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Mechanical and Barrier Properties of Biocellulose *Nata de Coco*-Based Edible Film: Influence of Purple Yam (*Dioscorea alata*) Flour and Glycerol Concentration

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Abstract. Edible films represent biodegradable food packaging. Potential material used as an edible film is biocellulose *nata de coco*, but flour made from purple yams needs to be added to increase the solubility of composites in water. The purpose of this research was to study the effects of purple yam flour and glycerol concentrations on moisture, ash content, thickness, barrier properties (WVTR), and mechanical properties biocellulose based edible film. Edible films made with the proportion of slurry biocellulose and purple yam flour were 100: 0; 98: 2; 96: 4% and glycerol were added 0, 1, and 2% and aquades was added to the total volume of 200 ml. The suspension is put in a mould and dried in a 40 ° C oven for 24 hours. Increased concentrations of purple yam flour at biocomposite and glycerol reduce tensile strength and modulus of elasticity, increase the thickness of the edible film. Interaction between biocellulose-purple yam flour and glycerol at high concentrations reduced mechanical strength as a result of network integrity disruption. Interaction between biocellulose-purple yam flour and glycerol reduced moisture content but increased ash content. Glycerol concentrations that were too low and high cause antiplastizicer properties that affected the elongation at break and WVTR edible film. This study demonstrated that proportion of biocellulose-purple yam flour 98:2% and glycerol 1% has good potential for producing the edible films.

Keywords: Biocomposites, Edible Film, Glycerol, Plasticizer, Purple yam flour

1 Introduction

The use of plastic derived from petroleum as a packaging that is not biodegradable has become one of the biggest problems for the environment; therefore, efforts are needed to develop biodegradable packaging namely edible film. The edible film development from renewable resources [1]. The most widely used plasticizer use of local Indonesian commodities as raw material for edible films is expected to be an option to reduce the petroleum plastics hazards [2].

Polysaccharide-based edible film has the potential to replace synthetic packaging, and many researchers have investigated and characterized it. (1; 3; 4). The commodity that can be utilized as raw material for polysaccharide-based edible films is biocellulose *nata de coco* (NDC).

NDC originates from the Philippines but is also well-known in Indonesia and Malaysia, Japan, Taiwan, and South-East Asia [5]. NDC comes from coconut water (*Cocos nucifera*) enriched in nitrogen and carbon through a process that is controlled by fermentation by *Acetobacter xylinum* to produce biocellulose. Biocellulose has a higher strength compared to pulp from plants, and chemically, the cellulose produced is free from lignin and hemicellulose [6]. Biocellulose has high crystallinity and regularity, so its structure does not dissolve in water; therefore it is necessary to modify it with other materials like a composite so that it matches the characteristics of edible film. [7] investigated the use of dried NDC as a filler in cellulose-canna starch biodegradable plastic, this study focused on wet NDC and added are yam flour (*Dioscorea alata*).

Purple yam tubers are a good source of starch for film production because starch contains high amylose, where amylose is important in the formation of starch films [8]. Yam tubers containing 5% mucus indicate high water-holding capacity [9], so it is expected to increase the solubility of composites in water, in addition to purple yam containing anthocyanin, which functions as an antioxidant as an added value of the edible film.

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Besides the addition of starch as a composite material, it is also necessary to add plasticizers to improve mechanical properties, especially the flexibility of making edible films is glycerol [10].

Edible film is applied to food as packaging. Food shelf life can be extended by using edible films as a barrier to gases and volatile compounds or by controlling water permeability [11]. Edible film mechanical characteristics are tensile strength (MPa) and strain at force maximum or elaticity (%) are the most important characteristics of edible films. Low tensile strength or elasticity can cause premature cracking during manufacturing, transportation, storage, and consumption [12].

The research aims to comprehensively study the effects of purple yam flour (PYF) and glycerol concentrations as plasticizers on biocellulose-based edible films on moisture content, ash, thickness, mechanical properties and Water Vapor Transmission Rate (WVTR). In this study, we aimed to determine the proportion of biocellulose-purple yam and glycerol that form the best edible film. In this study, we purposed to determine the formulation of biocellulose, purple yam, and glycerol that formed the best edible film.

