UTILIZATION OF ANTHOCYANIN IN EDIBLE FILM AS COCONUT MILK FRESHNESS INDICATOR

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Abstract

Technology in smart packaging continues to evolve, one of which is packages equipped with food freshness indicators to monitor food safety and quality. These indicators can be formed by immobilizing anthocyanin into edible film and then changing its color to pH changes. The research aims to determine the immobilization time, test the color stability of the edible film indicator to temperature changes, determine the characterizations of the edible film indicator based on the %relative standard deviation (%RSD) value, and apply the edible film indicators as coconut milk freshness indicators. The best immobilization time was in the 90th minute with %RSD=0%. The edible film indicators did not change color from 10 to 50°C with %RSD=0%. The characterizations of edible film indicators on pH 6, 7, 8, and 10 had response times of 6.68 minutes; 9.49 minutes; 3.57 minutes; and 4.37 minutes; periods of 14 days, 13 days, 14 days, and 16 days; and good reproducibility at pH 6 and 10 and not good at pH 7 and 8. Overall, based on anthocyanin color changes, an edible film indicator can be used as a freshness indicator in coconut milk but cannot indicate the pH value of coconut milk.

Keywords*: anthocyanin, edible film, immobilization, edible film indicator characteristics, coconut milk*

Introduction

Globalization has had a significant impact on human lifestyles, particularly in terms of technological advancements, one of which is food technology. As a result of consumer and business trends in food packaging, technology in the food industry continues to evolve. Food packaging advancements range from packaging that extends shelf life to packaging that includes food freshness indicators to monitor food safety and quality. Smart packaging is the term for this type of packaging.

Although smart packaging is not commonly applied in Indonesia, it has been observed by various institutes and government bodies. In the creation of smart packaging, Hasnedi (2009) examined the usage of chitosan-acetate, polyvinyl alcohol, and bromthymol blue as a tilapia fillet rot indication (Widiastuti, 2016). In the creation of smart packaging, Yanuariski (2019) studied the use of chitosan, cassava starch, and purple sweet potato *(Ipomoea batatas L.)* anthocyanin as a green chili *(Capsicum annuum L.)* freshness indicator. Imami (2019) examined the use of bacterial cellulose consumables and anthocyanin from hibiscus *(Hibiscus rosa-sinensis)* as a shrimp freshness indicator in the development of smart packaging.

Anthocyanin is a flavonoid chemical that can be found in orange, purple, and blue flowers and fruits (Grotewold, 2006). Purple sweet potato *(Ipomoea batatas L.)* is a brightly colored fruit with anthocyanin

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chemicals that can be utilized as pH indicators and natural dyes. The purple sweet potato *(Ipomoea batatas L.)* has anthocyanin pigments ranging from 84 to 600 mg per 100 grams of wet weight (Seftyani, 2019).

Seftyani (2019) has researched food freshness indicators to check the freshness of white oyster mushrooms. In this study, indicator characterization tests were performed, including response time, reproducibility, and period. The standard of anthocyanin color for changes in pH and color stability of the edible film indicator against temperature changes, however, has not been carried out.

Other food products must be tested to advance the use of edible film indicators. Coconut milk is a food product that is an emulsion of coconut oil in water, like milk (Board, 2012). According to Kumparan Food (2018), coconut milk that has been opened from its packaging has a shelf life of only two days before going stale. Meanwhile, coconut milk stored in a tightly sealed bottle or container in the refrigerator can last 7 to 10 days before going stale (CNN Indonesia, 2021). As a result, coconut milk was chosen because it spoils relatively quickly, allowing for quick research.

According to the above description, this study was carried out to characterize the edible film indicator, including response time, reproducibility, and period. It is performed to test the color stability of the edible film indicator against temperature changes to determine whether there is an effect of temperature on the color stability of the indicator. The freshness of coconut milk in a closed transparent glass bottle with a volume of 100mL was then determined using an edible film indicator.

