yeast can produce biocellulose layer, such as in kombucha fermentation (De Filippis *et al*., 2018; Amarasekara *et al*., 2020; Nguyen and Nguyen, 2022). Biocellulose is one of the hydrocolloid derivatives used to form a film matrix (Oliveira *et al*., 2019). Its quality

*Corresponding author.
Email: *nurhayati.ftp@unej.ac.id*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

depends on nutrition and environmental conditions. Unfavorable nutrients in the substrate can result in deficiencies such as being thinner and more easily tear (Santoso *et al*., 2020). This study aimed to improve the quality of bioedible nata film by developing the starter using a single culture (*A. xylinum*) or diverse culture (kombucha SCOBY).

2. Materials and methods

2.1 Substrate preparation of biocellulose fermentation

The substrate for biocellulose fermentation was prepared using coconut water obtained from the traditional market at Pasar Tanjung, Jember Regency, East Java, Indonesia. The fermentation was carried out using a starter of a single culture (*A. xylinum*) and kombucha SCOBY (symbiotic culture of bacteria and yeast).

The substrate was prepared by boiling coconut water and adding 0.75% sugar. It was then cooled until the temperature reached 30℃, and the pH of the medium was adjusted by adding 40% acetic acid solution to obtain a pH of 3-4. Subsequently, 15% of *A. xylinum* or 15% of kombucha SCOBY were inoculated and incubated at 30℃ for 14, 18 and 22 days.

2.2 Bioedible film production

The bioedible film was produced by washing the biocellulose, nata, boiled for 1-2 hrs using 1% NaOH until a pH of 7 was obtained and dried at 75℃ for 20-24 hrs, which were used as bioedible film after the drying process. The tensile strength, elasticity, surface structure by Scanning Electron Microscopy (SEM), and functional groups by FTIR (Fourier Transform Infrared Spectrophotometer) were analyzed (Herawati and Kamsiati, 2014).

2.3 Analysis of microbial growth during fermentation

Microbial growth during the fermentation of nata was determined using optical density (OD) based on substrate turbidity compared to before and after fermentation. A 1.5 mL of the sample substrate was placed in a cuvette, then the OD value was measured with a spectrophotometer at a wavelength of 620 (Stevenson *et al*., 2016).

2.4 Analysis of total titratable acid of residual medium

Analysis of total titratable acid (TTA) was carried out to determine the difference in pH values developed due to different types of starters and fermentation time. Approximately 2 to 3 drops of phenolphthalein were added to 10 mL of the remaining medium and were then titrated using 0.1 N NaOH solution until the solution changed color to pink. The TTA was calculated using the formula below (N: normality of 0.1 N NaOH; MW: the molecular weight of lactic acid (90); DF dilution factor: 1) (Dalu *et al*., 2019; Melia *et al*., 2022):

$$TTA(\%) = \frac{Volume\ NaOH\ \times\ N\ NaOH\ \times MW\ \times\ DF}{Volume\ sample\ \times\ 100} \times\ 100$$

2.5 Analysis of pH value of the residual substrate

The pH value of the residual substrate was analyzed before and after fermentation, and the value of the pH level was measured using a meter, where the use of the tool was first calibrated according to the pH. The calibrated tool is then inserted directly or immersed into the sample to read and know the acidity results (Santoso *et al.*, 2020).

2.6 Thickness analysis of bioedible film

The thickness of nata was measured using a caliper method, and the thickness value was measured with an accuracy of 0.000 mm and was repeated three times at different locations, then calculated as the average of the thickness value.

2.7 Tensile strength analysis of bioedible film

The tensile strength measurement of the bioedible packaging was performed to determine the tensile strength that the packaging can withstand. The tensile strength was measured using a computer and the Lloyd Instrument's Universal Testing Machine (UTM). Additionally, tensile testing was carried out using the ASTM D-638 standard with the TM 113 Universal 30 kN testing machine (Alsaadi *et al*., 2020).

$$Tensile\ strength\ \frac{kgF}{mm^2} = \frac{Tensile\ strength\ (F)}{Surface\ area\ (A)}$$

2.8 Elasticity analysis of bioedible film

The strength possessed by bioedible film was observed and measured using the Rheotex. The sample was placed under the Rheotex test needle at a depth of 2 mm and tested by pressing the start button for a few seconds until the jam sounded. The number shown by the Rheotex needle in g/mm was the result obtained from measuring the strength of the bioedible film (Pushpadass *et al*., 2019).

2.9 Surface analysis of bioedible film

The surface analysis of the bioedible film was identified using SEM, which provides data or information regarding surface morphology, particle size, length, and width. The preparation of bioedible film in suspensions at low concentrations to obtain fiber images with high individual fibers was examined. The bioedible film was placed on a plate and allowed to dry at room

https://doi.org/10.26656/fr.2017.8(4).225

temperature. After selecting a specific part of the sample and the desired magnification, the shooting was performed to obtain a good and clear image (Wu *et al.*, 2019).

2.10 Function groups analysis of bioedible film

The functional group of the bioedible film was analyzed using FTIR to show the organic and non-organic spectrum. Dried nata (0.001 g) was added with KBr in a ratio of 1:9 g into the mortar and stirred until smooth. The pellet mold was prepared, and the samples were with base, and table frame using chloroform. The mixed KBr sample was placed in a pellet mold set, then the mold was placed on a hydraulic pump and pressurized to 8 gauge. The KBr pellets formed were removed and placed in the table holder, and the functional groups of bioedible nata film were observed using FTIR at wavenumbers 400 cm^{-1} - 4000 cm^{-1} (Abd El-Rehim *et al.*, 2018).

