Development of Anthocyanin-Based Intelligent Packaging Film for Real-Time Monitoring of Food Freshness

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Abstract

Conventional plastic-based food packaging can neither reveal the internal quality of the packaged product nor reduce the huge volume of food that is discarded every year because consumers cannot judge freshness from the outside of a pack. In this study, an anthocyanin-based intelligent packaging film was developed by incorporating red cabbage (*Brassica oleracea L. var. capitata f. rubra*) anthocyanin extract (ACE) at four concentrations (0, 5, 10 and 15 % v/v) into a chitosan/polyvinyl alcohol (PVA) film-forming matrix through solution casting. The physicochemical, mechanical, barrier, antioxidant and colour-responsive properties of the resulting films were characterised, and the most sensitive formulation was applied to monitor the freshness of refrigerated shrimp (*Litopenaeus vannamei*) in real time. Fourier-transform infrared (FTIR) spectroscopy confirmed hydrogen bonding between the hydroxyl groups of anthocyanins and the chitosan/PVA backbone, while incorporation of ACE decreased tensile strength from 24.8 ± 1.1 MPa (control) to 15.2 ± 0.8 MPa and increased elongation at break from 18.4 ± 1.6 % to 47.5 ± 2.5 % at the highest loading. The films displayed a clear, reversible colour transition from reddish-pink at pH 2 to yellowish-green at pH 12 and showed high sensitivity to ammonia vapour. During shrimp storage at 4 °C, the film colour shifted progressively from purple-red to yellow-green over 10 days, and this visual change correlated strongly with the rise in total volatile basic nitrogen (TVB-N) from 8.2 to 43.1 mg N/100 g (R^2 = 0.97), successfully flagging the first-grade freshness limit (15 mg N/100 g) around day 3-4 and the spoilage limit (30 mg N/100 g) around day 7-8. These findings demonstrate that the anthocyanin-loaded chitosan/PVA film is a low-cost, biodegradable and visually interpretable tool for real-time, non-destructive monitoring of protein-rich food freshness, with clear potential for smart-packaging applications.

Keywords: anthocyanin; intelligent packaging; pH-sensitive film; colorimetric indicator; chitosan; food freshness monitoring

1. Introduction

Food loss and packaging-related plastic pollution remain two of the most pressing challenges facing the global food system. Conventional petroleum-derived packaging materials are largely non-biodegradable and provide no information about the internal condition of the food they contain, so consumers and retailers must rely on printed expiry dates rather than the real physiological state of the product (Kumar et al., 2020; Flórez et al., 2022). Intelligent packaging addresses this gap by incorporating indicators, sensors or data carriers that communicate directly with the packaged food and its immediate environment, allowing spoilage to be detected visually and non-destructively (Balbinot-Alfaro et al., 2019).

Among the many natural colourants explored for freshness indicators, anthocyanins have attracted particular attention because they are inexpensive, biodegradable, non-toxic and, most importantly, undergo pronounced and reversible colour changes as pH shifts from strongly acidic to alkaline conditions (Khoo et al., 2017; Wallace & Giusti, 2019). Structurally, anthocyanins exist predominantly as the red flavylium cation under acidic conditions, convert to a colourless carbinol pseudobase and blue-purple quinoidal bases at intermediate pH, and finally degrade to yellow chalcones under alkaline conditions (Cai et al., 2022). Because the pH of protein-rich foods such as meat, fish and shrimp rises steadily during spoilage as a result of ammonia and other volatile amines released by microbial and enzymatic activity, this colour-pH relationship can be exploited to build low-cost, naked-eye readable spoilage indicators (Echegaray et al., 2023; Neves et al., 2022).

A large body of recent work has embedded anthocyanins from diverse plant sources - red cabbage, purple sweet potato, roselle, mulberry, black rice, rose petals and others - into biodegradable polymer matrices such as chitosan, starch, polyvinyl alcohol (PVA), gelatin and cellulose derivatives to fabricate colorimetric freshness labels (Ebrahimi Tirtashi et al., 2019; Ge et al., 2020; Huang et al., 2020; Choi et al., 2023). Chitosan is especially attractive as a matrix because its free amino and hydroxyl groups readily form hydrogen bonds and electrostatic interactions with the phenolic hydroxyl groups of anthocyanins, improving pigment retention while imparting

inherent antibacterial activity (Li & Li, 2025; Bakshi et al., 2020). Blending chitosan with PVA further improves film flexibility, transparency and mechanical integrity because the abundant hydroxyl groups of PVA also hydrogen-bond with anthocyanin molecules (Boonmahitthisud et al., 2025; Xu et al., 2025).

