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RESEARCH ARTICLE

Development of Packaging Film from Purple Heart (*Tradescantia pallida*) Anthocyanin for Fish Freshness Indicator

Nurul Hidayah[1,*], Maura Syamsa Hawa[1], Bambang Purwono[2] and Safinta Nurindra Rahmadhia[1]

[1]*Department of Food Technology, Universitas Ahmad Dahlan, Tamanan, Banguntapan, Bantul 55191, Yogyakarta, Indonesia*
[2]*Department of Chemistry, Universitas Gadjah Mada, Bulaksumur, 55281, Yogyakarta, Indonesia*

([*]Corresponding author's e-mail: nurulhidayah@tp.uad.ac.id)

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Abstract

The pH sensitivity of anthocyanins enables their application as indicators of fish freshness, as spoilage is associated with an increase in pH due to the accumulation of volatile basic nitrogen compounds. Purple Heart (*Tradescantia pallida*) contain cyanidin-based anthocyanins as their primary pigment. In this study, different concentrations of Purple Heart anthocyanin extract (PHA) were incorporated into chitosan-cornstarch-based packaging films to evaluate their physical and chemical properties and to be applied as freshness indicators for tuna fillets. Five formulations with different concentrations of PHA (F0 - F4: 0 - 2 %) were evaluated for their physical properties (thickness, tensile strength, elongation, water vapor transmission rate (WVTR) and color) and chemical properties (antioxidant activity and total phenolic content). The concentration of PHA significantly influenced film thickness, elongation, and chemical properties ($p < 0.05$), while no significant effect was observed on tensile strength and WVTR ($p > 0.05$). Color analysis using a chromameter demonstrated that the film containing PHA was sensitive to pH changes. When applied to tuna fillets, the film exhibited a color shift from purple (fresh fish) to yellowish (spoiled fish), confirming its potential as a fish freshness indicator.

Keywords: Anthocyanin, Chitosan, Corn starch, Fish freshness, Indicator, Packaging film, *Tradescantia pallida*

Introduction

The consumption of fish has been shown to provide protection against certain cancers and cardiovascular diseases, as well as reduce the risk of coronary heart disease. The primary health benefits of fish consumption are largely attributed to its high content of polyunsaturated omega-3 fatty acids [1,2]. The majority of the Indonesian population tends to purchase fresh fish or seafood products rather than frozen ones [3]. Karuniawati *et al.* [4] reported that both working and non-working mothers in Bogor City, West Java, prefer to buy fresh fish that has been cleaned or is ready for cooking, such as filleted fish. However, a major challenge with fishery products is their susceptibility to spoilage and short shelf life [5,6]. Fish is highly perishable and loses its quality rapidly after being caught if not stored in refrigerated conditions or kept at temperatures below 5 °C [7-10]. The fundamental parameter for assessing fish quality is freshness. The total volatile basic nitrogen content, which includes ammonia, dimethylamine, and trimethylamine, plays a crucial role in the degradation of fish quality, contributing to the unpleasant odor resulting from microbial degradation. These compounds serve as key indicators for determining the level of fish freshness [11-14].

The Commission of the European Communities states that total volatile base nitrogen can be analyzed in the laboratory through several stages, including extraction, distillation, and titration of amines using hydrochloric acid [15]. In addition to this method, total volatile base nitrogen can also be determined photometrically via flow injection analysis [16], or chromatographically through solid-phase micro-extraction [17]. However, these methods require advanced instrumentation, complex sample preparation, and multi-step analytical procedures, resulting in relatively high costs. On the other hand, several studies have reported that fish freshness can be assessed using electronic nose technology [18] and gas chromatography-mass spectrometry (GC-MS) [19], although these techniques are not suitable for routine analysis [20].

Sensory evaluation of fish freshness includes the assessment of eye appearance, skin, gills, texture, color, and odor [21]. Among these, odor is often the primary parameter used by consumers to detect fish spoilage due to its ease of use. However, this method has been proven to be unreliable [22]. Given this background, innovative packaging solutions are needed to provide consumers with information about the condition of packaged fish products without requiring direct contact, relying instead on visual cues from the packaging. One such packaging innovation is dual-function packaging films that act both as protective packaging and as freshness indicators, allowing consumers to easily distinguish between fresh and spoiled products based on the visual color changes displayed by the packaging without opening it. Fish spoilage is characterized by an increase in pH due to the elevated concentration of volatile basic nitrogen compounds.

Anthocyanins are natural pigments capable of producing red, blue, or even black colors when present in high concentrations in plants [23,24]. These pigments are highly pH-sensitive, undergoing color changes in response to pH variations [25-27]. In acidic environments, anthocyanins appear pink, whereas in alkaline conditions, they turn purple or blue [28]. One potential source of anthocyanins is the Purple Heart (*Tradescantia pallida*) plant. To date, the utilization of Purple Heart (*Tradescantia pallida*) leaves in Indonesia remains limited to ornamental purposes, despite several studies exploring their potential use as a food colorant. One study reported that the anthocyanins extracted from Purple Heart leaves exhibit higher thermal stability compared to those from red cabbage and sweet potato [29,30]. In countries such as Pakistan, India, Bangladesh, and regions of Africa, *Tradescantia pallida* has been traditionally used as a remedy for inflammation, eye redness, and as an herbal supplement with antitoxic and antioxidant properties [31].