3. Results and discussion

3.1 Production of nata using different starters

Nata was produced from coconut water known as nata de coco (Santosa *et al*., 2020). The composition of the substrate for the production of nata was coconut water, sugar, minerals from inorganic fertilizers (urea), and acidic solutions such as acetic and citric acid, and the starter of nata can grow at acidic or low pH at pH 3-4. In this study, the nata starter used pure cultures (*A. xylinum*) and was compared with kombucha SCOBY and incubated in the glass jar for 14, 18 and 22 days (Figure 1). Other studies reported that SCOBY from cascara kombucha was produced after 14 days of fermentation (Urbahillah *et al*., 2021).

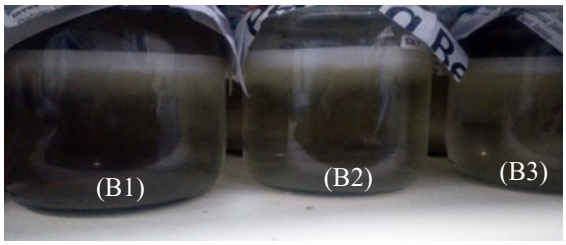

Figure 1. The fermentation process of nata de coco during 14 days (B1), 18 days (B2), and 22 days (B3).

The nata biofilm was entrenched into the layered substrate and thickened during the fermentation process for up to 3 weeks, and fresh nata with a white color has a chewy texture, which absorbs more water. Due to this condition, the moisture content of fresh nata is more than 90%. The processing of bioedible film was performed by drying the nata until the water content was less than 5% and had a white and soft brown color (Figure 2).

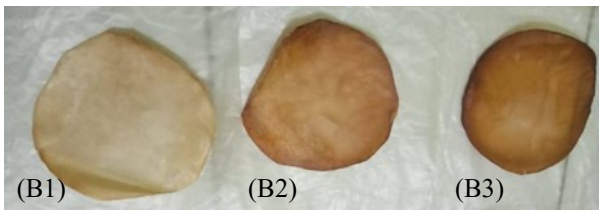

Figure 2. The dried nata was produced for 14 days (B1), 18 days (B2), and 22 days (B3).

3.2 The pH value of the residual substrate

The residual pH substrate's value decreased by the fermentation period to less than pH 3 (Figure 3). The lowest pH value was 22 days of fermentation, and the decreasing pH value was caused by acetic acid as the primary metabolite of acetic bacteria.

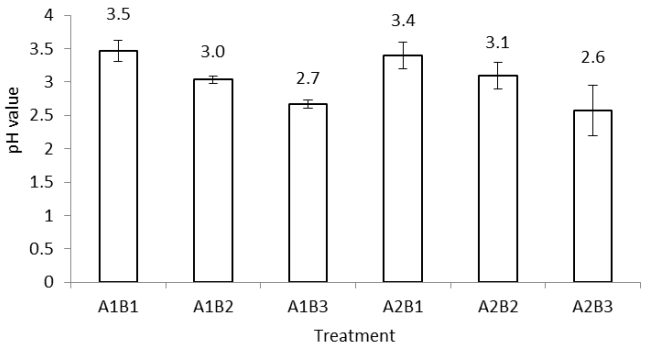

Figure 3. The pH value of the medium of nata fermented by *Acetobacter xylinum* (A1) and kombucha SCOBY (A2) during 14 days (B1), 18 days (B2), 22 days (B3) of fermentation.

Figure 3 shows that the pH of residual media indicated acidic pH. The residual substrate's pH on fermentation by kombucha SCOBY and *A. xylinum* (single culture) after 14 days was 3.400 and 3.467, while after 22 days was 2.567 and 2.667, respectively.

The pH decreased from 3.467 to 2.567 during the fermentation process, and the conversion of glucose to acetic acid took place. While the starter used in SCOBY can ferment glucose into alcohol, organic acids such as acetic acid lower the pH (Urbahillah *et al*., 2021). Another starter, such as lactic acid bacteria can reduce the pH from 7.5 to 5.8 after 7 days of fermentation (Azkiyah *et al.*, 2021). In addition, alcohol/ethanol has acidic properties that can decrease the pH value (Feng *et al*., 2021; Ma *et al.*, 2021).

3.3 Total titrated acid of residual substrate

The total acidity of the residual substrate increased during the fermentation process, and the total acid content of the residual substrate increased from 0.63 to 1.80% v/v (Figure 4). The higher TTA occurred after 22 days of fermentation, i.e., 1.16% by *A. xylinum* and 1.80% by kombucha SCOBY. During 14 days of fermentation, the TTA produced was 0.63% by *A.*

https://doi.org/10.26656/fr.2017.8(4).225

xylinum and 0.84% by kombucha SCOBY. The result showed that TTA was not significantly different from the starter, and using SCOBY as a starter can produce ethanol/alcohol that increases the TTA after seven days of fermentation (Ma *et al*., 2021). Some organic acids can decrease pH value and increase TTA (Chakravorty *et al.*, 2016). Elsewhere, other studies reported that kombucha SCOBY increases TTA to 1.1% after 6 days of fermentation for cascara kombucha (Urbahillah *et al*., 2021).

