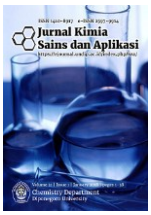
ISSN: 1410-8917
Jurnal Kimia Sains & Aplikasi
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 28 (6) (2025): 283-291

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa



Chitosan/Anthocyanin-Based Indicator Film with Carnauba Wax Addition for Monitoring Chicken Freshness

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https://doi.org/10.14710/jksa.28.6.283-291

Article Info

Article history:

Received: 24th January 2025
Revised: 21st June 2025
Accepted: 24th June 2025
Online: 05th August 2025

Keywords:
indicator film; chitosan; anthocyanin; carnauba wax; chicken meat

Abstract

Indicator films can be applied to food packaging to monitor the quality of food products. However, chitosan/anthocyanin films tend to have low hydrophobicity, which may compromise their performance as indicators and lead to dye migration. One approach to address this issue is to incorporate additional materials such as carnauba wax. This study investigates the effect of carnauba wax addition on the mechanical properties and water contact angle of chitosan/anthocyanin-based indicator films, as well as evaluates the performance of the resulting films in monitoring the freshness of chicken meat. Indicator films were prepared by adding carnauba wax at concentrations of 6, 9, 12, and 15% (w/w of chitosan). The results showed that the addition of carnauba wax improved the mechanical properties of the films, although the improvement was not statistically significant. The best mechanical performance was observed with the 15% wax formulation. Additionally, the water contact angle increased with higher concentrations of carnauba wax, with the highest value (71.630°) observed for the CS/ABT/CW15% film. The indicator films were applied to chicken packaging and evaluated based on color changes during storage as a measure of freshness. After 30 hours, the film's color changed from dark brown to green. The color change pattern during storage was similar for films with and without carnauba wax, indicating that the addition of carnauba wax does not affect the film's ability to monitor chicken freshness.

1. Introduction

Improperly stored food products are susceptible to biological, physical, and chemical degradation, which can lead to contamination and pose health risks to consumers [1]. Therefore, packaging plays a crucial role in maintaining the quality and safety of food products [2]. As consumer demand for fresh and safe food increases, various packaging innovations have been developed. One such innovation is the use of packaging equipped with pH-responsive indicator films that exhibit visual color changes, such as time–temperature indicators, freshness indicators, and leak indicators [3]. These smart packaging systems allow consumers to easily distinguish between fresh and spoiled food without opening the packaging.

Anthocyanins, which can be extracted from various plants, are known for their ability to change color under different pH conditions [4, 5, 6, 7, 8, 9, 10, 11]. They appear red in acidic conditions (pH < 4) and shift to yellow in alkaline environments (pH > 7). This makes anthocyanins effective for detecting food freshness, which is often associated with changes in pH. In addition to their pH sensitivity, anthocyanins can enhance the mechanical and thermal properties of indicator films through hydrogen bonding and intermolecular cross-linking mechanisms [12].

Polymers such as chitosan are commonly used as carriers for anthocyanins in indicator films due to their chemical stability and excellent film-forming ability [13]. However, chitosan has limitations, including poor water barrier properties and high sensitivity to moisture [14]. To address these limitations, hydrophobic materials such as carnauba wax can be incorporated. Carnauba wax is

known for its excellent hydrophobicity and mechanical strength, as well as its ability to improve the stability of composite films [15]. A study by Santos *et al.* [14] demonstrated that the incorporation of carnauba wax into a chitosan matrix increased the water contact angle, reduced water vapor permeability, and lowered the solubility of the film in water. Similarly, Hashim *et al.* [16] developed an indicator film using an agar–methyl cellulose (AM) matrix with sunflower wax (SFW) and purple cabbage anthocyanin (CPC). The addition of SFW not only enhanced the film's hydrophobicity but also improved its mechanical, thermal, and antioxidant properties, while reducing the release of anthocyanins. These findings indicate that incorporating wax into the film matrix can help protect anthocyanin dyes.

In this study, chitosan was combined with butterfly pea flower anthocyanins and carnauba wax to produce an indicator film with enhanced mechanical and water barrier properties. The resulting film was applied to chicken packaging to monitor freshness during storage. The film's performance was evaluated based on its color changes and the total volatile basic nitrogen (TVBN) content of the chicken meat.

2. Experimental

2.1. Tools and Materials

The tools used in this study included laboratory glassware, thin-walled containers (transparent food containers), syringes, a blender, oven, hotplate, rotary vacuum evaporator (Eleya), smartphone (Xiaomi Redmi Note 12 with a 50 MP rear camera), tripod, Conway cups, burettes, stands, analytical balance (Ohaus), UV-Vis spectrophotometer (Shimadzu UV Mini 1240), FTIR spectrometer (Perkin Elmer UATR Spectrum Two), and a Universal Testing Machine.