In this study, a bio-based film was developed using a combination of chitosan and corn starch. Starch, a polysaccharide, serves as an economical, environmentally friendly alternative to plastic polymers while providing desirable physical characteristics [32]. Corn starch is particularly rich in amylose, making it an excellent film-forming agent [33]. However, starch-based films tend to have low transparency, which is why they are combined with chitosan, a widely used thickening, stabilizing, gelling, and texturizing agent [34]. Additionally, chitosan possesses film-forming properties, is water-insoluble (hydrophobic), biodegradable, non-toxic, and enhances the transparency of the developed film [35].

Materials and methods

Materials

The purple heart plant (*Tradescantia pallida*) was obtained from Banguntapan, Bantul, Yogyakarta. Other materials used in this study included corn starch, chitosan, glycerol, glacial acetic acid, vitamin C standard, ethanol, methanol, DPPH solid, Na_2CO_3, Folin-Ciocalteu reagent, gallic acid, silica gel, NaCl, distilled water, buffer solution, and filter paper. The chemical reagents used were pro-analysis grade chemicals sourced from Merck and Sigma Aldrich.

Equipments

The equipment used in this study included glassware from Iwaki, a digital scale (SF 400), an analytical balance (Pioneer), a drying oven (Memmert SN30), a 0.01 mm micrometer screw gauge (Tricle brand), a hot plate (Thermo Scientific Cimarec 2), a rotary evaporator (IKA RV-10), a vortex mixer (VM-300), a Konica Minolta CR-400 chromameter, a Universal Testing Machine (Zwick), a spectrophotometer (B-One 19861-121-1), and an LC-MS system (Waters Xevo TQD).

Methods

Extraction of Purple Heart (*Tradescantia pallida*)

The extraction of purple heart began with drying fresh leaves (100 g) at 40 °C for 3 days. The dried samples were then crushed and macerated in absolute ethanol with a sample-to-ethanol ratio of 1:2 for 72 h. The maceration product was subsequently filtered, and the filtrate was evaporated at 50 °C [36].

Screening of major anthocyanins in purple heart extract (*Tradescantia pallida*)

A total of 200 µL of concentrated purple heart extract was diluted with 800 µL of ethanol and then filtered using a 0.22 µm Millex filter. After the preparation process was completed, 5 µL of the sample was injected into the LC-MS system. The mobile phase used was a gradient system consisting of a mixture of water - 0.1 % formic acid and CH_3CN-0.1 % formic acid, with a C18 column as the stationary phase.

pH sensitivity test of purple heart extract solution

The purple heart extract was distributed into 13 (5 mL vials with uniform volumes). Buffer solutions with pH values ranging from 2 to 14 were added to each vial containing the extract, and the resulting color changes were observed [37].

Preparation of packaging film

The preparation of the packaging film followed the casting method described by Silva-Pereira *et al.* [35] with modifications. A total of 0.4 g of chitosan was dissolved in 50 mL of 1 % glacial acetic acid and stirred using a magnetic stirrer for 24 h at 25 °C. Meanwhile, 2.5 g of corn starch was dissolved in 50 mL of distilled water at 70 - 80 °C with continuous stirring until the solution became thick and transparent. The chitosan and corn starch solutions were then mixed and stirred using a magnetic stirrer at 85 °C for 20 min. The mixture was allowed to cool to room temperature until it reached 50 °C. Once the desired temperature was achieved, purple heart extract (according to formulations F0 (0 %), F1 (0.5 %), F2 (1 %), F3 (1.5 %) and F4 (2 %) and 3 mL of glycerol were added and stirred until homogeneous. The final mixture was poured into a 20×15 cm glass tray and dried in an oven at 35 °C for 96 h.

Thickness measurement of packaging film

The thickness of the film was measured using a micrometer screw gauge with an accuracy of 0.01 mm. Measurements were taken at 5 different locations: The top-right corner, top-left corner, bottom-right corner, bottom-left corner, and center, with 3 repetitions for each location. The final thickness value was determined as the average of the 5 measurement points [38].

Tensile strength and elongation test of packaging film

The tensile strength and elongation properties of the film were measured following the ASTM D 882 standard method. The sample is cut into a rectangular shape measuring 2×10 cm, then tensile and elongation tests are conducted using a Universal Testing Machine [39].

Water Vapor Transmission Rate (WVTR) test of packaging film

The WVTR test for the packaging film was conducted based on the method described by Pranoto *et al.* [40]. The humidity inside the desiccator was maintained at a relative humidity (RH) of 70 - 75 % by adding a 40 % NaCl solution. Each film formulation was cut into 3×3 cm^2 pieces. A total of 5 g of silica gel was placed inside a porcelain crucible, and the film was sealed over the surface of the crucible to ensure no gaps along the edges before being placed inside the desiccator. The weight of the porcelain crucible was recorded every 24 h for 5 days. The water vapor transmission rate of the film was calculated using Eq. (1):

$$\text{WVTR}\ (\frac{g}{m^2}) = \frac{\Delta W}{t \times A} \quad (1)$$

where ΔW is slope (weight change of the sample over 5 days in g), t is time in 24 h, and A is surface area in m^2. The WVTR value was expressed in g/m^2. All samples were examined in triplicate.

