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Characteristics of the Anthocyanin Biosensor for Detecting Freshness of Gourami Fish *(Osphronemus Gourami)*

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ABSTRACT

Biosensors are a form of innovation that overcomes the quality assurance problem of fishery products by observing the level of product freshness through color changes on the Biosensor in real time, along with changes in the pH of the product. This research aims to obtain the best formulation of a starch-based biosensor with the addition of red dragon fruit peel anthocyanin extract as a freshness sensor and to determine the characteristics of a starch-based biosensor with the addition of anthocyanin extract as a freshness sensor. The starches used in this research were potato starch (A), cassava starch (B), corn starch (C), wheat starch (D), and sago starch (E). The results of this research are that the wheat starch biosensor (D) is the treatment with the characteristic results that most meet the JIS, namely the thickness value produced by wheat starch is 0.115 mm, the tensile strength value is 1,580 Mpa, and the elongation value is 109.489%. For the color characteristics of the best Biosensor, wheat starch has an L* value of 26, a* value of 24, a b* value of 25.2, and a Hue value of 16° hue.

Contribution to Sustainable Development Goals (SDGs):
SDG 2: Zero Hunger
SDG 3: Good Health and Well-being
SDG 9: Industry, Innovation, and Infrastructure
SDG 12: Responsible Consumption and Production
SDG 14: Life Below Water

1. INTRODUCTION

1.1. Research Background

The current development of packaging technology is smart packaging. Smart packaging is divided into two large categories: active packaging and intelligent packaging [1]. Smart packaging is an innovation in the packaging field that can monitor and provide information to producers and consumers regarding the quality of packaged products [2]. Intelligent packaging can monitor product freshness in real time. It can reduce losses due to errors in providing information regarding product damage so that it can increase food safety for consumers [3]. Intelligent packaging can detect a decrease in the quality of the product. It is packaged based on temperature changes, which affect changes in pH as indicated by the color changes on the packaging [4]. This packaging will react when it experiences changes either chemically or biologically in the packaging, which indicates product damage [5]. An active compound that has the potential to be developed as a freshness indicator is the extraction of anthocyanin from the skin of red dragon fruit.

Anthocyanin is a color pigment that is sensitive to changes in pH. The colour of anthocyanin at pH < 6 is red; at pH 6-7, anthocyanin will be pink; at pH 8, anthocyanin will be blue-purple; while at pH 9-11, anthocyanin will be green; and at pH 12, anthocyanin will be yellow, [6]. Anthocyanins from dragon fruit extract will change acid-base color from pink to green, making it easier for consumers to distinguish the freshness of fish [7]. The anthocyanin's color will change as the pH of the product changes [3]. It is hoped that red dragon fruit peel will be used as a natural anthocyanin extract to maintain food safety and reduce the use of chemical-based pH indicators. The application of freshness indicators can be combined into biosensors for packaging products.

Biosensors have the potential to be developed. One reason is that they are environmentally friendly and safe, as is the case with packaging that comes in direct contact with the product. Starch is one of the main ingredients in making biosensors—starch functions as a gelatinizer in forming texture and as a binder between other materials. Starch-based biosensors are expected to have good physical and chemical characteristics. Starch is also important in making biosensors as a thickener and binder, where amylose provides gel and amylopectin provides sticky properties [8].

1.2. Literature Review

Packaging technology is currently developing, and many innovations are being made, such as smart packaging or what is also called intelligent packaging. Smart packaging is an innovative form of packaging that can overcome the limitations of conventional/traditional packaging. Smart or intelligent packaging can provide active treatment in response to changes in external and internal conditions or can also be a means for consumers to find out the product's status in the packaging [9]. Intelligent packaging is included in intelligent packaging, which refers to packaging that can monitor and provide information about a product's condition or storage environment [10]. Intelligent packaging can detect the product's condition, the composition of the atmosphere in the packaging, and environmental conditions during transportation [11]. The working mechanism of intelligent packaging focuses on increasing communication capabilities. Intelligent packaging is included in the intelligent packaging category because it can provide information to producers, retailers, and consumers when the product's quality has decreased or the product is not stored in appropriate conditions [12]. Intelligent packaging can be combined into a biosensor.

