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Characteristics of Edible Film Based on Arrow Starch and Chitosan with Glycerol as Plasticizer

Eka Saputra[1]*, Ahmad S. Mubarak[1], Adriana M. Sahidu[1], Hapsari Kenconojati[2], Tiara M. Kasi[3], Anggari Y. Pramesti[3], Alifia Wahyuningtyas[3], Ariana Isthifarani[3] and Fitri Sil Valen[4]

[1]Department of Marine, Faculty of Fisheries and Marine, Universitas Airlangga, Campus C Universitas Airlangga Mulyorejo Surabaya 60115, Indonesia
[2]Department of Health and Life Sciences, Faculty of Health, Medicine and Life Sciences, Universitas Airlangga, Wijaya Kusuma Street 113, Banyuwangi 68425, Indonesia
[3]Undergraduated Program of Fisheries Processing Technology, Faculty of Fisheries and Marine, Universitas Airlangga, Campus C Universitas Airlangga Mulyorejo Surabaya 60115, Indonesia
[4] Department of Aquaculture, Agriculture, Fisheries and Marine Faculty, Universitas Bangka Belitung, Jl Kampus Terpadu UBB, Balunijuk 33127, Bangka Belitung, Indonesia
***Corresponding Author: ekasaputra@fpk.unair.ac.id**

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ABSTRACT

Packaging is one of the products used as a coating of foodstuffs that serves to protect the quality of products or foodstuffs. The use of plastic as packaging is one of the biggest contributors to world's waste because it is difficult to decompose in any media (non-biodegradable). Plastic packaging with synthetic polymers not only causes environmental damage but also endangers food, hence there is a need for an environmentally friendly packaging innovation that is good and safe for food, one of which is in the form of edible film. This study aimed to determine the physical characteristics of the edible film made from arrowroot starch with the addition of chitosan and glycerol. This research used the experimental method with the design of a complete randomized design model experiment. The treatments used were arrowroot starch 4%: chitosan 0% (P0), arrowroot starch 3.5%: chitosan 0.5% (P1), arrowroot starch 3%: chitosan 1% (P2), arrowroot starch 2.5%: chitosan 1.5% (P3) and arrowroot starch 2%: chitosan 2% (P4), with each treatment repeated 5 times. The main parameters in this study were the physical characteristics of edible film including thickness, tensile strength, and elongation at break. Data were analyzed using ANOVA (Analysis of variance) and continued with DMRT (Duncan Multiple Range Test) at a 5% level. The results showed that the formulation of edible film with the reduction of arrowroot starch and the addition of chitosan affected the physical characteristics, including thickness, tensile strength, and elongation at break. The best result was observed at arrowroot starch 2%: chitosan 2% (P4) treatment, which met the Japanese Industrial Standard (JIS).

INTRODUCTION

The use of plastic as packaging is one of the largest contributors to world waste since it is difficult to decompose (non-biodegradable) **(Babaremu *et al.*, 2022)**. This is due to the high level of plastic production. According to data from NPAP (The National Plastic Action Partnership), Indonesia produces around 6.8 million tons of plastic per year, and 61% of this has no proper management. NPAP also estimates that there will be an increase in the amount of plastic waste by 30% in 2025 if there is no treatment promoted by the government or the community. Plastic packaging with synthetic polymers not only causes environmental damage but also endangers food **(Kumar *et al.*, 2021)**. Therefore, an innovation is needed to make environmentally friendly packaging that is good and safe for food, one of which is edible film **(Kupervaser *et al.*, 2023)**.