Color measurement of packaging film

The L* value represents brightness, ranging from 0 (black) to 100 (white). A positive a* value (0 to 60) indicates a red hue, while a negative a* value (0 to −60) indicates a green hue. A positive b* value (0 to 60) corresponds to a yellow hue, whereas a negative b* value (0 to −60) corresponds to a blue hue. The total color difference (ΔE) of the film in response to pH changes was calculated using the following Eq. (2):

$$\Delta E = \sqrt{(L^* - L_0{}^*)^2 + (a^* - a_0{}^*)^2 + (b^* - b_0{}^*)^2} \quad (2)$$

where ΔE represents the total color variation; L^*, a^*, and b^*denote the color coordinates of the film at a given pH level; and $L_0{}^*$, $a_0{}^*$, and $b_0{}^*$ correspond to the color values of the reference sample. In this study, pH 2 was used as the reference value based on the initial appearance of the film before exposure to pH changes [41].

Total phenolic content test of packaging film

A total of 25 mg of the sample was dissolved in distilled water and diluted to a final volume of 100 mL. 1 mL of the sample solution was taken and further diluted in a 100 mL volumetric flask. A series of gallic acid standard solutions were prepared as reference standards. Both the sample solution and the gallic acid solutions were mixed with 1 mL of Folin-Ciocalteu reagent, 0.8 mL of Na_2CO_3 solution, and 3 mL of distilled water, followed by thorough mixing. The mixture was incubated in a dark room for 30 minutes, after which its absorbance was measured using a spectrophotometer at a wavelength of 753 nm [42]. The total phenolic content was calculated using Eq. (3):

$$\text{TPC}\left(\frac{\text{mg GAE}}{\text{g}}\right) = \frac{\text{x}\times\text{DF}\times\text{V}}{m} \quad (3)$$

where x is concentration of sample obtained from standard curve (mg/mL), DF is dilution factor, and V is sample volume, m is mass of sample in g. Total phenolic content (TPC) was expressed in mg per g in Gallic Acid Equivalent (GAE). All samples were examined in triplicate.

Antioxidant activity test of packaging film

Each sample (150 mg) was weighed and dissolved in methanol. A 2.8 mL aliquot of the sample solution was transferred into a test tube wrapped with aluminum foil, followed by the addition of 0.2 mL of 0.1 mM DPPH solution. The mixture was vortexed at 2,500 rpm for 1 min, then incubated for 1 h, and its absorbance was measured using a spectrophotometer at a wavelength of 517 nm [43]. The antioxidant activity was expressed as the percentage of radical scavenging activity (RSA) based on Eq. (4):

$$\text{RSA}(\%) = \frac{\text{A}_{\text{control}} - \text{A}_{\text{sample}}}{\text{A}_{\text{control}}} \times 100\ \% \quad (4)$$

where $\text{A}_{\text{control}}$ is absorbance value of the DPPH solution without the addition of the sample. A_{sample} refers to the absorbance value of the DPPH solution with the addition of the sample. All samples were examined in triplicate.

Application of packaging film on tuna fillet

The application of the packaging film was carried out at room temperature for 24 h to observe the appearance of both fresh and spoiled fish. Fresh tuna fillets were cut into cubes (2×2×2 cm^3) and wrapped with the packaging film embedded with Purple Heart anthocyanin extract (PHA) [20].

Data analysis

Each formulation underwent physical tests (thickness, tensile strength, elongation, and water vapor transmission rate) and chemical tests (antioxidant activity and total phenolic content), with 3 repetitions for each test, except for the color measurement and film application tests. The obtained data were analyzed statistically using a 1-way Analysis of Variance (ANOVA). If significant differences were found between treatments, further analysis was conducted using Duncan's Multiple Range Test (DMRT) at a 5 % significance level, utilizing SPSS version 22.0 software. The Pearson correlation was analyzed using SPSS Statistics version 30.

Results and discussion

Screening of major anthocyanins

The concentrated extract of *Tradescantia pallida* leaves was analyzed using LC-MS to screen for anthocyanin compounds. Based on the chromatogram and MS spectra, the peak at a retention time of 11.52 min was identified as an anthocyanin compound belonging to the cyanidin group. Previous studies by Shi *et al.* [29]; Baublis and Berber-Jiménez [44]; El-Hawary *et al.* [45] have reported that the primary anthocyanins in *Tradescantia pallida* belong to the cyanidin group.

Shi *et al.* [29] described that *Tradescantia pallida* contains 2 major anthocyanin compounds: Cyanidin-3,7,3'-triglucoside with 3 ferulic acid molecules and an additional terminal glucose unit, and a similar pigment lacking the terminal glucose unit. Baublis and Berber-Jiménez [44] further identified the major anthocyanin in *Tradescantia pallida* as tradescantin 3-O-[6-O-[2,5-di-O-(E)-α-L-arabinofuranosyl]-β-D-glucopyranosyl]-7,3'-di-O-[6-O-(E)-feruloyl-β-D-glucopyranosyl]-cyanidin, confirmed using ^{1}H-NMR and mass spectrometry.