The materials used in this study were chitosan (purchased from Chimultiguna; MW 168 kDa, DD 96.19%), dried butterfly pea flowers (*Clitoria ternatea* L.) (sourced from a local marketplace), carnauba wax (marketplace), glycerol (Merck), acetic acid (CH_3COOH, Merck), Tween-20 (marketplace), trichloroacetic acid (TCA, marketplace), boric acid (H_3BO_3), Conway indicator, saturated potassium carbonate (K_2CO_3) solution, hydrochloric acid (HCl, Merck), 70% ethanol, buffer solutions (pH 1–12 and pH 4.5, Merck), ammonia solution, petroleum jelly, distilled water, and chicken breast fillet (obtained from a traditional market).

2.2. Anthocyanin Extraction from Butterfly Pea Flowers

The dried butterfly pea flowers were cleaned by removing any remaining leaves and stems, then ground using a blender until a fine powder was obtained. A total of 100 grams of the powder was soaked in 200 mL of 70% ethanol for 24 hours. The mixture was then filtered, and the resulting filtrate was concentrated using a rotary vacuum evaporator to obtain a butterfly pea flower extract. The total anthocyanin content in the extract was determined using the pH differential method. Additionally, the color response of the butterfly pea flower anthocyanin extract was observed by adding it to buffer solutions with pH values ranging from 1 to 12.

2.3. Chitosan/Anthocyanin/Carnauba Wax Indicator Film Production

The chitosan/anthocyanin/carnauba wax indicator films were prepared based on the method described by Hashim *et al.* [16], with several modifications. First, 2 grams of chitosan were dissolved in 100 mL of 1% (v/v) acetic acid solution and stirred at 85°C. Then, 2 mL of glycerol was added as a plasticizer. Separately, carnauba wax was melted at 85°C and mixed with Tween-20 surfactant. The wax-surfactant mixture was then added to the chitosan solution at concentrations of 0, 6, 9, 12, and 15% (w/w of chitosan).

The temperature of the resulting mixture was then reduced to 65°C, and 5 mL of butterfly pea flower anthocyanin extract was added. The final solution was poured into petri dishes and dried in an oven at 60°C for approximately 48 hours [14]. Once fully dried and no longer tacky, the films were manually removed from the molds.

The resulting films were designated as follows: CS/ABT for the control film (without carnauba wax), and CS/ABT/CW6%, CS/ABT/CW9%, CS/ABT/CW12%, and CS/ABT/CW15% for films with the addition of 6, 9, 12, and 15% carnauba wax, respectively.

2.4. Characterization of the Indicator Films

The indicator films were characterized based on their functional groups, mechanical properties, water contact angle, and sensitivity to pH and ammonia. Mechanical properties were tested using a Universal Testing Machine at the Integrated Laboratory of Diponegoro University, Semarang. Each test was conducted in triplicate for every sample. Functional group analysis was carried out using an FTIR spectrometer (Perkin Elmer UATR Spectrum Two) within the wavenumber range of 400–4000 cm^{-1} at the same laboratory [17].

For water contact angle measurement, film samples (1 cm × 1 cm) were placed on a flat, level surface in a stable position. The camera was positioned parallel to the film surface. Distilled water was then slowly dripped onto the surface using a syringe, and the resulting droplets were immediately photographed. The captured images were analyzed using ImageJ software to determine the water contact angle.

The sensitivity of the indicator films to pH was assessed by cutting the films into 1 × 1 cm pieces, which were then treated with buffer solutions of varying pH values (pH 1–12). The films were left at room temperature for 15 seconds before being analyzed using an Android-based colorimeter to obtain L^*, a^*, and b^* values. The total color difference (ΔE) of the biofilm was then calculated using Equation (1).

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

Where, $\Delta L^* = L^* - L_0^*$; $\Delta a^* = a^* - a_0^*$; $\Delta b^* = b^* - b_0^*$; L_0^*, a_0^* and b_0^* are the initial gray values of the film; L^*, a^*, and b^* are the colors after adding the pH solution.

The sensitivity of the indicator film to volatile ammonia (100 mmol/L) was evaluated by measuring the film's RGB color values using a smartphone with the "Color Meter" application. Film samples (2 cm × 2 cm) were attached to the inside of the container lid, directly above 25 mL of ammonia solution, and stored for 1, 2, and 3 days to observe color changes. The percentage of RGB color change (S_{RGB}) was calculated using Equation (2).

$$S_{RGB} = \frac{|R_a - R_b| + |G_a - G_b| + |B_a - B_b|}{R_a + G_a + B_a} \times 100 \quad (2)$$

Where, R_a, G_a, and B_a are the red, green, and blue values of the film before ammonia exposure, and R_b, G_b, and B_b are the corresponding values after storage.

