

Potato starch/agar-based intelligent films infused with dried blackcurrant pomace anthocyanins for freshness monitoring of freshwater prawns

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Abstract

Recently, there has been an increasing focus on developing advanced packaging films using natural polymers. Therefore, the present work aimed to develop a potato starch/agar-based (PS/A) film incorporated with anthocyanins from dried blackcurrant pomace (DBP) to serve as a freshness indicator for monitoring freshwater prawns over three days of storage at room temperature ($25 \pm 1^\circ\text{C}$). PS/A films containing freeze-dried DBP powder at concentrations of 0, 0.0625, 0.125, 0.25, and 0.5% (w/w) were assessed for their physical properties and colour changes over a pH range of 2.0 to 12.0. The film with 0.125% (w/w) DBP was selected for the prawn freshness storage study due to its more suitable colour intensity compared to other concentrations. Also, the PS/A/DBP films showed no significant differences in terms of thickness, moisture content, and water vapour permeability (WVP) as the DBP concentration increased. During storage, the colour of 0.125% DBP film rapidly transitioned from purple-red on day 0 to dark purple on day 1, followed by purplish-blue on day 2, and ultimately to yellowish green on day 3. This sequence of colour changes indicated that spoilage began within the first 24 h. The PS/A film with 0.125% DBP anthocyanins proved to be an effective pH-indicating packaging for room temperature storage, changing colour in response to pH levels and volatile nitrogen compounds. Future work could assess lower storage temperature ($4 \pm 1^\circ\text{C}$) and other type of seafoods or seafood products to confirm the efficiency of the developed intelligent packaging film.

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Introduction

Intelligent packaging encompasses tools for monitoring both the packaged food and its surrounding environment, providing real-time information on food quality and safety without relying solely on expiration dates, which can be inaccurate (Yun *et al.*, 2019; You *et al.*, 2022). The importance of intelligent packaging lies in its ability to control and measure specific characteristics of the packaged food product by monitoring various parameters, such as temperature, humidity, pressure, and gas composition inside the package. This real-time monitoring helps ensure that the product remains in optimal condition, reducing the risk of spoilage, contamination, or damage. Currently, smart packaging films containing anthocyanins can

efficiently monitor food freshness in real-time. This is because anthocyanins stand out as the most notable pigment responsible for vibrant hues, such as red, orange, pink, and blue (Azman *et al.*, 2020; Ijod *et al.*, 2024).

Natural pigments, including anthocyanins, curcumins, chlorophylls, and carotenoids have gained significant interest as potential alternatives to chemical indicators in intelligent packaging films. In the United Kingdom (UK), more than 300 tons of blackcurrant by-products are produced annually, containing a substantial concentration of anthocyanins, ranging from 14 to 18 mg/g (Azman *et al.*, 2020). Blackcurrant pomace, the primary by-product of the juice pressing process, needs to be dried to enhance the stability of phytochemicals in the pigments, inhibit microbial growth, and minimise

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browning caused by both enzymatic and non-enzymatic reactions (Azman *et al.*, 2021). Several studies have incorporated dried blackcurrant pomace (DBP) anthocyanins into films. For instance, Pakulska *et al.* (2023) used single apple-derived pectin as a packaging film with DBP incorporated. Kurek *et al.* (2021) also studied the effect of DBP film based on single chitosan or pectin. However, these findings only demonstrated the effects of DBP-based film on the single component (pectin or chitosan) for film preparation. Furthermore, Yar *et al.* (2024) used carboxymethyl cellulose-xanthan gum and citric acid-based film with DBP for monitoring beef spoilage. While it is designed for improved precision and sensitivity to ammonia and pH fluctuations, it utilises modified polymers, which may have a higher cost compared to natural polymers. Additionally, limited studies have been reported on the synergistic effect of potato starch/agar-based films with an infusion of DBP prior to monitoring prawn freshness.

According to Khoo *et al.* (2017), the stability of anthocyanins is significantly influenced by the B-ring configuration and the presence of hydroxyl or methoxyl substituents. Specifically, the oxonium ion near C-2 renders these compounds vulnerable to reactions with nucleophiles, including sulphur dioxide, ascorbic acid, hydrogen peroxide, and water. Furthermore, environmental factors like metal ions, temperature, light, and oxygen can also compromise their stability. Anthocyanins exhibit distinct colour changes across pH levels. At pH 2.0, they appear bright red, shifting to purple around pH 6.0 - 7.0, and turning blue at pH 8.0 - 9.0. As the pH increases to 10.0 - 12.0, the pigments degrade, resulting in hues ranging from greenish-blue to yellowish (You *et al.*, 2022). This is because under acidic conditions, they exist as flavylium cations, resulting in vibrant red hues. As the environment approaches neutrality, these cations transition to uncharged quinonoid structures, manifesting as purplish or violet tones, which may also exhibit a bluish appearance. In alkaline conditions, degradation pathways are initiated, yielding blue, green, or yellowish hues, and ultimately leading to colour loss at elevated pH levels. This interplay between chemical structure and pH-responsive colouration renders anthocyanins valuable as natural indicators of acidity and alkalinity (Azman *et al.*, 2020; Ijod *et al.*, 2024).