Edible films represent a potential alternative to the development of environmentally friendly primary packaging. They are thin layers made from organic materials that can be consumed by consumers. The bio-compatible ability of edible film packaging is one of the advantages that make this packaging a future choice for food packaging. Edible film can be a barrier to oxygen and physical pressure that occurs on the product during storage **(Abdillah & Charles, 2021)**. Another function is as a preventative agent against the loss of volatile compounds in food products. Edible film is a thin layer made from polysaccharides and then printed in sheet form. One of the polysaccharides that can be used as the main ingredient for producing edible film is arrowroot starch. Arrowroot starch is a natural carbohydrate that has the ability to thicken twice, resulting in an edible transparent product **(Abdillah & Charles, 2021)**. It also has a fairly high amylose content of around 29% and an amylopectin content of around 70% **(Abdillah & Charles, 2021)**. High amylose levels can also increase inter-molecular bonds which can help in the formation of a dense matrix network so that it can thicken the solution **(Abdillah & Charles, 2021)**. However, the hydrophilic properties of most starches result in the formation of edible films that are unable to perform optimally in terms of water blocking. Therefore, chitosan (Shrimp and crabs) can be used as an additive in the preparation of edible film due to its moisture and water-holding properties (**Parra *et al.*, 2004; Kou *et al.*, 2021; Saputra *et al.*, 2023)**. The addition of glycerol is expected to enhance the quality of the resulting edible film. This is because glycerol serves as a plasticizer, reducing polymer stiffness and imparting elasticity to the film. According to the previous study, the film produced from starch exhibits a number of inherent characteristics that render it susceptible to damage, such as having high water vapor permeability and being less flexible **(Butler *et al.*, 1996; Gallo *et al.*, 2000; Perez *et al.*, 2011)**.

To our knowledge, no prior study has investigated the characteristics of edible films comprising a mixture of arrowroot starch, chitosan, and glycerol **(Abdillah & Charles, 2021)**. Therefore, this study evaluated the physicochemical characteristics of edible film made from arrowroot starch with the addition of chitosan and glycerol. In addition, it assessed whether the utilization of arrowroot starch and chitosan in edible film production can be used as the alternative processing of arrowroot tubers and chitosan development.

MATERIALS AND METHODS

1. Time and place

This study was performed during March-July 2024 in the Laboratory of Chemistry, Faculty of Fisheries and Marine, Universitas Airlangga.

2. Materials and equipment

The raw materials used in this study were arrowroot starch purchased from the Sooko Ponorogo production house and chitosan (crabs) obtained from Edu-Phymedia Malang. The tools used in this study include a digital micrometer (Syntek, Guangdong, China) and a universal testing machine (UTM) (Shimadzu EZ-SX, Kyoto, Japan).

3. SEM study

The AS/KC film samples were coated with a thin layer of gold using a vacuum gold sputtering machine, and the surface microstructure and cross-section film images were determined using a scanning electron microscope (SEM) (Hitachi S-300 N. Tokyo, Japan) at 500 × magnification with an accelerating voltage of 5 kV. The gold-coated AS/KC film samples were scanned using a laser scanning microscope (Keyence, VKX1000, Illinois, USA) to determine the micrometric 3D topography of the films' surface.

4. X-ray diffraction analysis

Crystallinity properties of AS/KC films, AS, and KC were investigated using a diffractometer (Bruker D8 Advance, Karlsruhe, German) with Cu K alpha radiation at 40kV and 40mA and a scan rate of 10°/min. The XRD patterns were expressed as 2θ in the range from 5 to 40°.

5. Edible films preparation

The edible films were prepared using the mixture of arrowroot starch (AS) and chitosan (CH) with the ratio of 4%:0% (P0); 3.5%:0.5% (P1); 3%:1% (P2); 2.5%:1.5% (P3); and 2%:2% (P4). The arrowroot starch was dissolved into 37.92mL of distilled water that was heated at 120-150°C and stirred at 200-400 RPM for ±20 minutes in a hot plate until a clear gel was formed. Then, chitosan was added to the solution and stirred for ±10 minutes. A total of 0.48ml of glycerol was added to the solution and stirred for ± 5 minutes. After the homogeneous solution was formed, it was cooled at room temperature and poured into a 9cm diameter circle mold. Then, the edible film was dried at 40°C for 24 hours using an oven. The edible film was released slowly from the mold and it was ready for the characterization test.

