

Colorimetric Intelligent Film Based on Carboxymethyl Cellulose/Sodium Alginate Incorporated Black Rice Bran Anthocyanins Co-Pigments for Shrimp Spoilage Monitoring

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Abstract

Colorimetric intelligent film for monitoring shrimp spoilage based on carboxymethyl cellulose (CMC)/sodium alginate (SA) and co-anthocyanin extract derived from natural source was fabricated. Juedanor (JD) rice, a black-purple variety cultivated and consumed in Pattani province, Southern Thailand, served as the primary source of anthocyanin in this study. Consequently, utilizing anthocyanin pigments from JD and other plants in indicator films for the detection of food deterioration is an effective approach. Mulberry (M) showed the highest anthocyanin content at 341.275 mg/L, followed by 170.730 mg/L in purple cabbage (PC) and 136.955 mg/L in JD rice. The intelligent films were then fabricated using CMC/SA loading with co-anthocyanin pigment (PC: JD) at a 1:4 ratio. The film incorporating JD extract and purple cabbage established the widespread colour change during pH sensitive test. Further tests were conducted on the film using an ammonia solution to assess shrimp spoilage. Film color (%S, ΔE) and pH showed a high association, suggesting that the films can be used as sensitive markers of shrimp freshness during storage time. The predicted ΔE values closely match the experimental values over the ammonia exposure duration, with a high R^2 coefficient of 0.985, which signifies a robust association. This study demonstrates the potential of utilizing anthocyanins from the indigenous JD rice variety for intelligent packaging and future food production. The value assigned to this native rice will aid in its utilization, conservation, and additional benefit to the community.

Keywords

Intelligent Packaging, pH-Sensitive, Juedanor Rice, Anthocyanin, Shrimp Spoilage, Carboxymethyl Cellulose (CMC)

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1. INTRODUCTION

The safety and the preservation of food quality are critical global concerns. The proliferation of chemical processes in microorganisms, such as lipid oxidation, along with physical alterations during storage, has raised concerns for human health and contributed to increased global food waste. Consequently, the regulation of food quality control and assessment throughout the supply chain has been established. The conventional approaches, like microbiological and laboratory chemical assays, yield precise results but they are labor-intensive, time-consuming, expensive, and unsuitable for real-time assessment (Ferone et al., 2020). In order to overcome this limitation, the intelligent packaging has been established to access and direct communicate food conditions to consumers (Xiong et al., 2024). Unlike conventional packaging, intelligent system gives information regarding the quality status of contained food by reflecting the surrounding environmental conditions of the package. Among other intelligent packaging, colorimetric in-

dicator films have attracted attention from researchers as a result of their simplicity, low cost, and capacity to provide direct visual information regarding food spoilage (Yang et al., 2025). Biopolymers, such as cellulose derivatives, chitosan (Roy and Rhim, 2020), gelatin, modified starch, polylactic acid (PLA) (Sobhan et al., 2022), and carboxymethyl cellulose (CMC)/sodium alginate (SA) (Yang et al., 2022), are mostly utilized as films matrix due to their sustainable properties beneficial to consumers and environment. In addition, natural pigments, particularly anthocyanins and its co-pigments, have been used as prospective pH-sensitive dye due to their exceptional colour-changing characteristics in response to pH variations (Welch et al., 2008; Yong and Liu, 2020).

Several studies have investigated the development of anthocyanin -based indicator films employing various biopolymers for food packaging applications. Intelligent indicator films utilizing nanocellulose derived from microorganisms and anthocyanins extracted from black carrots have been employed

for the freshness monitoring of fish fillets (Moradi et al., 2019). The integration of anthocyanins into chitosan and starch films markedly enhanced the antioxidant activity of the films, while also exhibiting discernible colour changes in reaction to pH variations linked to food degradation. The films effectively tracked the freshness of protein-rich samples, such as fish and meat products, by altering colour from reddish-purple in acidic to dark blue or black purple in alkaline environments within food packaging (Li et al., 2024). The incorporation of anthocyanins into various polymer matrices, such as gelatin, alginate, and cellulose derivatives, has been examined. The results demonstrated that polymer type significantly affected colour stability, mechanical properties, and environmental reactivity. Gelatin-based films exhibited notable flexibility and transparency, whereas cellulose-based films improved stability during storage time (Remedio and Parada Quinayá, 2024). Chitosan/PVA sensor films impregnated with roselle anthocyanins have been developed for the real-time visual assessment of chicken and shrimp freshness under various storage conditions. The anthocyanins were extracted by maceration of fresh roselle flowers using 75% (v/v) ethanol with a solid-to-solvent ratio of 1:10 at ambient temperature for 1–5 days. The polymer matrix was composed of chitosan and polyvinyl alcohol (PVA). Among the films examined, the Cs/PVA with 5-day roselle anthocyanins extraction showed the best visual clarity and responsiveness. As a result of structural changes in anthocyanins, the film displayed clear colour transitions across a wide pH range, and it effectively tracked changes in shrimp and chicken freshness over both ambient and chilled storage. These results show that Cs/PVA-R5 anthocyanin films could be used as smart packaging materials for real-time food freshness monitoring in an eco-friendly way (Hidayaty et al., 2026).

Anthocyanins are chemicals classified in the flavonoid group. Its water solubility is dependent upon variations in acid-base conditions. Anthocyanins exhibit colour alterations due to the cationic resonance of flavillium ions, resulting in pH-dependent changes in their characteristics (Rosalina et al., 2022). Anthocyanins are abundant in tropical flora such as mulberry, purple cabbage, sweet potato, dragon fruit, red banana, hibiscus, rosella flower including several types of black rice. Juedanor rice (JD) is an indigenous cultivar from Pattani province, Southern of Thailand, derived from eggplant rice and black glutinous rice. This is a unique new bleeding cultivar derived from local agricultural knowledge that has not yet received formal registration. This cultivar is authorized for eating and advised for cultivation across the region. The JD rice is regarded as an indigenous variety well adapted to the region climatic and resilience to illnesses and pests. The seed contained a diminutive grain with a black-purple pericarp, therefore, rich in anthocyanins, minerals, and phytochemicals as depicted in Figure 1.

