Characteristics of Biocellulose-Based Edible Film from Sago Wastewater (*Metroxylon sago* ROTTB.) on Various Glycerol Concentration

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ABSTRACT

This study aimed to investigate the the characteristics of biocellulose-based edible films from sago wastewater (*Metroxylon sago* ROTTB.) by incorporating varying concentrations of glycerol as a plasticizer. The edible films were made through a casting method using biocellulose from sago wastewater as the main ingredient. The treatments included the addition of varying glycerol concentrations to the edible films, namely 0.5, 1, and 1.5% (v/v), with three replications. The observed characteristics included physical (thickness, solubility, and moisture content of the film), mechanical (tensile strength, elongation percentage, and Young's modulus/modulus of elasticity) with a universal testing machine, chemical structure using FT-IR, and surface morphological characteristics through scanning electron microscopy (SEM). The results showed that increasing the glycerol concentration in the edible films improved the physical characteristics including thickness, solubility, and moisture. However, there was a decrease in the mechanical characteristics, namely tensile strength, elongation percentage, and Young's modulus of the film. The main components of edible films found were cellulose polymers, as indicated by characteristic functional groups, such as free-OH groups, aliphatic C-H, C-O, and β-1,4-glycosidic bonds. The surface morphology of the biocellulose edible film without glycerol was smoother compared to those containing glycerol. Biocellulose-based edible films from sago wastewater with glycerol concentrations of 0.5% and 1% showed physical-mechanical characteristics that fulfilled the Japanese Industrial Standard (JSI), indicating the potential for application as food packaging. These results indicated that incorporation of glycerol has an effect on the characteristics of the biocellulose-based edible films from sago wastewater.

Keywords: Biocellulose; edible film; glycerol; sago wastewater

INTRODUCTION

Plastic packaging has been the dominant option in the global food industry over the last two decades, displacing glass and cans containers. The use of plastic for packaging, storing, and wrapping food (Díaz-Montes & Castro-Muñoz, 2021) is attributed to its strong and lightweight characteristics, along with the resistance to corrosion and heat. This packaging can pose health issues when it comes into direct contact with food due to the migration of small molecules or substances from the plastic into the packaged food, potentially causing harm and introducing carcinogenic plastic chemical components. Furthermore, there is also an increase in plastic waste due to degradation difficulty, leading to environmental pollution (Díaz-Montes & Castro-Muñoz,

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2021). This shows the need for food packaging plastics that are both safe for health and environmentally friendly due to their ease of degradation.

The type of plastic safe for food materials is edible packaging in the form of thin films made from natural edible materials. The use of edible films as packaging inhibits the transfer of gases, water vapor, and soluble substances to protect the packaged material from mechanical damage. Furthermore, structural properties are maintained, preventing the loss of volatile compounds from certain food materials (Hammam, 2019).

The most promising polymer material for edible films is based on polysaccharides, including cellulose. One prospective form of cellulose with potential for development as a raw material for food packaging plastic is biocellulose, commonly known as *nata*. Biocellulose is a product of the acetic acid bacteria metabolism produced through fermentation processes using various carbohydrate-rich waste materials as production media, including coconut wastewater (Ismaya et al., 2021), sago wastewater (Yanti et al., 2017; Yanti et al., 2018), and tapioca wastewater (Sari et al., 2022). This material also has high potential for use as a packaging plastic due to its lignin-free nature, elevated mechanical properties, and ease of degradation, serving as an alternative to synthetic plastic packaging that is difficult to degrade. Biocellulose derived from sago wastewater has shown mechanical characteristics that meet the standards for use as a plastic raw material (Yanti et al., 2021a). Several edible films made from biocellulose have been reported, including edible films from *nata de siwalan* (Salsabila & Ulfah, 2017), *nata de coco* (Ismaya et al., 2021), and sago wastewater (Yanti, et al., 2021b).