The development of anthocyanin-based films is usually made up of four main components: solvents, plasticisers, matrices, and anthocyanins or

extracts containing anthocyanins (Xu *et al.*, 2024). Potato starch is a polysaccharide that exhibits excellent film-forming capabilities due to its chemical stability and edibility (Choi *et al.*, 2017; Gujral *et al.*, 2021). Compared to synthetic packaging, the biodegradability, safety, and non-toxicity of potato starch make it a suitable material for use in starch-based films, offering long-term benefits by enhancing safety and reducing plastic pollution. This aligns with the Sustainable Development Goals (SDGs) to enhance human well-being *via* innovation and reduce environmental pollution. The addition of agar to starch-based films can help form tough gels at extremely low concentrations. This unique property has led to agar being used extensively as a gelling agent in filmmaking. Glycerol acts as a crucial plasticiser in food packaging that improves the flexibility of bio-based films. While native starch films are too brittle, glycerol-plasticised versions offer good flexibility and processability, albeit with diminished water- and gas-barrier properties (Ben *et al.*, 2022). Agar-derived films, created through a water-based process with the addition of plasticising agents like glycerol or sorbitol, exhibit transparency and flexibility, rendering them a promising substitute for traditional plastic packaging in food applications (Mostafavi and Zaeim, 2020). Hence, the potato starch/agar-based (PS/A) film has the potential to be utilised as a biopolymer film.

However, there is currently no study on the application of DBP anthocyanins in films as a pH indicator for monitoring the freshness of prawns. From a polymer perspective, the addition of DBP enhances the formation of bridges or linkages between compounds in the film, owing to its high polyphenol content. In addition, Pakulska *et al.* (2023) demonstrated that the addition of DBP to a pectin film improved its tensile properties compared to the control film, suggesting that the addition of DBP enhances the structural integrity of the film. The high dietary fibre content in DBP can act as a reinforcing agent by filling the spaces in the film, which explains its structural improvement. Additionally, the high concentrations of cyanidin and delphinidin derivatives, anthocyanins, polyphenols, and antioxidants in DBP (Azman *et al.*, 2022) make it an excellent film indicator compared to other natural polymers. Furthermore, Kurek *et al.* (2021) reported that incorporating DBP into chitosan and pectin films serves as an effective pH-responsive indicator film. These films exhibit significant colour

changes at different pH buffers, indicating their potential as a visual indicator for product spoilage. The films also demonstrated consistent colour changes across different pH ranges (pH 2 - 12), suggesting their reliability as a film-pH indicator.

Therefore, the primary objective of the present work was to investigate the effect of varying concentrations of DBP anthocyanins on the quality of films. Additionally, it aimed to assess the colour changes and effectiveness of PS/A pH indicator films infused with DBP anthocyanins in monitoring the freshness of freshwater prawns during storage at room temperature ($25 \pm 1^\circ\text{C}$) for three days. The findings shall contribute to the broader field of sustainable materials science, providing insights into the development of more effective and reliable biodegradable pH sensors.

Materials and methods

Materials

The DBP were kindly provided by A&R House (BCL) Ltd., (Bleadon, Weston-super-Mare, UK). Potato starch flour and agar powder were obtained from AEON supermarket, Kuala Lumpur, Malaysia. Freshwater prawns were purchased from Seri Kembangan wet market, Selangor, Malaysia. The disodium hydrogen phosphate anhydrous was purchased from Supelco (Darmstadt, Germany), while citric acid was purchased from R&M Chemicals (Semenyih, Malaysia).

Extraction of anthocyanins from dried blackcurrant pomace (DBP)

The extraction of anthocyanins from DBP was carried out according to Azman *et al.* (2022) with slight modifications. The extraction procedure involved soaking approximately 12.5 g of DBP in 200 mL of a 50% (v/v) ethanol-water solution. Subsequently, the mixture was continuously shaken at 180 rpm in a shaking water bath (Wisebath, Seoul, Korea) for 2 h at 50°C . Then, the solution was vacuum-filtered using a Buchner funnel and Whatman No. 1 filter paper (Whatman, Buckinghamshire, UK). The extract was evaporated using a rotary evaporator (OSB-2000, Japan) to remove the ethanol. Then, the extract was freeze-dried for 48 h, and stored in a freezer at -18°C for further analysis.

Potato starch/agar/dried blackcurrant pomace (PS/A/DBP) film preparation

The PS/A colour indicator film was prepared using the casting method, as described by Saravanan *et al.* (2024). Initially, 2 g of potato starch and 2 g of agar were weighed separately and dissolved in 200 mL of distilled water as a film-forming solution. Then, 30% glycerol (based on the weight of the film base) was added, and the mixture was continuously stirred at 180 rpm in a shaking water bath (Wisebath, Seoul, Korea) at 90°C for 1 h to facilitate gelatinisation. After gelatinisation, the film solution was cooled to 50°C , and freeze-dried DBP powder (0.0625%, w/w, based on the weight of the film base) was added, following the method of Kim *et al.* (2022). The solution was stirred for 20 min to incorporate the DBP. Then, 25 mL of the mixed solution was cast onto a Petri dish, and dried in an oven at 35°C overnight. This reconstituted the PS/A/DBP films. The same procedure was repeated for other samples with different concentrations (0.125, 0.25, and 0.50%, w/w) of freeze-dried DBP powder added to the film solution.

UV-Vis spectroscopy of DBP anthocyanins in different pH solutions

UV-Vis spectroscopy was performed following the methods of Choi *et al.* (2017) and Chen *et al.* (2020), with slight modifications. The pH buffers (4 mL) with a range of 2.0 to 12.0 were separately mixed with 0.001 g of freeze-dried DBP powder, and then measured using a spectrophotometer in the range of 400 - 700 nm (Thermo Fisher, Malaysia). The maximum absorption wavelength (λ_{max}) and maximum absorbance (A_{max}) of anthocyanin solutions of DBP were measured and recorded.

