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Development of Smart Packaging Halochromic Film based on Jali Seed Flour (*Coix lacryma-jobi*) and Kencana Ungu Flower Extract (*Ruellia simplex*)

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Abstract

The spoilage of marine products such as fresh shrimp is characterized by an increase in pH as a result of protein degradation which produces biogenic amines which are harmful if consumed. Simple early detection of damage can be done using smart packaging halochromic film. The purpose of this research is the synthesis, characterization and ability testing of smart packaging halochromic film is based on jali seed flour (*Coix lacryma-jobi*) embedded with anthocyanin pigments of Ruellia simplex flower extract (EBRS) as a natural indicator. There are two stages in the research method, the first stage includes the extraction and characterization of anthocyanins from Ruellia simplex flowers. The second stage is the synthesis, characterization and testing of the halochromic film capability of jali seed flour-EBRS film. Measuring Total Anthocyanin Content content in Ruellia simplex flowers extract uses the differential pH methods the anthocyanin is 1.037 mg/L. Sensitivity Test of Ruellia simplex Flower Extract to pH indicate contains pigments and is sensitive to changes in pH from 1-12, and gradual discoloration from magenta to purple, colorless, blue, green, and yellow. Smart packaging halochromic film were made using varying EBRS concentrations of 6%, 8%, and 10% (w/w), The ability of the EBRS halochromic film to change pH from 1 to 12 was obtained by the 8% EBRS halochromic film being the best in inform of discoloration. Characterization of 8% (w/w) EBRS halochromic film compared with standard film showed that the addition of EBRS reduced tensile strength values, increased solubility properties, swelling but did not affect the thickness and water rate transmission properties. The EBRS 8% (w/w) indicator film test showed that the indicator film was able to communicate early detection of shrimp damage

Keywords: *Anthocyanin, halochromic film, natural indicator, Ruellia simplex, Coix lacryma-jobi*

1 Introduction

Early detection of shrimp damage is important because consuming rotten shrimp is prone to causing foodborne illness due to the accumulation of excess biogenic amines in shrimp [1]. In an effort to maintain food safety, research has recently been carried out for early detection of food spoilage, namely using food spoilage indicators [2].

Food spoilage indicators or indicator film are able to detect a decrease in food quality based on the physicochemical quality index pH value. The pattern of pH changes is caused by degradation of proteins and peptides by proteolytic enzymes and bacteria. The results of protein degradation are trimethylamine (TMA), dimethylamine (DMA), and ammonia (NH_3) which are alkaline and volatile, causing an increase in the pH of the shrimp from pH 6-8. The significant change in pH value indicates a decline in shrimp freshness, progressing toward the stage of spoilage [3].

Smart packaging is the latest food packaging technology that can monitor changes and provide information about the quality and freshness of packaged food products [4]. Innovative packaging application based on pH-sensitive natural dyes is also call halochromic film and is currently being developed. These smart systems quickly inform the food quality to the consumer and monitor fresh foods in real-time. Smart packaging protects food against ambiance risks and simultaneously sends

information to users for variations and alterations in the packaging settings [4,5].

Indicator films or halochromic films currently being developed consist of two main components, namely biopolymers and natural pigments. This material can detect pH changes that are specific depending on the nature of the natural pigment compound used [6,7]. Indicator film as food spoilage indicators can be made in film form, based on the properties of biopolymers as hydrocolloids that can be printed in sheet form. [8].

The most widely researched biopolymer raw material for film is starch, because capable of forming excellent films [9]. Starch is a biopolymer that is often used as raw material for packaging films because it has the advantages of being abundant in nature, cheap, safe and edible [7,10]. Starch is used as a base material for biopolymer films for smart food packaging [7,11].