Furthermore, several studies have shown that co-pigment markedly improves the pH range and sensitivity in contrast to individual anthocyanins. Prior research demonstrates that the combination of natural anthocyanins with phycocyanin,



Figure 1. Juedanor Rice Variety Harvested from the Growing Area in Pattani Province, Southern of Thailand

curcumin, oxalic acid, gallic acid, ferulic acid, and caffeic acid yields a wider range of color variations than a single dye. (Tavakoli et al., 2023; Huang et al., 2023; Kwak and Min, 2024; Chen et al., 2022; Hu et al., 2025). All the findings highlight the significance of co-pigmentation in enhancing the functional attributes of anthocyanins for practical applications in food freshness monitoring (Li et al., 2024; Ghoshal, 2018). Therefore, this work aims to develop innovative pH-sensitive intelligent films incorporating JD anthocyanin extract and other natural anthocyanin sources within a carboxymethyl cellulose (CMC)/sodium alginate (SA) biopolymer matrix. The film's physical properties and colour alterations due to exposure to different pH levels were evaluated. The effectiveness of the generated films for tracking the shrimp's deterioration during storage was also assessed.

2. EXPERIMENTAL SECTION

2.1 Sample Preparation

This investigation utilized six distinct samples; JD rice, mulberry fruit (M), purple cabbage (PC), butterfly pea flowers (BP), rose petals (R), and wakame seaweed (W). All samples were dried using a hot air oven at 50 °C (Kerbstadt et al., 2015), since moisture hinders extraction and can damage anthocyanins. Afterwards, the samples were blended into a fine powder using an appropriate grinder and kept in a desiccator for further analysis.

2.2 Plant Sample Extraction

The dried powdered sample was precisely weighed at 10.00 g. Fifty milliliters of a 50% (v/v) ethanol solution was added to each sample. Due to the capacity of ethanol to dissolve medium to high components, 50% ethanol was used to dissolve anthocyanin pigments for this study. The combination was macerated for 3 hours at 45 °C and 130 rpm in a shaking incubator. Then, the mixture was filtered using Whatman No. 4 filter paper. The extraction was repeated twice with 50 mL of 50% ethanol to extract the plant's residue. The extracted solvent was further concentrated using a rotary evaporator set to 130 rpm and 45 °C. The crude extract was weighed for determining the extraction yield, followed by the analysis of total anthocyanins in the sample. Finally, to prepare the concentrated extract for

subsequent analysis, the crude extract was diluted to 10% (v/v) with 50% ethanol.

2.3 Anthocyanin Content Using pH-Differential Method

The total amount of natural anthocyanin in crude extracts of Juedanor rice, mulberry fruit, purple cabbage, butterfly pea flowers, rose petals, and wakame seaweed was measured using the pH-differential method. Two buffers were prepared for this method. HCl was used to change the pH of the acidic buffer (0.025 M KCl) to 1.0, whereas acetic acid was used to adjust the pH of the control buffer (0.4 M sodium acetate) to 4.5. After diluting each extract with the two buffers at a ratio of 1:9, absorbance at 520 nm and 700 nm was measured (Teng et al., 2020).

2.4 Evaluating the pH Sensitivity of Anthocyanin Extracts

To determine how the anthocyanin extracts react to different pH levels, a range of buffer solutions from 1 to 12 was then prepared. These solutions were added to each extract in different ratios ranging from 1:1, 1:2, 1:3, 1:6, 1:10, and 1:20 to visualize the colour changes across the pH range. In addition, fresh solutions ranging in pH from 4 to 9 were made, and their absorbance was assessed using a UV-Vis spectrophotometer throughout a wavelength range of 380 to 780 nm. Thus, a UV-Vis spectrum was carried out for the λ_{max} evaluation (Estévez et al., 2021).

2.5 Preparation of Intelligent Films

Four film formulations were developed, as presented in Table 1. Following the approach described earlier (Yang et al., 2022), the films were prepared by combining 0.6 g of sodium alginate and 0.32 g of sodium carboxymethyl cellulose into 100 mL of distilled water under mechanical agitation until complete dissolution occurred. Subsequently, 0.2% (w/v) glycerol and 10% of each plant anthocyanin were added. The 40 mL film-forming solution was poured into petri dishes and dried in a hot air oven at 40 °C until dryness. The dried films were removed from the plates and stored at a constant temperature for further analysis.

Table 1. Composition of Intelligent Film Formulations Prepared in This Study

Formulation	Composition
1	Water + Glycerol + CMC + SA
2	Water + Glycerol + CMC + SA + JD
3	Water + Glycerol + CMC + SA + JD + PC
4	Water + Glycerol + CMC + SA + JD + BP

CMC = Carboxymethyl cellulose, SA = Sodium alginate, JD = JD extract, PC = Purple Cabbage extract, BP = Butterfly Pea extract.

2.6 Physical Properties of Intelligent Films

The film was weighed using an analytical balance. A thickness gauge was used to measure the film thickness at three distinct

positions on the film. The film moisture content was performed by cutting the film into 2×2 cm pieces and weighing before and after drying in a hot air oven at 105 °C until a steady weight. Moisture content was calculated using Equation 1.

MC (%) = (initial weight – final weight) / initial weight × 100 (1)

For water solubility, the samples were shaken for 24 hours while submerged in 100 mL of distilled water. The films were carefully removed and dried in a hot air oven at 105 °C until the weight obtained was steady. The water solubility of the film was determined using Equation 2.

WS (%) = (initial weight – final weight) / initial weight × 100 (2)

The Fourier Transform Infrared Spectroscopy (FTIR) was used to examine the film’s functional groups. Film samples were analyses using a 4 cm⁻¹ resolution scan in the 4000–400 cm⁻¹ range, as outlined in the approach of previous study (Ma et al., 2020).

The film’s surface morphology was investigated using a Scanning Electron Microscope (SEM). Before imaging, the film was covered with a gold coating. A magnification of 4,000× was used to picture the film’s center surface, while a magnification of 10,000× was used to capture the edge surface.

2.7 Colour Change of Intelligent Films in Ammonia Solution

A 1×1 cm square of each of the intelligent films was cut. A container lid served as a mounting point for each film sample. With the film spaced about 1 cm from the surface of the solution, 200 mL of a 0.002 M ammonia solution was added to the container. The film colour was recorded and shot four times in twenty minutes. To determine the film colour, image analysis software or a colorimeter was used to measure the RGB (Red, Green, Blue) values (Prietto et al., 2017; Yong and Liu, 2020). The following calculations were utilized to determine the colour change sensitivity (%S) and cumulative colour difference (ΔE) as described in Equations 3 and 4, respectively.

%S = (R_i – R_f) + (G_i – G_f) + (B_i – B_f) / (R_i + G_i + B_i) (3)

where: R_i, G_i, and B_i are the initial RGB values of the film, and R_f, G_f, and B_f are the final RGB values of the film after 20 minutes of exposure to ammonia.