Quality analysis of films

Thickness

The thickness of the film was determined using an Absolute Scale Digital Calliper (Mitutoyo, Japan) by measuring five random positions on each film. The average thickness of each film was then calculated and reported in mm.

Moisture content

The moisture content of the films was determined using a moisture analyser (SHS AND

MX-50, Tokyo, Japan) by cutting a square shape (6×4 cm), and drying the film until it reached a constant weight. The analyses were carried out in duplicate, and results were reported as the average moisture content percentage.

Colour measurement of film

Each film was evaluated using a colour spectrophotometer (Hunterlab, Reston, US). The results were expressed as L^* (lightness/darkness), $a^*/-a^*$ (redness/greenness), $b^*/-b^*$ (yellowness/blueness), chroma (C^*), and hue angle (h°) parameters to evaluate the colour of the film. The C^* , h° , and total colour difference (ΔE) were calculated using Eqs. 1, 2, and 3:

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (\text{Eq. 1})$$

$$h^\circ = \frac{b^*}{a^*} \quad (\text{Eq. 2})$$

$$\Delta E = \sqrt{(L^* - L_o)^2 + (a^* - a_o)^2 + (b^* - b_o)^2} \quad (\text{Eq. 3})$$

where, L^* , a^* , and b^* = colour values of the PS/A films incorporated with DBP; and L_o , a_o and b_o = colour values of the control film without DBP.

Water vapour permeability (WVP)

Each 100 mL crucible was filled with 40 mL of distilled water, and covered with 7.5×7.5 cm film samples. The initial weight of each crucible was recorded before it was placed in a desiccator with a relative humidity of $50 \pm 5\%$ at a room temperature of $25 \pm 1^\circ\text{C}$. The weight of the crucible was then measured and recorded hourly for 8 h. The WVP was measured with duplicates, and calculated using Eq. 4:

$$\text{WVP} = \frac{\Delta w \cdot l}{A \cdot t \cdot P} \quad (\text{g} \cdot \text{m}^{-1} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1}) \quad (\text{Eq. 4})$$

where, Δw = weight difference (weight initial - weight after 8 h); l = thickness of film (m); A = permeation area of film (m^2); t = time elapsed (8 h in s); and P = partial pressure of water vapour across the film (pressure (Pa) at a designated temperature) (Han Lyn et al., 2021).

Colour response of films to different pH levels

The films were cut into a square (1.5×1.5 cm),

and placed on a Petri dish. The pH buffer solutions (ten drops) in a range of pH levels from pH 2.0 to 12.0 were added to the films. After a 5-min incubation period, the changes in colour throughout the films were captured using a white background.

Application of films to monitor prawns' freshness

The films were used to monitor the freshness of freshwater prawns. The films were cut into a square (3×3 cm), and attached to the lid of the polypropylene (PP) container (8×4.5 cm). Each PP container contained two samples of whole fresh prawns, weighing approximately 80 g in total. The samples were stored at room temperature ($25 \pm 1^\circ\text{C}$) for 3 d, with all samples analysed every 24 h. The prawn samples were evaluated for their texture, pH values, and appearance by naked eyes. The changes in colour characteristics of films were recorded using HunterLab.

Texture profile analysis (TPA) of prawns

The texture profile analysis was conducted using texture analyser XT2i (Stable Micro System Ltd., Surrey, UK) with modifications as described by Wu et al. (2014). The prawn samples were cut into small pieces from different locations, and compressed to approximately 70% of their original shape at a speed of 115 mm/min with a compression load of 120 N using a cylindrical-shaped probe. The results were expressed as hardness (kg), cohesiveness, springiness (mm), gumminess (kg), chewiness (kg), and resilience of the prawn samples. This analysis was conducted over the 3-day storage period.

pH values for prawns during storage

The prawn samples were homogenised in 20 mL of distilled water for 5 min; then, the pH was measured using a pH meter (Mettler Toledo Seven Easy, UK). The pH meter was calibrated using buffer solutions with pH levels of 4.0, 7.0, and 10.0 before the measurements. The measurements were taken in triplicate.

Statistical analysis

All results were analysed using a One-way analysis of variance (ANOVA) in Minitab version 21.4, and expressed as mean $\pm$ standard deviation. The significance of the means was determined by the Tukey's test at $p < 0.05$.

Results and discussion

Characterisation of potato starch/agar-based dried blackcurrant pomace (PS/A/DBP) films

The physical properties of the film, including thickness, moisture content, and WVP were evaluated, and the results are presented in Table 1.

The addition of different concentrations of DBP did not result in any statistically significant differences ($p > 0.05$) in the measured properties of the film. This may be due to the limited concentration of anthocyanins in the films, which had insignificant impact on their thickness, moisture content, and WVP.

Table 1. Thickness, moisture content, and water vapour permeability (WVP) of PS/A/DBP film with different concentrations of freeze-dried DBP powder.

PS/A/% DBP in film (w/w)	Thickness (mm)	Moisture content (%)	WVP ($\times 10^{-11} \text{ g} \cdot \text{m}^{-1} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1}$)
0% (control)	0.139 ± 0.07^a	13.37 ± 1.12^a	1.89 ± 0.01^a
0.0625%	0.146 ± 0.05^a	15.80 ± 0.89^a	1.78 ± 0.03^{ab}
0.125%	0.157 ± 0.05^a	11.70 ± 0.45^a	1.65 ± 0.07^{bc}
0.25%	0.158 ± 0.06^a	16.82 ± 3.23^a	1.59 ± 0.03^c
0.50%	0.168 ± 0.06^a	15.56 ± 0.83^a	1.33 ± 0.01^d

Values are mean $\pm$ standard deviation of two separate determinations. Means with different lowercase superscripts in similar column are significantly ($p < 0.05$) different between each film. PS: potato starch; A: agar powder; and DBP: dried blackcurrant pomace.