Research Methods

Tools and materials

Oven, hotplate, 500 mL, 250 mL, and 50 mL beakers, thermometer, stirring rod, dropper, spatula, analytical balance, measuring cup, volume pipette, measuring pipette, suction ball, round bottom flask, stirrer motor, rotary evaporator, vacuum pump, glass bottle, and edible film mold were all used in this study.

Materials required for this study include corn starch (Maizena); gondola type purple sweet potato *(Ipomoea batatas L.)* from Cipendeuy, Garut; 96% technical ethanol (Kimia Mart); aquadest; chitosan (ChiMultiguna); PVA (Sigma-Aldrich); 99,7% acetic acid p.a (SMARTLAB); glycerol (Kimiapedia); sorbitol (Kimiapedia); and buffer solution pH 1-14 (Nitra Kimia).

Purple sweet potato (Ipomoea batatas L.) anthocyanin production

Ipomoea batatas L. (purple sweet potato) was peeled and chopped into small pieces. These pieces are blended with 96% ethanol. The ratio of purple sweet potato *(Ipomoea batatas L.)* to ethanol is 1:4 (w/v) (Seftyani, 2019).

Anthocyanins were extracted from purple sweet potatoes *(Ipomoea batatas L.) by* macerating them in 96 percent ethanol for 7 days. Extraction is followed by evaporation at 50^oC in a vacuum condition (Laksmiani et al., 2020).

Edible film production

Wet solvent processing was used to create the edible film. The edible film was created using chitosan, 1% acetic acid, corn starch, sorbitol, glycerol, and aquadest. In addition, the solution was molded with an edible film mold (Murni et al, 2013). The edible film was 0.08-0.15 mm thick. The edible film was then cut to a 3 cm x 1 cm size to be used as a supporting matrix for the indicator.

Anthocyanin immobilization into edible film

To create a coconut milk freshness indicator, anthocyanin must be immobilized in an edible film. The duration of immobilization ranged from 10 to 120 minutes. The best immobilization time was chosen based on the value of %RSD<5% (Seftyani, 2019).

Color stability of edible film indicator to temperature

This research develops its own method for determining color stability. Temperatures of 10°C, 25°C, 35°C, and 50°C were used to test color stability. When the percent RSD is less than 5, the color is considered stable.

Characterizations of edible film indicator of fresh and stale coconut milk at pH 6,7,8, and 10

1) Response Time

Response time analysis was performed by photographing the resulting indicator color every 1 minute until it became stable. This observation was made in triplets. The RGB value was used to identify the indicator color until it was close to a constant value (Seftyani, 2019).

2) Reproducibility

The color change of the edible film indicator was photographed in triples for reproducibility analysis. The RGB value expressed by RSD less than 5 percent was used to identify the indicator color (Seftyani, 2019).

3) Period

Period analysis was carried out by photographing the color changes of the edible film indicator daily. The period was identified by the resulting RGB value having less than 15% change percentage from the initial value (Seftyani, 2019).

Application of edible film indicator on coconut milk

This research develops its own method for testing the freshness level of coconut milk. The edible film indicator was hung in a closed transparent glass bottle containing coconut milk and displayed at room temperature. The analysis was carried out by taking photographs with the camera daily. An RGB value was assigned to the indicator color. Figure 1 shows an indicator edible film indicator on coconut milk.

Figure 1. Application of Edible Film Indicator on Coconut Milk

Results and Discussion

Anthocyanin immobilization time analysis which produces color stability on edible film indicator

To determine the best immobilization time, three stages of analysis were performed: changes in the color of purple sweet potato *(Ipomoea batatas L.)* anthocyanins to a buffer solution of pH 1-14, analysis of determining the value of red/green/blue (RGB), and analysis of determining the immobilization time.

1) Changes in Purple Sweet Potato (*Ipomoea batatas L.*) Anthocyanin Color to Buffer Solution pH 1–14.