2 Method

2.1 Preparation of Biocellulose NDC and PYF

Biocellose NDC films were prepared by slightly modified the method used by [5]. Coconut water was filtered from impurities. 1000 mL coconut water plus 10 grams of sugar and 1 gram of ammonium sulphate stirring until dissolved. The mixture was added glacial acetate to pH = 4 while heated, and then the mixture was poured into a fermented container that has been sterilized then immediately closed. The solution in the container was left to room temperature then 20 mL of Acetobacter xylinum starter media was added to each container. Fermented up to 7 days at room temperature. Biocellulose that was formed was then harvested.

Harvested biocellulose was washed and boiled in water by using NaOH solution (1%) to remove acidity, protein, and non-cellulose components and washed back to neutral pH. The slurry was prepared by weighing 500 g of biocellulose into small pieces and then adding 200 ml of water and blending for 5 minutes so that it takes the form of a slurry (Fig1a).

Yam flour, yam tubers were cleaned from the ground, peeled, washed, and sliced 2 mm thick, the tubers were dried in a drying oven at 50 ° C for 12 hours. Sliced dried yam tubers were ground and sieved through a 100 mesh sieve (Fig 1b).

2.2 Preparation of the Edible Films

Biocellulose slurry and yam flour weighed in the proportion of 100: 0; 98: 2; 96: 4% (w / w) with the addition of glycerol as much as 0, 1, and 2%. 100 ml of aquades heated then glycerol was added while stirring and biocellulose slurry was added and aquades added to 200 ml. Making an edible film with PYF as a treatment (98: 2 and 96: 4%). Yam flour was mixed with 100 ml of aquades and heated while stirring, added with glycerol, biocellulose slurry was added while stirring and added aquades to a total volume of 200 ml. The suspension was cast in a mould and dried in a oven 40 °C for 24 hours. Edible film in sheet form was released from the mould and analyzed (Fig 1c).

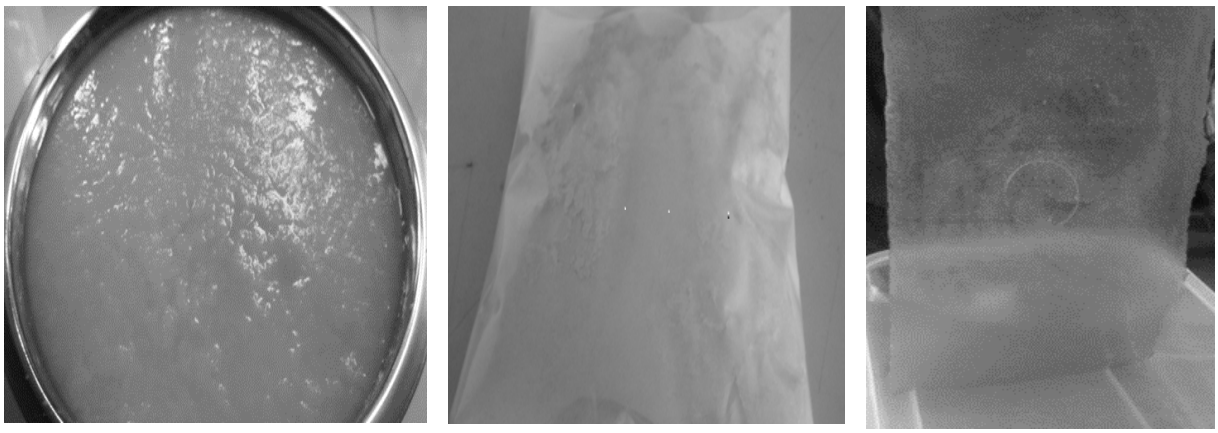

Figure 1. a. *Nata de Coco* b. Purple Yam Flour c. Edible Film

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2.3 Physical and Barrier Analysis