The general pattern of increase in thickness was evidenced when the percentage of DBP in the film increased up to 0.50% (w/w). The results agreed with those of Che Hamzah *et al.* (2024), who reported a significant enhancement in film thickness due to high solid loadings of red cabbage anthocyanin (RCA). As reported by Qin *et al.* (2019), increasing anthocyanin levels enhanced the complexity of the film-forming matrix, resulting in a greater film thickness. This supported the concept that higher solid loadings would directly contribute to the formation of thicker films.

The findings on moisture content showed that the incorporation of 0.125% DBP had the lowest moisture content level compared to the others. High humidity levels in meat and seafood packaging can adversely affect the colour change of a pH indicator, leading to inaccurate results. Reduced moisture content would enhance product protection and shelf life in films, whereas high moisture content would weaken them and increase the risk of microbial deterioration (Srinivasan and Rayappan, 2020). Kanani *et al.* (2017) suggested that films for primary food packaging should be designed with low water content to avoid contributing moisture that can negatively impact product quality. Therefore, a film with lower moisture content is more desirable. In the present work, PS/A/0.125% DBP film was considered the most suitable freshness indicator film

during a high-humidity ($50 \pm 5\%$) storage environment.

The WVP of the packaging material is a crucial parameter, as it directly affects the quality and shelf life of the packaged food (Chen *et al.*, 2023). WVP assesses a material's barrier properties by determining the mass of water vapour that permeates through it under a given pressure difference over a specified time and area ($\text{g/s} \cdot \text{m} \cdot \text{Pa}$). The solubility-diffusion model describes water permeation through a film as a process in which water molecules are first transported to the film surface before being adsorbed, dissolved, and finally diffused through the film until they are completely dissolved within the matrix (Cazón *et al.*, 2022). The concentrations of DBP significantly affected the WVP of the films. The WVP values showed significant differences ($p < 0.05$) between the films, where the increased concentration of DBP resulted in decreased WVP of the film.

Yar *et al.* (2024) also observed a decrease in WVP after being infused with a higher concentration of blackcurrant anthocyanin in a carboxymethyl cellulose-gum xanthan film. Films with increased anthocyanin concentration exhibited lower WVP due to the interactions between the extract, starch, and glycerine, which resulted in a denser film structure. The enhanced hydrogen bonding between film components and DBP improved the film's structure,

which explained the decrease in WVP as the DBP concentration increased (Yar *et al.*, 2024). Furthermore, the varied chemical compositions of the extracts influenced their interactions with starch. At the same time, the aromatic rings of anthocyanins potentially restricted the film's internal network, thus decreasing its water vapour affinity (Ge *et al.*, 2020). The addition of the extract can facilitate an interaction between phenolic compounds and the biopolymer, effectively filling the polymer matrix. This interaction creates a barrier to mass diffusion, consequently reducing the free volume and pathways available for water vapour to permeate (Avila *et al.*, 2022). Moreover, the incorporation of higher amounts of anthocyanins can increase the tortuosity and complexity of the diffusion pathways for water vapour, physically impeding its permeability through the film.

A study by Qin *et al.* (2019) also found that incorporating free anthocyanins or anthocyanin-loaded nanocomplexes into a starch/polyvinyl alcohol matrix decreased the material's ability to allow water vapour to pass through the film. Meanwhile, Kurek *et al.* (2021) found that the addition of 10% blackcurrant powder to chitosan-based films resulted in a lower WVP compared to a higher concentration. However, a reverse effect was observed in the pectin-based film, where the addition of 20% blackcurrant powder significantly reduced the WVP compared to the addition of only 10% blackcurrant powder. Furthermore, Yar *et al.* (2024) reported a reduction of WVP in a higher concentration of blackcurrant anthocyanins added to the carboxymethyl cellulose-gum xanthan and citric acid-based film, which corroborated the findings observed in the present work. The linkages interaction, such as the formation of new hydrogen bonds between anthocyanin and film components, can lower the WVP by reducing the presence of hydrophilic hydroxyl groups in the films, as reported by the studies of Qin *et al.* (2019) and Yun *et al.* (2019).

The results demonstrated that the film containing 0.125% DBP was the most suitable for monitoring applications. This formulation exhibited the lowest moisture content, moderate WVP, and an acceptable thickness compared to films with other DBP concentrations. The combination of low moisture absorption, controlled WVP, and balanced physical properties rendered 0.125% DBP film as the best choice for reliable and consistent performance.

Although higher DBP concentrations further reduced WVP, they also led to increased moisture content and thickness, potentially compromising the film's overall functionality.

Colour appearance of films

As shown in Table 2, the a^* values increased as the DBP concentration increased, indicating that the films were shifting toward a red hue. Similarly, the b^* values also increased, suggesting a shift toward a yellow hue. C^* value is the chroma or intensity of the colour, with higher values indicating more vivid, saturated, and high colour intensity.