In the indicator, the anthocyanins act as a pH-sensitive dye. The maceration method was used to extract anthocyanins from purple sweet potatoes *(Ipomoea batatas L.)* with 96% ethanol. The pH value was then subjected to the anthocyanin color change test. Anthocyanins can change color in response to a specific pH value, as shown in Table 1.

Table 1. Color changes of purple sweet potato (*Ipomoea batatas L.*) anthocyanin in variation of pH values

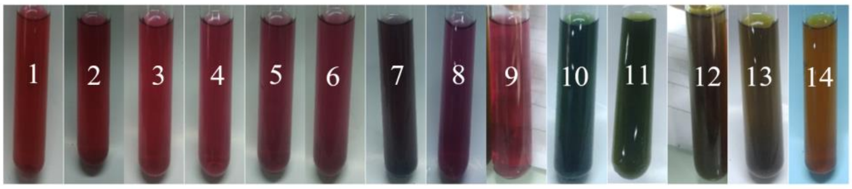
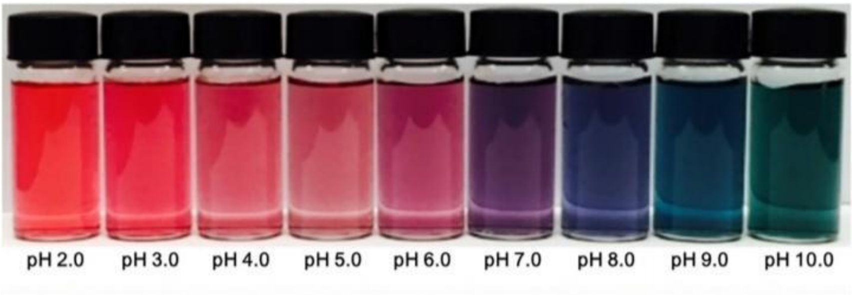
Manufacturing Anthocyanin Techniques	**Color Changes to pH**
Anthocyanins were extracted from purple sweet potato *(Ipomoea batatas L.)* by maceration using 96% ethanol. The ratio of purple sweet potato *(Ipomoea batatas L.)* to solvent is 1:4 (w/v). The evaporation process was then carried out in a vacuum at 50 °C.	 (Results)
Anthocyanins were extracted from purple sweet potato *(Ipomoea batatas L.)* by reacting it with 40% ethanol. The heat was applied at 60°C for 12 hours. The ratio of purple sweet potato *(Ipomoea batatas L.)* to solvent is 1:10 (w/v). Following that, the evaporation process was carried out under a vacuum condition at 50 °C.	 (Choi, 2017)

Table 1 shows that the color of anthocyanins changed when exposed to a buffer solution ranging from pH 1 to 14. There were color differences when compared to Choi et al. (2017) research, particularly pH 7, 8, and 9, the solvent concentration used, and extraction method.

2) Analysis of Determining the Value of Red/Green/Blue (RGB)

The value of red/green/blue (RGB) was chosen based on the coefficient of determination (R^2) being close to 1 (Whidhiasih, 2015). The coefficient of determination is calculated using the graph of the relationship between RGB values and pH. The RGB value was calculated from the color produced by anthocyanins after they were exposed to a buffer solution ranging in pH from 1 to 14. Figure 2 shows a graph created using a linear equation to determine the value of R^2.

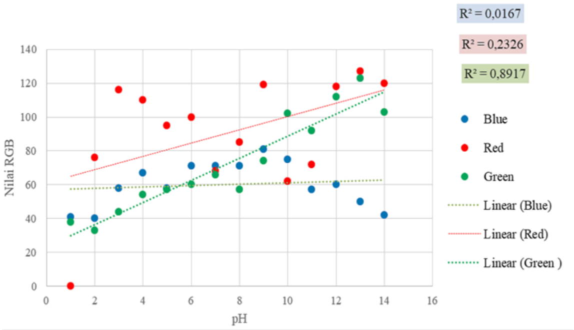


Figure 2. RGB Relationship to pH (Linear)

Green was chosen based on Figure 2 because it had a coefficient of determination R^2 = 0.8917. This means that the green had an 89.17 percent correlation with pH 1–14.

