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A paper-based Colorimetric Indicator Label using Natural Dye for Monitoring Shrimp Spoilage

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Abstract. Shrimp is a type of perishable food. This study developed a simple indicator label using colorimetric method for monitoring shrimp freshness. This indicator label was made from natural dye extract of *Ruellia simplex* flowers which immobilized on cellulose paper by dip coating method. The indicator labels were used for examining freshness of shrimp. In this experiment, shrimp were stored in sealed bottles that have been labeled using the indicator and stored at 13 °C, 25 °C, and 40 °C for a certain range of time. Color changes of the indicator labels were observed using digital photography after shrimp storage for 0 h, 2 h, 17 h and 24 h. The color changes that occur were quantified and analyzed using the ImageJ program. The color of the indicator label when detecting the fresh shrimp was pink and after the shrimp spoilage began, the color of the label changed to purple and then became yellow when the shrimp is badly spoilage. The color change rates of label indicator increases as the shrimp storage temperature increased. These results indicate that this label indicator can be used as an indicator of the freshness of shrimps and it is not toxic and safe for food.

Keyword. Colorimetric, dip coating, *Ruellia simplex* and shrimp spoilage.

1. Introduction

Shrimp freshness is the most basic indication in determining the quality of the shrimp, whether it can be consumed or not. Therefore, various methods have been developed by researchers to be able to determine the quality of shrimp. Methods to determine shrimp freshness include sensory methods, determination of microbial levels in shrimp, and determination of levels of Total Viable Count (TVC) or Total Volatile Basic-Nitrogen (TVB-N) levels [1]. Determination of quality of shrimp with the sensory method is the simplest way but become an obstacle when shrimp covered in a plastic package. Determining the amount of contamination through microbiological tests, such as measurement of TVC levels and TVB-N levels, takes a long time and should be done in the laboratory [2]. Therefore, it is necessary to develop a method that is simple, practical, and can determine the freshness of shrimp directly and integrated into shrimp packaging. Recently, a method to detect the freshness of perishable food using colorimetric indicator label has been developed. Research on this indicator has been done by other researchers by developing dye-colored labels, either using synthetic or natural dyes [3]. Research on this label by using environmentally friendly materials is very interesting to be developed further. Utilization of natural dyes as an indicator of the freshness of food such as the utilization of red cabbage extract [4], spinach [5], turmeric [1], and black rice [6] have been studied. Natural colorants that can be used for the indicator should have enough stability but can also be affected by factors such as acid-base to change its color, one of the famous natural dyes is anthocyanin [7].

The aim of this research is to develop an indicator label by using *Ruellia simplex* flower extract which is found in Indonesia as an ornamental plant. As the matrix of this dye extract is cellulose paper, all materials used in making this label are environmentally friendly. The indicator label is then tested to detect the freshness of the shrimp. The working principle of the label in the detection of the freshness of food used in this experiment is the colorimetric method.

2. Materials and methods

2.1. Materials
The chemical materials used in this experiment were Ethanol absolute purchased from JT Baker and Sodium Hydroxide and Chloride Acid which are both purchased from Merck with purity 99.99 %.

2.2. Method

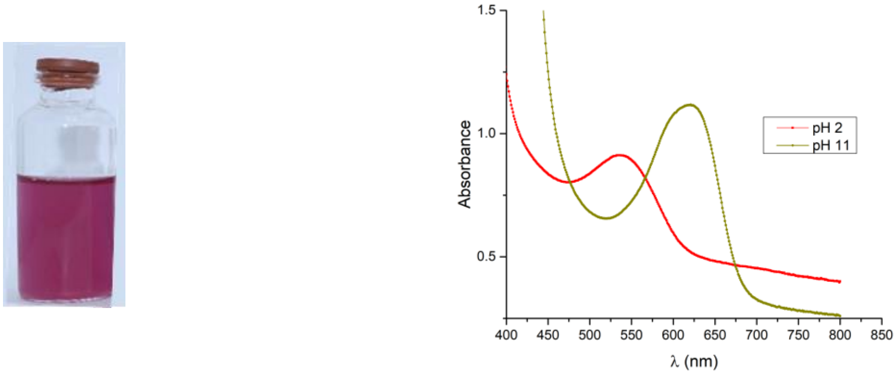
2.2.1. Preparation of Anthocyanin Extract from Ruellia Simplex flowers
The *Ruellia simplex* flower used in this experiment were collected from the plant and then washed with clean water and then drained. Flowers weighed as much as 80 g then put into a blender. After that the solution of ethanol/aquadest (7:3) as much as 100 mL mixed into the blender and then the flowers were chopped together. The solution mixture was then dropped with a solution of hydrochloric acid (HCl) until the pH of the solution becomes 2. The solution mixture then stored in a dark bottle for 24 h at 5 °C. After 24 h, the solution mixture was filtered using Whatman no. 41 paper. The resulting supernatant was stored in a dark bottle and used as a natural dye extract from the *Ruellia Simplex* flower. Part of the solution of the *Ruellia simplex* flower extract was further investigated its absorbance spectra in the UV-visible region using a UV-Vis Genesys 10S Thermo Scientific spectrophotometer.