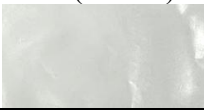
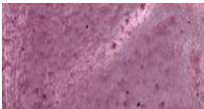
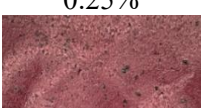
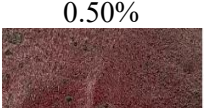
The film became more opaque as the DBP concentration increased. The hue angle (h°) value decreased with higher DBP concentrations, indicating a shift toward a more reddish-purple hue. Additionally, the total colour difference (ΔE) value increased, signifying that the colour differences between the films became more noticeable during the storage period.

In terms of appearance, the films became darker, and had lower transparency as the amount of DBP increased gradually. The anthocyanins present in the DBP are strong absorbers of visible light, particularly in the blue-violet region of the spectrum. This selective light absorption by the anthocyanins reduced the overall light transmission through the film, making it appear darker and less transparent. Similar to the research by Zhao *et al.* (2023), the transparency of the anthocyanin-containing films decreased gradually as the concentration of the anthocyanin solution increased.

Furthermore, the surface of the films appeared to be smoother and more uniform as the concentration of DBP increased. The anthocyanins and other phytochemicals present in the extract became more evenly dispersed and incorporated into the polymer matrix. This improved miscibility and compatibility between the pomace and the polymer reduced the tendency for phase separation or the formation of discrete domains. Homogeneous blending resulted in a more consistent and smooth appearance across the film surface.

The colour properties of PS/A/0.0625% DBP film, such as the lower L^* value (~ 77.87) and more negative a^* value of (~ -5.3), indicated a duller, less saturated red hue. This could make the colour change less visually striking for a pH indicator. On the other hand, a high level of darkness in the film colour of PS/A/0.25% DBP and PS/A/0.50% DBP would make

Table 2. Colour properties of PS/A/DBP film with different concentrations of freeze-dried DBP powder.

PS/A/% DBP in film (w/w)	L^*	a^*	b^*	C^*	h°	ΔE
0% (control) 	94.83 ± 0.01 ^a	-0.08 ± 0.01 ^e	0.99 ± 0.01 ^b	1.02 ± 0.01 ^e	94.46 ± 0.83 ^d	-
0.0625% 	77.87 ± 0.01 ^b	16.49 ± 0.00 ^d	-5.30 ± 0.02 ^d	17.60 ± 0.01 ^d	341.54 ± 0.05 ^c	24.52 ± 0.01 ^d
0.125% 	59.85 ± 0.01 ^c	36.51 ± 0.02 ^c	-7.37 ± 0.04 ^c	38.69 ± 0.00 ^c	347.68 ± 0.07 ^b	51.30 ± 0.00 ^c
0.25% 	36.24 ± 0.03 ^d	53.15 ± 0.02 ^a	-1.45 ± 0.06 ^c	58.01 ± 0.02 ^b	358.03 ± 0.08 ^a	79.20 ± 0.03 ^b
0.50% 	25.82 ± 0.02 ^e	51.61 ± 0.06 ^b	9.12 ± 0.04 ^a	62.07 ± 0.04 ^a	16.88 ± 0.11 ^e	86.60 ± 0.04 ^a

Values are mean ± standard deviation of two separate determinations. Means with different lowercase superscripts in similar column are significantly ($p < 0.05$) different between each film. PS: potato starch; A: agar powder; and DBP: dried blackcurrant pomace.

it difficult to provide a clear visual presentation of the product inside the package. The dark appearance could obscure or distort the view of the packaged contents, which is an important consideration for a pH indicator film application (Ge *et al.*, 2020). Therefore, based on the colour properties data and the practical application requirements, these three types of films would not be the best option as pH indicator films. Their less saturated, overly dark, and muted appearance would likely not meet the desired criteria for clear product visibility through the packaging.

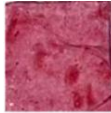
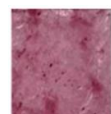
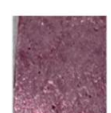
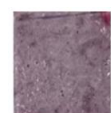
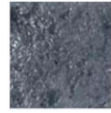
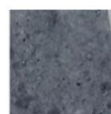
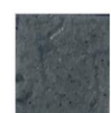
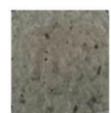
In contrast, the lighter, more saturated red hue with less brown could make the PS/A/0.125% DBP film better suited for a pH indicator application where a clean, vivid colour change is desirable. Additionally, the PS/A/0.125% DBP film appeared to maintain a lighter and more saturated appearance, offering a good balance of colour properties that could optimise the visual performance and functionality for the intended pH indicator film use case.

UV-Vis spectroscopy

Table 3 presents the results of the maximum absorption wavelength ($\lambda_{\max}$) and maximum absorbance ($A_{\max}$) of DBP anthocyanins in various buffer solutions, measured over a spectral range of 400 to 700 nm. Absorption in this region is typically responsible for the vibrant colour associated with anthocyanins, such as red, purple, and blue.

The data showed that the $\lambda_{\max}$ values for DBP were within the 490 - 550 nm range at pH values lower than 6, with the greatest light absorption ($A_{\max}$) occurring at pH 2.0. According to Dong *et al.* (2024), a typical UV-Vis spectrum of anthocyanins usually consists of two distinct wavelength regions: one in the ultraviolet (UV) range between 260 - 280 nm, and another in the visible range between 490 - 550 nm. The specific wavelength of $A_{\max}$ in this region can vary depending on the structural features and substitution patterns of the anthocyanin molecules. This suggested that the UV-Vis spectra of DBP are strongly pH-dependent, with the visible $A_{\max}$ shifting to shorter wavelengths as the pH decreases.