3) Immobilization Time Analysis

The percentage relative standard deviation (% RSD) was used to calculate the immobilization time. Table 2 shows the calculation of the percent RSD value for immobilization time.

Table 2. %RSD value calculation of immobilization

Immobilization Time (minutes)	Green Value			($\bar{X}$)	SD	%RSD
	Run 1	Run 2	Run 3			
10	123	123	102	116.00	12.12	10.45
20	119	98	93	103.33	13.80	13.35
30	90	82	82	84.67	4.62	5.46
40	107	99	82	96.00	12.77	13.30
50	74	99	99	90.67	14.43	15.92
60	66	107	74	82.33	21.73	26.40
70	97	99	99	98.33	1.15	1.17
80	74	90	37	67.00	27.18	40.57
90	**8**	**8**	**8**	**8.00**	**0.00**	**0.00**
100	99	90	82	90.33	8.50	9.42
110	90	57	49	65.33	21.73	33.27
120	33	90	77	66.67	29.87	44.81

Table 2 shows that the green at the 10th minute had the highest value. This occurs because anthocyanins were not well bound to the edible film by the 10th minute, so the resulting colors were not concentrated/still faded. The green value decreased over time, implying that the resulting colors became darker. However, the green rose again at the 100th minute, indicating that the anthocyanin colors in the edible film were not concentrated. This is because of the edible film's solubility on the liquid.

According to the calculations, the best immobilization time was at the 90th minute with a percent RSD value of 0%. As a result, by the 90th minute, the anthocyanin color produced on the edible film was stable at three repetitions and evenly distributed. According to Seftyani (2019), the best immobilization time was at the 90th minute with a percent RSD value of 0.016%. Yanuariski (2019) determined that the best immobilization time was at the 90th minute, with a percent RSD value of 2.382 percent. According to Imami (2019), the best immobilization time occurred at the 40th minute, with a percent RSD value of 0.42 percent.

Color stability analysis of the edible film indicator to temperature

According to Markakis (1982), anthocyanin color was stable at room temperature and low temperature, but relatively unstable at high temperature. A color stability analysis of the edible film indicator to temperature was not performed in Seftyani (2019). It is performed in this research.

The percent relative standard deviation (%RSD) was used to calculate the color stability analysis of the edible film indicator to temperature. When the percent RSD value is less than 5%, the anthocyanin color in the edible film is said to have no color change. Table 3 shows the calculation of the percent RSD value.

Table 1. %RSD value calculation of the edible film indicator color changes to temperature

Run	Green Value		($\bar{X}$)	SD	%RSD
Showcase Temperature (10ºC)					
	Early	**End**			
Run 1	66	66	66	0	0
Run 2	57	57	57	0	0
Room Temperature (25ºC)					
	Early	**End**			
Run 1	41	41	41	0	0
Run 2	57	57	57	0	0
Hot Temperature (Temperature 35°C)					
	Early	**End**			
Run 1	57	57	57	0	0
Run 2	57	57	57	0	0
Hot Temperature (50°C)					
	Early	**End**			
Run 1	57	57	57	0	0
Run 2	66	66	66	0	0

According to the calculation results in Table 3, the color of the edible film indicator did not change color at 10°C, 25°C, 35°C, and 50°C because it had a percent RSD value of 0%. Furthermore, the anthocyanin color in the edible film has not changed visually. According to the color stability analysis of the edible film indicator to temperature changes, the analysis can be carried out in conditions ranging from 10°C to 50°C to produce an accurate value in analyzing the effect of pH on color change.

Characterizations of edible film indicator of fresh and stale coconut milk at pH 6,7,8, and 10

The pH of coconut milk was measured in fresh and stale conditions before analyzing the characterizations of the edible film indicator. The pH of fresh coconut milk was measured after it had been opened aseptically from its packaging, whereas the pH of stale coconut milk was measured after it produced an unpleasant aroma or gas formation. After testing, fresh coconut milk had a pH of 6, whereas stale coconut milk had a pH of 7 or 8.