Biosensors are sensors that can monitor product quality during storage. Biosensors usually comprise biopolymers such as polysaccharides, lipids, and proteins. The polysaccharide groups that are widely used as materials for making biosensors are Starch and its derivatives, cellulose and its derivatives (methylcellulose, carboxyl methyl cellulose, hydroxy propyl methyl cellulose), pectin, marine algae extracts (alginate, carrageenan, agar), gum (gum arabic, gum karaya), xanthan, and chitosan [13] Starch has the potential to be used as a biosensor because starch functions as a thickener and binder, where amylose provides gel properties. Amylopectin provides sticky properties [8]. This Biosensor can also be used as alternative packaging that does not cause environmental problems, where the Biosensor has biodegradable properties so it does not cause ecological pollution like synthetic packaging materials [14]

1.3. Research Objective

This research aims to identify characteristics of starch-based biosensors with the addition of anthocyanin extract as a freshness sensor and obtain the best formulation for starch-based biosensors by adding anthocyanins.

2. MATERIALS AND METHODS

The research used chitosan, glycerin, red dragon fruit peel extract, cassava starch, corn starch, sago starch, potato starch, wheat starch, distilled water, 96% ethanol, and 10% citric.

2.1. Anthocyanin Extract

Taking anthocyanin extract from red dragon fruit skin goes through several stages. First, the red dragon fruit skin is washed, then sorted to separate the red dragon fruit skin, which is perfectly red and less red, and separates the fruit flesh from the still attached skin. Then, the red dragon fruit skin is washed and drained. The skin of the red dragon fruit is cut into ± 2mm pieces and then dried in an oven at 50°C for 6-7 hours. Dried red dragon fruit skin is ground into powder. The powder was macerated for 24 hours using a 1:5 (w/v) solvent. The solvents are 96% ethanol and 10% acetic acid (v/v). The macerated extract was then evaporated using a rotary vacuum evaporator at a temperature of 40 °C. The final result in the form of a concentrated extract is stored in a closed bottle in the refrigerator or ready to use.

2.2. Making Biosensors

The Biosensor manufacturing process uses the solvent casting method. The process of making a Biosensor is by mixing a 3% (w/v) chitosan solution into 50 ml of 1% acetic acid mixed with 50 ml of a 3% (w/v) starch solution and adding 1% (v/v) glycerin from the total volume—solution [15]. The solution was homogenized at 75°C for 15-20 minutes until gelatinized. The gelatinized solution was then printed with a size of 20x20 cm and dried at 40°C for 24 hours. After drying, the Biosensor is smeared thoroughly with 12 ml of anthocyanin on the surface of the edible film until it is evenly distributed [15].

2.3. Thickness Test

Thickness is an important parameter that influences film formation. Thickness also affects the mechanical properties of composite films. Film thickness was measured using a screw micrometer with a range of 0 – 25 mm. The test was carried out at five random points on the film surface, and then the average value was taken [16]

2.4. Tensile Strength Test

Tensile strength testing is a physical characteristic of the strength of the edible film to withstand physical damage when packaging food ingredients. The tensile strength value will show how much a film's stretch or tensile strength is. Good quality film has a high tensile strength value; the higher the tensile strength value, the better the film quality [16]. The tensile strength test was carried out by cutting edible film with a width of 4 cm according to the cross-sectional area of the tensile strain tester and a length of 7 cm. The tensile strength test is measured using a tensile strain tester by placing a piece of edible film on the cross-section; then, the tool will pull the edible film until it breaks.

$$\textbf{Tensile strength}\left(\frac{\mathbf{N}}{\mathbf{mm^2}}\right) = \frac{\textbf{Force}}{\textbf{Unit Area}\ (\mathbf{mm^2})}$$

2.5. Elongation Test

The elongation percentage is the percentage increase in the maximum length of the film compared to the initial length when the film is subjected to a tensile force until it breaks. Riceman measured the percentage of elongation by measuring the tensile strength value. Calculating the elongation of edible film is dividing it by the initial length of the edible film before withdrawal. Elongation of edible film refers to the ability of the edible film to stretch over time until it finally breaks or tears. The elongation percentage determines the elasticity of a film. The higher the film elongation percentage value, the more elastic the film.

$$\text{Elongation}\ (\%) = \frac{\text{Prolongation}\ \mathit{Biosensor}}{\text{Initial Lenght Biosensor}}\ X\ 100\%$$

2.6. Research Design

This research used a completely randomized design (CRD). The variations used consisted of 5 experimental treatments using different raw materials. The five experiments consisted of the first treatment for making edible film using potato starch (A), the second treatment using cassava starch (B), the third treatment using corn starch (C), the fourth treatment using wheat starch (D) and The fifth treatment used sago starch (E) with the addition of 3% starch each. The treatment was carried out 4 times to obtain 20 experimental units.