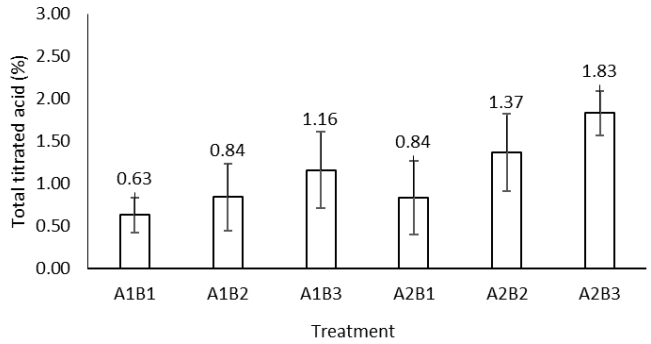

Figure 4. Titratable acidity of the medium of nata fermented by *Acetobacter xylinum* (A1) and kombucha SCOBY (A2) on different fermentation times, namely 14 days (B1), 18 days (B2), 22 days (B3).

3.4 The microbial growth during nata fermentation

Fermentation time and the type of starter during nata fermentation can affect the growth of microorganisms that produce nata as secondary metabolites. Microbial growth can be measured based on changes in OD value (optical density), which is associated with the growth of microorganisms (Figure 5).

The residual substrate OD values were not significantly different between the single culture and the SCOBY starter. Microbial growth was higher by *A. xylinum* fermentation after 22 days (1.4 OD) than by kombucha SCOBY (0.8 OD). The OD value was directly proportional to the number of cells in the medium.

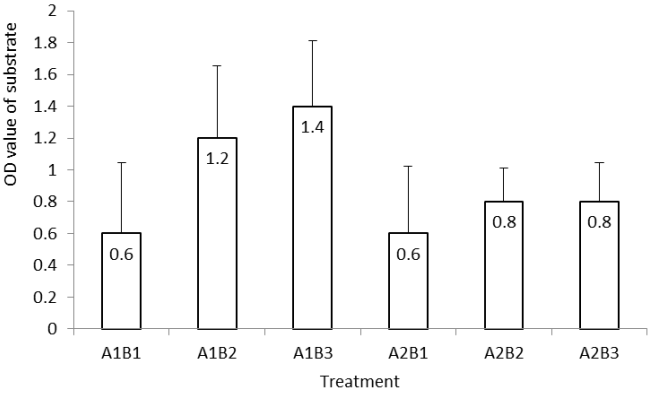

Figure 5. Microbial growth based on OD value of nata fermented by *Acetobacter xylinum* (A1) and kombucha SCOBY (A2) on different fermentation times, namely 14 days (B1), 18 days (B2), 22 days (B3).

The fermentation process was more adapted for *A. xylinum* than kombucha SCOBY. Other studies also reported that fermentation leads to the growth of bacteria or decreases due to the decrease in sugar content and the formation of acid as a metabolite of fermentation. The process and storage conditions/durability can affect the quality and quantity of bacterial cellulose.

3.5 Nata weight as bioedible film

The fermentation period and the type of starter can affect the weight of nata, which is produced to be proportional to the increase in thickness. This indicates the more extended the fermentation period was depleted, the heavier the nata, while the bacteria continued forming the layer. Figure 6 shows the weight of nata during 14, 18 and 22 days of fermentation.

The weight of nata at different fermentation periods with the starters of *A. xylinum* and kombucha SCOBY was proportional to the thickness. Figure 6 shows that fermentation affects the weight of nata by *A. xylinum* fermentation (34.6 g) and kombucha SCOBY (36.44 g) after 22 days. The microbial-producing cellulose matrix increases during fermentation until the substrate is exhausted (Nguyen *et al*., 2021).

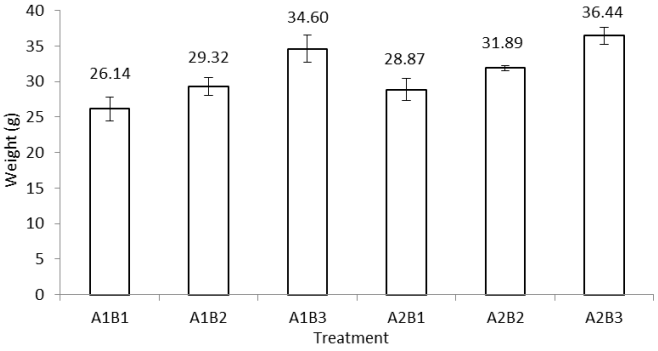

Figure 6. Weight of nata fermented by *Acetobacter xylinum* (A1) and kombucha SCOBY (A2) on different fermentation times, namely 14 days (B1), 18 days (B2), 22 days (B3).

3.6 Nata thickness as bioedible packaging

The thickness of the nata increased day by day during the fermentation process. Other studies reported that nata is bacterial cellulose that has high mechanical strength and viscoelasticity, which were contributed by their high cellulose concentration (Li *et al*., 2021). During fermentation can increase the thickness, elasticity, and texture of nata de coco. The thickness of the nata can then affect the resulting bioedible packaging. The results of the thickness of the nata with the effect of fermentation time and different types of starters can be seen in Figure 7.

The thickness of the nata increased during the fermentation process (Figure 7). The maximum thickness occurred after 22 days, i.e., 14.76 mm by *A. xylinum*

https://doi.org/10.26656/fr.2017.8(4).225

starter and 13.9 mm by the kombucha SCOBY. The activities of *A. xylinum* and SCOBY kombucha were affected by fermentation time, and after 22 days, a thick layer of nata was formed. *A. xylinum* bacteria can produce extracellular enzymes that place sugars on thousands of fibers of cellulose chains. In addition, the length of fermentation time affects the activity of *A. xylinum* during nata production. SCOBY (symbiotic culture of bacteria and yeast) can also form a cellulose matrix that floats and thickens (May *et al*., 2017).