2.5. Indicator Film Application to Monitor Freshness of Chicken Meat

Chitosan/anthocyanin/carnauba wax indicator film samples were applied to chicken meat packaging to monitor freshness during storage. A 2 × 2 cm film was placed inside the lid of a package containing 25 grams of fresh chicken meat. The packaged samples were stored at room temperature (25°C) for 3 days. Color changes (ΔE) of the indicator films were recorded using a smartphone with the Color Meter application at 0, 12, 24, and 30 hours of storage. Each measurement was performed in triplicate.

The TVBN content of the chicken meat was measured following the method described by Handayani *et al.* [18]. First, 5 grams of chicken meat were homogenized using a mortar. The ground sample was then mixed with 10 mL of 7.5% TCA solution and homogenized again. The mixture was filtered using Whatman No. 2 filter paper.

A Conway cup was prepared by coating the lid with petroleum jelly to prevent the escape of volatile bases. Then, 1 mL of H_3BO_3 solution and two drops of Conway indicator were added to the inner chamber of the cup. Subsequently, 1 mL of the filtered sample extract was pipetted into the outer chamber on the left side, and 1 mL of saturated K_2CO_3 solution was added to the outer chamber on the right side.

The Conway cup was tightly sealed and gently shaken to allow the sample extract and K_2CO_3 solution to mix and react. The cup was then incubated at 35°C for 2 hours. After incubation, the boric acid solution in the cup was titrated with 0.02 N HCl. The titration endpoint was indicated by a color change from green to pink. The TVBN content (mg/100 g) in the chicken meat was calculated using Equation (3).

$$TVBN = \frac{(V_{sample} - V_{blank}) \times 14.007 \times N_{HCl} \times 2}{Sample\ weight} \times 100 \quad (3)$$

3. Results and Discussion

3.1. Anthocyanin Extraction from Butterfly Pea Flowers

Anthocyanin extraction from butterfly pea flowers was performed using the maceration method with 70% ethanol as the solvent. The resulting extract was dark purple, reflecting the presence of anthocyanin compounds in the butterfly pea flowers. The anthocyanin content of the extract was then quantified using the pH differential method, yielding a concentration of 10,621.4 mg/L.

Figure 1 shows the color changes of anthocyanins in butterfly pea flower extract across a pH range of 1 to 12. Initially, the extract is deep blue, reflecting anthocyanins in their native form. Under highly acidic conditions (pH 1–3), the color shifts to pink due to molecular changes triggered by increased proton concentration. As pH rises to mildly acidic levels (4–6), the color transitions to purple, then to light blue near neutral pH (7–9), where quinoidal base forms dominate. Finally, in alkaline conditions (pH 10–12), the color changes to yellowish-green, indicating chalcone structures and degradation products. These progressive color shifts highlight anthocyanins' strong pH sensitivity and their potential as natural freshness indicators.

Anthocyanins' pH sensitivity arises from conjugated phenol rings and structural forms, including flavylium cations, pseudobasic carbinols, quinoidal bases, and chalcones. The hydroxyl groups undergo protonation or deprotonation depending on pH [19]. Under strongly acidic conditions (pH < 4), anthocyanins mainly exist as stable red flavylium cations. As pH rises to 4–5, these cations deprotonate and hydrate to form colorless pseudobasic carbinols. Between pH 6 and 8, anthocyanins shift to purple quinoidal base and blue anionic quinoidal base forms. In alkaline conditions (pH > 8), they predominantly appear as yellow chalcones [3, 20]. The complete structural changes of anthocyanins at different pH values are illustrated in Figure 2.

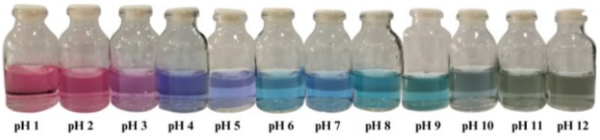

Figure 1. Color changes of butterfly pea flower anthocyanin extract across pH values from 1 to 12

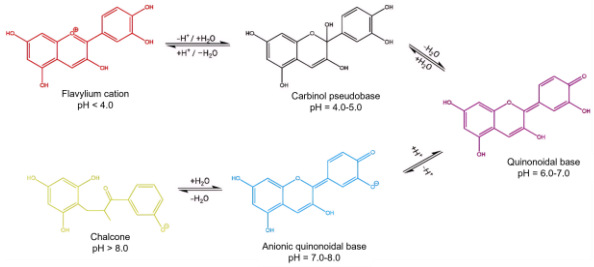

Figure 2. Structural forms of anthocyanin molecules at different pH levels

The color changes of anthocyanin extracts under various pH conditions were also quantitatively analyzed using the "Color Meter" colorimeter application on a smartphone. The results were expressed in L*, a*, and b* values based on the CIELab color system, as shown in Table 1. The L* value represents brightness, with higher values indicating lighter colors. The a* value represents the red-green axis, where positive values indicate red and negative values indicate green. The b* value corresponds to the blue-yellow axis, with negative values indicating blue and positive values indicating yellow [21].