The natural plant pigments most widely used as indicator films are anthocyanin compounds. Anthocyanins are able to change color in a wide pH range, are safe, have high abundance and can act as antimicrobial and antioxidant agents [12,13]. Several studies have used anthocyanins as the main compounds that function as halochromic indicators, with various anthocyanin sources such as berry anthocyanins [8], *Jacaranda cuspidifolia* anthocyanins [14], red cabbage [15]. The high anthocyanin content found in *Ruellia tuberosa l* flowers is 253.6 ± 58.9 mg/L [16]. Anthocyanins from *Ruellia simplex* flower have not been used as indicator film materials. Previous studies used *Ruellia simplex* flower anthocyanins as acid-base indicators [17].

Jali seeds (*Coix lacryma-jobi*) are a source of starch that is not used as a staple food, contains more than 60% starch-type polysaccharides [18,19]. Edible films made from jali seed flour produce good physical film properties [20,21]. So far, jali seeds have been widely cultivated in Asia for livestock and poultry feed purposes, but the use of jali seed flour as raw material for indicator films has not yet been implemented.

Previous research has only developed packaging films from jali seed flour and no one has developed a film combined with a pH indicator to monitor fish spoilage [20,21]. Adlay (*Coix lacryma-jobi* L.) has considerable potential as a raw material for indicator films because of its high starch content and favorable physicochemical properties. The structural characteristics of adlay starch support its application as a film-forming biopolymer, while starch-based materials have been widely recognized as sustainable and biodegradable alternatives for food packaging applications [22].

This study aims to reveal the characteristics and performance of Smart packaging halochromic film were made using jali seed fluor and *Ruellia simplex* flower extract.

2 Method

2.1 Extraction and Characterization of Ruellia simplex Flower Extract (EBRS)

The extraction of *Ruellia simplex* flowers refers to the research of [23] with modifications. Extraction was carried out by maceration of 58.66 g of *Ruellia simplex* flowers in 700 mL of 70% ethanol solvent and HCl (1:10). The residue is separated from the filtrate by simple filtration. Finally, the extract was concentrated by evaporating at a temperature of 60°C until the volume reached 70 mL. The results were stored in a dark container and in a refrigerator at a temperature of 4˚C.

2.2 Determination of Anthocyanin Content/Total Anthocyanin Content (TAC)

The total anthocyanin content (TAC) of the Ruellia simplex flower extract (EBRS) was determined using the differential pH method. Briefly, 0.05 mL of the concentrated extract was diluted to 5 mL using potassium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5). The absorbance of each solution was measured at 520 and 700 nm using a UV–Vis spectrophotometer. The total anthocyanin content was expressed as cyanidin-3-glucoside equivalents (C3G) and calculated using **Eq. 1** [24].

$$\text{TAC (mg/L)} = \frac{A \; x \; MW \; x \; DF \; x \; 1000}{\varepsilon \; xl} \qquad \text{(Equation 1)}$$

with

A (Absorbance) = $(A_{520}–A_{700})pH_1–(A_{510}–A_{700})pH_{4.5}$

MW: molecular weight of cyanidin-3-glucoside= 449.29 g/mol;

DF: dilution factor;

ε is the extinction coefficient of cyanidin- 3-glucoside = 26900 L/ (mol.cm)

l is the path length = 1 cm [24,25].

2.3 Color Characteristics of EBRS Extract at Various pH

Anthocyanin extract (0.05 mL) was added to various buffer solutions with pH values ranging from 1 to 12 with an extract to buffer ratio of 2:8 (v/v). Furthermore, *Ruellia simplex* flower extract solutions at various pH were measured using a UV-Vis spectrophotometer (Hitachi, Tokyo, Japan) at a wavelength of 300 to 750 nm [26].

2.4 Extract Color Changes at pH Variations

Anthocyanin extract (0.05 mL) was added to a buffer solution with a pH variation of 1-12 with an extract solution:buffer solution ratio of 2:8 (v/v). Next, the *Ruellia simplex* flower extract solution with various pH was measured using a UV-Vis spectrophotometer (Hitachi, Tokyo, Japan) in the range of 300-750 nm [26].