In a more recent study, El-Hawary *et al.* [45] reported that *Tradescantia pallida* contains anthocyanin derivatives, including petunidin-O-feruloyl, cyanidin-O-triferuloyl trihexosyl pentoside (rhoenin), and setcreasin. In the present study, the mass spectrum of the peak at 11.52 min was predicted to correspond to a cyanidin type anthocyanin, identified as the primary anthocyanin component in *Tradescantia pallida* extracted in this research. The peak at 287 m/z, as shown in **Figure 1**, is characteristic of cyanidin. The peak at 173.23 m/z corresponds to a ferulic acid molecule, while the peak at 622.60 m/z represents a cyanidin molecule conjugated with 1 glucose and 1 ferulic acid molecule. This result is consistent with the mass spectrometry findings of tradescantin by Baublis and Berber-Jiménez [44], however the molecular ion peak at 1,609 m/z was not observed.

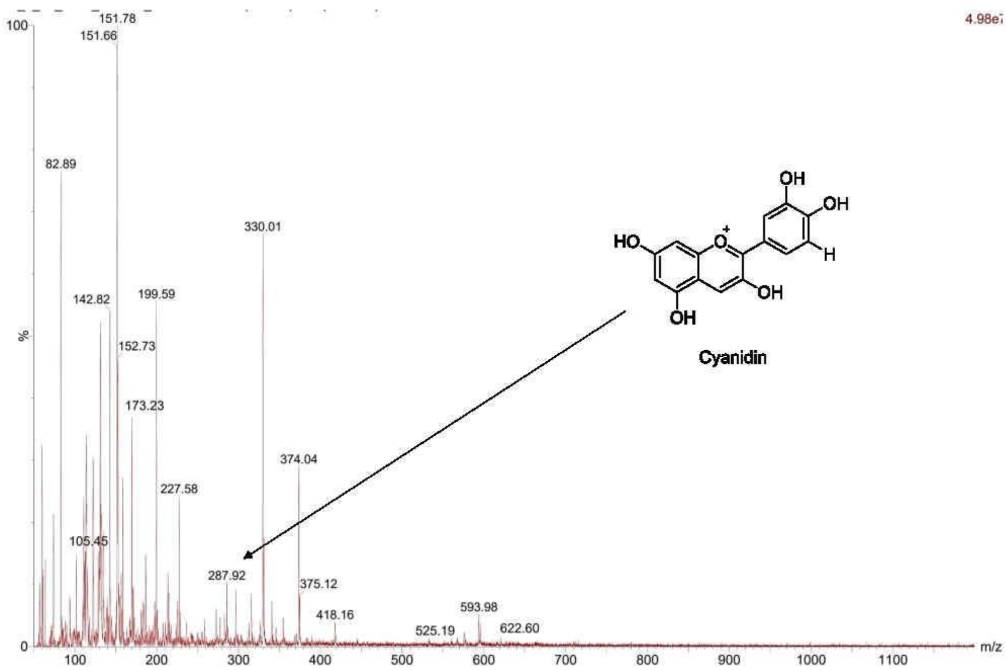

Figure 1 Mass spectrum of the major anthocyanin compound extracted from *Tradescantia pallida* (PHA).

pH sensitivity test

Anthocyanins are pigment compounds responsible for the blue, red, or purple colors found in plants, particularly in leaves, flowers, fruits, and tubers. Under acidic conditions, anthocyanins appear red, while under basic conditions, they turn blue [23]. This test aims to confirm that PHA contain anthocyanin compounds that are sensitive to pH changes. The anthocyanins in *Tradescantia pallida* exhibit a pink color in an acidic environment and a yellowish-green hue in a basic environment. At highly acidic conditions (pH < 2), anthocyanins exist in the flavylium ion form, appearing red. As the pH increases (pH 6 - 7), the flavylium ion transitions into a neutral quinonoid base, causing a color shift from red to purple. When the pH further increases (pH 7 - 8), the neutral quinonoid base transforms into an anionic quinonoid base. However, both neutral and anionic quinonoid bases are thermodynamically unstable, and the more stable species is found in the form of chalcone. Consequently, at basic pH levels (pH > 8), the purple color disappears and transitions to yellow [27,46].

The color change phenomenon observed in the anthocyanins of *Tradescantia pallida* differs from typical anthocyanin behavior. As documented in the pH sensitivity test of *Tradescantia pallida* extract shown in **Figure 2**, at pH 2, the extract solution appears pink. However, at pH 3-10, the solution retains a purple hue, and only at pH > 10 does the color shift to yellow-green. This phenomenon aligns with findings by Steingass *et al.* [30], who reported that *Tradescantia pallida* extract exhibits an unusual additional absorption peak at 585 nm (purple) under slightly acidic to neutral conditions (pH 1 - 10), producing a more intense red-to-purple color. This effect is attributed to intramolecular co-pigmentation, which enhances color intensity. The co-pigmentation is caused by the presence of ferulic acid moieties in tradescantin. The proposed reaction mechanism of *Tradescantia pallida* extract color changes in response to pH variation is illustrated in **Figure 3**.

Figure 2 Results of the pH sensitivity test of *Tradescantia pallida* extract (PHA).