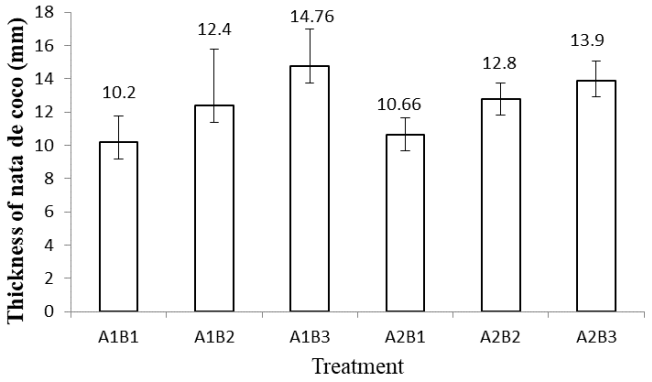

Figure 7. Thickness of nata fermented by *Acetobacter xylinum* (A1) and kombucha SCOBY (A2) on different fermentation times, namely 14 days (B1), 18 days (B2), 22 days (B3).

The bacteria used in the fermentation process have specific conditions to grow and produce the appropriate thickness of nata. Subsequently, certain factors can influence the ability of *A. xylinum* to produce cellulose, i.e., the culture method, carbon source, nitrogen source, pH, and temperature (Biyik, 2011). Using sugar as a source of food while SCOBY grows during fermentation can form a new layer (Jayabalan *et al*., 2014; Chakravorty *et al*., 2016). It can be seen that the length of fermentation time and the type of starter used affect the thickness of nata.

3.7 The surface profile of bioedible packaging from nata from starter fermented Acetobacter xylinum and kombucha SCOBY

The surface profile of bioedible packaging produced from nata was observed using the SEM, and the surface profile was examined to determine the structure of dried nata after 24 hrs of the drying process. The surface profile of nata bioedible packaging is shown in Figure 8.

The surface profile of bioedible packaging produced from nata shows the difference between bioedible fermented by *A. xylinum* with SCOBY kombucha on SEM observations with a magnification of 1500×. The surface profile of bioedible packaging produced from kombucha nata contains yeast colonies (Figure 8), which is known as SCOBY (Urbahillah *et al.*, 2021). While the surface profile of bioedible packaging produced from single culture (*A. xylinum*) nata does not contain yeast, and only the cellulose fibrils overlap to form a cellulose layer (Halib *et al*., 2012).

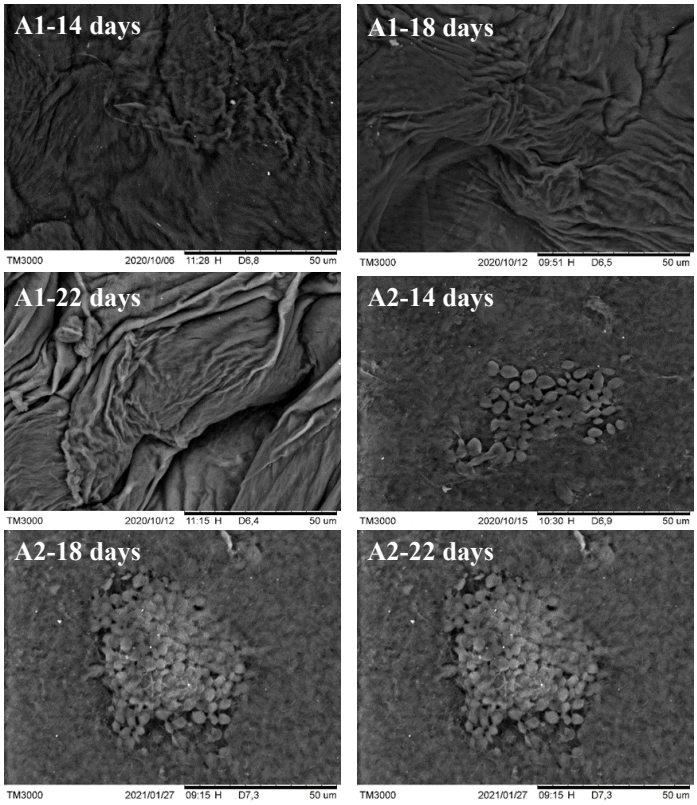

Figure 8. The surface profile of the bioedible film made from nata resulting from the fermentation of *Acetobacter xylinum* (A1) and kombucha SCOBY (A2).

3.8 The elasticity of bioedible packaging from nata from starter fermented Acetobacter xylinum and kombucha SCOBY

The elasticity of bioedible packaging was carried out to determine the strength of the sample or product. Elasticity is a measure of the strength of a material when it is getting stiffer (Chen *et al*., 2018). The elasticity value of bioedible packaging from nata de coco with differences in fermentation time and different types of starters can be seen in Figure 9.