Table 3. Maximum absorption wavelength ($\lambda_{\max}$), maximum absorbance ($A_{\max}$), and colour response of DBP anthocyanins and PS/A/0.125% DBP film in different pH levels of buffer solutions.

pH of buffer solution	UV-Vis		DBP anthocyanin	PS/A/0.125% DBP film (w/w)
	$\lambda_{\max}$ (nm)	$\lambda_{\max}$ (nm)		
2	515.75 $\pm$ 0.35 ^d	515.75 $\pm$ 0.35 ^d		
3	516.25 $\pm$ 0.35 ^d	516.25 $\pm$ 0.35 ^d		
4	520.00 $\pm$ 0.00 ^{cd}	520.00 $\pm$ 0.00 ^{cd}		
5	523.25 $\pm$ 0.35 ^c	523.25 $\pm$ 0.35 ^c		
6	529.50 $\pm$ 2.12 ^b	529.50 $\pm$ 2.12 ^b		
7	564.25 $\pm$ 1.77 ^a	564.25 $\pm$ 1.77 ^a		
8	564.25 $\pm$ 1.77 ^a	564.25 $\pm$ 1.77 ^a		
9	562.00 $\pm$ 2.12 ^a	562.00 $\pm$ 2.12 ^a		
10	563.50 $\pm$ 0.71 ^a	563.50 $\pm$ 0.71 ^a		
11	NA	NA		
12	NA	NA		

Values are mean $\pm$ standard deviation of two separate determinations. Means with different lowercase superscripts in similar column are significantly ($p < 0.05$) different between each pH level. PS: potato starch; A: agar powder; and DBP: dried blackcurrant pomace.

Another finding from Saha *et al.* (2020) suggested that the wavelength of $\lambda_{\max}$ for anthocyanins is typically observed around 510 - 520 nm in the visible region of the UV-Vis spectrum. Furthermore, Azman *et al.* (2022) reported that blackcurrant anthocyanins are most stable at acidic pH values, specifically below pH 3.0. The fact that the greatest light absorption ($A_{\max}$) for the DBP samples occurred at pH 2.0 and that the $\lambda_{\max}$ values were within the typical 510 - 520 nm range at these lower pH levels corroborated the findings that the DBP anthocyanins are most stable under acidic conditions.

There was a significant bathochromic shift (red shift) in the $\lambda_{\max}$ as the pH increased from acidic to basic conditions. At lower pH values (2.0 - 6.0), the $\lambda_{\max}$ fell within the 515 - 529 nm range, while at higher pH values (7.0 - 9.0), the $\lambda_{\max}$ shifted to the 562 - 564 nm range. However, the $A_{\max}$ values exhibited a more complex trend, with an initial decrease from pH 2.0 to 4.0, followed by a slight increase from pH 5.0 to 6.0, a significant increase at pH 7.0 and 8.0, before a slight decrease at pH 9.0. The $A_{\max}$ values were significantly different across the lower pH range (2.0 - 6.0), but became statistically equivalent at the higher pH levels of 7.0, 8.0, and 9.0. A possible explanation is that the DBP became increasingly unstable and prone to structural changes or degradation at higher pH levels, resulting in undetectable values of $\lambda_{\max}$ and $A_{\max}$ at pH levels of 11.0 and 12.0. The DBP degraded and became unstable under highly alkaline conditions.

Colour response of dried blackcurrant pomace (DBP) anthocyanins and films in different buffer solutions

The colour properties of DBP solutions were explored and the viability of employing the extract as a pH-sensitive dye was validated. Table 3 depicts the solutions containing DBP anthocyanins in different pH buffer solutions. As the pH increased from 2.0 to 12.0, DBP exhibited a gradual colour shift, transitioning from red to pink, then purple, blue, and finally yellow. These agreed with You *et al.* (2022), whereby blackcurrant anthocyanins exhibited a pH response similar to other anthocyanins. They reported that within the pH range of 2.0 to 12.0, the colour of the blackcurrant anthocyanins solution transitioned from red to white, and subsequently to yellow-green.

At low pH (pH < 3.0), the anthocyanins were primarily present in the highly stable red flavylium cation form. At around pH 4.0, the flavylium cation form of the anthocyanins predominated, resulting in a reddish colour. As the pH increased to around 5.0, the flavylium cation underwent rapid hydration, and the colourless carbinol pseudobase form of the anthocyanins was generated. When the pH was further increased in the range of 6.0 - 7.0, the flavylium cation was deprotonated, and the neutral quinonoid base form was formed. This neutral quinonoid base exhibited a purple-to-violet colour (Azman *et al.*, 2022). Lastly, at pH levels between 8.0 and 10.0, the anionic quinonoid base form of the anthocyanins was formed, resulting in the observation of a blue-purple colour. At higher pH (11.0 - 12.0), the anthocyanins underwent degradation, converting to chalcone compounds. As a result, the solution changed to a green or yellow colour (Qin *et al.*, 2019; Ijod *et al.*, 2024). Due to their sensitivity to changes in pH, which resulted in varied colouration from red to yellow-green, anthocyanin-containing DBP has potential applications in monitoring food quality.

The sequential colour transition of the films from bright red to yellowish green matched the expected colorimetric changes of DBP in different buffer solutions (see Table 3) as the pH of the solution increased from acidic to basic conditions. This can provide a visual representation of how the pH of the buffer solution can significantly influence the colour appearance of the films, which is an important consideration in various applications where pH-dependent colour changes are of interest.