PYF were analyzed for amylose by spectrophotometric method and starch by acid hydrolysis method (AOAC, 2011). PYF and biocellulose NDC and Edible films were analyzed for moisture and ash content using the thermogravimetric method (AOAC, 2011). The thickness, tensile strength (TS), modulus of elasticity (EM) and elongation (E) were determined in accordance with the methodology of [13]. Cut edible film into a dumbbell form. The thickness of the films was tested at 10 random points in the center of the dumbbell form, where failures typically occur. A hand micrometer was utilized for this task. The average of 10 measurements was used to determine film thickness. Tensile grips were employed to hold the films during the tests. The TS a material can withstand when stretched or pulled. The length of the edible film increases due to the pulling load until just before the edible film breaks. WVTR was determined using a modified gravimetric method, as suggested by Gonçalves *et al*. (2019).

2.4 Statistical Analysis

Data were analyzed by using ANOVA (Genstat 18.0). DMRT were used to determine the difference between treatments by using Genstat software.

Table 1. Some Chemical Constituent of the PYF and Biocellulose NDC

Material	Moisture content (%)	Ash content (%)	Starch content (%)	Amylose content (%)
Purple yam flour (PYF)	6.99±0.06	2.27±0.15	71.82±0.25	31.98±1.17
Biocellulose NDC	97.39±1.34	1,14±0.10	-	-

3 Results and Discussion

3.1 Moisture, Ash Content and Thickness of Edible Film

Edible films obtained are structurally intact, visually transparent, slightly opaque, purplish colour and smooth edible film surface. Table 2 shows the moisture content of edible film ranged from 11.93 ± 0.38 to 21.61 ± 0.47%, and the moisture content increased with the greater glycerol added to all treatments the proportion of biocellulose slurry and PYF. The addition of glycerol significantly increases the moisture content because glycerol has a hydroxyl group that tends to absorb water [2]. The greater the proportion of PYF in the biosellulose-purple yam composite, the lower the moisture content; This was because the hydrogen bonds with starch will bind the biosellulose more than water. In making edible films without glycerol, the addition of PYF causes an increase in moisture content, and this was a high amylose content in PYF causing the formation of rigid tissue and water will be trapped in the pores that are formed [13].

Ash content ranged from 1.29 ± 0.03 to 2.87 ± 0.12%, ash content was not affected by glycerol concentration and was only influenced by the proportion of biocellulose and PYF. Ash content increases with greater addition of PYF; this is because the raw material of PYF contains greater ash than biocellulose (Table 1).

The interaction of glycerol concentration and the proportion of biocellulose and PYF had a significant ($p < 0.05$) influence on the edible film thickness, the higher the concentration of glycerol and the proportion of PYF, the thicker the edible film. Edible film thickness varies from 0.032 to 0.211 mm.

Table 2. Moisture, Ash Content and Thickness of Edible Films

Biocellulose NDC: PYF (%) (w/w)	Glycerol (%)*	Moisture content (%)	Ash content (%)	Thickness (mm)
100 : 0	0	12.22±0.47 a	1.29±0.03 a	0.032±0.002 a
	1	30.92±0.64 f	1.28±0.06 a	0.079±0.001 b
	2	30.62±0.76 f	1.24±0.06 a	0.097±0.006 c
98 : 2	0	11.93±0.38 a	2.28±0.15 b	0.113±0.004 d
	1	16.97±0.58 c	2.34±0.05 b	0.109±0.006 d
	2	20.05±0.16 d	2.22±0.06 b	0.129±0.001 e
96 : 4	0	14.40±0.63 b	2.68±0.14 c	0.135±0.006 e
	1	21.61±0.47 e	2.87±0.12 c	0.184±0.005 f
	2	20.86±0.82 de	2.75±0.09 c	0.211±0.006 g

*compared to total biocellulose and purple yam flour. Statistically significant values ($p<0.05$) are indicated by different letters in the same column.

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Glycerol is hydrophilic so it can hold water, this causes edible films to have high moisture content and thickness, this is in accordance with the research of [14] samples with high glycerol lead to more increased film thickness whereas according to [10]. Glycerol can absorb water, thereby increasing the thickness of the film. Glycerol molecules will occupy pores in the matrix and interaction with the polymer, increasing the distance between the polymers and so increasing the edible film thickness (Table 2).

