THE EFFECT OF VARIATION OF IMMOBILIZATION TIME AND CONCENTRATION ROCELLA (*Hibiscus sabdariffa* L.) EXTRACT ON CHARACTERISTICS AND EVALUATION OF TIME TEMPERATURE INDICATOR

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Abstract. Anthocyanin is a type of colour pigment that has the potential as an indicator for making a Time Temperature Indicator (TTI). This is because anthocyanins are stable at low temperatures and change colour due to temperature changes (thermochromic). In this study, an analysis was carried out to determine the combination of various concentrations of roselle extract and immobilization time and to evaluate the colour stability of TTI. The research was carried out for 5 months at the Unsoed Agricultural Technology Laboratory. The combined treatment used RAK with roselle extract concentration factors of 17,5%, 20% and 22,5% and immobilization time factors of 1, 2 and 3 hours. Then the colour stability of a* TTI was observed. Variable measurements were carried out 3 times. Making TTI was using analysis of variance with a value of α 5% followed by DMRT test with α 5% if it was significantly different. While the evaluation of colour stability is analysis using linear regression. The selected TTI in this study was a combination of 22,5% concentration and 2 hours of immobilization. This treatment had a total absorption value of 1,56 g, total anthocyanin of 25,77 µg/mL, L* colour of 72.19, a* colour of 8,53, b* colour of 6.44 and ΔE of 10,98. Evaluation of TTI anthocyanin stability at 50^{o} C had the highest decrease in a* value of 2,798 and the lowest retention value at 12 hours of observation time of 91.58%.

Keywords: roselee anthocyanin, concentration, immobilization time, TTI, colour stability

1. Introduction

A common problem experienced by the food industry is the perishable nature of food products, so that the storage time for food products is limited and susceptible to quality degradation during distribution (1). The history of food products during the distribution process is difficult for consumers to know, such as the history of the product's environmental temperature. Based on (2), the quality of perishable products is difficult to maintain during distribution to consumers. Perishable food products generally have good nutritional content which can provide opportunities for microbial growth.

Packaging has an important role in protecting food products from the effects of physical and chemical contamination. However, nowadays packaging technology has developed rapidly. Smart packaging is packaging that is able to monitor the condition of the food in the package and provide information on the quality of the packaged food during distribution and storage (3).

Time Temperature Indicator (TTI) is a smart packaging innovation in the form of a label that is able to indicate changes that occur in a material in real-time based on changes in storage temperature over a certain time. TTI applications are generally for products that are perishable and require storage at low temperatures, for this reason an indicator that is stable at low temperatures is needed (4). To support color changes in TTI, it is necessary to have color pigments in the indicators that fulfill thermochromic elements, namely materials that can change color due to changes in temperature (2). Based on research by (5), roselle contains around 80-90% phenolic compounds in the form of anthocyanins.

Anthocyanins in plants are known as anthocyanidins because they are in the form of aglycones and anthocyanins are in the form of glycones as sugars that are linked glycosidically to form esters with monosaccharides (glucose, galactose, ramnose and pentose). Or by the hydrolysis process in the esterification reaction of an anthocyanidin (Aglycone) with one or more glycons (sugar groups) to form anthocyanin. Anthocyanin is a natural dye from the flavonoid group with the basic structure of 2-phenyl-benzopyrylium and the colors produced are red, purple and blue. Anthocyanins are unstable which can cause these compounds to easily undergo hydrolysis. Temperature is one of the factors that causes damage to the anthocyanin structure (6).

In this research, immobilization of anthocyanin into TTI was carried out by immersing the film in roselle extract. The immobilization process is carried out using the physical adsorption method, namely an immobilization technique that involves Van der Waals bonds or hydrogen bonds in binding reagent molecules to the supporting phase (7). Variations in immobilization time had an influence on total anthocyanin absorption. The low immobilization time means that the anthocyanin pigment cannot be completely bound, so the TTI color is not dark (8). Apart from that, in the research of (9), variations in concentration in the film matrix immobilization process have a real influence on the color of the film. The color of the film mobilized with an anthocyanin concentration of 0.3% showed a duller color compared to the film mobilized with an anthocyanin concentration of 1.2%.