Such anthocyanin-loaded films have been successfully applied to monitor the freshness of shrimp, fish, pork, chicken and milk, with colour changes tracking increases in total volatile basic nitrogen (TVB-N), pH and microbial load during storage (Chen et al., 2021; Dodange et al., 2023; Ji et al., 2025; Guo et al., 2025). However, the performance of these films is highly dependent on the anthocyanin source, extraction conditions, polymer matrix composition and pigment loading, which together govern the trade-off between colour sensitivity and mechanical/barrier performance (Huang et al., 2025). Red cabbage anthocyanins are of particular interest for large-scale application because red cabbage is an abundant, low-cost agro-industrial by-product with high anthocyanin content and comparatively good pH stability relative to other floral sources (Erna et al., 2022).

Despite the growing number of studies in this field, systematic reports that couple a full physicochemical and mechanical characterisation of a chitosan/PVA/anthocyanin film with a controlled real-time shrimp-freshness trial, correlated quantitatively against TVB-N, remain comparatively limited. The objective of the present study was therefore (i) to fabricate anthocyanin-loaded chitosan/PVA intelligent films at increasing anthocyanin extract (ACE) concentrations, (ii) to characterise their structural (FTIR), optical (UV-Vis, colorimetry), mechanical, barrier and antioxidant properties, and (iii) to evaluate the ability of the optimised film to monitor shrimp freshness in real time during refrigerated storage.

2. Materials and Methods

2.1 Materials

Fresh red cabbage (*Brassica oleracea L. var. capitata f. rubra*) was purchased from a local market. Chitosan (medium molecular weight, degree of deacetylation ≥ 85 %), polyvinyl alcohol (PVA, Mw 89,000-98,000, 99 % hydrolysed), glycerol, glacial acetic acid, and all buffer salts used for pH-sensitivity testing were of analytical grade. Fresh shrimp (*Litopenaeus vannamei*) used for the

freshness-monitoring trial were purchased on the morning of the experiment and transported to the laboratory under ice within 1 h of purchase.

2.2 Extraction of anthocyanins

Red cabbage leaves were washed, cut into small pieces and freeze-dried, then ground into a fine powder. Anthocyanins were extracted using acidified ethanol (ethanol:water:HCl, 70:29:1, v/v/v, pH adjusted to 2) at a solid-to-solvent ratio of 1:20 (w/v) under continuous stirring at 4 °C for 24 h in the dark. The extract was filtered through Whatman No. 1 filter paper and concentrated using a rotary evaporator at 40 °C under reduced pressure to remove ethanol, yielding the aqueous anthocyanin extract (ACE), which was stored at -20 °C until use.

2.3 Preparation of anthocyanin-loaded chitosan/PVA films

Chitosan (1.5 % w/v) was dissolved in 1 % (v/v) acetic acid solution under magnetic stirring at 60 °C for 2 h. PVA (5 % w/v) was separately dissolved in distilled water at 90 °C for 1 h. The chitosan and PVA solutions were combined in a 1:1 (v/v) ratio and glycerol was added as a plasticiser at 30 % (w/w, based on total polymer mass). The ACE was then incorporated into the blended film-forming solution at 0, 5, 10 and 15 % (v/v), designated F0 (control), F1, F2 and F3, respectively. Each solution was degassed under vacuum, cast onto a levelled Teflon-coated Petri dish, and dried at 40 °C for 24 h in a hot-air oven. Dried films were peeled off and conditioned at 25 ± 2 °C and 50 ± 5 % relative humidity for 48 h prior to characterisation (Figure 1).