Application of PS/A/DBP as freshness indicator film for prawns during storage

The colour characteristics of PS/A/0.125% DBP film for freshwater prawns significantly changed ($p < 0.05$) during three days of storage (Table 4). The L^* and b^* values of the films significantly ($p < 0.05$) decreased from day 0 until the end of storage time.

The increase in L^* values indicated that the film colour became lighter and less saturated throughout the 3-day storage period. Meanwhile, the b^* values shifted from negative to positive, transitioning from green and pale blue on day 2 to strong yellow by day 3, and ultimately reached a value of 7.6. This dramatic change in film hue from

Table 4. Colour characteristics and appearance of PS/A/0.125% DBP film and freshwater prawns during three days of storage at room temperature ($25 \pm 1^\circ\text{C}$).

Storage (day)	L^*	a^*	b^*	C^*	h°	ΔE	Films appearance	Prawns appearance
0	56.87 ± 0.01^d	38.93 ± 0.01^a	-5.83 ± 0.04^d	41.09 ± 0.02^a	350.62 ± 0.06^b	—		
1	65.88 ± 0.04^c	26.69 ± 0.11^b	-0.95 ± 0.06^c	27.66 ± 0.11^b	357.75 ± 0.15^a	15.96 ± 0.13^c		
2	72.48 ± 0.01^b	-0.16 ± 0.06^d	4.06 ± 0.04^b	4.66 ± 0.04^d	92.13 ± 0.81^c	43.22 ± 0.06^b		
3	76.66 ± 0.04^d	0.97 ± 0.03^c	7.60 ± 0.01^a	8.81 ± 0.01^c	83.31 ± 0.22^d	44.85 ± 0.03^a		

Values are mean $\pm$ standard deviation of two separate determinations. Means with different lowercase superscripts in similar column are significantly ($p < 0.05$) different between each storage day. PS: potato starch; A: agar powder; and DBP: dried blackcurrant pomace.

red to yellow likely reflected the deterioration of the prawns, which created an alkaline environment in the closed package, causing the film to absorb the volatile nitrogenous compounds released by the spoiled prawns. This interaction changed the colour of the DBP films on day 3, reflecting the basic nature of the environment. As reported by Mary *et al.* (2020), the colour changes observed in the indicator films are likely a result of their interaction with volatile compounds produced during the spoilage of the prawn samples.

Moreover, the a^* value decreased significantly from day 0 to 2, indicating that the films were becoming significantly less red and greener. However, this trend was accompanied by a significant decrease in the C^* value (*i.e.*, the measure of colour saturation) over the same period. This suggested that the films not only shifted toward greener tones, but their overall colour intensity was also significantly diminished on day 3. Furthermore, the combination of the steadily increasing ΔE value and the U-shaped trend in the C^* value demonstrated a complex picture of the films' colour evolution. While the overall colour difference from day 0 continued to grow, the colour's intensity first diminished, and then regained its vibrancy toward the end of storage.

The increasing intensity of the colour changes observed in the films toward the end of the storage

period likely corresponded with the development of obvious spoilage odours, as the production of volatile compounds intensified during the later stages of prawn deterioration. This is because the quality and shelf life of prawns are influenced by various factors during storage, including temperature, pH levels, and biochemical reactions. According to Das and Mishra (2023), the spoilage of shrimp after its death is attributed to several factors like autolysis resulting from endogenous proteinases during storage, growth of microorganisms that cause spoilage, degradation of adenosine triphosphate (ATP), melanin formation, and lipid peroxidation. Among these factors, the microbial by-product, total volatile basic nitrogen (TVB-N), is a significant contributor to the development of foul odours in spoiled prawns. Furthermore, the microbial activity on dead prawns also results in the production of trimethylamine, a biogenic amine and volatile compound, which is a primary cause of the "fishy odour" associated with spoiled prawn (Bevilacqua *et al.*, 2016). Microbial actions on prawn muscle can lead to the production of ammonia, a by-product of urea breakdown, which is another contributing factor to the unpleasant odour and flavour of spoiled prawns (Srinivasan and Rayappan, 2020).

Colorimetric changes in pH-sensitive films were conducted by pH changes in freshwater prawns

stored at $25 \pm 1^\circ\text{C}$ to evaluate the films as a reliable visual indicator of prawn spoilage. The results showed that the colour of the film changed sequentially from purple-red to dark purple, followed by a purplish-blue hue on day 2, and a yellowish-green tint on day 3. This indicated that this colorimetric pH-sensitive film can be used as a reliable method for visually monitoring the spoilage of prawn products. According to Alizadeh-Sani *et al.* (2021), the colour changes observed were attributed to a specific mechanism involving phenolic oxygen anions. As the seafood spoiled, volatile ammonia was released and diffused into the pH-sensitive film. The ammonia combined with water and formed hydrated ammonia, which then underwent hydrolysis. This process generated hydroxyl ions, and created an alkaline environment. The change in pH caused a structural transformation in the anthocyanins, ultimately resulting in observable colour variations across the pH scale.

The colour of the film also changes from red to blue over the two days of storage, indicating that the pH of the film was within the range of 7.0 - 9.0 (see Table 3). The reason for this colour transition can be attributed to research by Listyarini *et al.* (2018), which reported that the red flavylum cation converted to a blue quinonoid base due to the ammonia released during the decomposition phase of the prawn. This resulted in a shift in the bead's colour from purple to greenish blue.