2.5 Preparation and Optimization of Indicator Film

Indicator film Making refers to research by [20,21]. Jali seed flour, 10 grams, was dissolved in 100 ml distilled water and heated to the gelatinization temperature (54ºC). Then the glycerol solution was added with a concentration of 20% (v/w jali seed flour) and heated at 70ºC for 30 minutes with stirring using a magnetic stirrer. The mixture was cooled to 25ºC, then *Ruellia simplex* flower extract (EBRS) was added to the solution at 6%, 8% and 10% (w/w jali film solution). Then 8 mL of the film forming solution was molded into a plastic cup of the same size (90 mm × 15 mm), and dried at room temperature for 2 days. The film was stored at room temperature for 2 days before use.

2.6 Indicator Film Sensitivity Test to pH

The sensitivity of the film indicator to pH variations was measured by dripping 5 drops of pH 1-12 buffer solution for 1 minute on a film sample (0.5 cm × 1.5 cm). Then, the color change of the film sample was documented with a smartphone camera. The proper use of buffers is based on the presence of ionic strengt, wich in general higher ionic strength can accelerate anthocyanin degradation and decrease color intensity. Citrate-phosphate buffer (pH 1.0 to 7.0), phosphate buffer (pH 8.0), and glycine-NaOH buffer (pH 9.0 to 13.0) were prepared by protocol given by Wu et al.2022. The ionic strength data of citrate-phosphate, phosphate and glycine-NaOH buffers are 0.10 M-0.50 M, 0.15 M-0.2 M and 0.018 M to 0.05 M respectively. The ionic strength of the buffer used has a low value because it is less than 1 M, where the ionic activity is lower than the compound activity or anthocyanin concentration.

2.7 Effectiveness of Film in Detecting Shrimp Spoilage

The shrimp were placed in a petri dish, and EBRS 6%, 8%, 10% indicator film (size 1.5x1.5 cm) was attached to the lid of the petri dish [25]. Shrimp quality monitoring is carried out by measuring the pH of the shrimp periodically.

2.8 Characterization of Film Physical Properties Film Thickness

Film thickness was measured using a screw micrometer with a range of 0 – 25 mm. The test was carried out at five points on the film surface and then the average value was taken as the result. Measurements were carried out three times.

2.9 Film Solubility

The film was cut to a size of 1.5×1.5 cm then dried in an oven at 105°C for 2 hours then weighed to obtain the initial film weight (m_1). After that, the film was dissolved in 40 mL of distilled water at room temperature (25°C) for 24 hours. The insoluble film pieces were separated and dried again using an oven at 105°C for 2 hours and weighed again to obtain the final film weight (m_2). The test was carried out three times. The solubility of the film in water was calculated using **Eq. 2**.

$$\text{Film solubility (\%)} = \frac{m1-m2}{m1} \text{ x } 100 \quad \text{(Equation 2)}$$

2.10 Water Vapor Transmission Rate (WVTR)

The water vapor transmission rate (WVTR) of the film samples was measured according to the ASTM E-96 method. The test film sample was placed on a vial containing 10 g of distilled water. The plate is weighed every 1 hour for 7 hours and the WWTR of the film is calculated using **Eq. 3**.

$$\text{WVTR } (g/m^2\ jam) = \frac{w}{\text{A} \times \Delta\text{t}} \quad \text{(Equation 3)}$$

Where w is the weight (g) of the vial + film, Δt is the time interval (hours), A is the area of the film (m^2) covering the cup (27). WVTR value testing was carried out three times.

2.11 Swelling Films

The swelling level was carried out based on a modification of the method reported by [28]. The film was cut into a square shape (1.5 x 1.5 cm) and weighed as m_1. Then the film was soaked in 15 mL of distilled water at 25 °C for 10 minutes and weighed as m_2. The test was carried out three times. The swelling level is calculated based on **Eq. 4**.

$$Swelling\ \text{film}\ (\%) = \frac{m2-m1}{m1} \times 100 \qquad \text{(Equation 4)}$$

2.12 Mechanical Properties of Film

The mechanical properties of the film analyzed in this research include tensile strength (TS) and elongation at break (EAB). Tensile strength and elongation at break tests were carried out using an Instron 5985 Universal Testing Machine (UTM) following the ASTM D882 standard method. The film was cut (6 cm × 10 cm) and placed between the handles. The initial gauge length was set to 26.8 mm and the crosshead speed was set at 2mm/min. The initial length, force and extension distance were recorded during film extension until break.