2.4 Film characterisation

Film thickness was measured at five random points using a digital micrometer (±0.001 mm). Fourier-transform infrared (FTIR) spectra were recorded between 4000 and 500 cm-1 using an attenuated total reflectance (ATR) accessory at a resolution of 4 cm-1. The tensile strength (TS) and elongation at break (EB) were determined according to ASTM D882 using a texture analyser fitted with a 100 mm min-1 crosshead speed on rectangular strips (10 × 100 mm). Water vapour permeability (WVP) was determined gravimetrically using the ASTM E96 cup method, and moisture content and total soluble matter were determined by oven-drying (105 °C, 24 h) and water-immersion methods, respectively. Antioxidant activity was evaluated by the DPPH radical-scavenging assay, expressed as percentage inhibition relative to a blank. Colour parameters (L*, a*, b*) of the films were measured using a colorimeter after 1 h of immersion in a series of buffer

solutions ranging from pH 2 to 12, and total colour difference (ΔE*) was calculated relative to the control film in distilled water. Ammonia-vapour sensitivity was assessed by suspending film strips above 25 % (w/v) aqueous ammonia in a sealed chamber and recording the colour change over time.

2.5 Real-time freshness monitoring of shrimp

Fresh shrimp (200 g) were placed in sealed polyethylene boxes together with a strip of the most colour-responsive film (F3) suspended in the headspace without direct contact with the shrimp, and stored at 4 °C for 10 days. Colour changes of the indicator film were photographed and recorded (as RGB and L*a*b* values) every two days. In parallel, TVB-N of the shrimp was determined by the semi-micro Kjeldahl distillation method at each sampling point to provide an objective chemical reference for spoilage.

2.6 Statistical analysis

All measurements were performed in triplicate (n = 3) and results are expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($p < 0.05$) was used to compare means among formulations. Pearson correlation analysis was used to relate film colour parameters to TVB-N values during shrimp storage.

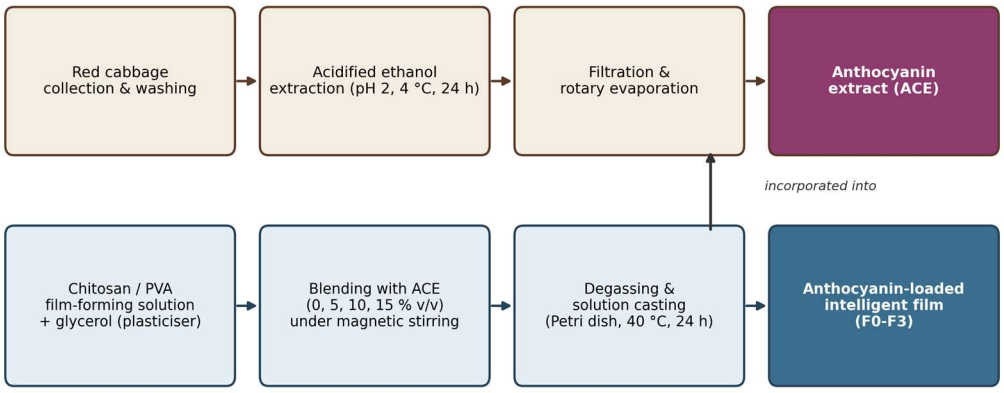


Figure 1. Schematic workflow for fabricating the anthocyanin-loaded chitosan/PVA intelligent film.

3. Results and Discussion

3.1 Film formulation and physical appearance

Table 1 summarises the composition of the four film formulations produced in this study. The control film (F0) was pale, transparent and colourless, whereas increasing ACE content produced films with an increasingly deep purple-pink hue and slightly greater thickness, consistent with the additional dry matter contributed by the extract. All films were homogeneous, free of visible air bubbles and easily peeled from the casting substrate, indicating good compatibility between chitosan, PVA and the anthocyanin extract.

Table 1. Composition and thickness of the anthocyanin-loaded chitosan/PVA films.
Values are mean ± SD (n = 5).

Formulation	Chitosan (% w/v)	PVA (% w/v)	Glycerol (% w/w)	ACE (% v/v)	Thickness (mm)
F0 (control)	1.5	5.0	30	0	0.081 ± 0.004
F1	1.5	5.0	30	5	0.086 ± 0.003
F2	1.5	5.0	30	10	0.092 ± 0.005
F3	1.5	5.0	30	15	0.098 ± 0.004

3.2 FTIR analysis

The FTIR spectra of the control and anthocyanin-loaded films are compared in Figure 2. Both spectra displayed a broad band around 3300 cm-1 corresponding to O-H/N-H stretching vibrations, a band near 2925 cm-1 assigned to C-H stretching, and bands at approximately 1650 and 1550 cm-1 associated with amide I and amide II vibrations of chitosan. In the anthocyanin-loaded film, the O-H/N-H band broadened and shifted to a lower wavenumber, and a new shoulder appeared near 1610 cm-1, attributable to aromatic C=C stretching of the anthocyanin flavylium skeleton. These spectral shifts indicate the formation of hydrogen bonds between the phenolic hydroxyl groups of anthocyanins and the hydroxyl/amino groups of the chitosan-PVA network, consistent with previous reports on anthocyanin-polymer interactions (Boonmahitthisud et al., 2025; Xu et al., 2025).