6. Characterization of edible film

The characteristics of the edible film, including thickness, tensile strength, and elongation at break, were determined using the method described by the previous study of **Abdillah and Charles (2021)**.

7. Statistical analysis

All the data were analyzed using ANOVA (Analysis of variance) and continued with DMRT (Duncan Multiple Range Test) at the 5% level of significance using IBM® SPSS statistic version 22.

RESULTS

1. Characteristic of edible film

The thickness, tensile strength, and elongation at the break of the edible films are presented in Table (1).

Table 1. Results of characteristic edible film

Parameters	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)
P0	0.256^{c}±.0114	2.995^{b}±.220	45.28^{a}±14.572
P1	0.242^{c}±.0278	2.847^{b}±.341	74.54^{a}±22.729
P2	0.220^{b}±.0122	2.136^{a}±.555	114.86^{b}±36.9
P3	0.198^{a}±.0084	2.525^{ab}±.454	153.48^{c}±9.296
P4	0.180^{a}±.0070	2.159^{a}±.685	148.98^{c}±21.203
P-value	<0.001	0.03	<0.001
JIS standard	<0.25	<3.920	>50

Data were presented as mean ± SD (n=5). Different superscript notation in the same column indicates significant difference (P<0.05). P0: arrowroot starch 4% and chitosan 0%; P1: arrowroot starch 3.5% and chitosan 0.5%; P2: arrowroot starch 3% and chitosan 1%; P3: arrowroot starch 2.5% and chitosan 1.5%; P4: arrowroot starch 2% and chitosan 2%.

2. Water solubility

The water solubility is presented in Fig. (1).

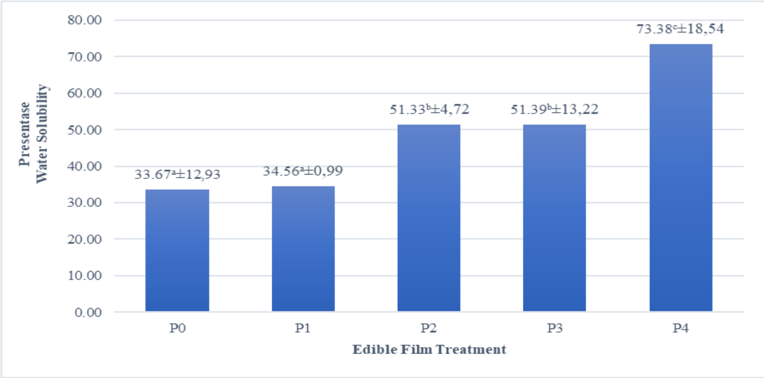


Fig. 1. Life specimen

P0: arrowroot starch 4% and chitosan 0%; P1: arrowroot starch 3.5% and chitosan 0.5%; P2: arrowroot starch 3% and chitosan 1%; P3: arrowroot starch 2.5% and chitosan 1.5%; P4: arrowroot starch 2% and chitosan 2%

3. Water vapor transmission rate

The water vapor transmission rate is presented in Fig. (2).