Table 1. Colorimeter measurement results of butterfly pea flower anthocyanin extract at different pH levels

pH	Color	L*	a*	b*
1		24.142	44.529	32.048
2		18.126	37.477	20.559
3		7.206	22.182	1.875
4		7.208	20.533	-20.181
5		19.056	22.370	-37.063
6		7.779	22.149	-24.480
7		25.931	-6.452	-14.408
8		17.546	26.086	-40.647
9		24.642	-5.548	-9.916
10		6.112	16.334	-16.563
11		13.020	15.231	-28.840
12		7.805	12.991	-16.459

The colorimetric measurements using the CIELab system confirmed the visual observations of color changes in the extract in response to different pH levels. As shown in Figure 1 and detailed in Table 1, the anthocyanin extract from butterfly pea flowers exhibited significant and distinct color changes across the pH range. This confirms its potential suitability for use in fabricating indicator films designed to monitor the freshness of chicken meat.

3.2. Chitosan/Anthocyanin/Carnauba Wax Indicator Film Production

Chitosan-based indicator films were prepared by incorporating anthocyanin extract and varying amounts of carnauba wax using a simple mixing method on a hot plate. The carnauba wax was added at concentrations of 6, 9, 12, and 15% w/w relative to chitosan. The resulting indicator films appeared dark black due to the presence of anthocyanin extract from butterfly pea flowers. The addition of carnauba wax imparted a slightly brownish tint to the films. The visual characteristics of the prepared films with different wax contents are summarized in Table 2.

The control film without carnauba wax exhibited a smooth and uniform surface. In contrast, films containing carnauba wax displayed uneven surfaces, which may have resulted from wax recrystallization as the solution temperature was lowered. Carnauba wax has a relatively high melting point of approximately 85°C, while the film solution was cooled to 65°C to protect the anthocyanins from thermal degradation during mixing. This temperature difference likely caused the wax to solidify and create surface irregularities. Additionally, the wax-containing films showed small holes, possibly formed by trapped air bubbles during mixing.

Table 2. Visual and physical characteristics of chitosan/anthocyanin indicator films with varying concentrations of carnauba wax

Film variation	Before drying	After drying
CS/ABT		
CS/ABT/CW6%		
CS/ABT/CW9%		
CS/ABT/CW12%		
CS/ABT/CW15%		

3.3. Characterization of Chitosan/Anthocyanin/Carnauba Wax Indicator Films

3.3.1. Characterization of Functional Groups by FTIR

The intermolecular interactions among chitosan, butterfly pea flower anthocyanin extract, and carnauba wax in the indicator films were analyzed using FTIR spectroscopy. The FTIR spectra for all prepared indicator film samples are presented in Figure 3.

The FTIR spectra in Figure 3 show a broad absorption band between 3600 and 3000 cm^{-1}, corresponding to the overlapping stretching vibrations of hydroxyl groups (- OH) and amine groups (-NH_2). In the control film spectrum, characteristic chitosan bands are observed at 3298 cm^{-1}, representing hydrogen-bonded NH stretching; 2920 cm^{-1}, corresponding to aliphatic CH stretching vibrations; 1602 cm^{-1}, associated with C=O stretching; 1038 cm^{-1}, indicating interactions between the glycerol plasticizer's OH groups and the chitosan matrix; and 926 and 846 cm^{-1}, attributed to C=C and CH bond vibrations, respectively [2, 22, 23, 24].