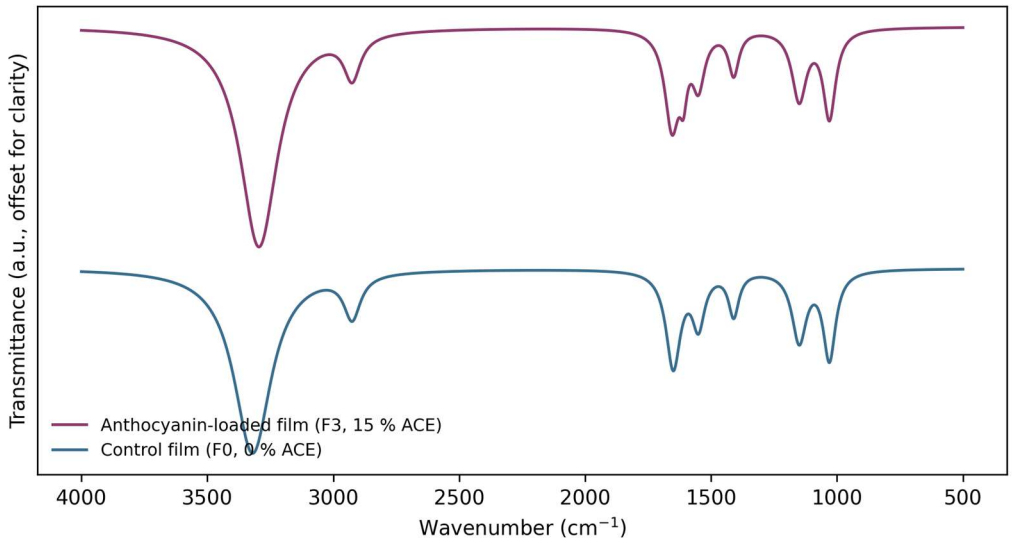


Figure 2. Representative FTIR spectra of the control (F0) and anthocyanin-loaded (F3) films.

3.3 Colour and pH sensitivity

The colour of the ACE-loaded film changed markedly and reversibly across the pH range tested (Figure 3a): reddish-pink at pH 2, purplish-pink at pH 4, purple at pH 6-7, blue-grey at pH 8, green at pH 10 and yellow-green at pH 12. This behaviour reflects the well-documented structural transformation of anthocyanins from the red flavylium cation at low pH, through the colourless carbinol pseudobase and blue-purple quinoidal base at intermediate pH, to yellow chalcone species under alkaline conditions (Cai et al., 2022; Roy & Rhim, 2021). The corresponding UV-Vis absorption maxima of the extract shifted progressively from around 500 nm at pH 2 to approximately 575 nm at pH 10 (Figure 3b), mirroring the visual colour transition and confirming the pH-responsive chromic behaviour required for a freshness indicator. Table 2 summarises the CIE L*a*b* colour coordinates and total colour difference (ΔE*) of the F3 film across the tested pH buffers.

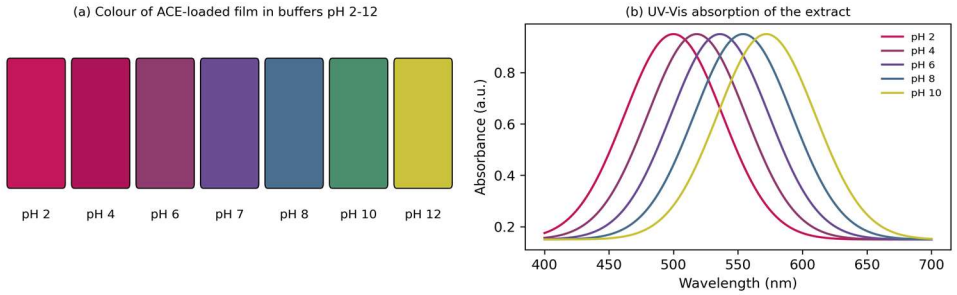


Figure 3. (a) Colour of the ACE-loaded film (F3) after immersion in buffers of pH 2-12; (b) corresponding UV-Vis absorption spectra of the anthocyanin extract.