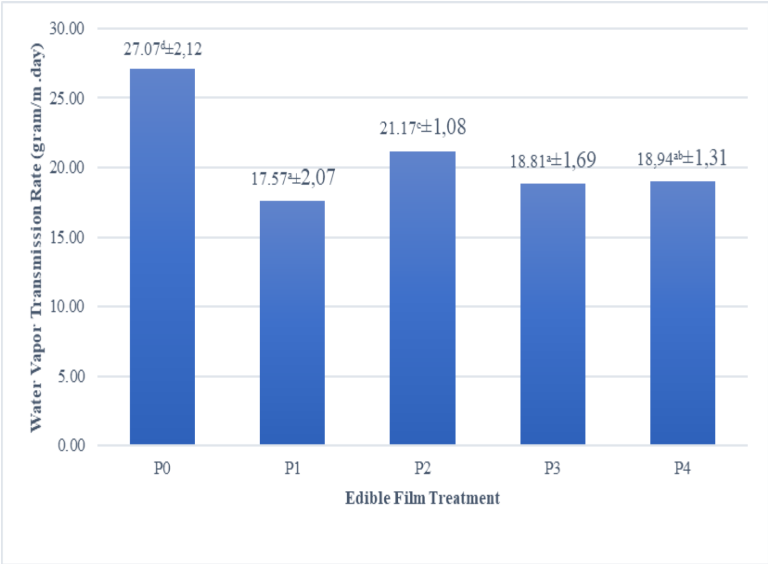


Fig. 2. Water vapor transmission rate

P0: arrowroot starch 4% and chitosan 0%; P1: arrowroot starch 3.5% and chitosan 0.5%; P2: arrowroot starch 3% and chitosan 1%; P3: arrowroot starch 2.5% and chitosan 1.5%; P4: arrowroot starch 2% and chitosan 2%

4. X-Ray Diffraction (XRD)

The X-ray diffraction (XRD) is presented in Fig. (3).

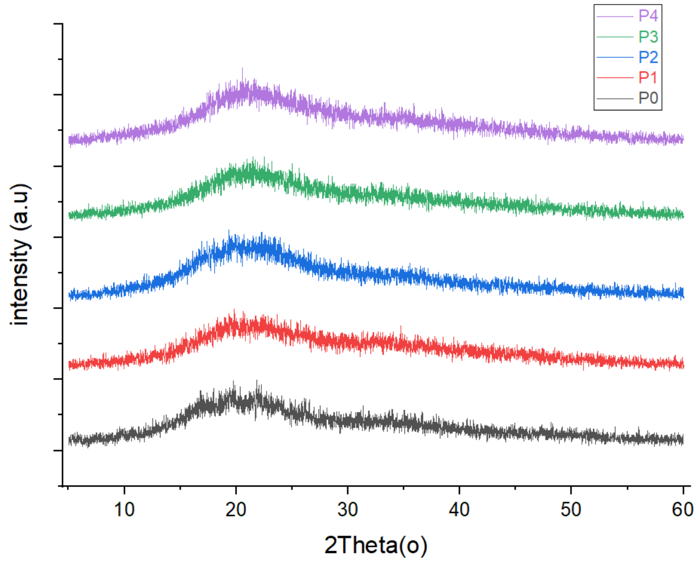
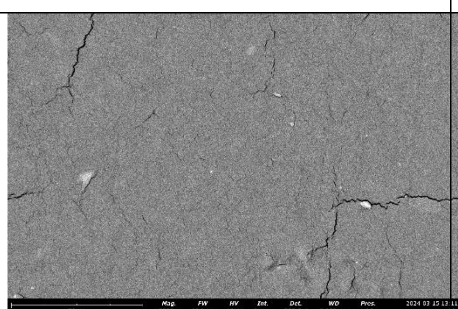
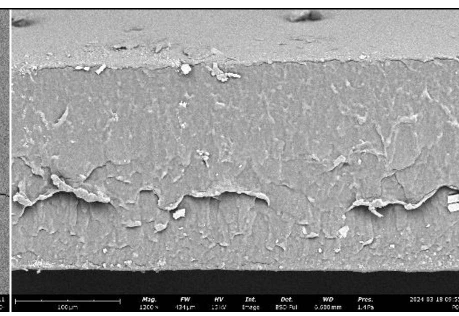
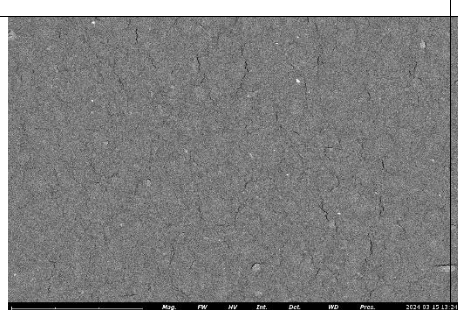
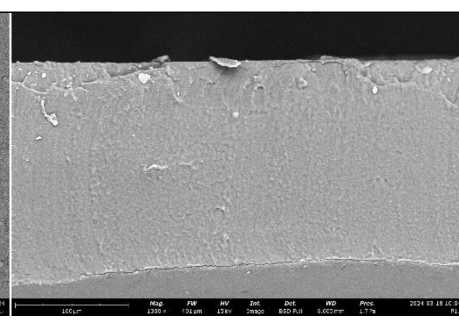