2.2.2. Indicator Label Preparation
The prepared indicator labels were composed of the *Ruellia simplex* dye extract and the matrix made of cellulose-based paper. The cellulose paper used in this research was Whatman no 1 cat.1001. The indicator label was made by cutting the cellulose paper with the size of 10 cm × 1 cm, then dipped into the solution of flower extract of *Ruellia simplex* with pH 2 for 30 s. The indicator label then dried at room temperature and ready for use.

2.2.3. Shrimp freshness detection test
In this study, the shrimp species used for freshness testing were fresh *Fenneropenaeus merguiensis* obtained from the local market. The shrimp after purchased directly transported to the laboratory and immediately prepared for the observation. For the preparation of the test, the shrimp weighed as much as 10 g and then put into a transparent glass bottle. The indicator label was hung on the bottle cap containing the shrimp. Label indicator was placed not in direct contact with the shrimp. The distance between the label and the shrimp was approximately 5 cm. The bottle was then tightly closed. Similar samples were prepared like this and then the bottles were tested for observation in various conditions of shrimp storage temperature 13 ºC (in refrigerator) temperature, 25 ºC (room temperature), and temperature of 40 ºC (in the oven). Color changes that occur on the indicator label due to the decay process experienced by the shrimp were observed after a storage time of 2 h, 17 h, and 24 h at each storage temperature condition. The color change of the label indicator was photographed using the Canon EOS 750D camera and for subsequent color changes was analyzed using the ImageJ program.

3. Results and discussion

The *Ruellia simplex* flower extract produced in this study has a red color at pH 2 as shown in Figure 1.a. The UV-visible spectrum of this extract is shown in Figure 1.b. The red color of this extract shows that under conditions of pH 2 the dominant compound present in anthocyanin is in the form of stable flavylium cation [8]. In the visible spectrum, the *Ruellia simplex* flower extract has an absorbance peak at a wavelength of about 535 nm. This wavelength value is similar to the wavelength value when the maximum absorbance is found in the *Bauhinia blakeana dunn* flower extract [9] or also grape skin extract [10]. The UV-visible spectrum for the *Ruellia simplex* flower extract on base condition (pH value of 11) is also measured (Figure 1.b). This characterization is used as a preliminary detection to determine the color change of the extract. The color change of the extract from the acidic pH 2 condition to the base condition is likely to occur when the anthocyanin detects a rotten shrimp. When the shrimp decay, the condition is base. In the base condition, the color of the extract is yellow and the wavelength at the maximum absorbance value shifts to the right to about 625 nm.



(a) (b)

Figure 1. The color of the Ruellia simplex flower extract conditioned on the pH value of 2 (a), the UV-visible spectrum of the *Ruellia Simplex* flower extract in buffer pH value 2 and pH value 11 (b).

0 hour 2 hours 17 hours 24 hours

Figure 2. Indicator label's color changes upon detecting gas emitted by the shrimp when spoilage process occurs. Measurement is performed at 25 ºC.

The indicator label made of cellulose paper that has been dyed into the *Ruellia simplex* flower extract produces a purplish pink color. This color has not changed significantly and is still similar to the color of the extract solution. This is also found in the Zhai *et al* 2017 study that uses anthocyanins from roselle flower extract using starch / polyvinyl alcohol matrix, the color of the early label is purplish pink, in accordance with the color of roselle flower extract at pH 2 [11].

Testing the indicator label to detect shrimp freshness is done by hanging the label inside the shrimp filled bottle, as shown in Figure 2. The bottle containing the indicator and shrimp label is then stored in three different storage temperature conditions, at 13 ºC storage temperature, room temperature at 25 ºC, and extreme heat conditions with a temperature of 40 ºC . Observations of color changes that occur on the label indicator performed simultaneously with an organoleptic test of shrimp. The grading for the shrimp organoleptic conditions is based on the SNI 01-2728.1-2006 standard on fresh shrimp part 1-specification. The results of an organoleptic test of the shrimp are shown in Table 1.

Based on the standards used, shrimp come into the fresh category and can be consumed if it has a value of 7 to 9 [12]. Thus, based on organoleptic test results, storage at 13 ºC for up to 24 h indicates that shrimp can still be consumed even though the quality has decreased. While at 25 ºC, after being stored at 17 h or more, the shrimp is not worth consuming. At a temperature of 40 ºC when the shrimp has been stored for 17 h or more, the shrimp should not be consumed or have been very rotten.