Glycerol is a plasticizer material in edible films, which functions to enhance flexibility and reduce brittleness while also improving durability, particularly when stored at low temperatures (Alfatahillah et al., 2021). The addition of glycerol with varying concentrations to edible films can influence the physical-mechanical characteristics of the edible packaging (Wattimena et al., 2016). Therefore, this study aimed to investigate the characteristics of biocellulose-based edible films made from sago wastewater, with the addition of glycerol as a plasticizer at varying concentration.

METHODS

Materials

The materials in this study included biocellulose produced through the fermentation of sago wastewater substrate (*nata de sago*) using *Acetobacter xylinum* LKN6 bacteria (Yanti et al., 2017). The chemicals used were comprised of glycerol (Merck, 85%), carboxy-methyl cellulose (CMC) (food grade), and NaOH (Merck, 99%).

The equipment used included a universal testing machine (Admet, Norwood, MA, USA), FT-IR Spectra 2000 (Perkin Elmer, Waltham, MA, USA), Scanning Electron Microscopy (Tescan Vega III Easyprobe, Brno, Czech Republic), and a micrometer (Mitutoyo, Japan).

Procedure

Edible film was made following the method described by Yanti et al. (2021b). Sheets of biocellulose obtained from the fermentation of sago wastewater substrate (*nata de sago*) were washed with a 1% NaOH solution to remove non-cellulose components. Subsequently, *nata de sago* was transformed into a cellulose slurry by adding water to biocellulose at a 1:4 ratio and homogenized using a blender. The cellulose slurry was added with 1.5% (w/v) CMC and glycerol using varying concentrations of 0, 0.5, 1, and 1.5% (v/v). The edible films solution was stirred for 1 minute and heated using a hot plate stirrer at 70 °C for 15 minutes until homogenized. The solution was left to stand for 5 minutes and heating was continued using a hot plate at 100 °C for 1 hour without stirring to remove gas from the solution (degassing). After completion, 50 mL of the solution was poured into a glass mold measuring 15 × 15 cm and dried at 40°C in an oven overnight. The resulting film was stored at room temperature in a container with a relative humidity of 50% before analysis.

Characterization of Film Physical-Mechanical Properties

Film thickness Measurement

The thickness of film was measured at five different locations, namely the left, right, top, bottom, and center utilizing a manual micrometer with a precision of 0.001 mm, and averaged.

Film solubility measurement

Film solubility was measured based on the method reported by Yanti, et al., (2021b). In this method, Film samples measuring 4 x 4 cm were dried for 24 hours using an oven at 105°C and then weighed to obtain the initial dry weight. The dried films were soaked in 50 mL of water for 24 hours, while periodically stirred at 100 rpm. After 24 hours, the samples were screened using filter paper, then the undissolved film was dried using an oven at 105 °C for 24 hours. Subsequently, the samples were weighed to obtain the final dry weight, with three replications. Solubility was counted using the following formula (Equation 1).

$$\text{Solubility (\%)} = \frac{initial\ dry\ weight - final\ dry\ weight}{initial\ dry\ weight} \times 100 \quad (1)$$

Measurement of film moisture content

The moisture content of the samples measurement was carried out based on the method described by Yanti et al. (2021b). Film samples, uniformly cut to 2 x 2 cm, were weighed for their initial weight and dried for 24 hours in the oven at 105°C, and then the final dry weight of the sample was weighed. Measurement of sample moisture content was carried out with three replications. The moisture content was calculated using the formula (Equation 2).

$$\text{Moisture Content (\%)} = \frac{initial\ weight - final\ dry\ weight}{initial\ weight} \times 100 \quad (2)$$

Measurement of mechanical characteristics

The mechanical characteristics of the film included tensile strength, elongation percentage, and Young's Modulus. In this study, the tensile strength and elongation measurements were carried out using a Universal Testing Machine (Admet, Norwood, MA, USA), following ASTM D638-14 (2014) standards. Young's Modulus reflects the slope of the linear portion of the stress-strain curve. Meanwhile, data analysis indicates the average of three replicates for each sample.

Chemical Structure Characterization Using Fourier Transformed Infrared (FT-IR) Spectroscopy

Film samples were taken using FT-IR (Spectra 2000), while spectra were recorded in the wavenumber range of 500-4000 cm^{-1}.