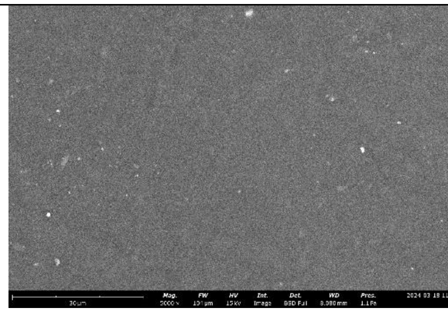
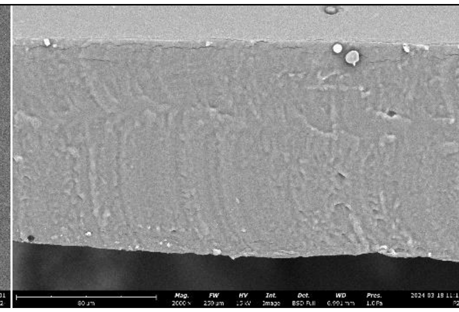
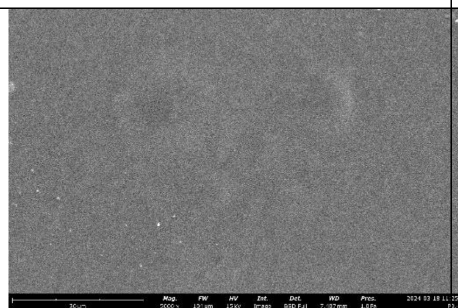
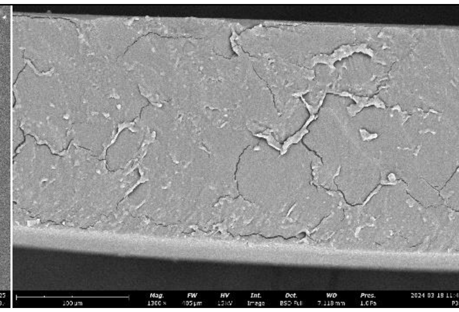
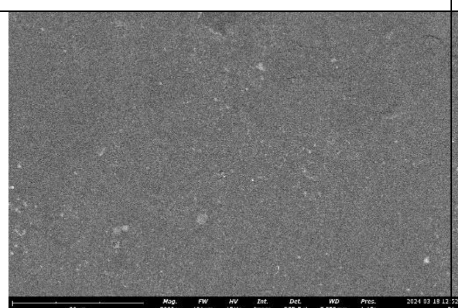
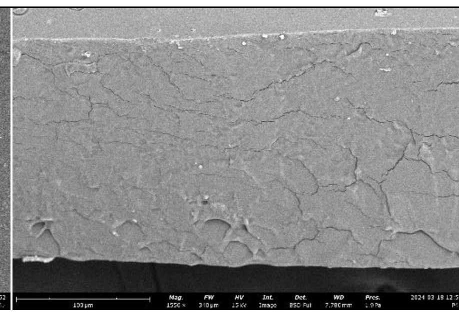
Fig. 3. The X-ray diffraction (XRD)

5. Scanning electron microscopy

The scanning electron microscopy is presented in Table (2).