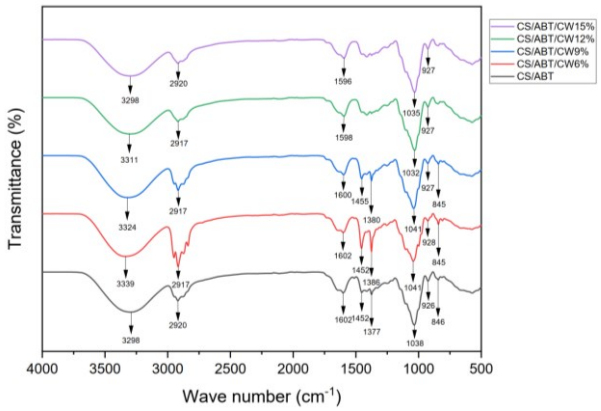

Figure 3. FTIR spectra of indicator films

The addition of carnauba wax to the chitosan-anthocyanin mixture induces a shift in the broad band from 3298 cm^{-1} to 3339 cm^{-1}, suggesting the formation of hydrogen bonds among chitosan, butterfly pea anthocyanin, and carnauba wax. Furthermore, a decrease in intensity and shift of the C=O stretching band from 1602 to 1596 cm^{-1} indicates interactions between the aglycone-pyranoside C=O group and the aromatic ring's C=C stretching vibration of anthocyanin with the NH stretching vibration of chitosan's amide groups.

Additional absorption peaks associated with carnauba wax are also evident. The presence of fatty acid chains in the wax results in a sharp peak near 2920 cm^{-1}, corresponding to asymmetric and symmetric methylene stretching vibrations of aliphatic CH groups typical of lipids [24, 25].

3.3.2. Analysis of Mechanical Properties of Indicator Films

Tensile strength is a critical parameter that indicates the maximum tensile force a film can withstand before breaking, reflecting its ability to protect products from mechanical damage [26]. Based on Table 3, the addition of carnauba wax slightly increased the tensile strength of the films, although the improvement was not statistically significant. The highest tensile strength (0.695 MPa) was observed in the film containing 15% carnauba wax, while CS/ABT exhibited the lowest tensile strength of 0.585 MPa. However, all measured values remain considerably lower than the Japanese Industrial Standard (JIS) benchmark of 40 MPa [27]. The results of the analysis of the mechanical properties of the indicator films are presented in Table 3.

Table 3. Mechanical properties of indicator films

Sample	Tensile strength (MPa)	% Elongation	Young's modulus
CS/ABT	0.585	196.30	0.0030
CS/ABT/CW6%	0.615	191.35	0.0032
CS/ABT/CW9%	0.685	199.65	0.0034
CS/ABT/CW12%	0.460	199.85	0.0023
CS/ABT/CW15%	0.695	199.50	0.0035

Table 4. Water contact angle of the indicator films

Sample	Water contact angle (°)
CS/ABT	32.11
CS/ABT/CW6%	56.76
CS/ABT/CW9%	58.58
CS/ABT/CW12%	61.99
CS/ABT/CW15%	71.63

Elongation at break reflects the film's ability to stretch before rupture, calculated as the percentage increase in length at break compared to the initial length [26]. The CS/ABT/CW6% film showed a slightly lower elongation (191.35%) than the CS/ABT film (196.30%). Films with 9, 12, and 15% wax content exhibited similar elongation values around 199%, indicating minimal impact from wax addition on this property. All films met the JIS minimum elongation standard of 50% [27].

Young's modulus measures the film's stiffness and resistance to deformation under stress. The highest Young's modulus value (0.0035 MPa) was recorded for the CS/ABT/CW15% film, whereas the lowest (0.0023 MPa) was observed for the CS/ABT/CW12% film. These values are significantly below the JIS standard of 0.35 MPa for a good film material [27]. Overall, while carnauba wax addition slightly enhanced some mechanical properties of the chitosan/anthocyanin films, the values remain far from industrial standards, indicating room for further optimization.

3.3.3. Water Contact Angle Analysis of Indicator Films

The water contact angle indicates the water resistance properties of an indicator film. A material is considered hydrophobic if its contact angle exceeds 90° and hydrophilic if it is below 90°. A contact angle above 150° classifies the material as superhydrophobic [22]. The water contact angle measurement results of the indicator film are presented in Table 4.

The CS/ABT film exhibited the lowest water contact angle (32.11°), attributed to the abundance of hydrophilic groups in chitosan that readily interact with water molecules. In contrast, the incorporation of carnauba wax into the chitosan matrix increased the water contact angle in line with the wax concentration. The water contact angle values for films containing 6, 9, 12, and 15% wax were 56.76°, 58.58°, 61.99°, and 71.63°, respectively. Despite this increase, the films cannot be classified as hydrophobic materials, as a water contact angle above 90° is required for such classification [16].

3.3.4. Analysis of Indicator Film Sensitivity to pH

Figure 4 shows the color response of the indicator films to buffer solutions with pH values ranging from 1 to 12. The color changes observed in the films across the pH spectrum differed slightly from those in the butterfly pea flower extract solution alone. This suggests that the anthocyanin compounds from the butterfly pea flower interacted with other components in the film matrix, namely chitosan and carnauba wax, which may also contribute to the color characteristics of the films.