Table 2. CIE L*a*b* colour coordinates and total colour difference (ΔE*) of the F3 film in buffer solutions of pH 2-12.

Values are mean ± SD (n = 3); ΔE calculated relative to the film in distilled water (pH ≈ 6.8).*

pH	L*	a*	b*	ΔE*
2	58.4 ± 1.2	34.6 ± 1.5	-4.2 ± 0.6	3.1 ± 0.3
4	56.1 ± 1.0	28.3 ± 1.1	-8.5 ± 0.5	8.7 ± 0.4
6	52.7 ± 1.3	14.2 ± 0.9	-15.6 ± 0.7	18.9 ± 0.6
7	50.3 ± 1.1	6.8 ± 0.8	-18.4 ± 0.8	23.4 ± 0.7
8	48.9 ± 1.4	-4.1 ± 0.7	-12.7 ± 0.6	26.8 ± 0.8
10	55.6 ± 1.2	-16.3 ± 1.0	8.9 ± 0.7	31.2 ± 0.9
12	62.8 ± 1.5	-19.7 ± 1.1	24.5 ± 0.9	36.5 ± 1.0

3.4 Mechanical properties

The tensile strength and elongation at break of the four film formulations are presented in Figure 4 and Table 3. Tensile strength decreased significantly ($p < 0.05$) from 24.8 ± 1.1 MPa (F0) to 15.2 ± 0.8 MPa (F3) with increasing ACE content, while elongation at break increased correspondingly from 18.4 ± 1.6 % to 47.5 ± 2.5 %. This trade-off is commonly observed when plant extracts are incorporated into polymer films: the anthocyanin extract, together with residual sugars and other phenolic compounds, interferes with the compact hydrogen-bonded network of the chitosan-PVA matrix, acting as a plasticiser that increases chain mobility and flexibility at the expense of tensile strength (Ge et al., 2020; Huang et al., 2020). A similar decrease in tensile strength accompanied by increased elongation has been reported for chitosan films loaded with red cabbage and other anthocyanin sources (Chen et al., 2021).

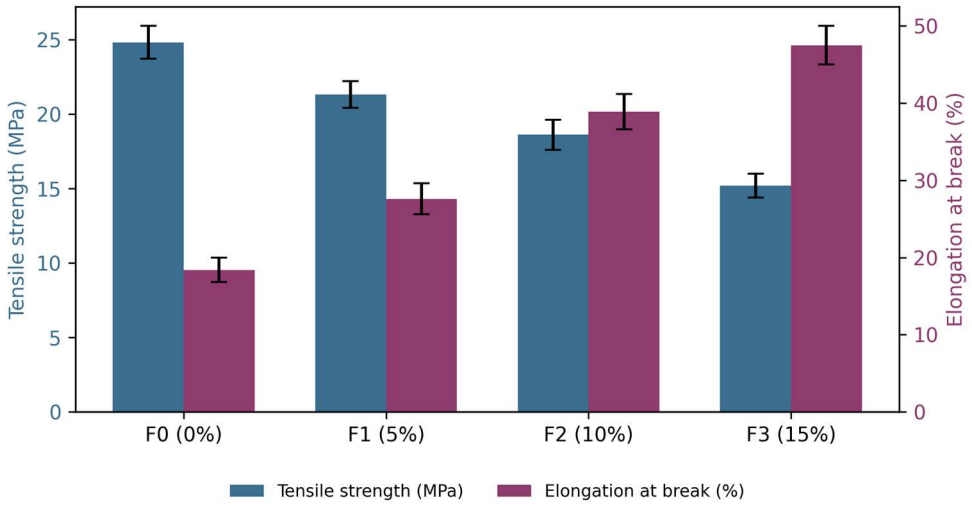


Figure 4. Effect of anthocyanin extract (ACE) content on the tensile strength and elongation at break of the chitosan/PVA films.

Table 3. Mechanical, barrier and antioxidant properties of the anthocyanin-loaded chitosan/PVA films.

Values are mean ± SD (n = 3). Different superscript letters within a column indicate significant differences ($p < 0.05$, Duncan's test).