An analysis of the response time, reproducibility, and period was required to determine the characterizations of the indicator. The edible film indicator characterizations were examined at pH 6, 7, 8, and 10. pH levels of 6, 7, and 8 were chosen based on pH changes in fresh and stale coconut milk. Meanwhile, the pH 10 was chosen based on research conducted by Seftyani (2019), who discovered that edible film indicators turned green when the food being analyzed was stale/rotten.

1) Response Time

A response time analysis was performed to determine how quickly the edible film indicator can respond to changes in color to pH. The response time was calculated three times. According to Table 4, the average response times at pH 6, 7, 8, and 10 were 6.68 minutes, 9.49 minutes, 3.57 minutes, and 4.37 minutes, respectively. These edible film indicators responded faster than Seftyani (2019)'s and Yanuariski (2019)'s, which had response times of 6 and 8 minutes, respectively, but took longer than Imami (2019)'s, which had a response time of 3 minutes.

2) Reproducibility

Analysis of reproducibility was carried out to determine the accuracy of the indicator in analyzing color changes. Based on Table 5, edible film indicators had good reproducibility at pH 6 and 10 because they showed the %RSD values of 0% and 3.24%, respectively. So, the possibility of error in the measurement was 0% and 3.24%. In contrast to pH 7 and 8, the edible film indicators had poor reproducibility because they produced %RSD values of 5.46% and 5.29%, respectively.

3) Period

A period analysis was performed to determine how long the edible film indicator can be used. The period of the edible film indicator was determined by the color changes of the indicator daily. Table 6 shows the average change percentages at pH 6, 7, 8, and 10.

Table 4. Response time of edible film indicators at pH 6, 7, 8, and 10

Green Value				
Run	**pH 6**	**pH 7**	**pH 8**	**pH 10**
Run 1	109.25	55.80	22.80	63.10
Run 2	62.70	71.95	7.60	54.55
Run 3	70.30	72.35	15.20	63.10
Response Time T_{95} (Minutes)				
Run	**pH 6**	**pH 7**	**pH 8**	**pH 10**
Run 1	664	10.93	3.85	5.68
Run 2	5.87	8.74	2.95	4.85
Run 3	7.54	8.79	3.90	3.68
Average (Minutes)	6.68	9.49	3.57	4.73

Table 5. Calculation of %RSD value for reproducibility

pH	**Green Value**			**Average ($\bar{X}$)**	**SD**	**%RSD**
	Run 1	**Run 2**	**Run 3**			
6	99	99	99	99	0	0
7	82	82	90	87,33	4,62	5,46
8	82	90	90	93	5,20	5,29
10	140	140	148	142,67	4,62	3,24

3) Period

A period analysis was performed to determine how long the edible film indicator can be used. The period of the edible film indicator was determined by the color changes of the indicator daily. Table 6 shows the average change percentages at pH 6, 7, 8, and 10.