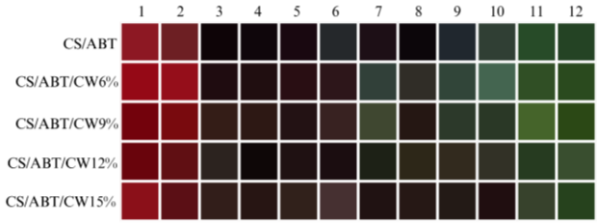

Figure 4. Sensitivity of indicator films to pH 1–12

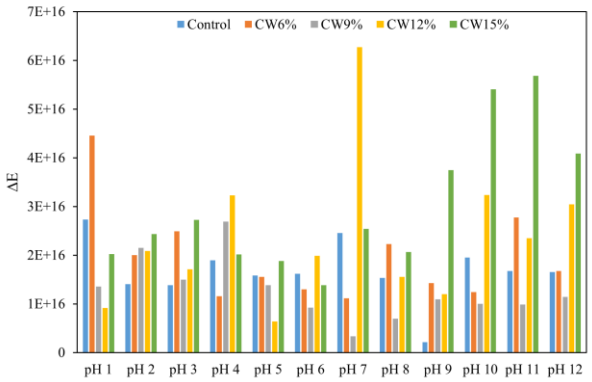

Figure 5. Color change (ΔE) of indicator films in buffer solutions at pH 1–12

Under strongly acidic conditions (pH 1 and 2), all films exhibited a red hue due to flavylium cations. At pH 3–5, the films shifted to purplish. Near neutral (pH 6–7), a greenish tint appeared. As pH increased (8–10), the green became more pronounced but remained relatively dark. At pH 11–12, the films showed a more intense green, likely from the coexistence of quinoidal (blue) and chalcone (yellow) forms [16, 22]. This ability to undergo visible color changes across a wide pH range demonstrates the potential of the films as pH-responsive indicators for monitoring the freshness of chicken meat.

The pH sensitivity of the indicator films, supported by colorimeter data in Figure 5, was evaluated using ΔE values, which represent the degree of color change before and after exposure to buffer solutions. A higher ΔE indicates greater sensitivity to pH variations. The CS/ABT/CW6% film exhibited the highest ΔE at strongly acidic pH (pH 1), while the CS/ABT/CW15% film showed the most significant color changes at pH 2, 3, 5, and 9-12. The CS/ABT/CW12% film recorded the highest ΔE values at pH 4, 6, and 7, and the CS/ABT/CW6% film again led at pH 8. Overall, the CS/ABT/CW15% film demonstrated the greatest ΔE across most pH levels, indicating higher pH responsiveness and making it the most promising candidate as a pH-sensitive indicator film.

3.3.5. Sensitivity Analysis of Indicator Films to Ammonia

A sensitivity analysis of the indicator films to ammonia was conducted to evaluate their response to ammonia vapor prior to application in monitoring chicken meat freshness. As chicken meat deteriorates, it releases volatile nitrogen compounds such as ammonia, dimethylamine, and trimethylamine [28]. Previous studies have demonstrated that ammonia can induce visible color changes in colorimetric films containing anthocyanins [22].

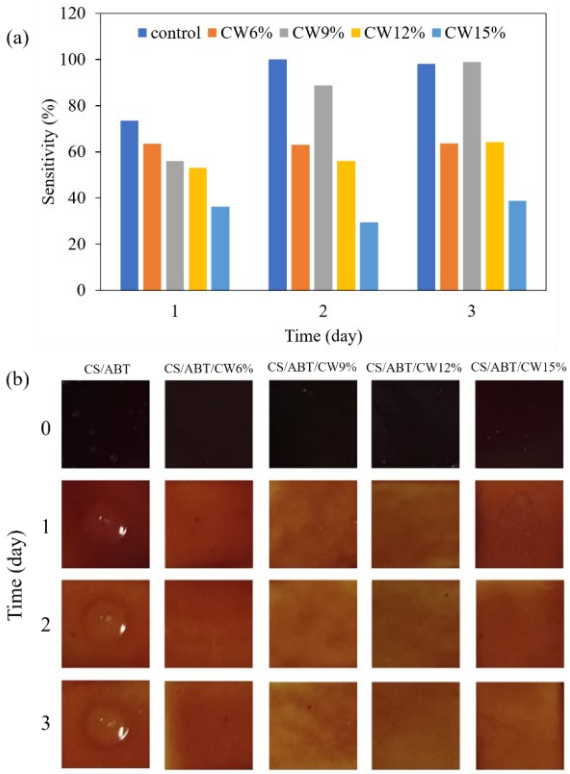

Figure 6. (a) Sensitivity of indicator films to ammonia vapor over time and (b) visual color changes of indicator films exposed to ammonia solution

The analysis results are presented as Mean RGB values, which were then used to calculate the sensitivity of the indicator films. Figure 6 illustrates the sensitivity of the films to ammonia vapor over a 3-day exposure period. During the first 24 hours, the sensitivity of the films decreased with increasing carnauba wax content. On the second and third days, sensitivity values fluctuated; however, the CS/ABT film consistently exhibited the highest sensitivity across all three days. In contrast, the film containing the highest concentration of carnauba wax demonstrated the lowest sensitivity throughout the testing period.