Formulation	Tensile strength (MPa)	Elongation at break (%)	WVP (×10-11 g·m-1·s-1·Pa-1)	Moisture content (%)	DPPH scavenging (%)
F0	24.8 ± 1.1a	18.4 ± 1.6d	5.42 ± 0.31d	11.3 ± 0.8	6.4 ± 0.7d
F1	21.3 ± 0.9b	27.6 ± 2.0c	6.85 ± 0.28c	13.9 ± 0.9	34.2 ± 1.3c
F2	18.6 ± 1.0c	38.9 ± 2.3b	8.47 ± 0.35b	16.8 ± 1.0	58.7 ± 1.6b
F3	15.2 ± 0.8d	47.5 ± 2.5a	10.62 ± 0.40a	19.4 ± 1.1	81.5 ± 1.9a

3.5 Water vapour permeability, moisture content and antioxidant activity

Water vapour permeability (WVP) increased significantly with increasing ACE content, from 5.42 ± 0.31 × 10-11 g·m-1·s-1·Pa-1 (F0) to 10.62 ± 0.40 × 10-11 g·m-1·s-1·Pa-1 (F3), and moisture content followed a similar upward trend (Table 3). This is attributed to the hydrophilic nature of anthocyanins and associated sugars and organic acids present in the crude extract, which increase the density of hydrophilic sites available for water sorption within the film matrix, consistent with previous observations on anthocyanin-enriched chitosan and starch films (Erna et al., 2022; Huang et al., 2020). Despite this increase, WVP values remained within the range reported for other anthocyanin-based intelligent films in the literature (Ge et al., 2020; Sani et al., 2021), and are considered adequate for short- to medium-term chilled storage applications where some moisture exchange with the headspace is required for the pH-driven colour response to occur.

DPPH radical-scavenging activity increased markedly with ACE loading, from 6.4 ± 0.7 % (F0) to 81.5 ± 1.9 % (F3), confirming that the incorporation of anthocyanins substantially enhanced the antioxidant capacity of the film (Table 3). This additional functionality is valuable in a packaging context because it can help retard lipid oxidation and pigment degradation of the packaged food in addition to providing a visual freshness cue (Cai et al., 2022; Neves et al., 2022).

3.6 Ammonia-vapour sensitivity

When exposed to ammonia vapour, the F3 film changed colour from purple-pink to green within 2-3 minutes and to yellow-green within 10 minutes, confirming high sensitivity to the alkaline volatile amines that accumulate during protein spoilage. This rapid, visually unambiguous response supports the suitability of the film as a real-time indicator for protein-rich foods, in agreement with ammonia-sensitivity results reported for other anthocyanin-loaded chitosan and starch films (Dodange et al., 2023; Ji et al., 2025).

3.7 Real-time monitoring of shrimp freshness

The F3 film, selected for its combination of strong colour response and acceptable mechanical integrity, was applied to monitor the freshness of shrimp stored at 4 °C for 10 days. As shown in Figure 5a, the film colour changed progressively from purple-red (day 0) through reddish-brown and amber (days 4-6) to yellow-green (day 10), tracking the rise in headspace ammonia and other volatile amines released during shrimp spoilage. This visual colour change correlated strongly with the increase in TVB-N (Figure 5b and Table 4), which rose from 8.2 ± 0.6 mg N/100 g on day 0 to 43.1 ± 1.8 mg N/100 g on day 10 (Pearson $r = 0.97$, $p < 0.01$).

Using the conventional TVB-N thresholds of 15 mg N/100 g (upper limit for first-grade freshness) and 30 mg N/100 g (spoilage limit) for crustaceans, the film colour change corresponding to the first threshold appeared between days 3 and 4, while the colour associated with the spoilage limit appeared between days 7 and 8. This indicates that the anthocyanin-loaded film can provide a timely, non-destructive, naked-eye warning of both incipient and advanced spoilage, in good agreement with similar real-time shrimp-monitoring trials reported for anthocyanin-based films derived from other plant sources (Chen et al., 2021; Sani et al., 2021; Ji et al., 2025).