Based on the background above, it is necessary to carry out research to analyze the effect of variations in immobilization time and concentration of roselle extract on the physical characteristics of TTI. Seta knows the best combination of variations and its stability over variations in temperature and observation time.

2. Methods

The research was carried out from November 2022 to May 2023. Located at the Agricultural Technology Laboratory, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto. The materials used in this research include roselle flower petals obtained from Beringharjo Yogyakarta, porang tuber flour, pectin, glycerol, HCL, KCL, Na, Acetic Acid, distilled water and a buffer solution of pH 2, 4, 8.

This research is divided into 2 parts, namely making TTI and evaluating color stability. The method used in making TTI was a Randomized Block Design (RBD), with 2 factors, namely roselle extract concentration (17.5, 20 and 22.5%) and immobilization time (1, 2 and 3 hours). Next, the best TTI was determined using the effectiveness index test in priority order ΔE: 1, L: 0.9, a: 0.8 and b: 0.7. The second part is evaluating color stability using a Randomized Block

Design (RBD) with 2 factors, namely observation temperature (30, 40 and 50° C) and observation time (3, 6, 9 and 12 hours).

2.1 TTI Manufacturing Process

The film was made based on research by (10) modified, heating 200 ml of distilled water to a temperature of 70°C for 30 minutes. After that, mix 2 grams of porang flour and 3 grams of pectin in a glass beaker and stir with a magnetic stirrer for 20 minutes at 70°C until thickened. Glycerol was added and stirred until homogeneous for 5 minutes. The next stage is to carry out the drying process on glass pallets by pouring 80 grams of mixture per pallet. Next, drying was carried out using a cabinet dryer at a temperature of 50° C for 12 hours. The next stage is the process of immobilizing the indicator film with roselle flower extract on an indicator film measuring 5.25 cm x 2 cm. The immobilization process was carried out in roselle extract solutions with concentrations of 10%, 12.5% and 15% for 1 2 and 3 hours at room temperature. Then the indicator film is aired until the container is dry and stored at freezer temperature (±10° C) so that the dye can stick and merge with the film.

2.2 Data Analysis Stage

The data obtained will be analyzed using the Analysis of Variance test (ANOVA) at a confidence level of 95% (α=0.05%). If there are significant differences in the data, then the Duncan Multiple Range Test (DMRT) will be continued with a confidence level of 95% (α=0.05%).

After that, an effectiveness index test was carried out to determine the best TTI. The effectiveness index test was carried out with the following weighting variables, ΔE: 1, L: 0.9, a: 0.8 and b: 0.7. Next, the best TTI will be evaluated regarding its color stability

2.3 Analysis of TTI Characteristics

The variables measured in this study are divided into two parts. The first part, namely making TTI, is tested for total absorption (11), total anthocyanin (12), color test (L, a, b) (3) and ΔE of the indicator film (13). Meanwhile, in evaluating color stability, color retention measurements were carried out (14).

3. Results And Discussion

The results of analysis of various concentrations of roselle extract and immobilization time on total absorption, total anthocyanin, color L*, a* and b* are presented in Table 1.

Table 1. ANOVA results for making TTI

No	Measurement	Variable		
		A	B	AXB
1	Total absorption	*	**	ns
2	total anthocyanins absorbed	ns	*	ns
3	Color L*	*	*	ns
4	Color a*	**	**	ns
5	Color b*	ns	ns	ns

Note: A= concentration of roselle extract; B= mobilization time; AxB = interaction between roselle extract concentration factors and mobilization time, ns = not significant; *= significant; **= high significance

The immobilization technique used in this research is physical adsorption which involves Van der Waals bonds or hydrogen bonds in binding reagent molecules to the film matrix (15).