3. RESULT AND DISCUSSION

3.1. Biosensor

A biosensor is a type of smart packaging that can monitor the quality of packaged food products during transportation and storage. It provides real-time information about product quality, including microbial growth or chemical changes in the food. In this study, the Biosensor was applied as a freshness sensor for gourami fish. The color changes of the anthocyanin-based Biosensor during storage at a chiller temperature of 4°C can be observed in Figure 1.

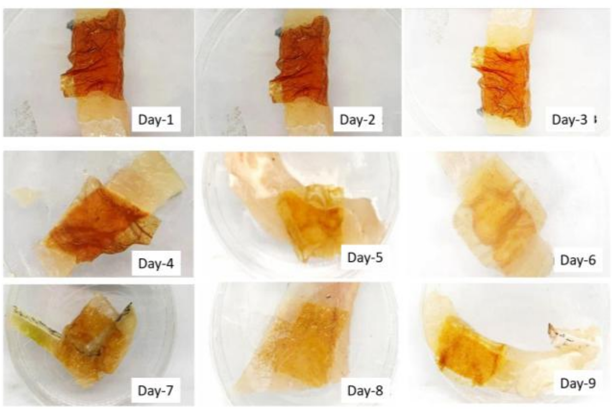

Figure 1. Color Changes of the Biosensor During Product Storage

Color changes in the Biosensor during storage and product application. These color changes indicate the condition of gourami fish stored in a chiller at 4°C for 9 days (Figure 1). The Biosensor changed color from reddish-brown to yellow-green as the freshness of the gourami fish declined.

3.2. Thickness

Thickness is an important characteristic of edible film because it is a barrier against water vapor. Thickness testing on starch-based edible films was carried out using the microcell messmer method, which was carried out in research using a screw micrometer [17]. This thickness measurement is done by measuring five different points on the Biosensor and then getting the results from the average of the measurements from the five other points. The average value of biosensor thickness for various types of Starch can be seen in Figure 2.

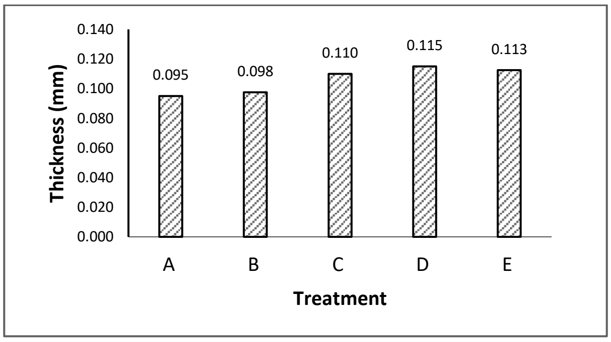

Figure 2. Thickness of Biosensor

Description: A: Potato starch; B: Cassava starch; C: Corn starch; D: Wheat starch; E: Sago starch

Based on Figure 2, the thickness value ranges from 0.095-0.115. The average values are sequentially from highest to lowest. Namely treatment D 0.115 mm, treatment E 0.112 mm, treatment C 0.110 mm, treatment B 0.098 mm and treatment A 0.095 mm. [18] stated that wheat starch contains 0.7-1.4% fat, this fat content is higher compared to other types of Starch such as sago starch 1.3% [19], corn starch 0.36%, cassava starch 0.03% and 0.01% potato starch [20]. The significant fat content in wheat starch means that wheat starch edible film can form complex compounds that bind to amylose, making the edible film hydrophobic. The hydrophobic ability to hold water vapor can maintain the thickness characteristics of wheat starch edible film by adding anthocyanin extract to the surface of the edible film. [15] stated that the coloring process on edible film causes the thickness value to decrease by 0.04 mm and is not much different from before coloring the edible film. [21] stated that another factor that can cause an increase in the thickness value of edible film is the interaction between Starch and plasticizer during the gelatinization process so that it can produce thicker edible film. Japanese Industrial Standard [22], the maximum thickness value for edible film is 0.25 mm. The results obtained show that even though they have different thickness values, all treatments D, treatment E, treatment C, treatment B, and treatment A have met the standards that have been determined.