ΔE = √(L* – L₀^{*})² + (a* – a₀^{*})² + (b* – b₀^{*})² (4)

where: L*, a*, and b* are the colour values of the film at a given time, L₀^{*}, a₀^{*}, and b₀^{*} are the initial colour values of the film. The colour alteration model was established by further analyzing the obtained ΔE values.

2.8 Colour Change of Intelligent Films for Shrimp Freshness Monitoring

The intelligent films were cut into 1×1 cm squares. A container lid served as a mounting point for each film sample. In every container, 100 grams of shrimp were placed then the container were put in the refrigerator at 4°C (aerobic conditions). The film's colour was captured and tracked for 7 days. It is important to note that the colour measurements were conducted at 6, 12, 18, and 24 hours on the initial day. Subsequently, measures were documented daily from Day2 to Day7. The cumulative colour difference (δE) over time was calculated using Equation 4, to validate the visual observation. The acquired δE values were further analyzed to develop the color modification model.

3. RESULTS AND DISCUSSION

3.1 Extraction Yield and Total Anthocyanin Content from Different Plants

The results showed significant differences in the extraction yield amongst the various plants. A high extraction yield of 58.09% was obtained in butterfly pea (BP) followed by mulberry (M) exhibited a yield of 54.57%, rose (R) at 36.154%, purple cabbage (PC) at 22.17%, wakame seaweed (W) at 13.34%, and JD at 4.44% which exhibits the minimal extraction yield among all samples. The findings indicate that mulberry had the highest total anthocyanin concentration at 341.28 mg/L, followed by purple cabbage at 170.730 mg/L, as depicted in Table 2. These differences in anthocyanin concentration can be related to the variation in plant matrix composition and pigment distribution among the sources. Previous research has indicated that combination of ethanol and water is the most effective solvent for extracting anthocyanins from berry plants while preserving their antioxidant properties (Roy and Rhim, 2021). Thus, choosing the suitable solvent is essential for optimizing anthocyanin extraction while maintaining the properties and quality of the compounds. Table 2 shows anthocyanin concentration of the plants used, thus, affirming the antioxidant properties of all plant sources, as anthocyanins are acknowledged for their antioxidant efficacy. No anthocyanins were found in wakame (W) (*Undaria pinnatifida*) seaweed, which is consistent with prior report. The main pigment extracted from wakame was blue-green pigments so-called phycocyanin. Phycocyanin is a pigment-protein complex that gives blue color due to its absorption of red to orange visible light wavelengths (Thevarajah et al., 2024). It was successfully employed as a co-anthocyanins pigment to broaden the pH range of intelligent film for food monitoring (Tavakoli et al., 2023). The concentration of anthocyanins in butterfly pea flowers and roses is lower even though the extraction yield is relatively high. It was demonstrated that extraction yield is not correlated with anthocyanin content. The 50% ethanol-water solvent used in this work can efficiently extract anthocyanins while simultaneously extracting other substances such as sugars, tannins, organic acids, and polysaccharides. Furthermore, tannins and polysaccharides in plant tissues may interact with anthocyanins or block the release of anthocyanins from cells which can cause

a decrease in the concentration of anthocyanins. The purpose of this study was to compare the total anthocyanin content of different plant extracts and evaluate their potential for intelligent film development. Therefore, the pH-differential method was selected as a rapid, reliable, and widely recognized in the evaluation of total monomeric anthocyanins and relies on the reversible structural change of anthocyanins under different pH conditions (Giusti and Wrolstad, 2001). However, the analysis of individual anthocyanins in plant sources using advanced separation techniques such as HPLC-DAD or LC-MS is necessary to confirm the specific anthocyanin species in the plant extract that affect the performance of the intelligent film production (Welch et al., 2008; Enaru et al., 2021).

3.2 Optimizing Anthocyanin Extracts for pH-Sensitive Colorimetric Indicators

This study examined the optimal conditions for measuring pH using plant extracts. It was found that the most visible changes in color occurred across a broad pH range (1.0 to 12.0) when a ratio of 20:1 of pH solution to plant material was utilized, particularly for PC and BP. The color changes were significantly enhanced when PC or BP was combined with JD extract in a 1:4 ratio, as illustrated in Figures 2a-2e. This indicates that a combination of various anthocyanin pigments functions synergistically to enhance the indicator's sensitivity. These results are crucial for the development of intelligent food packaging. These indicators contain the capacity to enhance food safety and reduce food waste by offering consumers a clear visual cue. This study highlights the necessity of modifying the pH solution-to-plant extract ratio in the creation of effective colorimetric indicators. Furthermore, it demonstrates the ability to combine various plant extracts to improve the sensitivity and perception of these indicators. Since the environment in food packaging changes from weakly acidic to weakly basic, the mixed anthocyanin of JD rice extract to BP and PC extract at pH levels ranging from 4 to 9 were subjected to further examination using UV-Vis spectrophotometry. The UV-Vis spectrophotometry analysis provides significant evidence for the pH-responsive properties of the combined extracts, confirming the observed color shifts and substantiating their suitability as colorimetric indicators in intelligent food packaging applications, as illustrated in Figures 3a-3d. Anthocyanins rapidly altered their color across pH values, transitioning from red to blue as the solution shifted from acidic to alkaline. This resulted from the structural transformation between flavylium cations (red) and quinonoidal bases (blue). The UV-Vis absorption spectra revealing an isosbestic point that illustrates the interconversion of the two forms. When NaOH was added, the absorption band at 528 nm, associated with flavylium cations, rapidly decreased, whilst the band at 607 nm, related to quinonoidal bases, intensified. The introduction of hydroxide ions (OH^-) causes flavylium cations to deprotonate, resulting in the formation of quinonoidal bases and a subsequent color change (Tang et al., 2019). The pH sensitivity of six plant extracts against various pH levels indi-

Table 2. Extraction Yield and Anthocyanin Content of Different Plant Sources Used

Plant Source	Extraction Yield (%)	Anthocyanin Content (mg/L)
Juedanor Rice (JD)	4.44	136.96 ± 0.01
Purple Cabbage (PC)	22.17	170.73 ± 0.02
Butterfly Pea (BP)	58.09	105.41 ± 0.01
Rose (R)	36.15	64.58 ± 0.01
Mulberry (M)	54.57	341.28 ± 0.01
Wakame Seaweed (W)	13.34	Not Detected

cated that the color change for each extract was not significantly different. Research studied by [Tavakoli et al. \(2023\)](#) indicated that the combination of multiple plant extracts increased sensitivity to color alteration. Figure 2a demonstrates that the combination of anthocyanins from JD rice with PC extract at a ratio of 1:4 results in varying visual colors of the solution across pH levels 4 to 12. Consequently, this ratio was selected and utilized for subsequent analysis.