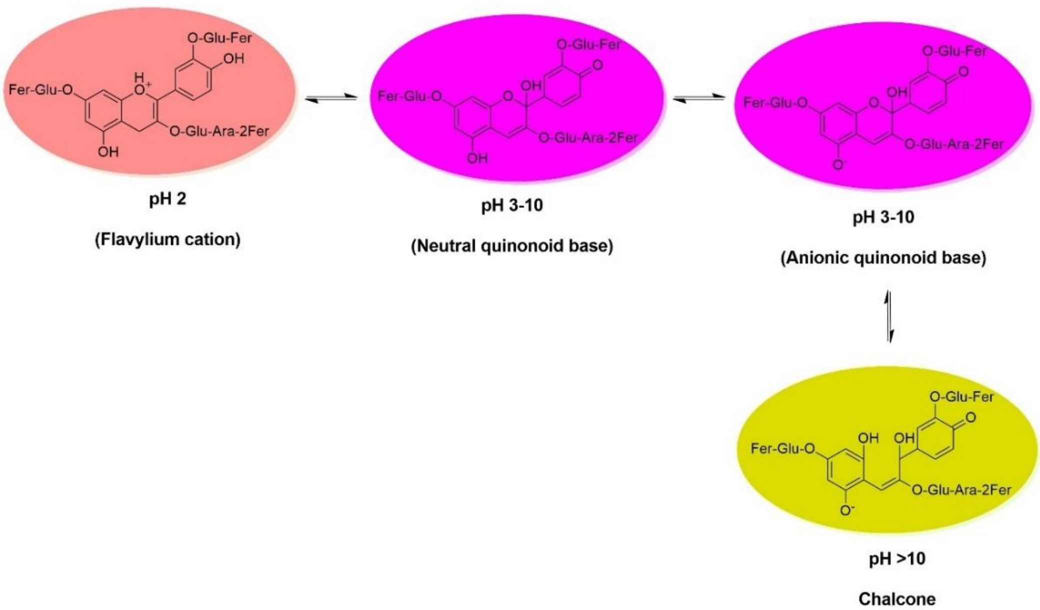

Figure 3 Possible reaction mechanism of *Tradescantia pallida* extract (PHA) color changes in response to pH variation (Ara: Arabinose, Fer: Ferulic acid and Glu: Glucose).

Thickness, tensile strength, elongation, and WVTR

The physical properties of the packaging film, including thickness, tensile strength, elongation, and water vapor transmission rate (WVTR), are presented in **Table 1**. Based on **Table 1**, it can be observed that variations in the concentration of Purple Heart Anthocyanin Extract (PHA) incorporated into the packaging film for each formulation did not significantly affect the tensile strength and WVTR parameters (p > 0.05). Conversely, differences in *Tradescantia pallida* extract concentrations had a significant effect on the thickness and elongation of the packaging film. The film sheets from formulations F0 to F4 are shown in **Figure 4**, where F0 appears colorless, while the films from F1 to F4 progressively exhibit a more purplish hue.

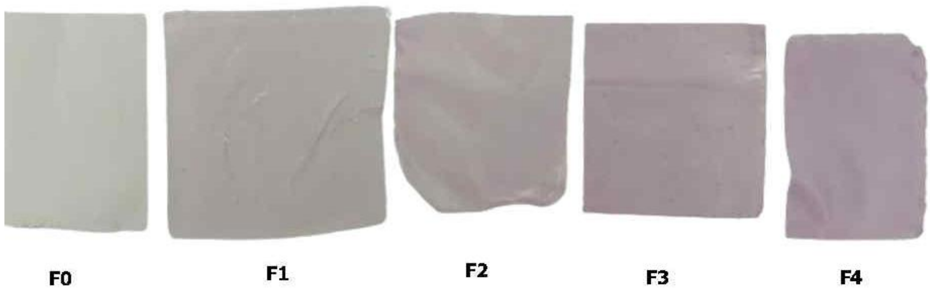

Figure 4 Appearance of film formulations F0, F1, F2, F3, and F4.

Based on **Table 1**, the higher the concentration of Purple Heart Anthocyanin Extract (PHA) added to the packaging film, the greater the film thickness. The addition of PHA at concentrations of 0 - 1.5 % did not significantly affect (p > 0.05) film thickness. However, the addition of 2 % PHA resulted in a significantly higher thickness (p < 0.05) compared to other formulations. This phenomenon may be attributed to the strong hydrogen bonding between anthocyanins and the chitosan-cornstarch polymer matrix [37,47]. Anthocyanins are flavonoid compounds that contain hydroxyl (-OH) and carbonyl (-C=O) groups, where the hydroxyl groups act as hydrogen bond donors, while the carbonyl groups serve as acceptors. Chitosan and cornstarch are polysaccharides with numerous hydroxyl (-OH) groups capable of forming hydrogen bonds with anthocyanins. If anthocyanins are well dispersed within the polymer, the resulting film will be more compact and uniform, as observed in film formulations F0 - F3 (PHA < 2 %). However, when anthocyanin content is high, as in the F4 film formulation, aggregation may occur, leading to an increased solid concentration in the film, which contributes to the observed increase in thickness [39].

The difference in PHA concentration in packaging films (F0 - F4) significantly affected (p < 0.05) the film's elongation percentage. Elongation is an indicator of film flexibility. Elongation increased with higher PHA concentrations in the packaging film (ranging from 55 to 84 %). The phenolic groups in the PHA may reduce intermolecular interactions between chitosan and cornstarch by acting as plasticizers, thereby increasing film flexibility, as indicated by the higher elongation percentage.