Table 2. Results of scanning electron microscopy

Treatment	Surface	Cross
P0 (control)		
P1		

P2		
P3		
P4		

P0: arrowroot starch 4% and chitosan 0%; P1: arrowroot starch 3.5% and chitosan 0.5%; P2: arrowroot starch 3% and chitosan 1%; P3: arrowroot starch 2.5% and chitosan 1.5%; P4: arrowroot starch 2% and chitosan 2%

DISCUSSION

This present study successfully produced an edible film made from a combination of arrowroot starch and chitosan with glycerol as plasticizers. The addition of chitosan to the arrowroot starch-based film affected the characteristics of produced edible film, including thickness, tensile strength, and elongation at break. Based on the thickness test analysis, it showed that the thickness of the edible film decreases with the addition of chitosan concentration and the reduction of arrowroot starch concentration. The highest edible film thickness (0.256c±.0114mm) was observed in the P0 treatment (arrowroot starch 4% and chitosan 0%), while the thinnest edible films were found in the P4 treatment (arrowroot starch 2% and chitosan 2%) at 0.180a±.0070mm. The thickness of the edible film serves as a physical parameter, which are related to the product's shelf life. The thickness of the film is directly correlated with the product's shelf life **(Saputra *et al.*, 2023)** as a thicker film provides a greater barrier to gas migration, thereby extending the product's shelf life. The increase in the film's thickness may be attributed to the increased amount of dry material used. This is supported by a previous study **(Putri *et al.*, 2023)** which stated that the thickness value of the edible film decreases with the reduction of starch concentration. The previous study reported that the addition of iota-carrageenan ranges from 0.066 to 0.084mm with a decreasing graph as other polymers increase **(Abdillah & Charles, 2021)**.

The thickness of the edible film will affect other physical properties of edible films, such as tensile strength and elongation at break. The tensile strength test is used to determine how strong films are when pulled apart, with a higher value indicating a stronger film. As a food packaging material, edible film must have a tensile strength that is in line with the standard for product protection from external factors. Meanwhile, the elongation test is used to determine the ability of a film to become more elastic with the higher elongation indicating the more elastic the film **(Ansori *et al.*, 2020)**. In our study, there is an inverse correlation between tensile strength and elongation of the produced film. The tensile strength decreased in line with the reduction of arrowroot starch and the addition of chitosan, while the elongation at the break of the film increased. This proved that adding chitosan concentration can increase the tensile strength and decrease the elongation value of the film **(Li *et al.*, 2024)**. According to **Rosseto *et al.* (2019)**, a higher starch concentration results in thicker composite films with enhanced mechanical properties. This improvement is likely due to the increased polymer content, which strengthens the film structure. Chitosan can generate hydrogen bonds between chains, giving the film a dense surface that is difficult to break because it requires large energy. While the decreasing value of elongation is assumed to be due to the size of the chitosan particles being quite large, it does not dissolve perfectly during gelatinization and causes instability in the formation of chemical bonds between polymers **(Ansori *et al.*, 2020)**. Another study also suggested that the weakening of the bonds between the polymers causes the tensile strength of the film to decrease while the mobility of the polymer increases so that the elongation value increases **(Abdillah & Charles, 2021)**. This is due to the factor of decreasing the concentration of arrowroot starch. Previous study **(Abdillah & Charles, 2021)** suspects that the low tensile strength of the film is caused by arrowroot starch undergoing gelatinization during the heating process so that it becomes amorphous which is easily deformed. Tensile strength measurements are followed by film elongation measurements where the maximum elongation is experienced by the edible film when testing tensile strength until the film breaks. Reducing the concentration of arrowroot starch and adding chitosan in this study resulted in a greater percentage value of film elongation, so it can be concluded that this formulation can improve the elongation properties of the film. The presence of glycerol is understood to interact with polysaccharides. This is because glycerol can disperse starch and chitosan molecules to produce an elastic film. Glycerol can disrupt the compactness of the starch structure, reduce intermolecular interactions, and increase polymer mobility, resulting in more elastic film **(Ansori *et al.*, 2020)**.