2.13 FTIR Test

The functional groups of jali seed flour, control jali film, optimum EBRS indicator film and EBRS were identified using Fourier Transform Infrared Spectroscopy (FTIR), and its spectrum was analyzed in the range 4000-400 cm^{-1}.

3 Result and Discussion

3.1 Characteristics of Jali Seed Flour

The characteristics of jali seed flour as a starch source were observed based on the FTIR spectrum compared to standard jali seed starch (29). FTIR data are shown in **Fig. 1**. The FTIR spectrum of the jali seed flour (a) is similar to the FTIR spectrum of the jali seed starch extract (b). The FTIR spectrum at wavenumbers 3550-3200 cm^{-1} shows the OH^- (hydroxyl) group, the C-H group is shown at wavenumbers 3000-2840 cm^{-1}, the C=O (carbonyl) group is shown at wavenumbers 1630-1750 cm^{-1}. This is in accordance with the starch structure which has carbonyl and hydroxyl groups. Meanwhile, the wavenumbers 1045 and 1022 cm^{-1} are related to the crystallinity and amorphous regions in the starch [29]. These results confirm that starch dominates the content of jali seed flour [19].

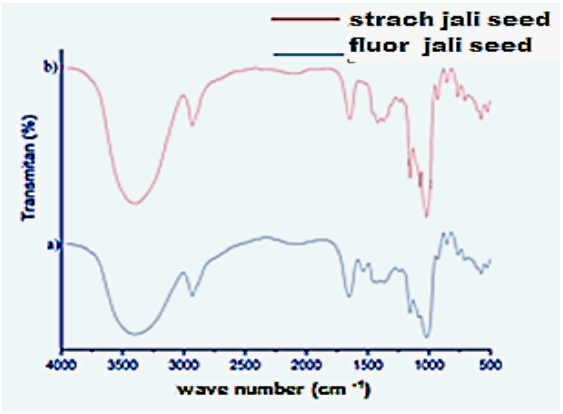


Figure 1. FTIR Spectrum of strach and fluor jali seed

3.2 Characteristics of Ruellia simplex Flower Extract (EBRS)

Extraction was carried out to obtain anthocyanins found in Ruellia simplex flower petals. During the extraction process, 0.1 N HCl was added to achieve a pH of 2, which maintains the stability of the anthocyanins in the form of flavylium cations [30].

Ruellia simplex flower maceration was carried out in a closed container for 4x24 hours at 4°C to prevent anthocyanin degradation. This is consistent with research by [31], which found that anthocyanin degradation was very low under acidic and dark storage conditions. Extraction of anthocyanins from 58.66 g of Ruellia simplex flowers in 700 mL of 70% ethanol + HCl resulted in 77 mL of a concentrated red liquid.

3.3 Anthocyanin Content/Total Anthocyanin Content (TAC) of Ruellia simplex Flower Extract (EBRS)

Anthocyanin levels were measured using the differential pH method based on the principle that anthocyanin structure changes in solutions with different pH values, resulting in differences in absorbance between the two solutions [31]. The extract solution at pH 1 was red, with a maximum absorbance of 0.731 at 520 nm and 0.047 at 700 nm. The extract solution at pH 4.5 was colorless, with a maximum absorbance of 0.154 at 520 nm and 0.091 at 700 nm. This occurs because at pH 1, anthocyanins exist in the colored oxonium or flavylium forms, while at pH 4.5, most anthocyanins exist in the colorless pseudobasic carbinol form [31]. Based on the calculation

results, the anthocyanin content in Ruellia simplex flowers is 1.037 mg/L.