The absorbance value is assessed at a wavelength range of 380 to 780 nm to verify the color variation of the mixed anthocyanin solution at pH levels ranging from 4 to 9. The variation in λ_{max} indicates the colors differ. The spectrum shifts in Figures 3a to 3d illustrate that pH substantially influences the electronic configuration of the pigments. Bathochromic shifts (red shifts) illustrated in Figures 3a and 3b signify enhanced conjugation and stabilization of chromatic species at intermediate pH, while hypsochromic shifts (blue shifts) depicted in Figures 3c and 3d imply chromophore breakdown and elimination at elevated pH levels ([Welch et al., 2008](#); [Enaru et al., 2021](#)). The results indicate that pigment stability and optical characteristics are significantly affected by protonation state and molecular environment, which is critical for applications involving pH-sensitive colorimetric materials and active packaging systems ([Cai et al., 2022](#)).

3.3 Physicochemical Properties of Anthocyanin-Loaded Intelligent Films

This study evaluated the physical properties of four different intelligent films designed to change color in response to pH variations. The films included a control film with sodium alginate (SA), a film incorporated with JD, a film incorporated with JD and purple cabbage (JD+PC), and a film loaded with JD and butterfly pea extract (JD+BP). The analysis of the films revealed that the JD film had the greatest weight (0.6112 g), while the JD+BP film demonstrated the highest thickness (0.0817 mm). The JD+PC film exhibited the highest moisture content (50.79%), obviously explained by the hydrophilic characteristics of the contained anthocyanins. The JD film exhibited the maximum water solubility at 99.60%, signifying its quick dissolution in water. Table 3 demonstrates that the films with plant extracts (JD, JD+PC, and JD+BP) displayed a slightly acidic pH, varying from 6.529 to 6.743 upon dissolution. This differed from the SA control film, which exhibited a normal pH of 7.160. The results indicate that the inclusion of anthocyanin-rich plant

extracts significantly influences the physical properties of the films, such as weight, thickness, moisture retention, water solubility, and pH. This information may be employed to alter intelligent films with specific properties for particular applications, notably in food packaging, where these characteristics are vital to preserving food quality and freshness. The findings align with other reports indicating that anthocyanin can alter film thickness, moisture content, and water solubility by changing intermolecular interactions within a polymer matrix. The incorporation of butterfly pea extract into a chitosan/PVA film increased film thickness and changed the structural properties of the polymer matrix ([Phanumong, 2021](#)). Additionally, anthocyanins extracted from purple cabbage have been shown to enhance the thickness and water solubility of chitosan-starch films by adding hydrophilic molecules rich in hydroxyl groups into the film matrix ([Pawde et al., 2026](#)). This study’s results indicate that the physicochemical properties of intelligent films are substantially affected by the composition of the incorporated extracts and their interactions with the polymer matrix.

The FTIR spectra of the sample films display notable peak shifts and intensity variations, confirming the existence of intermolecular interactions among the film components, as depicted in Figures 4a and 4b. Key alterations should be determined in the hydroxyl (O–H), carbonyl (C=O), and polysaccharide fingerprint regions, which suggesting hydrogen bonding and chemical compatibility within the film matrix. The absorption band detected at 3272 cm^{-1} is corresponds to O–H stretching vibrations. This peak broadens and shifts slightly toward lower wavenumbers in modified films compared to the control film. The red shift (bathochromic shift) indicates stronger hydrogen bonding interactions between the hydroxyl groups of the polymer chains and the anthocyanins extract. A signal at 2919 cm^{-1} is attributed to C–H stretching vibrations. Minor shifts and intensity changes in the C–H stretching bands indicate alterations in the molecular environment of aliphatic groups. Suggest that the plant extract was successfully incorporated into the polymer matrix. The absorbance peak ranging from 1650 to 1550 cm^{-1} is corresponds to the symmetric and asymmetric stretching vibrations of the carboxylic group in CMC ([Hosseini and Jafari, 2020](#)). This confirms molecular interactions between the film compositions and higher degree of compatibility among the components. Peaks in the range 1637–1400 cm^{-1} , exhibiting multiple shoulders, may correspond to the presence of aro-