Solubility or water solubility is an important characteristic of degradable properties. The formulation of arrowroot starch reduction in edible film affects the water solubility value in each treatment. The results of the water solubility analysis increased with the decrease in arrowroot starch concentration and the addition of chitosan concentration which can be seen in (Fig. 1). The increase in water solubility results in this study is thought to affect the use of arrowroot starch as the basic material for edible film and glycerol as a plasticizer. Similarly, films formulated with higher concentrations of arrowroot starch exhibited decreased water solubility, suggesting a more compact and organized polymer structure that resists water penetration **(Nogueira *et al.*, 2021)**.

Water solubility testing is greatly influenced by the source of the film. The starch-based edible film is influenced by the bonds of starch hydroxyl groups, where the weaker the bonds of the starch hydroxyl groups, the higher the solubility. Solubility in water can also be related to the amylose content of starch. The higher the amylose content, the greater the solubility index. The solubility index depends on the origin of the starch.

In the study, the study of edible films with a mixture of corn starch and chitosan also experienced an increase in the results of water solubility tests. This may be due to the type of chitosan used, namely the LMW-CH type. LMW-CH is a type of chitosan with more flexible interactions between polymer-plasticizer and polymer-solvent in the formulation of films with low molecular weight chitosan. Overall, the results of the edible film water solubility test produced in this study have met the standards **(Butler *et al.*, 1996; Karbowiak *et al.*, 2006; Fernando *et al.*, 2011)** with a minimum edible film solubility of 50%. The high edible film indicates that the film can be easily consumed. Higher solubility indicates lower water resistance. However, high solubility will be an advantage for some applications. Packaging that can dissolve easily is convenient for use in ready-to-eat products when melted in boiling water.

Water vapor transmission is the amount of water vapor passed through a unit area of material per unit of time. Water is an important factor in food spoilage, so an important feature of edible film is its ability to prevent moisture exchange between the environment and the food matrix. A low water vapor transmission rate indicates that the product has a shorter shelf life. The decrease in the water vapor transmission rate in P1 (3.5% arrowroot starch; 0.5% chitosan) and P3 (2.5% arrowroot starch; 1.5% chitosan) is thought to be influenced by the properties of amylose and amylopectin on the water vapor transmission rate. Another research focused on arrowroot starch films observed that films with a 2% starch concentration exhibited the lowest WVTR. The study suggested that the amylose and amylopectin ratio plays a crucial role in determining the film's barrier properties, with higher amylose content contributing to reduced water vapor permeability **(Nogueira *et al.*, 2021)**.

The increase in water vapor permeability in P2 (3% arrowroot starch; 1% chitosan) and P4 (2% arrowroot starch; 2% chitosan) may be due to a decrease in film hydrophilicity and a decrease in the polymer order of the film which allows more water vapor to pass through **(Fernando *et al.*, 2011)**. The increase in water vapor transmission values at low chitosan proportions may be due to differences in the hydration layers of the two polymers which make the chitosan-containing matrix more open to water transfer **(Karbowiak *et al.*, 2006)**.

The rate of water vapor transmission decreases as the concentration of arrowroot starch decreases because the amylose content is lower than amylopectin. **Fadholly *et al.* (2021)** stated that starch with low amylose content will increase the rate of water vapor transmission. This is due to the crystallization process that occurs in the amylose chain, where amylose shows type B crystals while amylopectin is completely amorphous. Water vapor diffusion occurs more easily in the amorphous form than in the crystalline form **(Ansori *et al.*, 2021)**.