The greater the concentration of PYF added will increase the solute solid so that the thickness of the edible film increases, this is in accordance with the results of the study of [14] increasing concentrations of agar cause total dissolved solids to increase and produce thick films, whereas according to [15], amylose content was positively correlated with film thickness. Amylose PYF content 31.98 ± 1.17% (Table 1) causes an increase in the concentration of yam flour will increase the thickness of edible film.

3.2 Mechanical Properties

Tensile strength (TS). The addition of PYF and glycerol to the biocellulose edible film caused significant changes ($p < 0.05$) in tensile strength (TS), elongation (E) and modulus of elasticity (EM). Table 3 shows edible films in the biocellulose treatment without the PYF and glycerol addition showed the highest TS among the treatments, which was 87.48 ± 3.39 MPa, this showed that biocellulose NDC had a strong hydrogen bond [6]. TS in the biocellulose treatment without the addition of PYF and glycerol was slightly higher than Yudianti *et al*. [16] research, which was 64.8 MPa due to differences in edible film thickness.

Table 3. Mechanical and Barrier Properties of Edible Films

Biocellulose NDC: PYF (%) (w/w)	Glycerol (%)*	TS (MPa)	E (%)	EM (MPa)	WVTR (g/m^2)
100 : 0	0	87.48±3.39 f	3.58±0.03 b	2444.15±72.21 d	27.48±0.31 d
	1	1.94±0.01 ab	17.50±1.00 f	11.08± 0.58 a	44.31±0.60 g
	2	0.46±0.03 a	9.99±0.49 d	4.57± 0.06 a	45.82±0.95 h
98 : 2	0	18.27±0.36 e	2.08±0.08 a	876.64±17.72 b	9.43±0.49 a
	1	6.20±0.33 d	10.65±0.48 d	58.20± 2.23 a	39.93±0.40 f
	2	3.71±0.02 bc	12.61±0.72 e	29.48± 1.59 a	31.11±0.11 e
96 : 4	0	18.84±0.86 e	1.81±0.09 a	1038.65±32.60 c	8.85±0.42 a
	1	5.26±0.24 cd	10.49±0.29 d	50.12± 0.84 a	21.18±1.55 b
	2	2.30±0.16 ab	7.11±0.13 c	32.42± 2.24 a	23.09±0.13 c

*compared to total biocellulose and purple yam flour. Statistically significant values ($p<0.05$) are indicated by different letters in the same column. (TS): Tensile Strength, (E): Elongation, (EM) modulus of elasticity, (WVTR) Water Vapour Transmission Rate.

The addition 1 and 2% glycerol of proportion of biocellulose slurry PYF 98:2 and 96:4 increased TS compared without the PYF addition (100:0) while the treatment of of proportion of biocellulose slurry PYF 98:2 and 96:4 did not cause a significantly different TS ($p > 0.05$). An increase in TS due to an increase in raw material concentration was also reported by [8] in making edible films from starch yam and [14] in making edible films with the addition of agar concentration. This is thought to be the formation of hydrogen bonds between starch from PYF and biocellulose. [17] and [18] also reported that the formation of hydrogen bonds between starch and agar molecules causes TS to increase, resulting in a compact film structure. TS is due to a greater number of biocellulose-starch and starch bonds or stronger hydrogen bonds.

The addition of glycerol to the biocellulose treatment without the addition of PYF caused a sharp decrease in TS, even in the treatment of 2% glycerol addition showed the smallest TS (0.46 ± 0.03 MPa) among all treatments. In the treatment of adding PYF the greater the glycerol causes a decrease in TS, increasing the concentration of glycerol causing a decrease in TS was also observed by [14] where the plasticizer concentration decreases the strength between adjacent macromolecules. [19] explained that plasticizers form hydrogen bonds with starch molecules so that the intramolecular attraction force of starch chains and the formation of hydrogen bonds between starch chains are reduced and allows for greater and lower TS.