In this process, the roselle extract not only adheres to the surface of the film, but also enters the pores of the film matrix. The results of measurements of the concentration of roselle extract showed that the total absorption decreased along with the high concentration of roselle extract (Table 2). According to (16), a dye concentration that is too large will result in the film matrix being insufficient to adsorb all the colors. Thus, the dye desorption process will occur in the film matrix.

Table 2. Roselle extract concentration on TTI characteristics

Concentration (%)	Total Absorption (g)	L*	a*
17.5	1,763[a]	73.16 [a]	3.58 [c]
20.0	1.671 [a]	72.76 [a]	5,68 [b]
22.5	1.405 [n]	70,54 [b]	8.43 [a]

Meanwhile, the results of measurements of the immobilization period showed that the highest total absorption occurred at an immobilization period of 2 hours, amounting to 1,893 g and the lowest total absorption at an immobilization period of 1 hour, amounting to 1,348 g (Table 3). The lowest total absorption at an immobilization time of 1 hour occurred as a result of the color pigment not being absorbed optimally during the short absorption time (8). However, the matrix adsorption to dyes has an optimum contact time. When the contact time exceeds the maximum time, the dye is released back into the solution (16). This is in accordance with the total absorption at an immobilization period of 3 hours which has a lower total absorption value than at an immobilization period of 2 hours.

Table 3. Time of immobilization on TTI characteristics

Time of immobilization (hours)	Total Absorption (g)	Total Anthocyanin (µg/mL)	L*	a*
1	1.348[c]	19.70[c]	73.47[a]	2.24[c]
2	1.893[a]	27.43[a]	70.76[b]	5.95[b]
3	1.597[b]	23.45[b]	72.24[a]	7.50[a]

Total anthocyanins showed an increase in the treatment with an immobilization period of 2 hours compared to an immobilization duration of 1 hour (Table 3). This is because the binding of anthocyanin is not high in the film with a short immobilization time (8). However, treatment with an immobilization period of 3 hours showed lower results than an immobilization period of 2 hours (Table 3). In (17), the polyphenol and flavonoid compounds present in anthocyanin extract have a role as a bridge between the polymer chains of the film matrix through intermolecular H-bonds. The formation of hydrogen bonds between anthocyanins and the matrix causes a decrease in the number of hydrophilic groups interacting with solution molecules. This indicates the release of anthocyanin pigments in the film matrix.

Based on Table 2, it is known that the L* color value decreased along with the high concentration of roselle extract. The highest L* value was found at a 17.5% roselle extract concentration of 73.16, while the lowest L* value was at a 22.5% roselle extract concentration of 73.16. This is because, at a high concentration of roselle extract, it will increase the density of the film color which reduces the brightness value (L*) on TTI (9). Based on the length of immobilization, it is known that the highest L* value is found at an immobilization time of 1 hour, amounting to 73.47, while the lowest L* value is at an immobilization time of 2 hours, amounting to 70.76 (Table 3). Based on (18), a decrease in the L* value indicates color density along with an increase in the amount of anthocyanin. However, there was an increase in the L* value monitored at the contact time which was associated with a decrease in the adosrptivity of the film matrix.

The a* color value shows an increase along with the high concentration of roselle extract (Table 2). The a* color value shows the lowest value at 17.5% roselle extract concentration of 3.58, while the highest value is found at 22.5% roselle extract concentration of 8.43. This is because roselle extract contains the natural color pigment anthocyanin which gives a red color or a* color value (19). Research by (9) explained that samples with higher adsorption concentrations will produce higher a* color values than samples adsorbed at low concentrations.

The immobilization time factor also shows an increase in the a* color value along with increasing immobilization time (Table 3). The lowest color a* value was found at an immobilization period of 1 hour at 4.24, while the highest color a* value was at an immobilization period of 3 hours at 7.5. The increase in a* value on TTI is not in line with the total anthocyanin absorbed at an immobilization period of 3 hours which should have a lower a* value compared to an immobilization period of 2 hours. This can occur due to the absorption of other compounds which can increase the a* value on TTI. Based on (20), tannin is a group that can bring brown color or can increase the a* value. Research by (21) stated that the tannin content in roselle flower petals was 13.73%. Thus, the tannin content is high enough to increase the a* value on TTI.