On the other hand, variations in PHA concentration did not significantly affect (p > 0.05) the tensile strength and water vapor transmission rate (WVTR) of the film. Although the effect was not statistically significant, **Table 1** shows that the tensile strength values tended to increase with increasing anthocyanin extract concentration in the film (F0 - F3). However, tensile strength decreased in the film containing the highest concentration of anthocyanin extract (F4). This increase in tensile strength may be attributed to the presence of phenolic groups in anthocyanins, which enhance intermolecular interactions between chitosan and cornstarch polymers through hydrogen bonding [48]. Conversely, at a certain point, hydrogen bonding interactions between anthocyanins and the chitosan-cornstarch polymer matrix may disrupt the existing intermolecular bonds within the chitosan-cornstarch polymer itself, leading to a decline in tensile strength.

Water vapor transmission rate (WVTR) measures the amount of water vapor that can pass through a film over a given period. A low WVTR value indicates that the film material provides an effective barrier against water vapor, thereby preventing moisture ingress and extending product shelf life. Conversely, a high WVTR value suggests that the film is more susceptible to moisture. Based on **Table 1**, the variation in anthocyanin extract concentration in the film did not significantly affect WVTR values ($p > 0.05$). However, as the concentration of PHA in the film increased, the WVTR value decreased. This reduction may be due to the formation of a dense and compact network through hydrogen bonding interactions between PHA and the chitosan - cornstarch matrix, thereby reducing the availability of hydroxyl groups to interact with water vapor [49-51].

Table 1 Physical properties of packaging film (thickness, tensile strength, elongation and WVTR).

Parameters	Formulation				
	F0 (0 %PHA)	**F1 (0.5 %PHA)**	**F2 (1.0 %PHA)**	**F3 (1.5 %PHA)**	**F4 (2.0 %PHA)**
Thickness (mm)	0.12 ± 0.02^a	0.13 ± 0.02^a	0.15 ± 0.03^a	0.16 ± 0.015^a	0.19 ± 0.10^b
Tensile strength (MPa)	0.47 ± 0.01^a	0.55 ± 0.10^a	0.60 ± 0.10^a	0.68 ± 0.22^a	$0.54 \pm 0,01^a$
Elongation (%)	55.00 ± 5.00^a	60.67 ± 4.04^{ab}	73.67 ± 4.93^{bc}	79.67 ± 15.63^c	84.00 ± 9.17^c
WVTR ($g \cdot m^{-2} \cdot day^{-1}$)	0.47 ± 0.02^a	0.45 ± 0.02^a	0.44 ± 0.01^a	0.44 ± 0.02^a	0.43 ± 0.03^a

Note: The values are calculated as the mean of 3 repetitions ± standard deviation. Different letters (a, b and c) indicate significant differences ($p < 0.05$).

Total phenolic content and antioxidant activity

The evaluation of total phenolic content in the film aims to determine the number of phenolic compounds embedded within the film. Total phenolic content and antioxidant activity are key components of phytochemical properties [52]. The total phenolic content of a substance correlates with its antioxidant activity [53,54]. The correlation between total phenolic content and antioxidant activity was statistically analyzed using Pearson correlation, as presented in **Table 4**. Based on the table, the correlation coefficient was found to be r = 0.970, indicating a very high correlation between phenolic content and antioxidant activity. The *p*-value < 0.05 suggests that the correlation is statistically significant**.** According to Kuckartz *et al.* [55], the strength of the correlation is interpreted as follows: r ($0.0 < r < 0.1$) indicates no correlation, r ($0.1 < r < 0.3$) indicates a low correlation, r ($0.3 < r < 0.5$) indicates a moderate correlation, r ($0.5 < r < 0.7$) indicates a high correlation, and r ($0.7 < r < 1.0$) indicates a very high correlation. Therefore, the obtained r value of 0.970 clearly falls within the very high correlation category [55].

Table 4 Correlation between total phenolic content and antioxidant activity.

		Antioxidant activity	**Total phenolic content**
Antioxidant activity	Pearson correlation	1	0.970**
	Sig. (2-tailed)		0.006
	N	5	5
Total phenolic content	Pearson correlation	0.970**	1
	Sig. (2-tailed)	0.006	
	N	5	5

Note: ** Correlation is significant at the 0.01 level (2-tailed).

Phenolic compounds can act as antioxidants through several mechanisms [56]. The first mechanism is hydrogen donation (radical scavenging), in which hydroxyl (-OH) groups within the phenolic structure donate hydrogen atoms to neutralize free radicals. The second mechanism is metal ion chelation, wherein phenolic compounds bind metal ions such as Fe^{2+} and Cu^{2+}, which play a role in radical formation reactions.

The third mechanism involves inhibition of lipid oxidation, where phenolic compounds prevent lipid peroxidation, a key process in cellular damage caused by oxidative stress. Klinger *et al.* [57] reported in their research that anthocyanins reduce lipid oxidation through a radical scavenging mechanism. According to Villanueva-Toledo *et al.* [36], the phenolic compounds with the highest antioxidant activity in *Tradescantia pallida* belong to the anthocyanin group.

Based on **Figures 5** and **6**, the higher the concentration of PHA added to the film, the greater the total phenolic content and antioxidant activity. Antioxidant activity, which can counteract free radicals, is a crucial property for food packaging. Free radicals can cause changes in the physical characteristics of fresh products, rancidity, and the development of undesirable flavors in packaged foods. Seafood is a highly perishable commodity due to its high content of unsaturated fats, making it susceptible to oxidation. Food packaging incorporating antioxidant compounds such as phenolic compounds can inhibit oxidation, thereby extending shelf life [39,43,58].