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3.3. Tensile Strength

Tensile strength is the maximum pull that edible film can achieve before breaking. Tensile strength measurements aim to determine the magnitude of the longitudinal force resulting from the maximum pull over an area. The tensile strength can be calculated by dividing the resulting tensile force by the area of the edible film being tested. The average tensile strength value of edible starch film can be seen in Figure 3.

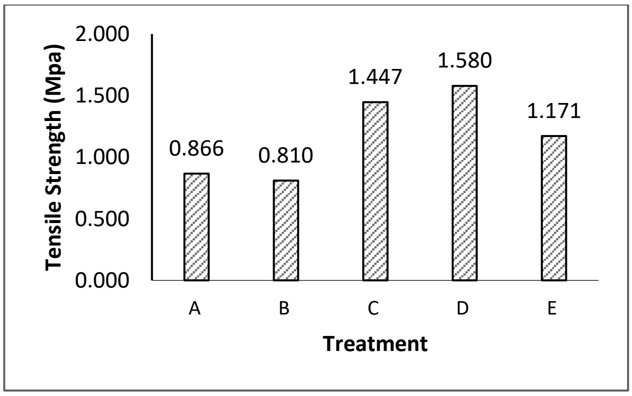

Figure 3. Tensile Strength of Biosensor
Description: A: Potato starch; B: Cassava starch; C: Corn starch; D: Wheat starch; E: Sago starch

Based on Figure 3, the average values obtained sequentially from highest to lowest are treatment D 1.580 Mpa, treatment C 1.447 Mpa, treatment E 1,171 Mpa, treatment A 0.866 Mpa, and treatment B 0.810 Mpa. Based on the Japanese Industrial Standard [22], the minimum tensile strength value for edible film is 3.2966 Mpa. Ref. [23] stated that high amylose content can produce strong starch biosensor characteristics. Biosensor with wheat starch has the highest tensile strength and contain amylose at 29%. Amylose content in other starches, such as potato starch, is 21% [23], cassava starch is 8.92% [24], corn starch is 24-26% [25], and sago starch is 21.7 [26]. The added anthocyanin can also influence the tensile strength value of the Biosensor. [27] stated that adding anthocyanin to edible film can reduce the tensile strength value of edible film because anthocyanin extract contains dissolved substances that enter the film matrix, weakening the bonds between the film polymers. Another influence is the humidity conditions in the room before the tensile strength test is carried out [28]

3.4. Elongation

The elongation test measures the Biosensor's elongation value until it breaks during testing. Riceman measured the percentage of elongation by measuring the tensile strength value. Elongation is flexibility, one of the important characteristics of edible film. A good elongation value based on JIS is > 70%. The average elongation value of Starch edible film can be seen in Figure 4.

Based on Figure 4, the average values obtained sequentially from highest to lowest are D 109.489%, treatment E 97.254%, treatment C 94.096%, treatment B 85.560%, and treatment A 73.397%. Based on JIS, good tensile strength with a value of > 70%. [29] stated that protein can be used to produce edible film with good elasticity. [27] stated that the elongation value can decrease due to the influence of the addition of anthocyanin to biosensors and the biosensor storage process.

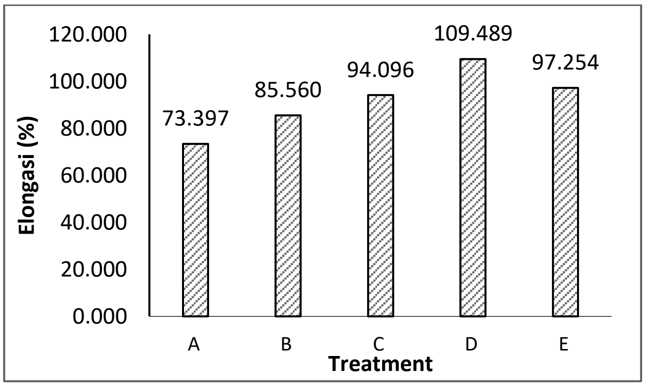

Figure 4. Elongation of Biosensor
Description: A: Potato starch; B: Cassava starch; C: Corn starch; D: Wheat starch; E: Sago starch

4. CONCLUSION

The conclusion is the best formulation is found in treatment D (wheat starch), which has the most characteristics that meet the requirements for edible film in JIS, namely with an average thickness value of 0.115 mm, tensile strength of 1.052 Mpa, elongation of 10.949 %

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