The color b* shows yellow if the value is positive and blue if the value is negative. Color b* does not show any real effect because the anthocyanin in roselle extract has a dominant red color group. Based on (9), the color values of L* and a* change in line with the amount of RCA (Red Cabbage Anthocyanin) absorption, while the color of b* experiences fluctuations.

Based on the effectiveness index test, TTI with the best treatment was obtained, namely A3B2, namely TTI with a roselle extract concentration of 15% and an immobilization time of 2 hours. This treatment combination has the largest weight value compared to other treatments, namely 0.639. Treatment A3B2 had the highest change value of 10.98, L value of 72.19, color a of 8.53 and color b of 6.44. (17) explained that, when applying TTI, color changes in indicators play a role in indicating the influence of environmental temperature on the freshness of food ingredients.

Figure 1 shows that at an observation temperature of 30^{o} C, it shows an increase in the a* value of 6.425 at an increase of one observation unit. Based on (22), the color response of TTI stored at room temperature did not show a decrease in chromatic value and the color response showed a decrease after storage for 36 hours. Research by (23) showed that there was no significant decrease in anthocyanin levels at a heating temperature of 30^{o} C. Apart from that, there is a possibility of an increase because the water content in TTI with an observation temperature of 30^{o} C has not evaporated completely even though an adjustment process was carried out before the observation, namely drying at a temperature of 50^{o} C for 30 minutes.

Meanwhile, at observation temperatures of 40^{o} and 50^{o} C, it shows a decrease in the a* value. At a temperature of 40^{o} C the decrease was 1.055 for every increase of one unit of observation time, while at a temperature of 50^{o} C there was a decrease of 2.798 for every increase of one unit of observation time. Based on the results above, it can be seen that the decrease in the a* color value occurs more rapidly at a temperature of 50^{o} C compared to a temperature of 40^{o} C. This decrease is caused by the degradation of anthocyanin compounds due to temperature treatment. Degradation of anthocyanin color occurs due to hydrolysis of the anthocyanin glycosidic bonds which creates an open aglycone ring structure. Thus forming various labile aglycones as well as colorless carbonyl and chalcone groups (Priska et al., 2018). The decrease in a* color values is in accordance with the research of Halász et al. (2023), a striking color change occurs starting from a storage temperature of 40^{o} C. Thus, TTI roselle extract has an effective temperature for application, namely at an environmental temperature above 40^{o} C.

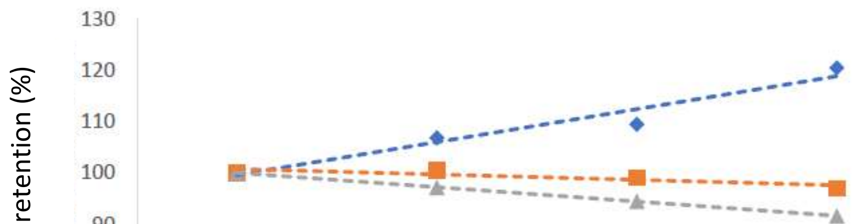

Figure 1. The effect of temperature and observation time on the color stability of TTI anthocyanins

4. Conclusion

The results of this study showed that the TTI of roselle extract showed the highest total absorption at a concentration of 17.5% of 1.763 g. Meanwhile, the immobilization time of 2 hours had the highest total absorption effect of 1.893, and the highest total anthocyanin absorption was 27.43. The TTI chosen in this study was a combination of a concentration of 22.5% and an immobilization period of 2 hours. This treatment had a total absorption value of 1.56 g, total anthocyanin of 25.77 µg/mL, color L* of 72.19, color a* of 08.53, color b* of 6.44 and ΔE of 10. 98. Evaluation of the stability of TTI anthocyanin at a temperature of 50°C had the highest reduction in a* value of 2.798 and the lowest retention value at 12 hours of observation time was 91.58%.

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