The color map of the indicator label for the observation of the color change in the three storage temperature conditions and the effect of the storage time given in Figure 3.

Table 1. The results of shrimp organoleptic test at 13 ºC, 25 ºC and 40 ºC storage temperature as a function of storage time.

Storage temperature	Results of organoleptic test (score) 0 h	2 h	17 h	24 h
13 ºC	The shape is unchanged, the color of the flesh is clear luminescent, smells very fresh, very elastic texture, compact and solid (9)	The color of the flesh is less clear, fresh smell, elastic texture (8)	The clear color of the flesh is somewhat lost, specific odor, less elastic texture (7)	
24 ºC			The color of the flesh is rather pink, arising ammoniac smell, not elastic texture (5)	The color of the flesh becomes red, smell of strong ammonia, soft texture (3)
40 ºC		The clear color of the flesh is somewhat lost, specific odor, less elastic texture (7)	The flesh become very dull red color, dark spots, strong ammonia smell and foul odor, soft and watery texture (1)	

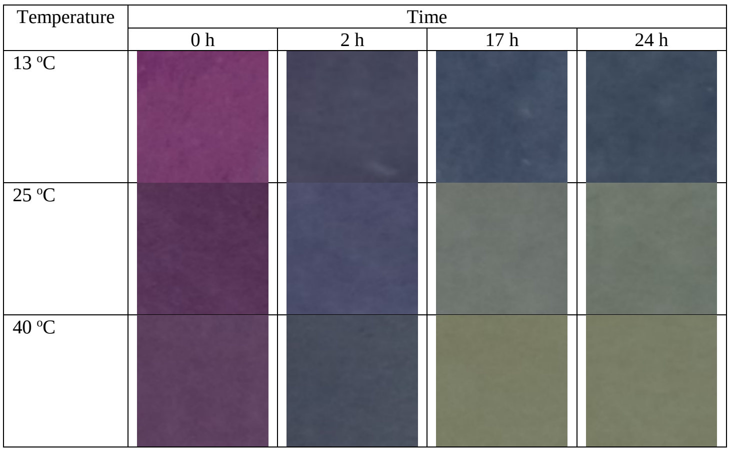


Figure 3. Map of color change of the indicator labels on shrimp storage temperature of 13 ºC, 25 ºC and 40 ºC as a function of time.

After 2 h of storage time, the indicator label for all observed storage temperature conditions changes its color from purplish pink to purplish blue. At a storage temperature of 13 ºC, the color of the indicator label with increasing storage time up to 24 h has not undergone significant changes in

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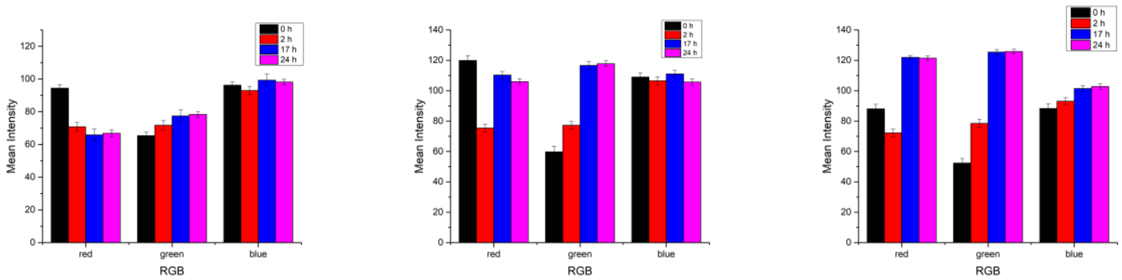
purplish blue color. On the other hand, the color of the indicator label when measuring shrimp freshness at storage temperatures of 25 ºC and 40 ºC for storage time up to 17 h, changes further from purplish-blue to greenish gray. The more prolonged storage time of up to 24 h in both conditions of this temperature causes the color of the label to change further into a yellowish gray.

Based on the observations for the color change of the indicator label shown on the color map in Figure 3 and the organoleptic observation results of the shrimp shown in Table 1 it can be concluded that under conditions of storage temperature of 25 ºC and 40 ºC for 17 h storage time has made the shrimp become rotten and should not be consumed. The color change of this indicator label occurs due to ammonia released during the decomposition process of the shrimp [2]. Ammonia vapor will shift the equilibrium of the anthocyanin form contained in the dye extract in the indicator label. The shift in anthocyanin equilibrium when affected by ammonia corresponds to a shift of the cation of flavylium having a red color to a quinoidal base having a blue color. So the color of the indicator label that originally purplish pink color change to purplish blue. With increasing time, the ammonia produced from the decomposition process will increase steadily so that the anthocyanin that has changed to the quinoidal base will continue to change to a calcon form that has a yellow color [8]. The phenomenon of shifting from this form of anthocyanin is observed when the shrimp become very rotten, the color of the indicator label becomes yellowish gray. The change to yellowish color is also found when the Ruellia simplex flower extract is mixed in the buffer base solution with a pH value of 11 as shown in Figure 1.b. The results of this experiment indicate that the color change of the indicator label due to the changing environmental conditions that became the base for this case due to the ammonia released from the process of shrimp decomposition.