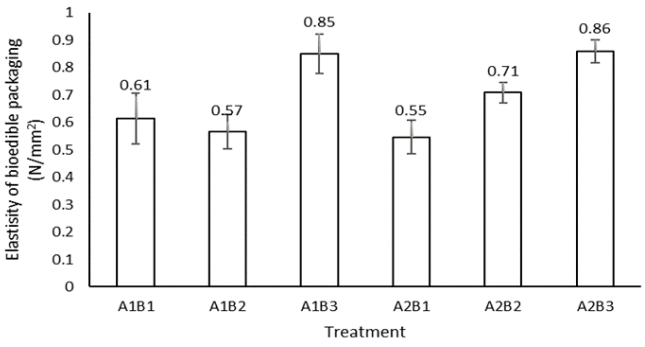

Figure 9. The elasticity of bioedible film fermented by *Acetobacter xylinum* (A1) and SCOBY kombucha (A2) on different fermentation times, namely 14 days (B1), 18 days (B2), 22 days (B3).

https://doi.org/10.26656/fr.2017.8(4).225

Figure 9 shows that the elasticity value of the bioedible packaging produced from the two types of starters was similar after 22 days of fermentation and later resulted in the highest value, i.e., 0.85 N/mm^2 and 0.87 N/mm^2 from *A. xylinum* and SCOBY kombucha, respectively. In comparison, the lowest elasticity was produced by a 14-day fermentation.

The increase in the strength of the bioedible elasticity of nata was caused by the interaction of polymer fibers. The elasticity indicates the rigidity of the material, and if the elasticity value is high, it will be stiff or complex (Fransiska *et al*., 2021).

3.9 The tensile strength value of bioedible packaging from nata from starter fermented Acetobacter xylinum and kombucha SCOBY

Tensile strength or bioedible elongation was determined to evaluate the bioedible quality. Table 1 shows the tensile strength of bioedible packaging produced from nata de coco using different fermentation times and starter types. Subsequently, the tensile strength of biodegradable packaging from nata de coco showed that the greater the value, the longer the fermentation time.

Table 1. Tensile strength of bioedible film.

Treatment	Tensile strength (MPa)	Elongation (%)
A1B1	34.21	1.2
A1B2	44.60	2.4
A1B3	52.01	2.6
A2B1	34.02	2.3
A2B2	32.69	1.7
A2B3	38.04	2.3

The bioedibility of nata with *A. xylinum* as a starter has a higher tensile strength value (stress 34.21-52.01 MPa and 1.2-2.6% of elongation if fermented during 22 h). Meanwhile, the tensile strength value of edible nata with SCOBY kombucha starter had a stress value of 34.02-38.04 MPa and 2.3% of elongation. Suppose the value of tensile strength is higher, therefore, it will be more robust, indicating the ductility value of the biodegradable when the material is fractured or broken. When the elongation at break is greater than 1.9%, it will show higher tensile strength, indicating the higher the strain value, the higher the increase in stress (Oliver-Ortega *et al*., 2021) and the higher the force produced, the greater the edible strength (Saputra *et al*., 2021). It also shows the higher the tensile strength, the more the qualitative value of the biodegradable material is determined (Zaki *et al*., 2021). Therefore, nata has high mechanical properties compared to cellulose from wood, implying the higher the tensile strength value, the stronger the mechanical properties of the biodegradable packaging and the less likely it is to break.

3.10 Function group of bioedible packaging from nata from starter fermented Acetobacter xylinum and kombucha SCOBY

The functional group of the nata bioedible packaging was determined by FTIR to perform a bioedible packaging wavelength at a frequency of 4000-400 cm^{-1}. The results showed that multiple peaks with different characteristics were generated (Figure 10). Characteristic peaks at wavelengths of 3400-3440 cm^{-1} (hydroxyl group), 2800-2900 cm^{-1} (methylene), 1620-1640 cm^{-1}, and 1420-1440 cm^{-1} (carbonyl group), followed by C-O-C and C-O-H at wavelengths of 1040-1068 cm^{-1} (Gayathry and Gopalaswamy, 2014).

Bioedible nata packaging has a functional group detected at 1636 cm^{-1} as the wavelength of the cellulose component with the cellulose water molecule (Gayathry and Gopalaswamy, 2014). In addition to the cellulose detected, O-H groups stretched at 3316.50 cm^{-1} and C-H stretched at 2898.88 cm^{-1} (Table 2). These results indicate carboxymethyl substitution of the molecule contained in the material as a cellulose compound. However, an increase in the nanocomposite may indicate

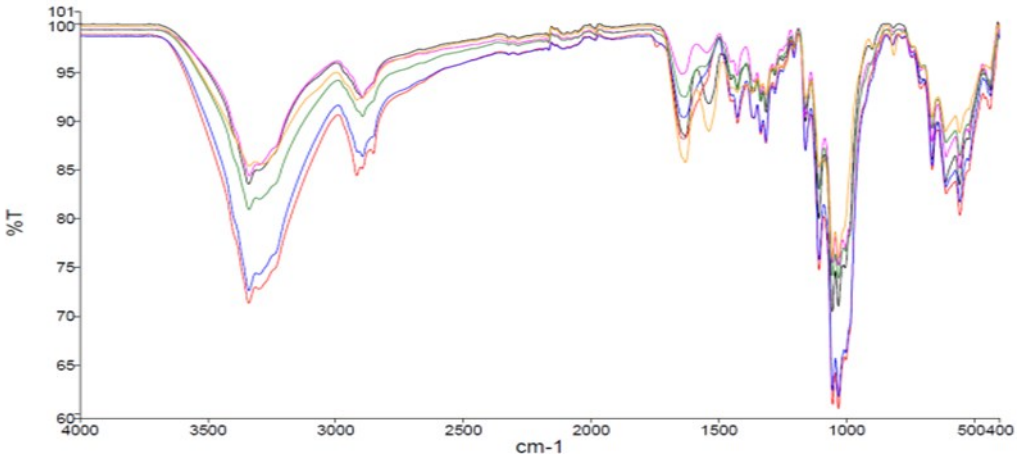

Figure 10. The profile of functional groups of nata bioedible film fermented by *A. xylinum* (A1) or SCOBY kombucha (A2) during fermentation 14 days (B1), 18 days (B2), and 22 days (B3).

https://doi.org/10.26656/fr.2017.8(4).225

Table 2. Wavenumber of peak function groups of nata bioedible film.