Film Surface Morphology Characterization

The characteristics of the film surface morphology were analyzed using Scanning Electron Microscopy (SEM). Subsequently, the film samples were coated with gold and examined at a magnification of 5000 times and a voltage of 15 kV.

RESULTS AND DISCUSSION

Physical Characteristics of Edible Film

The physical characteristics of biocellulose-based edible films made from sago wastewater, including thickness, solubility, and moisture content, are presented in Table 1.

Film thickness is a crucial physical properties and an essential parameter for assessing the suitability of film for food packaging. The thickness can affect the rate of water vapor, gas transmission, and mechanical properties such as tensile strength and elongation (Lan et al., 2018). As presented in Table 1, the thickness of biocellulose-based edible films increased from 0.058 mm to 0.083 mm, with elevated glycerol concentration in the film. This suggested that the addition of glycerol to edible films led to an increase in thickness, as indicated by previous reports (Nemet et al., 2010; Anandito et al., 2012; Alfatahillah et al., 2021; Ismaya et al, 2021; Yanti, et al., 2021b). The addition of glycerol at high concentrations increased the total solids in the solution, thereby improving the polymer matrix, and enhancing the edible film thickness (Nemet et al., 2010).

The thickness of biocellulose-based edible films made from sago wastewater with the addition of glycerol as a plasticizer, ranged from 0.075 to 0.083 mm, as presented in Table 1. These values met the standard requirements based on the Japanese Industrial Standard (JIS), with a maximum of 0.25 mm (JIS, 2019). Consequently, biocellulose-based edible films from sago wastewater are highly suitable for use as food packaging. The thickness exceeding 0.25 mm can affect water permeability properties, impacting the capability of the edible film to preserve the packaged product (Díaz-Montes & Castro-Muñoz, 2021).

Generally, Solubility reflects the water-resistance properties of a substance. Based on Table 1, an increasing concentration of glycerol enhanced the solubility of biocellulose-based edible films. This result was consistent with Purnavita et al. (2020) and Anandito

Table 1. Physical characteristics of biocellulose-based edible films made from sago wastewater with various glycerol concentrations

Glycerol concentration (%)	Thickness (mm)	Solubility (%)	Moisture (%)
0 (Control)	0.058±0.004a	20.15±1.20a	9.89±0.28a
0.5	0.075±0.003a	24.22±0.96b	10.02±0.39a
1	0.082±0.007a	29.76±1.03c	13.30±0.72b
1.5	0.083±0.006a	33.92±1.19d	13.96±0.69b

Values followed by different letters showed significant differences ($p<0.05$)

et al. (2012), who reported that the existence of glycerol in edible films led to increased solubility. The increase in solubility of edible film in the presence of glycerol is due to the hydrophilic nature of glycerol (Sitompul & Zubaidah, 2017; Jafarzadeh et al., 2018).

Based on Table 1, the moisture content increased with the rising glycerol concentration in the film, from 9.89% (control) to 13.96% (with 1.5% glycerol addition). Rangel-Maron et al. (2013) reported that the ability of glycerol to bind water contributed to increase moisture content in edible films. Glycerol is the simplest glyceride compound with hydroxyl groups, making it hydrophilic and hygroscopic, facilitating easy binding with water and increasing the moisture content (Díaz-Montes & Castro-Muñoz, 2021). Moisture content is a limiting factor in using edible films as food packaging, as a higher value can trigger the growth of spoilage microorganisms, making the packaged food more susceptible to deterioration (Díaz-Montes & Castro-Muñoz, 2021; Yanti, et al., 2021b).

Mechanical Characteristics of Edible Film

The characteristics of mechanical of biocellulose-based edible films made from sago wastewater, including tensile strength, elongation, and Young's Modulus, are shown in Table 2.