3.4 Ruellia simplex Flower Extract Sensitivity Test (EBRS) to pH

Ruellia simplex flower extract contains anthocyanins that are sensitive to changes in pH. The color changes gradually from magenta to purple, colorless, blue, green, and yellow from pH 1 to 12 (**Fig. 2**).



Figure 2. discoloration of *Ruellia simplex* Flower Extract to pH changes

Discoloration of anthocyanin can be explained by the structural changes that occur in the anthocyanin molecule along with changes in the H^+ concentration in the solution. As shown in **Fig. 3**, anthocyanins can exist in the form of flavylium ion, carbinol pseudobase, quinonoid, anionic quinonoid, cis-chalcone and trans-chalcone [32].

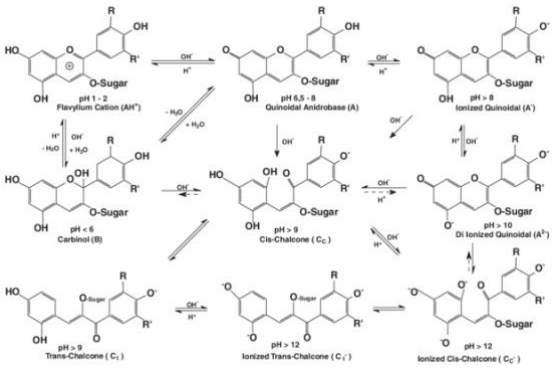


Figure 3. Anthocyanin structure based on pH changes

The UV-Vis test results show that the extract at pH 1-7 has an absorption peak with a wavelength range of 503-532 nm, are shown in **Fig. 4**.

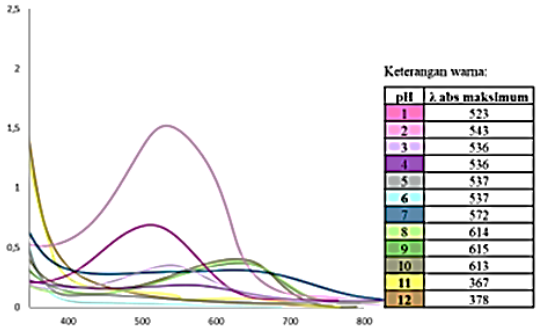


Figure 4. Anthocyanin UV-Vis spectrum

The EBRS solution at pH 1-4 has a maximum absorption in the range 523-543 nm, and the color of the magenta–purple EBRS solution corresponds to its complement. The maximum absorption at 540 nm indicates that the anthocyanin is mostly in the form of flavylium cation [30]. The color of the EBRS solution fades as the pH increases and becomes colorless at pH 5-6, due to the hydration of flavylium into a colorless carbinol pseudobase. The EBRS solution at pH 7 and 8, the color of the solution changes to blue, marked by maximum absorption at wavelengths of 572 and 614 nm. , the color change indicates the role of blue quinonoids. The change in wavelength (λ) of maximum absorption is due to the deprotonation of flavylium which is accompanied by two shifts in the maximum absorbance λ. The first deprotonation converts favylium to a neutral quinonoid, causing a shift in the maximum absorbance λ of 20-35 nm. The second deprotonation converts the neutral quinonoid to an anionic neutral quinonoid, causing a shift in the maximum absorbance λ of 40–60 nm.

3.5 Characteristics of Film-EBRS Interactions

The specific infrared wavelengths absorbed can be used to detect functional groups in compounds. FTIR spectra of jali seed flour, jali control film, 8% EBRS indicator film, and Ruellia simplex flower extract (EBRS) are shown in **Fig. 5**.