These findings confirm that the CS/ABT film, without carnauba wax, exhibited the highest sensitivity to ammonia vapor. The sensitivity analysis was conducted to assess whether carnauba wax affects the film's ability to detect chicken meat freshness through ammonia exposure. Although the addition of carnauba wax slightly reduced the film's sensitivity, all variations still showed clear color changes, indicating their suitability for freshness monitoring. Thus, while carnauba wax primarily enhances mechanical properties and water resistance, it does not significantly compromise the film's effectiveness as an ammonia-sensitive indicator.

3.4. Indicator Film Application to Monitor Chicken Meat Freshness

The prepared and characterized chitosan/anthocyanin/carnauba wax indicator film was subsequently applied to monitor the freshness of chicken meat during storage. Freshness assessment was based on the TVBN content of the chicken meat and the ΔE value of the indicator film. While TVBN content serves as an

objective measure of the meat's freshness, the ΔE value reflects the indicator film's sensitivity in detecting freshness changes. The results of TVBN and pH measurements of the chicken meat during storage are presented in Figure 7.

Chicken meat is considered fresh when its TVBN content does not exceed 25 mg/100 g, as higher values indicate the accumulation of nitrogenous compounds resulting from microbial and enzymatic degradation [16]. As shown in Figure 7, the TVBN content increased gradually with storage time, reflecting ongoing spoilage processes. Initially, the TVBN level was 2.24 mg/100 g, indicating that the chicken was still fresh. After 12 hours, the value rose to 9.80 mg/100 g, and further increased to 19.61 mg/100 g at 24 hours, still within the acceptable freshness range but approaching the threshold. By 30 hours, the TVBN content reached 37.54 mg/100 g, surpassing the freshness limit and indicating that the chicken had undergone significant deterioration and was considered spoiled. These findings confirm that TVBN is a reliable chemical indicator of freshness loss in chicken meat during storage.

The decline in chicken freshness was also reflected in changes in pH values. According to SNI 3924-2009, fresh chicken typically exhibits a pH range between 6.0 and 7.0 [29]. In this study, the initial pH was measured at 6.0, confirming the meat's freshness. After 12 to 24 hours of storage, the pH increased to 7.0, and further rose to 8.0 by 30 hours, indicating spoilage. This pH elevation is attributed to the microbial degradation of proteins, which produces volatile basic nitrogen compounds such as trimethylamine (TMA), ammonia (NH_3), and dimethylamine (DMA) [28].

The pH shift was effectively detected by the indicator film through visible color changes, as illustrated in Figure 8. Initially, the film appeared dark brown and remained stable until the 12th hour, although it exhibited slight moisture absorption and a damp texture. At 24 hours, the film began to transition to a green hue, signaling the onset of spoilage. By 30 hours, the green coloration became more intense, clearly indicating advanced spoilage of the chicken meat. These visual changes confirm the film's potential as a practical freshness indicator responsive to pH fluctuations associated with meat deterioration.