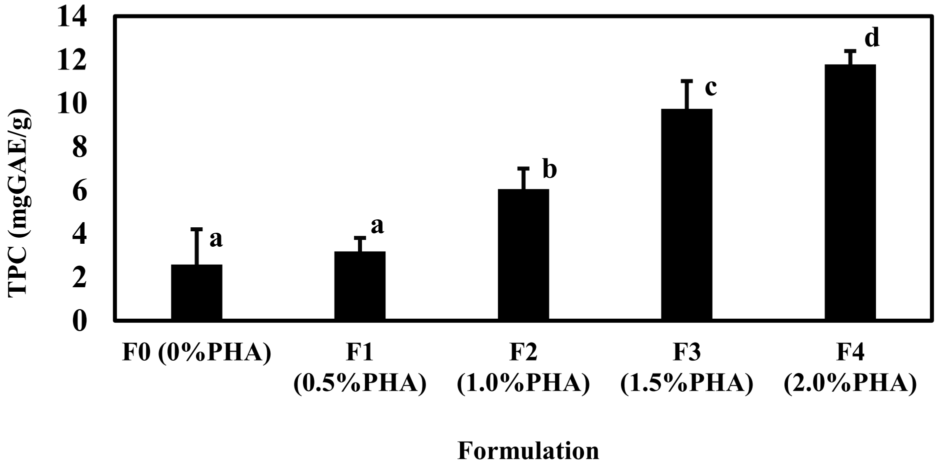

Figure 5 Total phenolic content of the packaging film.

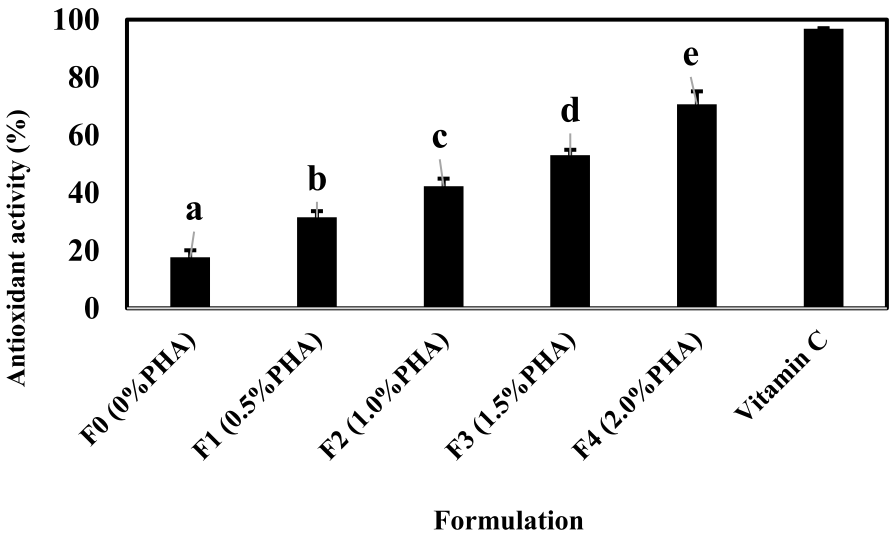

Figure 6 Antioxidant activity of packaging film.

Color test

Films formulated without the addition of purple heart anthocyanin PHA (F0) and films with PHA addition (F4) were exposed to acidic-to-basic conditions (pH 2 - 12) to evaluate their sensitivity to pH changes. This assessment is closely related to the application of the packaging film as both a food wrap and a freshness sensor for seafood products. The loss of freshness in seafood is indicated by an increase in pH (alkaline conditions) due to the rising concentration of total volatile base nitrogen, a byproduct of protein decomposition. Film F4 was selected for color analysis as a representative of films containing PHA, based on its superior physical properties (relatively high tensile strength and elongation, along with a low WVTR value) and chemical properties (the highest total phenolic content and antioxidant activity). The results of the color test, including L*, a*, and b* values, were converted into R (Red), G (Green), and B (Blue) values to visualize the film's color. ΔE* is a numerical measure used to indicate the extent of color change. A higher ΔE* value signifies a greater difference in film color compared to the initial color at pH 2, reflecting the film's sensitivity to pH changes, which can be associated with the freshness level of fish. According to Quilez-Molina *et al.* [59], a ΔE* value greater than 5 indicates that the color change is visible to the naked eye, whereas a ΔE* value less than 5 suggests that the color change is not perceptible without instrumentation. Based on the data presented in **Tables 2** and **3**, all ΔE* values for films without PHA addition were below 5. In contrast, films containing PHA exhibited ΔE* values greater than 5 when exposed to pH levels of 8 - 12. This indicates that color changes in these films are easily observable to the naked eye at pH 8 - 12.