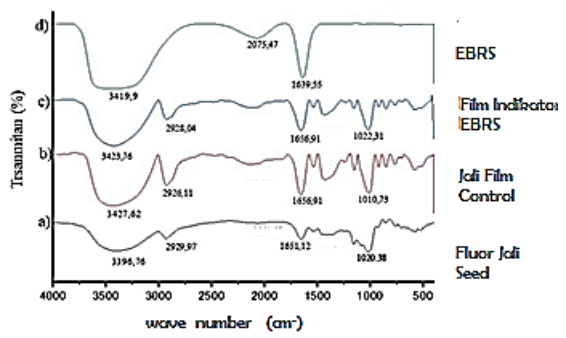


Figure 5. FTIR Spectrum EBRS, Film Indikator-EBRS, Jali Film control and fluor jali seed

Based on the FTIR test results, the gelatinization process of jali seed flour to form the jali control film did not form new absorptions in the FTIR spectrum of the jali control film (b). Likewise, the addition of EBRS to the jali film does not form new absorptions in the FTIR spectra of the 8% EBRS indicator film (c). This shows that the process of making indicator films did not form new functional groups. The high peak intensity of the FTIR EBRS spectrum (d) shows a typical cluster in EBRS. If the four spectra are compared, there are several differences in the relative intensities and band positions, a band shift can be seen which means there is a chemical interaction between the components [33].

FTIR spectrum shows that the four samples have OH (hydroxyl) groups because there is absorption at wave numbers 3550-3200 cm^{-1}; has a C-H group because there is absorption at wave numbers 3000-2840 cm^{-1}, except in EBRS where there are aromatic C-H groups that appear at wave numbers 2000-1650 cm^{-1}. All four have the C=O group because there is absorption at wave numbers 1630-1750 cm^{-1}. C-O groups appeared in jali seed flour, jali control film and 8% EBRS indicator film because there was absorption at 1300-1000 cm^{-1} [34].

Absorption at wave numbers 3400–3200 cm^{-1} originates from stretching vibrations of free hydroxyl (OH) groups, intermolecular or intramolecular hydroxyl groups (33). The band around 3400 cm^{-1} (OH stretching) on the EBRS indicator film is wider and shifted compared to the jali control film. Interaction between anthocyanins and starch are illustrated in **Fig. 6** as per research by Qin et al. (2019).

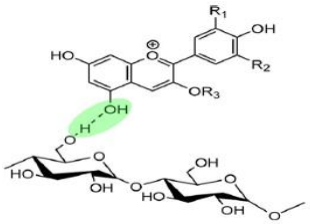


Figure 6. Intermolecular interactions between anthocyanins and starch (Qin et al., 2019)

These results indicate that anthocyanins from Ruellia simplex flower extract (EBRS) were successfully immobilized in an indicator film matrix based on jali seed flour.

3.6 Indicator Film Sensitivity Test to pH changes

Film sensitivity testing was carried out by dripping a solution of pH 1-12 onto an indicator film (0.5 x 1 cm) with concentrations of 6%, 8% and 10%, then observing the color changes shown by the film. The color response of the EBRS indicator film in the pH range 1–12 for the three concentrations is listed in **Table 1**.

Table 1. Indicator film sensitivity

pH	Film color change and total color difference value (ΔE)		
	Film Indicator EBRS 6%	Film Indicator EBRS 8%	Film Indicator EBRS 10%
initial			
1	44,71	38,26	29,92
2	39,3	38,29	21,48
3	43.94	18,30	16,76
4	38,02	24,64	19,06
5	29,86	23,36	30,51
6	17.95	19.53	20,51
7	16,31	31,02	29,85
8	37,31	44,60	55.92
9	43,56	60,73	61,26
10	40,40	48,99	51,28
11	45,09	47,32	50,29
12	38,78	51,78	37,32

The ΔE value indicates a significant color change of the 6%, 8%, and 10% EBRS indicator films when the pH value increases from 6.0 to 8.0, because a ΔE value greater than 3 indicates a significant color difference [35]. So the color change of the EBRS indicator film is suitable for detecting shrimp spoilage directly. This is based on the reason that an increase in pH from 6 to 8 is a condition for the process of changing the freshness of the shrimp until it rots [3].