Treatment	Wavenumber	Bond type	Compound type	Frequency area
A1B1	3316.50	O-H	Amine	3200-3600
	2898.88	C-H	Alkanes	2850-2970
	1636.80	C=C	Alkene	1610-1680
	1426.83	C-H	Alkanes	1340-1470
A1B2	3319.11	O-H	Amine	3200-3600
	2902.45	C-H	Alkanes	2850-2970
	1636.37	C=C	Alkene	1610-1680
	1426.61	C-H	Alkanes	1340-1470
A1B3	3315.90	O-H	Amine	3200-3600
	2912.68	C-H	Alkanes	2850-2970
	1636.68	C=C	Alkene	1610-1680
	1426.41	C-H	Alkanes	1340-1470
A2B1	3306.41	O-H	Amine	3200-3600
	2910.52	C-H	Alkanes	2850-2970
	1634.55	C=C	Alkene	1610-1680
	1539.38	C=C	Aromatic ring	1500-1600
A2B2	3322.95	O-H	Amine	2850-2970
	2895.85	C-H	Alkanes	1610-1680
	1427.74	C=C	Alkene	1340-1470
A2B3	3325.82	O-H	Amine	3200-3600
	2899.76	C-H	Alkanes	2850-2970
	1635.38	C=C	Alkene	1610-1680
	1539.06	C=C	Aromatic ring	1500-1600

hydrogen bonding (Rachtanapun and Rattanapanone, 2011; Oliver-Ortega *et al.*, 2021). An aromatic ring group (C=C) at a wavelength of 1539 cm^{-1} was found in nata fermented by SCOBY kombucha starter. Other studies (Marsh *et al*., 2014) reported that the yeast found in SCOBY kombucha bacteria can produce several organic acids and alcohols, which also contribute to forming aroma, taste, and flavor. This functional group also indicates that the bioedible nata is degradable, it can be degraded or easily decomposed. It has also been reported (Fransiska *et al*., 2021) that the carbonyl functional group in bioplastic materials is easily biodegradable. Nata can applied as edible coatings of fresh-cut jackfruit quality to increase their shelf life under refrigerated conditions (10°C) until 3 weeks (Yasa *et al*., 2023).

Other studies have also confirmed that nata-based plastics can decompose in the soil (Yasa *et al*., 2023). This study showed that the resulting biodegradable packaging has high elasticity and strong tensile strength and can be decomposed in the soil because there is a carbonyl functional group derived from the primary material used as biodegradable packaging material. In addition, the resulting biodegradable packaging includes environmentally friendly packaging, which was produced from nata which medium is derived from coconut waste that was abundant in Indonesia.

4. Conclusion

This environmentally friendly bioedible packaging was produced from nata using coconut water as a substrate called nata de coco with a minimum fermentation time of 14 days using a single starter *A. xylinum* or SCOBY kombucha. The best properties of bioedible nata packaging were produced from SCOBY starter during 22 days of fermentation compared to using a single starter (*A. xylinum*). Subsequently, the nata yield reached 34.6% w/v - 35.44% w/v with a thickness of 14.76-13.9 mm and a diameter of 5 cm. The characteristics of bioedible nata packaging have a profile of functional groups i.e OH, CH, C=C, and an aromatic ring, 34.02-52.01 MPa of tensile strength, 2.3-2.6% of elongation, and the elasticity of 0.86 N/mm^2. The data obtained in this study can be used as an approach for a smart solution in utilizing organic waste in biodegradable packaging and in particular supports the zero-waste process to reduce plastic pollution.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors express profound gratitude to the CDAST laboratory for supporting the conduct of this study and special appreciation to the SEM technician at

https://doi.org/10.26656/fr.2017.8(4).225

the Faculty of the Pharmacy University of Jember for their help during SEM analysis.

References

Abd El-Rehim, H., Kamal, H., Hegazy, E.S.A., Soliman, E.S. and Sayed, A. (2018). Use of gamma rays to improve the mechanical and barrier properties of biodegradable cellulose acetate nanocomposite films. *Radiation Physics and Chemistry*, 153, 180-187. https://doi.org/10.1016/j.radphyschem.2018.08.007

Alsaadi, M., Erkliğ, A. and Abbas, M. (2020). Effect of clay nanoparticles on the mechanical and vibration characteristics of intraply aramid/carbon fiber reinforced epoxy composite. *Polymer Composites*, 41(7), 2704-2712. https://doi.org/10.1002/pc.25568

Amarasekara, A.S., Wang, D. and Grady, T.L. (2020). A comparison of kombucha SCOBY bacterial cellulose purification methods. *SN Applied Sciences*, 2, 240. https://doi.org/10.1007/s42452-020-1982-2

Azkiyah, M., Laga, A., Mahendradatta, M. and Shimomura, R. (2021). The effect of rice types on chao properties during fermentation. *Canrea Journal: Food Technology, Nutrition and Culinary Journal*, 4(2), 83-90. https://doi.org/10.20956/canrea.v4i2.380

Biyik, H. (2011). Evaluation of different pH and temperatures for bacterial cellulose production in HS (Hestrin-Scharmm) medium and beet molasses medium. *African Journal of Microbiology Research*, 5(9), 1037-1045. https://doi.org/10.5897/AJMR11.008