(a) (b) (c)

Figure 4. The RGB histogram of the indicator label as a function of shrimp storage time at the temperature of (a) 13 ºC, (b) 25 ºC, and (c) 40 ºC

The color of the indicator label is further analyzed to quantify the color change using the ImageJ program. The results of the analysis are histograms of RGB as shown in Figure 4. RGB values at various storage temperatures show different trends for changes in the value of each color component. A more regular pattern of changes in color values is indicated by the green color component. At a storage temperature of 13 ºC the value of the green color component becomes larger as the length of storage time increases. The trend of increasing values of the green color component is also found for storage temperatures of 25 ºC and 40 ºC. Based on Figure 4, the green color component can be used to indicate a marker of increased shrimp decomposition process. Figure 5 shows the relationship between the intensity of the green color component as a function of storage time for various storage temperatures.

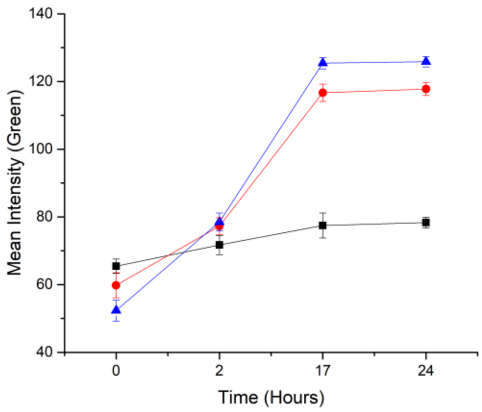


Figure 5. Intensity of green color components as a function of time for various storage temperature (—■— for 13 ºC , —●— for 25 ºC, and —▲— for 40 ºC)

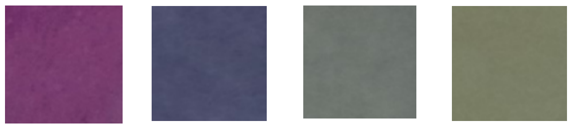


Figure 6. Recommended color for label indicator used to detect shrimp freshness

The intensity of green color components at storage temperatures of 13 °C did not increase significantly compared to the storage temperatures of 25 °C and 40 °C. Storage conditions at cold temperatures can slow down the shrimp process so that the ammonia released by the shrimps is small as a result the label of the indicator does not change color dramatically. When the storage temperature is high, the shrimp become more rapidly decomposed so that the amount of ammonia vapor released increases and the production is faster. This phenomenon causes the labels to change color significantly. The storage time also has an effect on the speed of the indicator color change. The longer of shrimp storage time, the shrimp freshness condition decreased causing the amount of ammonia released increased and caused the label to change color.

Based on the results of the observations described earlier the recommended indicator label is applied to the consumer along with an explanation of the color change can be seen in Figure 6. In Figure 6 for the real application can be given four types of color indicators to monitor the shrimp freshness condition: when the shrimp is in fresh condition, the shrimp can still be consumed even though the quality has decreased, the shrimp is not proper to be consumed, and the shrimp is very rotten. This simple indicator label that simply looks at the color change when it detects the shrimp and compares the color with the given color explanation can help the consumer to quickly identify the freshness quality of the shrimp, whether the shrimp is still fresh or rotten. A similar color change phenomenon was observed in a study of Zhang *et al* 2014 using colorimetric labels from Bauhinia and chitosan flowers extracts to observe the freshness of the fish. Colors of colorimetric labels changed from pink to brown after contact with fish for 12 h. Then change from brown to green after 16 h at 25 ºC. The label color turned green after the fish sample was stored over 20 h [9].

4. Conclusions

A simple method of detecting shrimp freshness has been developed using indicator labels using colored cellulosic paper with *Ruellia simplex* flower extract. The color change of the indicator label from the purplish pink color to the purplish blue then turns further into greenish gray and becomes yellowish gray can be used to express the quality of the shrimp from the fresh to the already rotten sequentially. Quantitatively the quality of shrimp freshness can also be expressed by measuring changes in the intensity of the green color component of the RGB analysis of the indicator label color. Practically this indicator label can be further developed by determining four color indicators that are given color information describing the condition of the shrimp.

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