The fluctuating ΔE values as seen in **Tables 2 and 3** indicate the influence of anthocyanin concentration and pH. The combined effects of pH

and anthocyanin concentration on the color and spectral characteristics of aqueous solution. Anthocyanin concentration directly affects the intensity, stability, and shade of colors in a solution. Increased concentration generally leads to higher saturation, deeper colors, and improved color stability, while lowering it can reduce color intensity and alter the shade. van Buren, Hrazdina, and Robinson (1974) defined how changes in the concentrations of anthocyanins can impact the color space parameters. In their study, van Buren et al. (1974) showed that as pH increased for a given anthocyanin concentration there was a corresponding increase in lightness and a decrease in a* and b* significantly affecting the strength of color gradation. The effect of Gradation results in a decrease in color saturation, making colors appear more faded, less intense, and closer to gray/desaturated.

Anthocyanins are stable at low pH, increased pH level can affect the rate of destruction. In solution, anthocyanins molecules are present in an equilibrium between the colored cationic form and the colorless pseudo base. This equilibrium is directly influenced by pH, so that pH is very important for the color of anthocyanins. The reason for this is right here this structure of the anthocyanins (red circle) is called flavylium cation the reason, at low pH the cyanidin molecule is protonated and forms a positive ion or cation, as the pH increases the molecules become deprotonated, at high pH the molecule forms a negative ion or anion. This is the reason that most colorants containing anthocyanins can only be used at pH values below four [36].

3.7 Optimization of Ruellia simplex Flower Extract (EBRS) Concentration on Indicator Film in Monitoring Shrimp Damage

Response of EBRS indicator film to shrimp damage showed that the three types of EBRS indicator film (6%, 8%, 10%), as in **Table 2**. showed changes in the color of the EBRS indicator film at 6%, 8%, and 10% during monitoring of shrimp damage stored at room temperature.

An increase in pH from 6 to 8 is a condition for the process of changing the freshness of the shrimp, leading to rotting, which is indicated by the shrimp pH having passed pH 8 [3]. Based on Table 2, the pH of the shrimp exceeds pH 8 after storing the shrimp at room temperature for 13 hours. This is in accordance with the research of Abu Bakar et al. (2008) who recommend not storing shrimp at room temperature for more than 12 hours.

Tabel 2. EBRS indicator monitoring data at room temperature storage

Time (hour)	pH	discoloration Conc. EBRS 6%	8%	10%	ΔE Film Conc. EBRS 6%	8%	10%
0	6.840						
2	6.877				13.08	13.89	16.81
4	6.920				3.30	4.24	9.38
6	6.937				5.25	6.62	2.70
8	7.193				2.99	6.13	8.93
9	7.730				1.94	3.29	3.46
13	8.033				5.62	13.80	9.93
14	8.170				3.28	382	4.59
16	8.237				2.65	5.66	1.31
ΔE Average					4.76	7.18	7.14

Discoloration of the EBRS indicator film 6% and 10% from purple to gray corresponds to a mixture of flavylium and carbinol forms, with an average ΔE (total color difference) value of 4.76 and 7.14. The blue Colorants hues introduced by the diversification of flavylium cations into the quinoidal bases in solution are virtually reflected in combination pigments with a deterioration of saturation until the color appears in grey tones at a deposition pH value of 7 (37) Meanwhile, the color of the 8% EBRS indicator film changed from purple to gray, then to green with an average ΔE value of 7.18. The average ΔE value of the three types of indicator films shows very different color changes (ΔE>3) (35), and the average ΔE value of the EBRS indicator film is 8% greater than the EBRS indicator film 6% and 10%. Based on ΔE data, storing shrimp at room temperature is the most effective type of EBRS 8% indicator film.

The performance of 6%, 8%, and 10% EBRS indicator films during damage monitoring for shrimp stored at a chiller temperature of 4 ºC is listed in **Table 3**. The pH of the shrimp exceeded pH 8 after storing the shrimp in a chiller at room temperature 4 ºC for 6 days. This is in accordance with the research of Mendes et al. (2005) who reported a shelf life of shrimp of 6 days when stored at 0–4 °C. The color changes of the three types of EBRS indicator films at a chiller

temperature of 4 °C were like those observed at room temperature, and the 8% (w/w) EBRS indicator film showed better color changes than the other two types of films. The color change of the 8% EBRS indicator film (w/w) from purple to gray, then to green with an average ΔE value of 8.69. Based on ΔE data, storing shrimp at 4^0C is the most effective type of EBRS 8% indicator film.