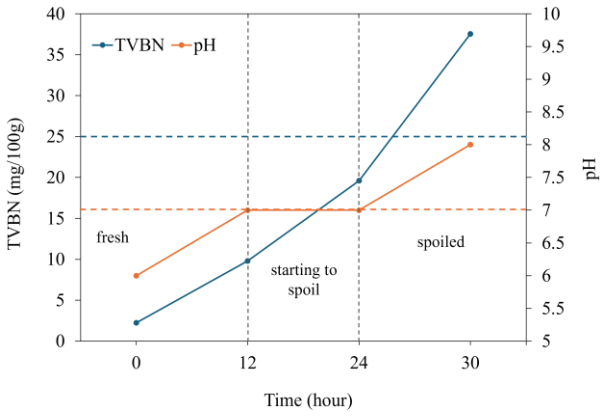

Figure 7. Changes in TVBN content and pH levels of chicken meat during storage

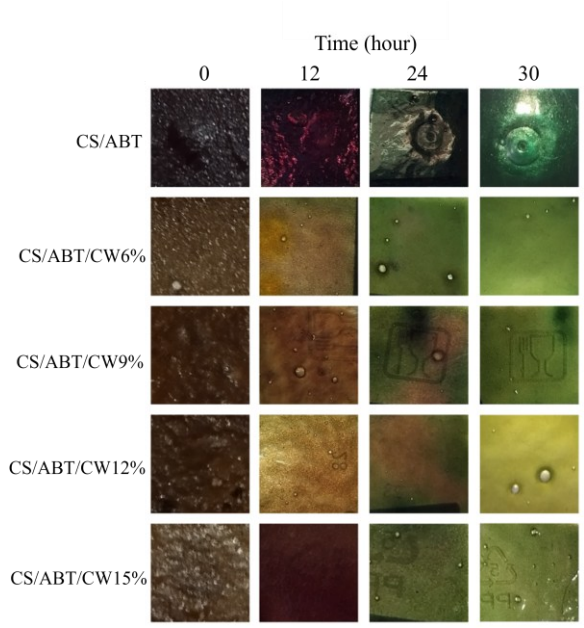

Figure 8. Color change of the chitosan/anthocyanin/carnauba wax indicator film during chicken meat storage

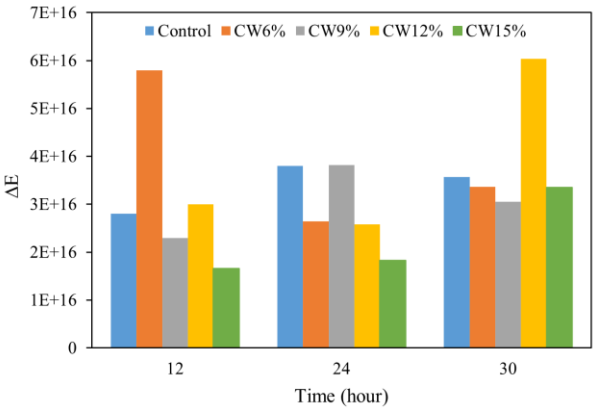

Figure 9. The ΔE value of the indicator film during chicken meat storage

The color change of the indicator film was also quantified by measuring the ΔE value using a colorimeter application, where a higher ΔE indicates greater sensitivity to color changes. The measurement results are shown in Figure 9. Over the storage period, the ΔE values of the films fluctuated. Within the first 12 hours, the CS/ABT/CW6% film displayed the highest sensitivity, showing the greatest color difference among the tested films.

At 24 hours, the CS/ABT/CW9% film exhibited a higher ΔE value, while by 30 hours, the CS/ABT/CW12% film showed the most pronounced color change. These fluctuations may be influenced by inconsistencies in ambient lighting during the colorimeter measurements. Despite this, visual observations in Figure 8 confirmed that all films experienced noticeable color changes throughout storage. Therefore, it can be concluded that the indicator films developed in this study are effective for monitoring chicken meat freshness over time. However, the addition of carnauba wax did not

significantly enhance the films' pH sensitivity or overall performance as freshness indicators.

4. Conclusion

Based on the results of this study, it can be concluded that the mechanical properties of the indicator films—including thickness, tensile strength, and percent elongation—increased with the addition of carnauba wax, although these improvements were not statistically significant. The film with 15% carnauba wax (CS/ABT/CW15%) demonstrated the best mechanical performance. Additionally, the water contact angle of the films increased as the concentration of carnauba wax increased, from 32.11° for the film without wax to 71.63° for the film with 15% wax, indicating enhanced water resistance. The chitosan/anthocyanin indicator films containing carnauba wax effectively monitored chicken meat freshness, as shown by the color change from dark brown to green during storage. This color change corresponded with the increasing TVBN content, which indicated spoilage. However, the addition of carnauba wax did not significantly enhance the film's sensitivity or performance as a freshness indicator. Future research could explore other hydrophobic materials to improve the sensitivity and stability of the indicator films. Furthermore, combining anthocyanins with other natural dyes may broaden the color change range, improving the films' responsiveness. Optimizing the film production process—such as applying ultrasonication or using dual surfactants—could also promote better additive dispersion and enhance film properties.

Acknowledgement

We would like to express our sincere gratitude to the Directorate General of Higher Education, Research, and Technology, Ministry of Education, Culture, Research, and Technology for funding this research through the Budget Implementation List (DIPA) of the Directorate of Research, Technology, and Community Service, Directorate General of Higher Education, Research, and Technology for the 2024 fiscal year, under SP DIPA-070/E5/PG.02.00.PL/2024 dated June 11, 2024. This funding was channeled through the Budget Implementation Document (DPA) of the Institute for Research and Community Service (LPPM), Semarang State University (UNNES), under DPA Number 023.17.2.690645/2024.10. The fund recipient is F. Widhi Mahatmanti.

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