Based on Table 2, increasing glycerol concentration reduced mechanical characteristics, including tensile strength, elongation percentage, and Young's Modulus. However, edible films without glycerol produced the lowest mechanical properties compared to those added with glycerol. This indicated that glycerol can affect the mechanical properties of biocellulose-based edible films made from sago wastewater. Similarly, Ballesteros-Mártinez et al., (2020) and Ismaya et al.)2021) reported that the addition of glycerol at certain concentrations in edible films could enhance their mechanical properties. Excess concentrations might reduce the intermolecular forces among the film matrix components, leading to a decrease in the stability of the solid dispersion system and a reduction in mechanical strength (Jafarzadeh et al., 2018; Lintang et al., 2021).

An increase in glycerol concentration reduced the tensile strength from 39.07 MPa (film with 0.5% glycerol concentration treatment) to 7.92 MPa (film with 1.5% glycerol concentration), as illustrated in Table 2. This reduction occurred because glycerol, as a plasticizer, can reduce the interactions between molecules and decrease bonding between biopolymer molecules, thereby reducing the tensile strength (Hammam, 2019). Ismaya et al. (2021) also stated that the supplementation of inadequate glycerol resulted in a brittle and less elastic edible film, but an excessive amount produced a soft and sticky film, which was difficult to remove from molds.

Elongation percentage represented the percentage of length change of the film when pulled until breaking (Pereda et al., 2012). As illustrated in Table 2, an increase in glycerol concentration decreased the percentage of elongation, ranging from 93.59% (film with 0.5%) to 55.93% (film with 1.5%). Similarly, Anandito et al. (2012) and Alfatahillah et al. (2021) stated that increasing glycerol in edible films reduced elongation. This indicated that increasing glycerol concentration tended to reduce film elongation due to the saturation of the plasticizer molecules, causing the presence of excess molecules outside the polymer phase, and a decrease in intermolecular forces between polymer chains (Hammam, 2019).

Young's Modulus or modulus of elasticity is the measure of elasticity indicating the rigidity of the material and the flexibility of the film. A high Young's Modulus value indicates that the film material is stiffer, while a low value represents more elasticity (Nandane & Jain, 2015). According to Table 2, an increase in glycerol concentration reduced the Young's Modulus, ranging from 41.76 MPa (film with 0.5%) to 16.16 MPa (film with 1.5%). Yanti et al. (2021b) also reported that higher glycerol concentrations in bacterial cellulose-based edible films lowered the Young's Modulus value. This was supported by Asl et al. (2017), where

Table 2. Mechanical properties of biocellulose-based edible film from sago wastewater with varying glycerol concentrations

Glycerol concentration (%)	Tensile strength (mpa)	Elongation (%)	Young's Modulus (mpa)
0 (control)	4.09±0.19a	49.16±0.88a	8.32±0.33a
0.5	39.07±0.70d	93.59±0.88d	41.76±1.12d
1	13.72±0.60c	72.18±0.91c	19.02±1.09c
1.5	7.92±0.27b	55.93±0.58b	14.16±0.62b

Values followed by different letters showed significant differences (p<0.05)

plasticizers were easily interpolated between polymer chains, creating a "cross-linker" effect that bring down the free volume and the movement of polymer segments, leading to reduced mechanical strength and Young's Modulus.

The addition of glycerol as a plasticizer in edible films can enhance elasticity and elongation at break (Dick et al., 2015; Lintang et al., 2021). However, in this study, glycerol added with edible films increased elasticity but decreased elongation percentage. This phenomenon was also reported by Medina-Jaramillo et al. (2016); Asl et al. (2017); and Abdullah et al. (2019), due to the anti-plasticization action of glycerol. Anti-plasticization happened when plasticizer molecules exceeded a critical value, correlating with the reduced mobility of macromolecules which is blocked by stronger interactions between the plasticizer and the polymer. This caused weaker interactions between polymer molecules and decreased cohesive forces in the polymer chains, causing a decline in film elongation (Hammam, 2019; Abdullah et al., 2019). The decrease that occurred in elongation could also be attributed to phase separation caused by glycerol migration from the polymer matrix, limiting the mobility of polymer chains (Asl et al., 2017; Abdullah et al., 2019).