Tabel 3. EBRS indicator monitoring data at room temperature of 4^0C

Day	pH	Discoloration			ΔE Film		
		Conc. EBRS			Conc. EBRS		
		6%	8%	10%	6%	8%	10%
0	6.637± 0.006						
1	6.713± 0.006				14.04	16.0	11.7
2	6.86 ± 0.010				5.41	7.91	6.22
3	6.987± 0.006				1.66	4.87	3.59
4	7.217± 0.006				1.94	4.48	7.65
5	7.790 ± 0.010				3.29	4.78	1.88
6	8.023 ± 0.006				3.34	11.3	2.46
7	8.163 ± 0.015				6.05	11.5	9.96
		ΔE Average			5.10	8.69	6.21

Discoloration is associated with the formation of protein degradation products such as trimethylamine (TMA), dimethylamine (DMA), and ammonia (NH_3) which are basic and volatile. The results of protein degradation until ammonia is formed causes pH increase of the shrimp atmosphere from pH 6-8 [3].

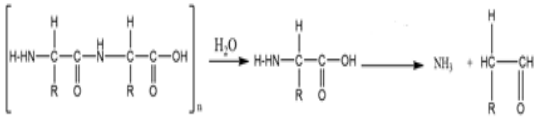


Figure 7. Protein Degradation Reaction

Volatile ammonia diffuses into the film matrix and combines with water molecules, forming $NH_3.H_2O$ interactions and then decompose to produce NH_4^+ and hydroxyl ions (OH^-) which causes an pH of the film [38]. The initial pH of the film was 2.32 with a purple color film and most of the anthocyanin was in the form of flavylium cations. The increase in pH changes the color of the film to gray, indicate that anthocyanin is in the form of colorless carbinol pseudobase. As more ammonia diffuses into the film, the pH becomes more alkaline and changes to a green color, indicating that there are anthocyanins in the form of quinonoid bases (30).

3.8 Physical characteristics of EBRS Control Film and Indicator Film

Characterization of the physical properties of the film consists of mechanical properties and structural properties. The test was carried out by comparing the properties of the jali control film and the EBRS 8% (w/w) indicator film as the optimum film. Information regarding the properties of this indicator film is important when the film is to be further developed into a packaging film.

The results (**Table 4**) showed that the addition of EBRS 8% (w/w) to the control jali film reduced the tensile strength value, increased the solubility, swelling and elongation at break values, but did not affect the thickness and water vapor transmission rate of the film.

Table 4. Physical characteristics of EBRS Control Film and Indicator Film

Parameter	Control jali Film	Indicator Film EBRS 8%
Thickness (mm)	0.165 ± 0.008	0.159± 0.006
Solubility (%)	26.70% ± 0.0009	35.13% ± 0.003
WVTR (g/m^2 jam)	43.9744 ± 2.6492	47.8632 ± 2.9160
Swelling (%)	209.23% ± 0.0436	280.66% ± 0.0827
Tensile Strength (Mpa)	1.09	4.01
Elongation at break (%)	0.72	7.78

4 Conclusion

The characteristics of anthocyanins extracted from Ruellia simplex flowers give them unique properties as natural halochromic pigments. The total anthocyanin content in Ruellia simplex flowers is 1.037 mg/L. Synthesis of indicator films using a matrix base on jali seed starch with EBRS has been made. halochromic film with an EBRS concentration of 8% showed sensitivity to changes in solution pH and shrimp damage. The addition of 8% EBRS to the film matrix did not significantly change the physical, mechanical and structural characteristics of the film. The 8% EBRS indicator film can still maintain its function as an indicator of food freshness when part of the anthocyanin is released from the film matrix. Therefore, 8% EBRS indicator film can be used as a component to form smart halochromic film packaging.

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