The addition of chitosan reduces water vapor transmission. This is because chitosan has higher hydrophobicity properties compared to starch. In addition, hydrogen bond interactions between starch and chitosan reduce the availability of hydrophilic groups, thereby reducing interactions with water molecules. Therefore, it can reduce the rate of water vapor transmission **(Butler *et al.*, 1996)**. Edible films made from polysaccharides and proteins generally have high water vapor transmission values and low oxygen permeability. This is because polysaccharides and proteins are classified as polar polymers and have a high number of hydrogen bonds so that they can produce water absorption at high humidity. The rate of water vapor transmission of a material is influenced by the chemical properties and structure of the forming material, the concentration of plasticizer, and environmental conditions when testing such as humidity and temperature. Water vapor transmission is greatly influenced by RH and temperature when testing, thickness, type, and concentration of plasticizer and the properties of the edible film former.

The use of chitosan types can affect the resulting edible film. Chitosan with low molecular weight or Low Molecular Weight Chitosan (LMW-CH) tends to increase the water vapor transmission value significantly compared to films with high molecular weight or High Molecular Weight Chitosan (HMW-CH). This is because water diffusion occurs more easily through films made with LMW-CH and produces a less compact structure when compared to edible films with high molecular weight. In other words, the higher the molecular weight of chitosan, the more compact the film structure is, resulting in a low water vapor transmission value. This concept is in accordance with other tests, where the water solubility test value will be higher if using low molecular weight chitosan **(Proboningrat *et al.*, 2022)**.

X-ray diffraction abbreviated as XRD is one of the most important instruments for analyzing the content of a material, namely montmorillonite in edible composites

(Widyananda *et al.*, 2021). X-ray diffraction (XRD) on edible films with the addition of arrowroot starch, and arrowroot starch with the addition of chitosan is found in Fig. (8). The type of crystallin produced by arrowroot starch is classified as type A crystallin, this is indicated by the 2 theta peaks, namely 15°, 17°, 20°, and 23° **(Faridah *et al.*, 2014)**. This is in accordance with the XRD test in this study which also showed type A crystallin. Heating the arrowroot starch solution in the film preparation will form gelatinized starch where the starch granules swell and absorb water molecules so that they destroy the starch structure crystals and then form an amorphous structure **(Kharisma *et al.*, 2021)**.

The results of the XRD test of edible films with the addition of chitosan made the peak intensity smaller compared to edible films without the addition of chitosan, where when two film-forming components between starch and chitosan are mixed, the peak increase still exists but the intensity becomes smaller. The increase in crystal peaks with increasing chitosan content can be explained by the occurrence of molecular miscibility between the two components. The film that was mixed with chitosan had a lower peak than starch-based films.

The results of the edible film analysis using SEM (Scanning Electron Microscopy) show that the surface and cross cracks and folds that are visible become fainter along with the reduction in the concentration of arrowroot starch and the addition of chitosan concentration. The cracks and folds seen in the SEM results indicate the inhomogeneity of the materials used, in other words, there are large particles that cannot be completely dissolved. These cracks can cause water to be easily absorbed, making the edible film less effective when used in food packaging. The addition of glycerol as a plasticizer is also indicated to reduce cracks and pores in the resulting film. It can be concluded that the materials used in the edible film formulation can be homogeneous so that they have good mechanical properties with an intact matrix structure. No cracks and folds indicates that the resulting microstructure is good because the materials produce strong interactions and adhesion at the interface with perfect dissolution.

The physicochemical characteristics of edible film in this study showed results that qualified within the Japanese Industrial Standard (JIS), with a maximum standard of less than 0.25mm for the film thickness, tensile strength with at least 3.92 MPa and elongation at break value with more than 50% **(Abdillah & Charles, 2021)**. It can be concluded that adding chitosan is possible to produce edible films that meet the JIS.

CONCLUSION

The addition of 2% arrowroot starch and 2% chitosan (P4) in the manufacture of edible films resulted in the best edible film with the physiochemical characteristics that meet the JIS.

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