Table 6. Period calculation of edible film indicators at pH 6, 7, 8, and 10

pH	Day	Green Value			Average ($\bar{X}$)	SD	%RSD	ΔMean *Green*
		Run 1	Run 2	Run 3				
6	1	74	57	57	62,667	9,815	15,662	0,000
	2	66	66	74	68,667	4,619	6,726	9,574
	12	74	49	49	57,333	14,434	25,175	-8,511
	13	82	57	49	62,667	17,214	27,470	0,000
	14	**82**	**90**	**57**	**76,333**	**17,214**	**22,552**	**21,809**
	15	74	57	74	68,333	9,815	14,363	9,043
	16	74	57	66	65,667	8,505	12,952	4,787
7	1	57	66	66	63,000	5,196	8,248	0,000
	2	57	57	66	60,000	5,196	8,660	-4,762
	11	57	49	82	62,667	17,214	27,470	-0,529
	12	57	57	90	68,000	19,053	28,018	7,937
	13	**57**	**49**	**49**	**51,667**	**4,619**	**8,940**	**-17,989**
	14	57	66	74	65,667	8,505	12,952	4,233
	15	66	57	74	65,667	8,505	12,952	4,233
8	1	82	82	57	73,667	14,434	19,593	0,000
	2	82	74	57	71,000	12,767	17,982	-3,620
	12	74	57	66	65,667	8,505	12,952	-10,860
	13	74	90	49	71,000	20,664	29,104	-3,620
	14	**74**	**66**	**41**	**60,333**	**17,214**	**28,532**	**-18,100**
	15	74	66	57	65,667	8,505	12,952	-10,860
	16	74	66	41	60,333	17,214	28,532	-18,100
10	1	90	107	107	101,333	9,815	9,686	0,000
	2	115	107	115	112,333	4,619	4,112	10,855
	14	115	115	115	115,000	0,000	0,000	13,487
	15	115	107	123	115,000	8,000	6,957	13,487
	16	**140**	**107**	**115**	**120,667**	**17,214**	**14,266**	**16,022**
	17	140	107	115	120,667	17,214	14,266	19,079
	18	140	115	115	123,333	14,434	11,703	21,711

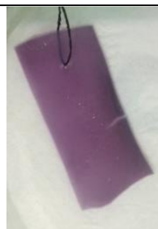
According to Table 6, the pH 6 period was 14 days, the pH 7 period was 13 days, the pH 8 period was 14 days, and the pH 10 period was 16 days. The period of the white oyster mushrooms' freshness indicator only lasted 13-14 days, which is similar to Seftyani (2019). The green chili freshness indicator only lasted 13-17 days, which is similar to Yanuariski (2019). In contrast to Imami (2019), the shrimp freshness indicator only lasted for 26-28 days.

Analysis of edible film indicator applications on coconut milk

The color changes of edible film indicators containing anthocyanins were used to test food freshness. When a change in pH in foodstuffs is detected, anthocyanin-based freshness indicators can change color. Coconut milk was chosen for this study because there has been no research on the use of edible film indicators to detect its freshness.

An edible film indicator was applied to coconut milk to determine whether it can indicate the freshness of food ingredients. When applied to coconut milk, the edible film indicator had a pH of 7, whereas the fresh coconut milk had a pH of 6. The color changes of the edible film indicators were observed during the analysis, as shown in Table 7.

Table 7. Comparison of produced colors and anthocyanin color standards

pH Value and Condition of Coconut Milk	Coconut Milk Storage Time	Color Indicator of Edible Film Hanging in A Coconut Milk Bottle	Color Standards Indicators of Edible Films Containing Anthocyanins.
4 (Stale)	11 Days		
Green Value		66	0
5 (Stale)	5 Days		
Green Value		74	33
6 (Fresh)	1 Day		
Green Value		74	8
7 (Stale)	3 Days		
Green Value		49	81

According to Table 7, when the coconut milk becomes stale, the edible film indicators inserted into the bottles change color. However, when viewed visually, the colors on the edible film indicators differed from the standard color indicators and the green value. As a result, while the edible film indicator can detect the freshness of coconut milk, it cannot indicate the pH value of coconut milk.

Conclusion

The following conclusions can be drawn from the research that has been conducted. The best immobilization time of anthocyanins into edible film producing color stability on the edible film indicator was 90 minutes with a percent RSD of 0%.

The edible film indicator was stable at a temperature from 10°C-50°C with a percent RSD of 0%.

Response times for edible film indicators at pH 6, 7, 8, and 10 were 6.68 minutes, 9.49 minutes, 3.57 minutes, and 4.37 minutes, respectively; periods for 14 days, 13 days, 14 days, and 16 days; and good reproducibility at pH 6 and 10 tests with percent RSD values of 0 percent and 3.24 percent, respectively, whereas poor reproducibility at pH 7 and 8 with percent

RSD values of 5.46 percent and 5.29 percent, respectively.

The edible film indicator can be used as an indicator of the freshness of coconut milk but cannot indicate the pH value of coconut milk.

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