The yellowish-grey colour that appeared on day 3 indicated that the pH of the film was around 11.0 - 12.0. It was possible that the colour may change to a yellowish-green hue due to anthocyanins degrading readily at high pH and temperature after three days of storage, causing the film to turn green or yellow. The prawn had an overwhelmingly pungent, acrid odour that was immediately noticeable upon opening the package. It emanated a strong, repulsive stench of rotten eggs and ammonia. There was bacterial spoilage, resulting in the production of various volatile, nitrogenous compounds that can serve as indicators of shrimp freshness and quality.

pH values and texture profile analysis (TPA) of freshwater prawns during storage

The initial pH of the prawns was low (6.65), indicating that they were relatively fresh. It later increased significantly ($p < 0.05$) by 15.49%, reaching a pH of 7.68 by day 3. This trend aligned with the significant increase in pH observed for both

tiger prawns and freshwater prawns during the storage period (Saravanan *et al.*, 2024).

The gradual increase in pH level indicates advanced spoilage, as the bacterial breakdown of proteins leads to the production of alkaline compounds. Prawns produce biogenic amines like histamine, tyramine, cadaverine, and putrescine. These biogenic amines are formed when the microorganisms convert free amino acids into these compounds through a process called decarboxylation, which occurs within the prawn's body (Siripongpreda *et al.*, 2020).

Another reason for the increase in pH level is that room temperatures would likely accelerate the spoilage of freshwater prawns by promoting faster microbial growth, thus enhancing enzymatic activity, increasing oxidation, and accelerating autolysis. These processes can collectively lead to changes in the pH, potentially causing an initial decrease followed by an eventual increase as spoilage progresses. As shown in Table 5, the general texture of freshwater prawns did not undergo a significant change, even though decreasing trends were observed in terms of hardness, chewiness, springiness, and gumminess. In contrast, other properties, such as cohesiveness and resilience, exhibited increasing trends during the 3-day storage period.

The observed decreasing trends in terms of hardness, chewiness, springiness, and gumminess in freshwater prawns could be due to changes in the protein structure and disruption of the prawn meat's internal network. Throughout three days of storage, the prawn meat's structural proteins and connective tissues may break down, leading to a softer texture and decreased hardness. The cohesive bonds within the prawn meat also weaken, leading to a less tough and chewy texture. Since the internal network of the prawn meat was disrupted, it reduced the meat's ability to regain its original shape after deformation, leading to decreased springiness.

The cohesiveness and resilience of prawns increased by 7 and 6%, respectively, at the end of the storage period. This may be due to the prawns producing a natural mucus that helps them adhere to one another and to surfaces. An increase in mucus production during storage could lead to greater cohesiveness. Chemical changes in the prawns' exoskeletons or other tissues during storage could lead to increased cohesiveness. For example, the cross-linking of proteins in the exoskeleton can make it more rigid and resistant to separation.

Table 5. pH values and texture profile analysis (TPA) of freshwater prawns during three days of storage at room temperature ($25 \pm 1^\circ\text{C}$).

Storage (day)	pH	Hardness (kg)	Springiness (mm)	Cohesiveness	Gumminess (kg)	Chewiness (kg)	Resilience
0	6.65 ± 0.01^d	10237 ± 2941^a	0.274 ± 0.035^a	0.204 ± 0.052^a	2100 ± 791^a	585 ± 248^a	0.121 ± 0.037^a
1	6.89 ± 0.02^c	10959 ± 3641^a	0.281 ± 0.010^a	0.187 ± 0.041^a	2144 ± 1161^a	596 ± 301^a	0.110 ± 0.028^a
2	7.40 ± 0.02^b	11014 ± 2155^a	0.260 ± 0.036^a	0.173 ± 0.031^a	1906 ± 507^a	496 ± 143^a	0.098 ± 0.021^a
3	7.68 ± 0.02^a	8359 ± 2310^a	0.230 ± 0.027^a	0.219 ± 0.031^a	1826 ± 495^a	423 ± 127^a	0.129 ± 0.023^a

Values are mean $\pm$ standard deviation. Means with different lowercase superscripts in similar column are significantly ($p < 0.05$) different between each storage day.

Conclusion

In the present work, PS/A intelligent films were successfully developed by incorporating DBP anthocyanins. Adding 0.0625 to 0.50% (w/w) DBP resulted in increased film thickness and moisture content, along with a decrease in WVP. The film with 0.125% DBP had a balanced colour, neither too dark nor too light, and was therefore selected for monitoring the freshness of prawn products during storage. The PS/A/0.125% DBP film shifted from red (indicating freshness) to yellow-green (signalling spoilage) as freshwater prawns deteriorated rapidly during three days of storage at $25 \pm 1^\circ\text{C}$. The spoilage of freshwater prawns began within 24 h of storage, as evidenced by significant changes in colour from purple-red to dark purple. The colour-changing properties of PS/A/DBP films are related to the pH levels, as spoilage of freshwater prawns releases volatile ammonia that diffuses into the pH-sensitive film, leading to the generation of hydroxyl ions, an alkaline environment, and structural transformations in anthocyanins, which cause colour variations across the pH scale. Overall, PS/A/DBP films demonstrated promising potential as eco-friendly pH indicator films, effectively monitoring the freshness of freshwater prawns. For future studies, the monitoring frequency should be increased, potentially to every five hours, and prawns should be stored at 4°C to extend their shelf life, and enhance the accuracy of the results. Overall, these findings could have significant implications for the development of smart packaging systems, particularly in the seafood industry, where real-time monitoring of product freshness is crucial.

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