Elasticity at break (E). E is a percentage increase in length before breaking because pressure reflects a degree of flexibility [2]. E ranged from 1.81 ± 0.09% to 17.50 ± 1.00%. The elasticity of edible film increased with the greater glycerol concentration, this was in accordance with the results reported by [20]; [10] and [2]. Glycerol as a plasticizer serves to reduce stiffness and increase the flexibility of the film based on two mechanisms, namely increasing the mobility of polymer bonds and increasing the capacity of the water reservoir by attracting water molecules into the polymer network [21].

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The interesting thing was seen in the treatment of proportion of biocellulose slurry and PYF 98:2 and 96:4, the addition of 1% glycerol concentration causes an increase in E compared without the addition of glycerol (0%), but the addition of 2% glycerol concentration causes a decrease in E. Similar results were reported by [13] and [22] were at certain concentrations glycerol causes antiplasticization effects. According to [23] when the concentration of plasticizer in the polymer material is too low or high, causing the interaction between the polymer tissue and the plasticizer is less or excessive so the flexibility of the film is modified. In contrast, according to [24], too high levels of plasticizer cause stronger interactions between plasticizers and biopolymers, leading to a loss of macromolecular mobility. PYF contains high amylose, namely 31.98% (Table 1). According to Yanti *et al*. [25], the amylose content that causes edible films has higher TS and E. This is because amylose has glucose units with α (1-4) glycosidic bonds, resulting in gel strength and solid film formation. According to Ningrum et al. [26], the minimum TS level of the Japanese Industrial Standard (JIS) is 3.92 MPa, and if the percent elongation is less than 10%, edible film is categorized as low quality. Edible film with the addition of 1% glycerol in the proportion of biocellulose slurry PYF 98:2 and 96:4 meets JIS standards.

Modulus of elasticity (EM). EM as an index for 'stiffness' Muscat *et al*. [13], shows a decrease with greater glycerol concentration (Table 3). Films produced from starch with high amylose content such as PYF have higher TS and EM and lower E because of higher gelatinization temperatures and glass transition temperatures. The results are in accordance with the research of [8] have reported decreasing TS and EM and E increasing with increasing glycerol in starch films.

3.3 WVTR

The interaction of glycerol concentration and the proportion of biocellulose and PYF had a significant ($p < 0.05$) on WVTR edible film. The addition of glycerol 1 and 2% caused an increase in WVTR edible film compared to the film without the glycerol addition of in both biocellulose without PYF or biocellulose with the addition of PYF (Table 3). WVTR increases with greater concentration of glycerol added. The same phenomenon was reported by [22] and [20]. According to [27]. The mechanism of glycerol as a plasticizer is to draw water molecules into the polymer tissue so that the plasticization effect is seen in films with the addition of glycerol to have a higher moisture content. The addition of glycerol causes an increase in free volume and moisture content resulting in an increase in permeability to water and oxygen [27]. According to [28][1], the small and hydrophilic glycerol molecule size causes glycerol trapped between polymers to facilitate the migration of water molecules.

The greater the concentration of yam flour added the WVTR decreases. The bond between biocellulose and starch in PYF is thought to be stronger than the bond with water thereby reducing the permeability of the film to water, this is evident in the low moisture r content in edible film with the addition of PYF. These results are in accordance with the research of [29] which states WVTR decreased with the addition of arrowroot starch to the carrageenan film.

4 Conclusion

The combination of PYF and glycerol added to biocellulose-based edible films affected water content, ash content, thickness, tensile strength, elongation, modulus of elasticity, and Water Vapor Transmission Rate. Glycerol concentrations that are too low or too high cause antiplasticizer properties thereby reducing elongation at break. Increasing the concentration of PYF can reduce the Water Vapor Transmission Rate. so it is suitable to be used as a package which provides good moisture barrier properties. Edible films prepared from biocellulose-PYF concentration 98:2% and glycerol 1% showed good moisture barrier properties, elongation, tensile strength, modulus of elasticity which were reasonable and did not show antiplasticize properties.

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