Blanco, P.F.G., Santoso, S.P., Chou, C.C., Verma, V., Wang, H.T., Ismadji, S. and Cheng, K.C. (2020). Current progress on the production, modification, and applications of bacterial cellulose. *Critical Reviews in Biotechnology*, 40(3), 397-414. https://doi.org/10.1080/07388551.2020.1713721

Chakravorty, S., Bhattacharya, S., Chatzinotas, A., Chakraborty, W., Bhattacharya, D. and Gachhui, R. (2016). Kombucha tea fermentation: Microbial and biochemical dynamics. *International Journal of Food Microbiology*, 220, 63-72. https://doi.org/10.1016/j.ijfoodmicro.2015.12.015

Chen, S.Q., Lopez-Sanchez, P., Wang, D., Mikkelsen, D. and Gidley, M.J. (2018). Mechanical properties of bacterial cellulose synthesised by diverse strains of the genus *Komagataeibacter*. *Food Hydrocolloids*, 81, 87-95. https://doi.org/10.1016/j.foodhyd.2018.02.031

Dalu, K.C.A., Nurhayati, N. and Jayus, J. (2019). In vitro modulation of fecal microflora growth using fermented "Pisang Mas" banana and red guava juices. *Current Research in Nutrition and Food Science Journal*, 7(2), 449-456. https://dx.doi.org/10.12944/CRNFSJ.7.2.14

De Filippis, F., Troise, A.D., Vitaglione, P. and Ercolini, D. (2018). Different temperatures select distinctive acetic acid bacteria species and promotes organic acids production during Kombucha tea fermentation. *Food Microbiology*, 73, 11-16. https://doi.org/10.1016/j.fm.2018.01.008

Feng, Y., Ma, X., Kong, B., Chen, Q. and Liu, Q. (2021). Ethanol induced changes in structural, morphological, and functional properties of whey proteins isolates: Influence of ethanol concentration. *Food Hydrocolloids*, 111, 106379. https://doi.org/10.1007/978-981-16-0320-4

Fransiska, D., Irianto, H.E., Priambudi, P., Abdullah, A.H.D., Nissa, R.C., Asri, P.P.P. and Handoyo, W.T. (2021). Effects of Water on Hydrophobization and Mechanical Properties of Thermoplastic Agar. *IOP Conference Series: Earth and Environmental Science*, 715(1), 012057. https://doi.org/10.1088/1755-1315/715/1/012057

Gayathry, G. and Gopalaswamy, G. (2014). Production and characterization of microbial cellulosic fiber from *Acetobacter xylinum*. *Indian Journal of Fibre and Textile Research*, 39(1), 93-96.

Halib, N., Amin, M.C.I.M. and Ahmad, I. (2012). Physicochemical properties and characterization of nata de coco from local food industries as a source of cellulose. *Sains Malaysiana*, 41(2), 205-211.

Herawati, H. and Kamsiati, E.W.S. (2014). Physic-chemical characteristic of nata de coco. *IOP Conference Series: Earth and Environmental Science,* 458(1), 012014. https://doi.org/10.1088/1755-1315/458/1/012014

Jayabalan, R., Malbaša, R.V., Lončar, E.S., Vitas, J.S. and Sathishkumar, M. (2014). A review on kombucha tea—microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. *Comprehensive Reviews in Food Science and Food Safety*, 13(4), 538-550. https://doi.org/10.1111/1541-4337.12073

Kremer, I., Tomić, T., Katančić, Z., Hrnjak-Murgić, Z., Erceg, M. and Schneider, D.R. (2021). Catalytic decomposition and kinetic study of mixed plastic waste. *Clean Technologies and Environmental Policy*, 23(3), 811-827. https://doi.org/10.1007/s10098-020-01930-y

Li, Z., Chen, S. Q., Cao, X., Li, L., Zhu, J. and Yu, H. (2021). Effect of pH buffer and carbon metabolism

on the yield and mechanical properties of bacterial cellulose produced by *Komagataeibacter hansenii* ATCC 53582. *Journal of Microbiology and Biotechnology*, 31(3), 429-438. https://doi.org/10.4014/jmb.2010.10054

Ma, X., Wang, J., Gao, M., Wang, N., Li, C. and Wang, Q. (2021). Effect of pH regulation mode on byproduct ethanol generated from the lactic acid fermentation of *Sophora flavescens* residues. *Journal of Cleaner Production*, 279, 123536. https://doi.org/10.1016/j.jclepro.2020.123536

Marsh, A.J., O'Sullivan, O., Hill, C., Ross, R.P. and Cotter, P.D. (2014). Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. *Food Microbiology*, 38, 171-178. https://doi.org/10.1016/j.fm.2013.09.003

May, A.N., Medina, J., Alcock, J., Maley, C. and Aktipis, A. (2017). Kombucha as a model system for multispecies microbial cooperation: theoretical promise, methodological challenges and new solutions 'in solution'. *BioRxiv*, 2017, 214478. https://doi.org/10.1101/214478

Melia, S., Juliyarsi, I. and Kurnia, Y.F. (2022). Physicochemical properties, sensory characteristics, and antioxidant activity of the goat milk yogurt probiotic *Pediococcus acidilactici* BK01 on the addition of red ginger (*Zingiber officinale* var. *rubrum rhizoma*). *Veterinary World*, 15(3), 757. https://doi.org/10.14202/vetworld.2022.757-764