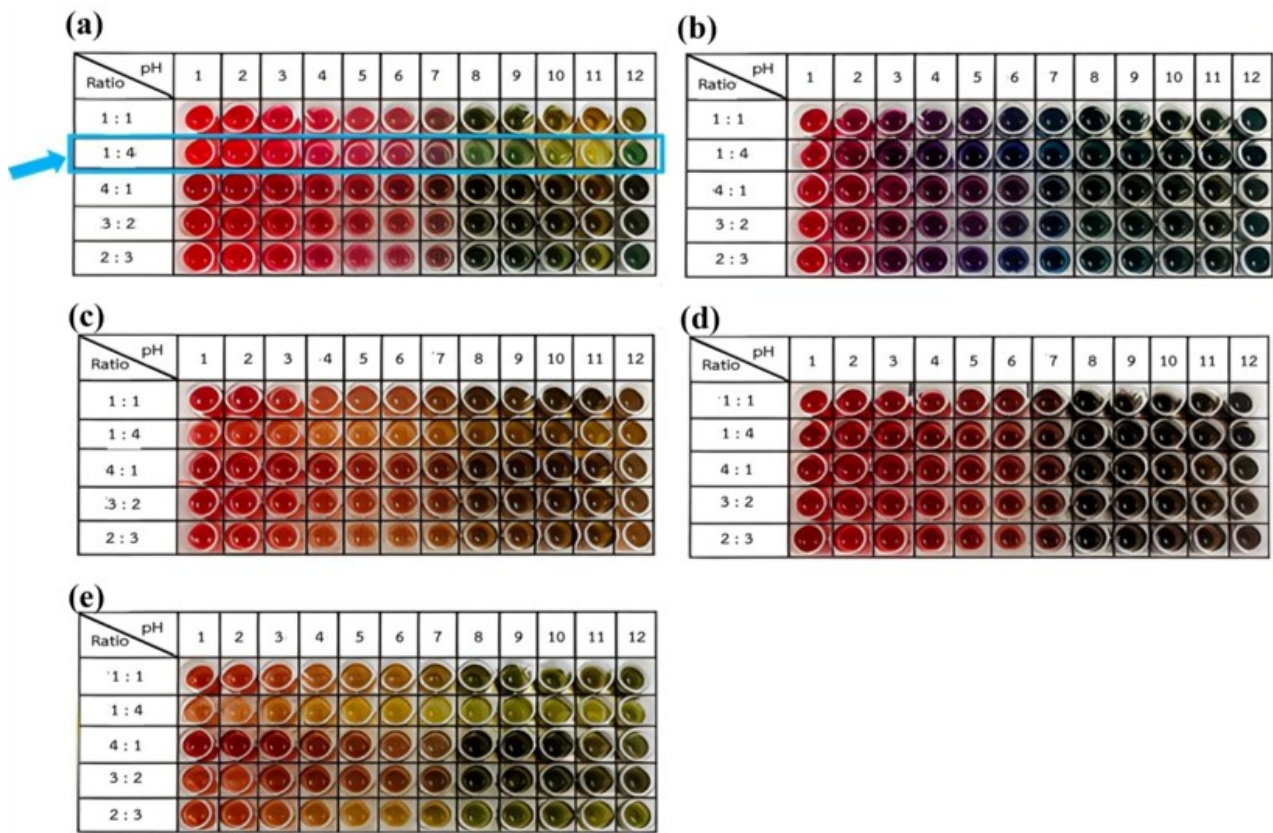


Figure 2. Color Changes when JD Anthocyanin was Combined with the Other Sources in a 1:4 Ratio. (a) JD Mixed PC, (b) JD Mixed BP, (c) JD Mixed R, (d) JD Mixed M, (e) JD Mixed W

Table 3. Physical Properties of Intelligent Films Developed in the Study

Formulation	Weight (g)	Thickness (mm)	%MC	%WS	pH of Film Solution
S.A	0.5263 ± 0.0003	0.0517 ± 0.0029	41.97	94.84	7.160 ± 0.0026
JD	0.6112 ± 0.0002	0.0783 ± 0.0076	39.95	99.60	6.536 ± 0.0017
JD + PC	0.5966 ± 0.0004	0.0583 ± 0.0028	50.79	95.16	6.743 ± 0.0010
JD + BP	0.5349 ± 0.0001	0.0817 ± 0.0104	44.87	88.66	6.529 ± 0.0020

matic groups linked to anthocyanins (Yang et al., 2015). The FTIR peak shifts indicate intermolecular interactions, especially hydrogen bonding among the film components. These interactions possibly enhance structural cohesiveness and effect the mechanical properties (e.g., tensile strength, elongation at break) of the films.

Scanning electron microscopy (SEM) was used to analyses the microstructure of the films, which showed clearly that there were differences in surface morphology and edge characteristics. SEM analysis demonstrated variations in surface morphology throughout all film formulations. Essentially, intelligent film analysis typically involves the qualitative observation of film morphology and structural uniformity. Consequently, pore size and roughness were not quantitatively evaluated in this study. A mixture of JD+BP was examined with a 4000× magni-

fication. The film’s surface derived from JD rice extract exhibit numerous holes and little fissures, displayed in Figure 5a in comparison to the other two types films. The extract was evenly distributed throughout the film, as shown in Figure 5b, which indicates that the surface is homogeneous and free of visible defects or holes. Regarding the JD+PC extract, the film had a smooth surface, but there were signs of cracking. These cracks were likely caused by stress during sample preparation (Figure 5c). The edge analysis was conducted using a 10,000× magnification. Extract from JD, Figure 5d shows that the film’s edge had an irregular thickness. JD+PC, in comparison to JD, this film’s edge thickness was more consistent (Figure 5e). Figure 5f demonstrates that among the three films, JD+BP exhibited the most uniform thickness at the edges. The mechanical characteristics, barrier properties, and overall performance of the

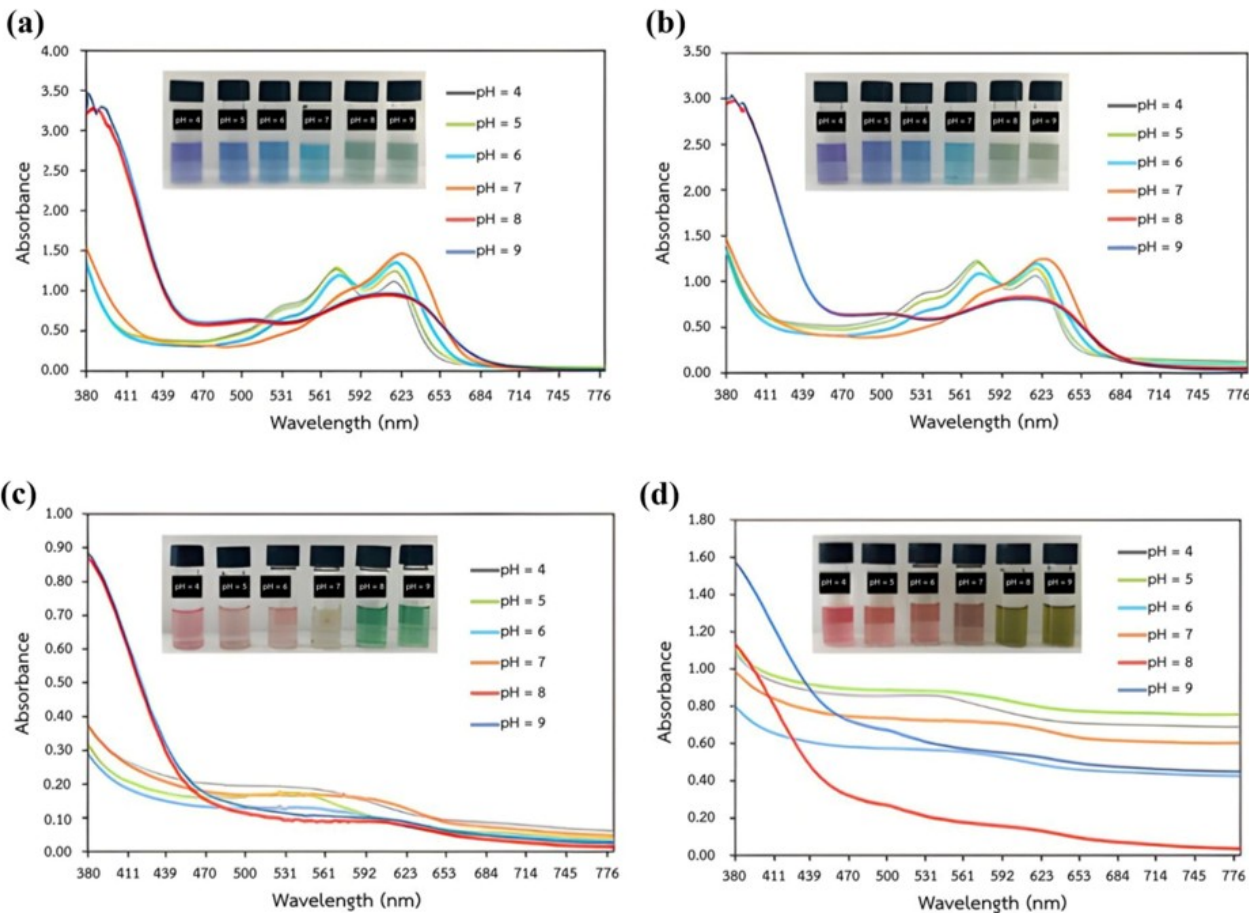


Figure 3. UV-Vis Spectra of (a) BP Extract at pH 4.0 to 9.0, (b) JD rice extract mixed with BP at pH 4.0 to 9.0, (c) PC Extract at pH 4.0 to 9.0, (d) JD rice Extract Mixed with PC at pH 4.0 to 9.0