As shown in Table 2, the tensile strength of glycerol-free biocellulose-based edible films was 4.09 MPa, while those containing glycerol ranged from 7.92 to 39.07 MPa, meeting the minimum tensile strength standard by JIS, at 0.3 MPa (JIS, 2019). Based on the JIS standard for the elongation characteristics of edible films, which specified a minimum of 70% (JIS, 2019), the edible films with 0.5% and 1% glycerol concentrations caused elongation at break of 93.59% and 72.18%, respectively. These values met the standard, while the film with a 1.5% glycerol concentration of 55.93% did not meet the standard. Therefore, edible films with glycerol concentrations of 0.5% and 1% were potentially suitable for use as food packaging.

Chemical Structure Characteristics of Edible Film

The chemical structure characteristics of the edible film were examined using FT-IR based on the functional groups constituting the film. A comparison of FT-IR spectra of biocellulose-based edible films made from sago wastewater with varying glycerol concentrations of 0, 0.5, 1, and 1.5%, is shown in Figure 1.

Based on the FT-IR spectra shown in Figure 1, absorption peaks in the range of 3410-3447 cm^{-1}, 2922-2950 cm^{-1}, 1041-1161 cm^{-1}, and 850-860 cm^{-1} represented O-H, C-H, C-O, and the presence of β-1,4 glycosidic bonds, respectively. Skoog et al. (2018) stated that FTIR absorption peaks in the range of 3200-3600 cm^{-1}, 2850-2970 cm^{-1}, and 1050-1300 cm^{-1} represented the O-H, C-H, and C-O groups, respectively. The characteristic film components based on the presence of specific functional groups, such as free O-H groups, aliphatic C-H, C-O, and β-1,4 glycosidic bonds,

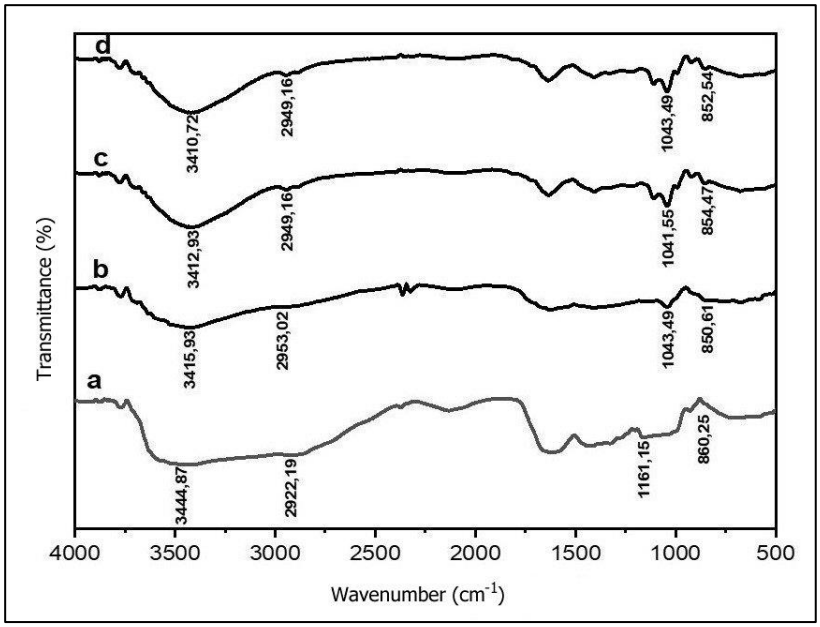

Figure 1. FTIR spectra of biocellulose-based edible films from sago wastewater with varying glycerol concentrations. a. without glycerol, b. 0.5% glycerol concentration, c. 1% glycerol concentration, d. 1.5% glycerol concentration.