Nguyen, H.T., Saha, N., Ngwabebhoh, F.A., Zandraa, O., Saha, T. and Saha, P. (2021). Kombucha-derived bacterial cellulose from diverse wastes: A prudent leather alternative. *Cellulose*, 28(14), 9335-9353. https://doi.org/10.1007/s10570-021-04100-5

Nguyen, Q.D. and Nguyen, N.N. (2022). Effects of different hydrocolloids on the production of bacterial cellulose by *Acetobacter xylinum* using Hestrin–Schramm medium under anaerobic condition. *Bioresource Technology Reports*, 17, 100878. https://doi.org/10.1016/j.biteb.2021.100878

Oliveira, F.M., da Silva Barud, H., Torres, E.C., Machado, R.T.A., Caetano, G.F., Leite, M.N., Marco, A.C.F., Signey, J.L.R. and Berretta, A.A. (2019). Development, characterization and pre-clinical trials of an innovative wound healing dressing based on propolis (EPP-AF®)-containing self-microemulsifying formulation incorporated in biocellulose membranes. *International Journal of Biological Macromolecules*, 136, 570-578. https://doi.org/10.1016/j.ijbiomac.2019.05.135

Oliver-Ortega, H., Geng, S., Espinach, F.X., Oksman, K. and Vilaseca, F. (2021). Bacterial cellulose network from kombucha fermentation impregnated with emulsion-polymerized poly (Methyl methacrylate) to form nanocomposite. *Polymers*, 13(4), 664. https://doi.org/10.3390/polym13040664

Pushpadass, H.A., Emerald, F.M. and Balasubramanyam, B.V.P.S. (2019). Rheological properties of milk-based beverages. In Milk-Based Beverages, p. 373-396. USA: Woodhead Publisher, https://doi.org/10.1016/B978-0-12-815504-2.00011-6

Rachtanapun, P. and Rattanapanone, N. (2011). Synthesis and characterization of carboxymethyl cellulose powder and films from *Mimosa pigra*. *Journal of Applied Polymer Science*, 122(5), 3218-3226. https://doi.org/10.1002/app.34316

Rusianto, T., Yuniwati, M. and Wibowo, H. (2020). Effect carrageenan to biodegradable plastic from tubers. *Jurnal Bahan Alam Terbarukan*, 8(2), 148-155. https://doi.org/10.15294/jbat.v8i2.22975

Salhofer, S., Jandric, A., Soudachanh, S., Le Xuan, T. and Tran, T.D. (2021). Plastic recycling practices in Vietnam and related hazards for health and the environment. *International Journal of Environmental Research and Public Health*, 18(8), 4203. https://doi.org/10.3390/ijerph18084203

Santosa, B., Wignyanto, W., Hidayat, N. and Sucipto, S. (2020). The quality of nata de coco from sawarna and mapanget coconut varieties to the time of storing coconut water. *Food Research*, 4(4), 957-963. https://doi.org/10.26656/fr.2017.4(4).372

Saputra, E., Tjahjaningsih, W. and Abdillah, A.A. (2021). Characteristics of edible film from chitosan as biodegradable packaging. *IOP Conference Series: Earth and Environmental Science*, 718(1), 012078. https://doi.org/10.1088/1755-1315/718/1/012078

Sharma, R., Garg, P., Kumar, P., Bhatia, S.K. and Kulshrestha, S. (2020). Microbial fermentation and its role in quality improvement of fermented foods. *Fermentation*, 6(4), 106. https://doi.org/10.3390/fermentation6040106

Stevenson, K., McVey, A.F., Clark, I.B., Swain, P.S. and Pilizota, T. (2016). General calibration of microbial growth in microplate readers. *Scientific Reports*, 6, 38828. https://doi.org/10.1038/srep38828

Urbahillah, A., Jayus, J. and Nurhayati, N. (2021). Improving SCOBY starter using co-culture of tapai and bakery yeast. *Biodiversitas Journal of Biological Diversity*, 22(10), 4617-4624. https://doi.org/10.13057/biodiv/d221055

Wang, C., Gong, C., Qin, Y., Hu, Y., Jiao, A., Jin, Z. and Wang, J. (2022). Bioactive and functional biodegradable packaging films reinforced with

nanoparticles. *Journal of Food Engineering*, 312, 110752. https://doi.org/10.1016/j.jfoodeng.2021.110752

Wu, H., Lei, Y., Zhu, R., Zhao, M., Lu, J., Xiao, D. and Li, S. (2019). Preparation and characterization of bioactive edible packaging films based on pomelo peel flours incorporating tea polyphenol. *Food Hydrocolloids*, 90, 41-49. https://doi.org/10.1016/j.foodhyd.2018.12.016

Yasa, I.W.S., Basuki, E., Alamsyah, A. and Sari, L.G.D. (2023). The Application of Nata de coco-based Coatings to Fresh-cut Jackfruits during Refrigerated Storage. In Proceedings of the 7th International Conference on Food, Agriculture, and Natural Resources (IC-FANRES 2022), p. 119-129. USA: Atlantis Press. https://doi.org/10.2991/978-94-6463-274-3_10

Zaki, D.Y., Safwat, E.M., Nagi, S.M., Salem, H.N., Hamdy, T.M., Moharam, L.M., Mohammad. L.H. and Hamzawy, E.M.A. (2021). A novel dental re-mineralizing blend of hydroxyethyl-cellulose and cellulose nanofibers oral film loaded with nepheline apatite glass: Preparation, characterization and in vitro evaluation of re-mineralizing effect. *Carbohydrate Polymer Technologies and Applications*, 2, 100035. https://doi.org/10.1016/j.carpta.2021.100035