films in their intended applications can be greatly impacted by these aspects.

3.4 Evaluating the Colour Alteration of Intelligent Films in the Presence of Ammonia and Spoiling Shrimp

An examination of the colour sensitivity of JD mixed PC intelligent films exposed to ammonia solutions was carried out. The RGB values were analyzed with the Colour Tool application, and the average δE and %S values were calculated and reported in Table 4. The investigation revealed that JD mixed PC intelligent films exhibited a favorable response to ammonia.

The film’s time-sensitive colour responses to ammonia vapor were evaluated, as demonstrated by the ΔE and %S values shown in Table 4. This test mimicked food packaging that contained ammonia due to food decomposition. The ΔE (0.00–14.05) and %S (0.00–31.69) values of the intelligent film loaded with JD mixed PC dyes exhibited a pronounced increase in first 4 min of exposure, reflects the fast diffusion and rapid interaction between anthocyanins and ammonia vapor in the film matrix. Subsequently, ΔE gradually increased at 8, 12, and 16 min before reaching a near-plateau at 20 min, showing a weakened response during the longer exposure time,

Table 4. ΔE and %S of JD Combined PC Intelligent Film Responds to Volatile Ammonia

Time (min)	ΔE	S (%)
0	0.00 ± 0.00	0.00 ± 0.00
4	14.05 ± 0.65	31.69 ± 1.85
8	17.15 ± 0.72	36.31 ± 1.94
12	19.96 ± 0.81	41.54 ± 2.13
16	22.85 ± 0.77	32.62 ± 2.06
20	23.44 ± 0.68	30.15 ± 1.88

this indicates a saturation behavior as the equilibrium reaction between anthocyanin molecules and ammonia is achieved. The results demonstrate the strong pH responsiveness of the anthocyanin-loaded film, allowing for the quick detection of ammonia within minutes, hence underscoring its potential application as a food freshness indicator (Yong and Liu, 2020; Alizadeh-Sani et al., 2020).

Additionally, to clarify the change in film colour over time due to ammonia exposure, the ΔE response was modeled using

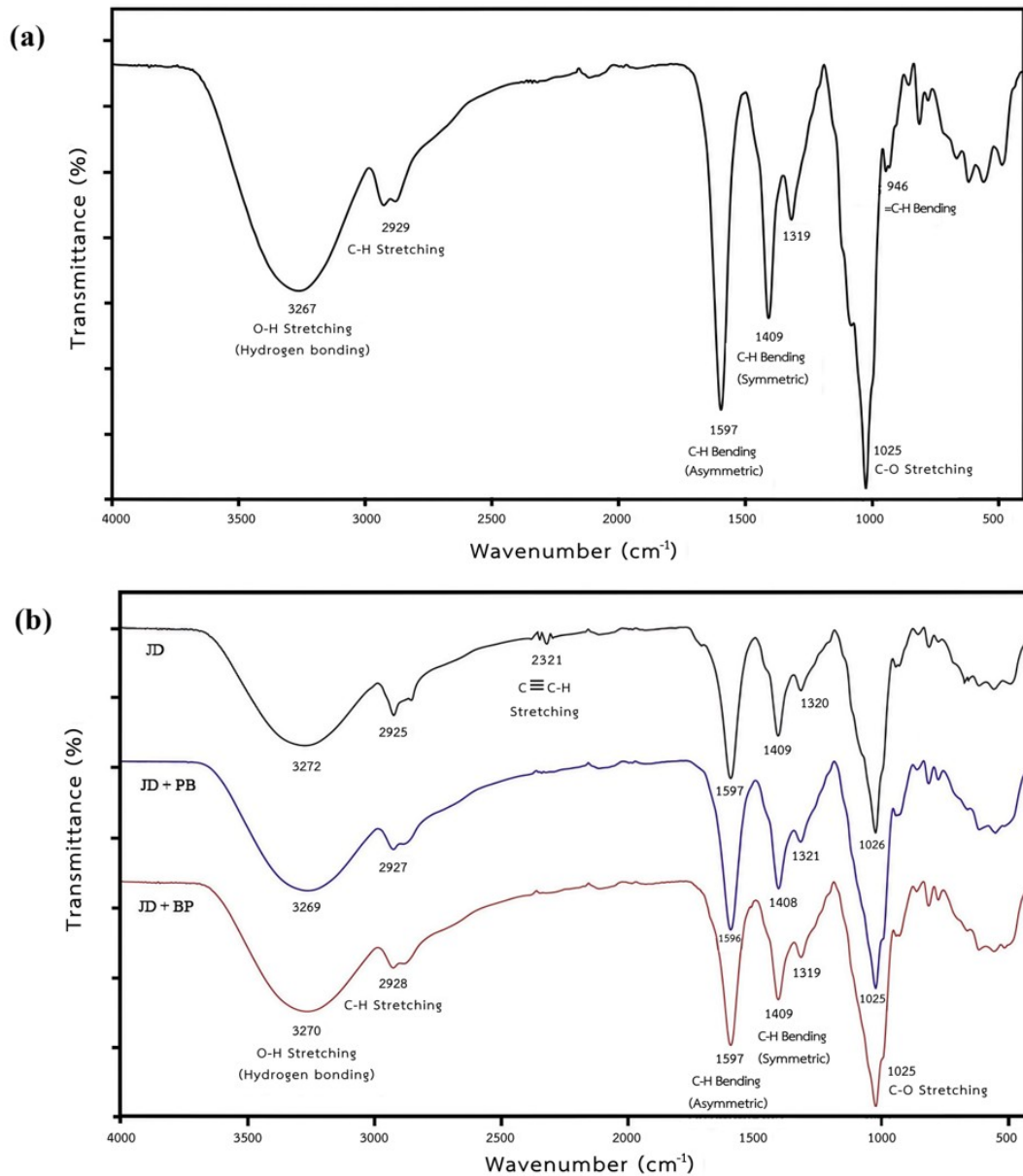


Figure 4. FTIR Spectra of (a) Control Film Containing CMC and SA, (b) Films with Various Anthocyanin Extracts, Which are JD, JD Mixed PC, and JD Mixed BP

a pseudo-first-order equation, as demonstrated in Equation 5.