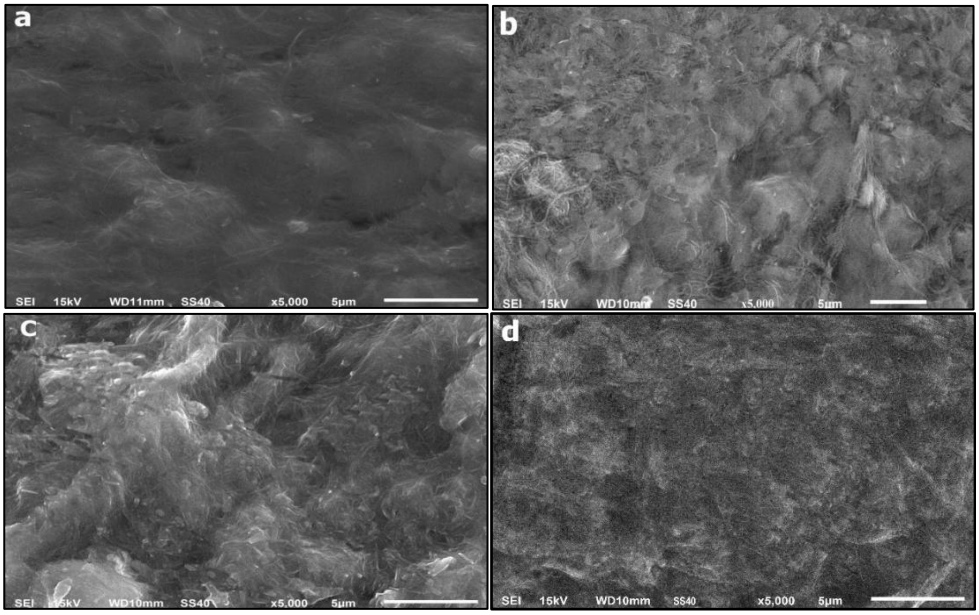

Figure 2. Surface morphology of biocellulose-based edible films from sago wastewater with varying glycerol concentrations. a. without glycerol, b. 0.5% glycerol concentration, c. 1% glycerol concentration, d. 1.5% glycerol concentration

suggested that the main components of the edible film were cellulose polymers. According to Partomo & Rohaeti, (2015), the characteristic functional groups for cellulose are free O-H groups, aliphatic C-H, C-O, and β-1,4 glycosidic bonds. In films containing glycerol, the absorption peak of O-H groups decreased to lower wavenumbers, ranging from 3410-3415 cm^{-1} (Figures 1b-d) compared to the control which had a value of 3444 cm^{-1} (Figure 1a). This decrease in wavenumber in biocellulose-based films containing glycerol was due to structural changes in cellulose. Atta et al. (2016) suggested that peaks in the range of 3410-3420 cm^{-1} were derived from the stretching of O-H groups in cellulose and glycerol.

Surface Morphology Characteristics of Edible Film

Scanning using an electron microscope was conducted to determine the micro-surface structure. The surface morphology of biocellulose-based edible films from sago wastewater with varying glycerol concentrations is shown in Figure 2.

Figure 2a shows that the surface morphology of films without glycerol is relatively smoother and uniform without cracks on the film surface compared to those containing glycerol, as presented in Figures 2b-2d. Similarly, Dick et al. (2015) reported that the surface morphology of edible films from chia seed mucilage (*Salvia hispanica* L.) without glycerol was smoother and more uniform than those containing glycerol. In this study, figures 2b-2d demonstrated the surface morphology of films with different glycerol concentrations, without significant differences. However, the edible film with a 1.5% glycerol concentration (Figure 2d) showed a smoother and more homogeneous surface morphology compared to films with lower glycerol concentrations (0.5% and 1%) (Figures 2b and 2c). Caicedo et al., (2022) also reported that composite-based edible films made from corn starch and chitosan, with varying concentrations of glycerol, demonstrated surface morphology that was nearly similar with slight differences.

CONCLUSION

In conclusion, this study showed that the addition of glycerol with higher concentrations in biocellulose-based edible films made from sago wastewater increased physical characteristics such as thickness, solubility, and moisture content. However, there was a decrease in mechanical characteristics, including tensile strength, elongation percentage, and Young's modulus. Based on functional group analysis, the main component of the edible film was found to be cellulose polymer. Considering both physical and mechanical characteristics, edible films with the most potential for use as food packaging consisted of 0.5% and 1% glycerol concentrations.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest with any party in this study.

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