Based on the color test data of the film without PHA addition (F0) and the film with PHA addition (F4) presented in **Tables 2** and **3**, it is evident that the film without PHA does not undergo significant color changes in response to pH variations. In contrast, the film with PHA addition (F4) exhibits significant color changes in response to pH variations. These color changes occur due to the structural transformation of anthocyanin molecules in response to pH shifts. At pH 2 - 5, the film appears purple; at pH 6 - 10, it tends to turn bluish-purple; and at pH 11 - 12, it shifts to yellow. As previously mentioned, anthocyanin color changes result from the molecular transformation of anthocyanins from the flavylium ion to quinonoid structures. The yellow coloration of the film occurs as quinonoid structures transition into chalcones. The ability of the PHA-containing film to change color in response to pH variations makes it suitable for application as both food packaging material and a freshness indicator for seafood products.

Table 2 Color test results of the film without PHA addition (F0).

Formulation	pH	L*	a*	b*	ΔE*	Color
Film without the addition PHA (F0)	2	35.15 ± 0.13	−1.90 ± 0.11	−0.28 ± 0.11	0.00	
	3	33.55 ± 0.20	−1.49 ± 0.06	−0.37 ± 0.03	1.65	
	4	32.51 ± 0.30	−0.77 ± 0.08	−0.29 ± 0.01	2.87	
	5	33.00 ± 0.38	−0.31 ± 0.08	−0.31 ± 0.20	2.67	
	6	32.06 ± 1.17	0.27 ± 0.13	−0.77 ± 0.21	3.81	
	7	34.74 ± 0.45	0.77 ± 0.06	−0.73 ± 0.36	2.74	
	8	35.10 ± 0.11	1.34 ± 0.15	−1.19 ± 0.05	3.37	
	9	34.63 ± 0.05	1.78 ± 0.04	−0.53 ± 0.11	3.72	
	10	33.81 ± 0.04	2.35 ± 0.05	−0.84 ± 0.01	4.49	
	11	31.17 ± 0.22	−1.43 ± 0.07	1.33 ± 0.05	4.32	
	12	31.76 ± 0.05	−1.51 ± 0.01	1.33 ± 0.04	3.77	

Note: The L*, a*, and b* values represent the average values from 2 repetitions ± standard deviation.

Table 3 Color test results of the film with PHA addition (F4).

Formulation	pH	L*	a*	b*	ΔE*	Color
Film with the addition PHA (F4)	2	34.10 ± 0.03	7.40 ± 0.02	−4.71 ± 0.03	0.00	
	3	33.34 ± 0.30	7.33 ± 0.16	−4.39 ± 0.08	0.83	
	4	32.21 ± 0.16	7.30 ± 0.30	−4.42 ± 0.29	1.91	
	5	33.39 ± 0.42	6.52 ± 0.30	−4.22 ± 0.37	1.23	
	6	33.30 ± 0.13	5.03 ± 0.01	−4.38 ± 0.16	2.52	
	7	33.50 ± 0.40	4.49 ± 0.40	−4.15 ± 0.23	3.02	
	8	29.63 ± 0.06	4.28 ± 0.06	−5.01 ± 0.54	5.46	
	9	29.93 ± 0.09	2.39 ± 0.15	−5.08 ± 0.21	6.53	
	10	29.57 ± 0.52	1.11 ± 0.06	−5.07 ± 0.75	7.76	
	11	26.74 ± 0.21	−0.05 ± 0.10	4.84 ± 0.31	14.17	
	12	26.57 ± 0.05	−0.40 ± 0.26	5.65 ± 0.47	14.99	

Note: The L*, a*, and b* values represent the average values from 2 repetitions ± standard deviation.

Application of packaging film on tuna fillet

As shown in **Figure 7**, fresh tuna appears red when wrapped with the PHA-embedded film, which initially exhibits a purple color. However, as the tuna darkens, the PHA-embedded film changes to a yellowish hue, indicating that the fish is no longer fresh (spoiled). This suggests that the film containing PHA can be effectively used as food packaging based on its physical and chemical characteristics, as well as a freshness sensor for seafood products.

Figure 7 Application of PHA-embedded film on tuna fillet as packaging.

Conclusions

The extract of purple heart (*Tradescantia pallida*) contains cyanidin-based anthocyanins as its primary pigment. The anthocyanins in *Tradescantia pallida* are sensitive to pH changes, appearing red at pH < 2, purple at pH 3 - 10, and yellow at pH >10. This color change is attributed to structural modifications of the anthocyanin molecules. The variation in PHA concentration incorporated into chitosan-corn starch-based film significantly affects the film's thickness, elongation, total phenolic content, and antioxidant activity. However, it does not significantly influence the tensile strength and water vapor transmission rate (WVTR). Increasing the PHA concentration in the film leads to greater thickness, elongation, and tensile strength, while WVTR values decrease. These variations in film properties are influenced by hydrogen bonding interactions between anthocyanins and the chitosan-corn starch film matrix. Based on the chromameter-based color analysis, PHA-enriched packaging film exhibits pH sensitivity. Additionally, in its direct application to tuna fillets, the film undergoes a noticeable color shift from purple (fresh) to yellow (spoiled), indicating its potential use as both food packaging and a freshness sensor for fish products. This study reports a novel approach involving the use of *Tradescantia pallida* as a freshness indicator embedded within a chitosan-corn starch polymer film. Currently, there are no commercially available fish packaging systems equipped with integrated freshness indicators in Indonesia. However, when compared to petroleum-based polymer packaging, this biopolymer film still requires further improvement, particularly in enhancing its physical properties such as tensile strength, elongation, and water resistance.

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