$$\Delta E_t = \Delta E_{\max} (1 - e^{-kt}) \quad (5)$$

where ΔE_t is the colour difference at time t , $\Delta E_{\max}$ is the maximum colour difference, t is time in minute and k is the apparent rate constant. The fitted parameters yielded a maximum colour difference ($\Delta E_{\max}$) of 23.30 and an apparent rate constant (k) of 0.194 min⁻¹, with a coefficient of determination (R^2) of 0.985, demonstrating excellent agreement between the model and the experimentally observed data.

To evaluate the efficacy of intelligent films as indicators of food spoilage, the JD mixed PC film was exposed to decaying shrimp, and the film's colour changes were monitored during 7 days storage period at 4 °C. Nonetheless, the ammonia concentration was not directly measured in the present study. The observed colour changes most likely reflect the cumulative of volatile decomposition metabolites that accumulated during storage. An increasingly unique and consistent colour change occurred during storage time, demonstrating a gradual accumulation of volatile basic compounds and enhanced contact with the anthocyanin-based indicator film. Table 5 presents

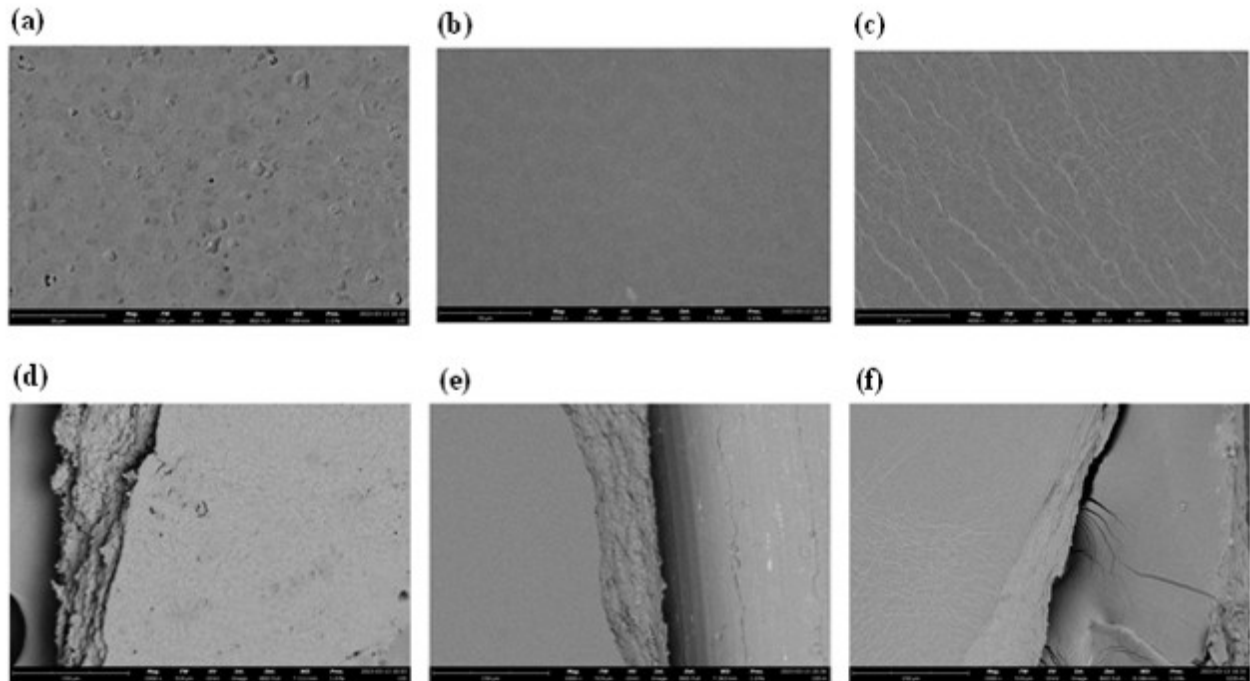


Figure 5. SEM Micrographs of the Central Surface (5a-5c) and Cross-Sectional Edge (5d-5f) of Films Loaded with (a) Central Surface-JD Extract (b) Central Surface -JD Extract with BP (c) Central Surface -JD Extract with PC (d) Cross-Sectional Edge -JD Extract (e) Cross-Sectional Edge -JD Extract with BP (f) Cross-Sectional Edge -JD Extract with PC