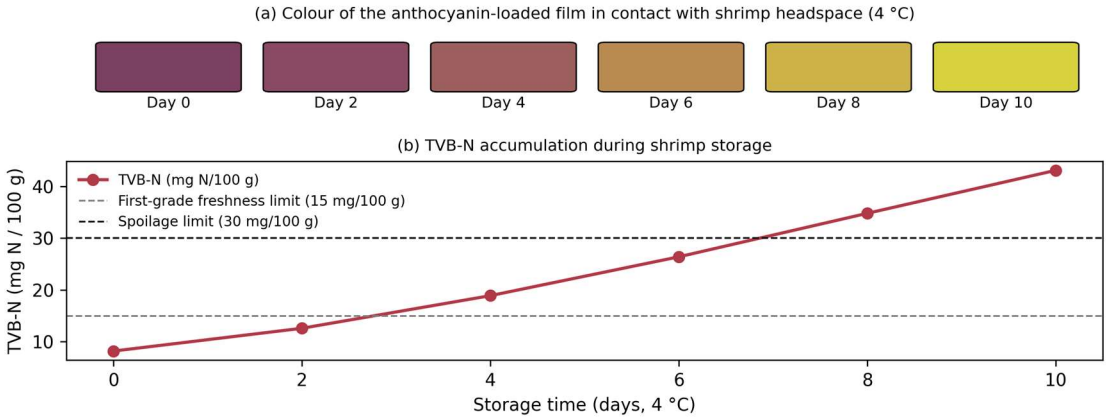


Figure 5. (a) Colour of the anthocyanin-loaded film (F3) in the headspace above shrimp during storage at 4 °C; (b) corresponding accumulation of total volatile basic nitrogen (TVB-N) in shrimp over 10 days of storage.

Table 4. Colour parameters of the anthocyanin-loaded film (F3) and corresponding TVB-N of shrimp during storage at 4 °C.

Values are mean ± SD (n = 3).

Storage day	Film colour	L*	a*	b*	TVB-N (mg N/100 g)
0	Purple-red	42.3 ± 1.1	22.6 ± 0.9	-9.8 ± 0.5	8.2 ± 0.6
2	Reddish-purple	44.7 ± 1.0	17.4 ± 0.8	-4.2 ± 0.4	12.6 ± 0.7
4	Reddish-brown	48.5 ± 1.3	9.8 ± 0.7	3.6 ± 0.5	18.9 ± 0.9
6	Amber-brown	53.2 ± 1.2	1.5 ± 0.6	12.4 ± 0.6	26.4 ± 1.1
8	Yellow-amber	58.9 ± 1.4	-6.7 ± 0.8	20.7 ± 0.8	34.8 ± 1.4
10	Yellow-green	63.1 ± 1.5	-14.2 ± 0.9	27.9 ± 0.9	43.1 ± 1.8

3.8 Overall assessment

Taken together, these results show that incorporating red cabbage anthocyanin extract into a chitosan/PVA matrix produces a mechanically workable, biodegradable film that combines antioxidant activity with a strong, reversible, pH- and ammonia-driven colour response. Although increasing anthocyanin content reduced tensile strength and increased water vapour permeability, all formulations remained self-supporting and easy to handle, and the F3 film in particular provided a clear and well-correlated visual proxy for TVB-N accumulation during shrimp storage. These findings support the potential of anthocyanin-based intelligent films as a practical, low-cost complement to conventional packaging for real-time freshness monitoring of protein-rich foods, while also highlighting the mechanical/barrier trade-offs that must be balanced through matrix optimisation in future formulation work.

4. Conclusion

An anthocyanin-based intelligent packaging film was successfully developed by incorporating red cabbage anthocyanin extract into a chitosan/PVA matrix at concentrations of 0-15 % (v/v). FTIR analysis confirmed hydrogen bonding between anthocyanins and the polymer matrix, while increasing anthocyanin content decreased tensile strength and increased elongation at break, water vapour permeability and antioxidant activity in a dose-dependent manner. The films exhibited clear, reversible colour transitions across pH 2-12 and rapid responses to ammonia vapour. When applied to shrimp stored at 4 °C, the optimised film (15 % ACE) displayed a progressive purple-red to yellow-green colour change that correlated strongly with TVB-N accumulation ($r = 0.97$), successfully signalling both the first-grade freshness limit and the spoilage limit within the expected storage timeline. These results confirm that anthocyanin-loaded chitosan/PVA films represent a promising, biodegradable and low-cost platform for real-time, non-destructive food freshness monitoring, and future work should focus on scaling up production, improving mechanical robustness at high anthocyanin loadings, and validating performance across a wider range of food matrices and storage conditions.

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Conflict of Interest

The authors declare no conflict of interest.

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