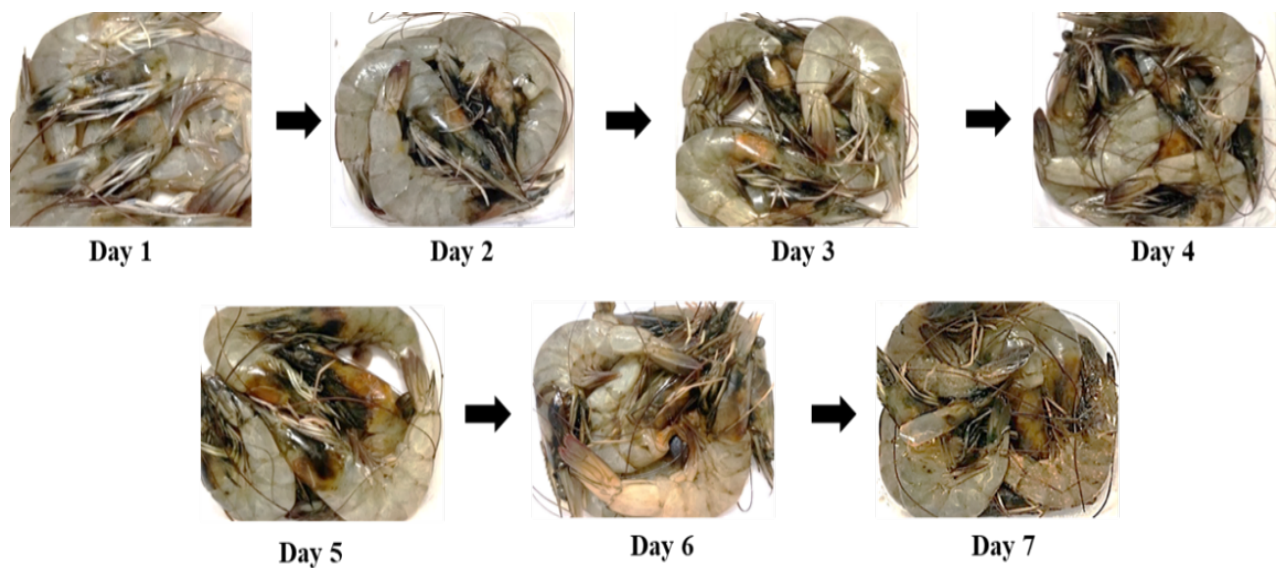


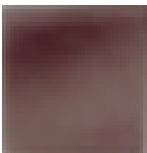
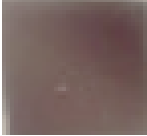
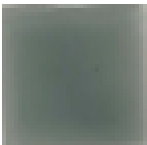
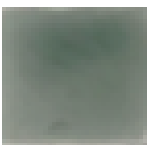
Figure 6. The Decomposition of Shrimp Throughout a 7-Day Storage Period

the ΔE values corresponding to the JD mixed PC film color at various time intervals during the shrimp rotting process, providing a visual record of the observed colour alterations. To improve the accuracy and effectiveness of these films as reliable indicators of shrimp spoilage, adjustments, or optimizations in experimental settings, such as pH calibration, temperature

control, or film formulation, may be necessary.

The colour difference (ΔE) of the JD + PC film increased progressively during shrimp storage, indicating its ability to monitor freshness changes in real time. A noticeable colour change was observed within the first 24 h, with ΔE reaching 6.69, which exceeds the threshold for visual perception. Then,

Table 5. Total Colour Difference (ΔE), the Predicted ΔE and Visual Colour Change of Intelligent Films Exposed to Spoiling Shrimp (7 Days)

Time	ΔE of JD + PC Indicator Film	Predicted ΔE	Film Image
0 hours	0.000	0.000	
6 hours	5.536	1.691	
12 hours	3.919	3.274	
24 hours	6.690	6.144	
2 days	11.149	10.864	
3 days	13.418	14.490	
4 days	15.846	17.276	
5 days	19.677	19.416	
6 days	21.940	21.060	
7 days	22.395	22.324	

ΔE increased steadily from 11.15 on day2 to 22.40 on day7, suggesting the continuous accumulation of volatile basic compounds generated during shrimp spoilage (Liu et al., 2021; Nadi et al., 2023). The rapid increase in ΔE during the early storage period may be attributed to the initial production of ammonia and other volatile amines, while the gradual stabilization after day6 indicates that the film response approached saturation. These results demonstrate that the anthocyanin-based film possesses sufficient sensitivity to provide a visual indication of freshness deterioration throughout refrigerated shrimp storage.

To evaluate the response kinetics of the indicator film during shrimp storage, the ΔE values were fitted using an exponential rise-to-maximum model. The correlation between colour difference and storage duration is expressed by the equation

$$\Delta E_t = 26.51(1 - e^{-0.264t}) \quad (6)$$

where t represents storage duration in days. The model exhibited a strong fit to the experimental data, evidenced by a R^2 value of 0.965, signifying that the colour change adhered to a saturation-type kinetic behavior. The estimated maximum colour difference ($\Delta E_{\max}$) was 26.51, while the rate constant (k) was 0.264 day⁻¹. These results suggest that the anthocyanin-based film responded progressively to the accumulation of volatile spoilage compounds during storage and approached a stable colour state after approximately 6–7 days. The predicted ΔE values exhibited closely match the experimental values over the ammonia exposure period, with a high R^2 coefficient of 0.985 indicating a strong correlation. The results demonstrate that the colour response of the anthocyanin-based film may be well characterized by the resulting kinetic equation.

The spoilage of shrimp in the container in contact with JD mixed PC intelligent film over a period of 0 to 7 days is illustrated in Figure 6. The visual colour changes in shrimp appearance after a storage duration of 7 days. Fresh shrimp on Day1 exhibit a brilliant grayish colour and a translucent appearance, indicating freshness and exhibiting good initial quality. Colour discoloration and a decrease in transparency were progressively seen over the storage duration, starting on Day3. The shrimp's surface developed a darker and more milky appearance, the overall appearance suggested a gradually deterioration. The development of a distinct light orange hue and soften texture by Day5–7 indicated the decomposition process had reached an advanced spoilage. The ΔE values of the film also increase along with the storage time and show consistent with the visual changes, suggesting that the film effectively responded to spoilage-related volatile metabolites. Increased values in ΔE from 11.15 on Day 2 to 22.40 on Day 7 corresponded well with the progressive visual deterioration of the shrimp, illustrating the developed film's capacity to offer a real-time assessment of freshness degradation.

However, the dissolution of the film after six days suggests that its water resistance may be insufficient for prolonged application in high-moisture food packaging systems. The film

modifications using calcium chloride (CaCl₂) which is ionic crosslinking could strengthen the polymer network through the formation of intermolecular ionic bridges and reduce water solubility (Rhim, 2012; Priyadarshi and Rhim, 2020). In addition, reinforcement with nanomaterials or blending with more hydrophobic biopolymers may further enhance the mechanical strength and moisture resistance of the film while maintaining its colorimetric sensing performance (Yavari Maroufi et al., 2021).

Maintaining strict oversight of food quality and safety can help promptly detect problems arising during production or delivery. Co-anthocyanins derived from JD rice and PC demonstrate potential as innovative materials for intelligent food packaging, owing to their colour flexibility and sensitivity to pH changes. The use of co-anthocyanins in films frequently modifies their properties in various ways. The research indicates that co-anthocyanins can be utilized in intelligent packaging to monitor food quality and freshness, hence extending their shelf life (Chen et al., 2023).

4. CONCLUSIONS

The study indicated that anthocyanin-based CMC/SA intelligent films have proven effective for real-time visual monitoring of shrimp freshness. The use of co-pigments of JD rice and PC anthocyanins increased the stability and sensitivity of the film's colour response. The selection of pigment source plays a critical role in intelligent packaging development. The robust correlation between the visual deterioration of shrimp and different colour changes in films underscores the potential of anthocyanin-based indicator as effective tools for monitoring freshness in seafood packaging applications. Furthermore, kinetic modeling providing quantitative evidence of the film's ability to track spoilage progression over time. This study offers significant insights into the production and characteristics of JD rice extract-based intelligent films employing natural anthocyanins, highlighting their considerable potential for food packaging applications.

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