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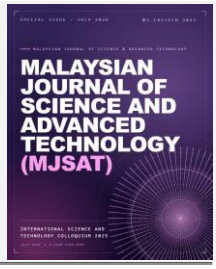

Development and Characterization of pH-Sensitive Starch/Pectin/DES Films with Purple Cabbage Anthocyanins for Food Spoilage Detection

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ABSTRACT

Innovations in food packaging are increasingly employing smart indicators to boost safety, monitor quality, and minimize waste. However, most of synthetic dyes in indicator systems can leach harmful substances into food. This study developed a natural, biodegradable pH-indicator film using starch-pectin matrix plasticized with a deep eutectic solvent (DES) and functionalized with anthocyanin extract from purple cabbage. The films were formulated with varying anthocyanin concentrations (3%, 6%, and 9%) and evaluated for mechanical properties, water vapor transmission rate (WVTR) and sensitivity to pH and ammonia vapor. FTIR analysis confirmed molecular interactions between the biopolymer matrix, DES and anthocyanins. Increasing anthocyanin concentration to 9% enhanced film flexibility (20.66 ± 3.19%) and increased WVTR (86.22 g/m²·day), but produced darker hues that reduced the visibility of color changes. In contrast, the film with 3% anthocyanin (S/P/DES/3A) exhibited clearly color transitions from bright pink to bright green at high pH level (pH 10). It also had the lowest WVTR (78.11 g/m²·day) and was flexible enough. Furthermore, this film effectively detected ammonia vapor, a crucial spoilage marker; via a visible shift to dark green. These results suggest that S/P/DES/3A is the most effective formulation and offers a promising natural alternative for real-time food spoilage detection.

1. INTRODUCTION

Food waste is a significant global concern because it affects not only the environment but also the economy. Food spoilage has become a significant concern in the industry, as it leads to product loss and ultimately contributes to waste, threatening overall food security. Food packaging prevents spoilage by protecting food from pathogens and limiting exposure to air [1]. Indicator compounds such as nanomaterials, synthetic pigments, and dyes from various plant extracts are added to the film matrix to produce films that responds to changes in the packaged product [2]. Chemical indicators such as bromothymol blue, bromocresol green, violet, and red are synthetic pH indicators [3] which frequently used in food packaging and various applications to identify changes in acidity or basicity that indicate spoilage [4]. However, these chemical dyes can leach into the food as potential carcinogens and create environmental hazards by releasing toxic by-products as they decompose [5]. Consequently, these synthetic pigments are not suitable as indicators for intelligent food-packaging applications.

Anthocyanin, a natural pigment and non-toxic natural dye, is usually found in plants and is sensitive to pH variation [3]. The incorporation of natural anthocyanins into food packaging enhances pH responsiveness. Faisal et al. [8] successfully prepared biodegradable pH-indicator films using anthocyanin from purple cabbage as an indicator and pure amylase extracted from a barley cultivar as a type of polymer. However, the amylase content affected the interaction with purple cabbage anthocyanins, which degraded the mechanical strength and thermal properties.

Deep eutectic solvents (DES) have gained attention as eco-friendly and effective plasticizers for bioplastic production, owing to their properties and compatibility with natural polymers. It has been considered a green solvent and a new class of ionic liquids (IL) because they share many characteristics and properties with ILs [9]. The DES solution contains a mixture of hydrogen bond acceptors and donors, which form a eutectic mixture that results in a lower melting point compared to the individual components [10]. The

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presence of DES not only increases the mechanical properties of bioplastics but also stabilizes the anthocyanin components in the bioplastic [11]. A previous study by Kurek et al. [12] showed that DES stabilized anthocyanin components. The ability to form hydrogen bonds may increase the stability of anthocyanins by reducing their degradation rate during storage. In 2022, Thakur et al. [13] successfully produced edible pH-responsive polyvinyl alcohol (PVOC) films by combining anthocyanins and natural deep eutectic solvents (DES). However, the formation of an irregular fleck structure in the morphology results in a lack of bioplastic potential as a smart indicator. Thakur et al. [14] reported that a higher concentration of anthocyanin resulted in better elongation, but impaired tensile strength. Excess or insufficient amounts of anthocyanins may negatively affect the stability, quality, and effectiveness of an indicator. Therefore, concentration optimization is crucial for balancing the performance of the films and their practical applications in food packaging.

This study aimed to extract and quantify the anthocyanin content in purple cabbage, as well as to prepare, assess, and evaluate the properties and potential of pH indicator bioplastic films composed of starch, pectin, and DES at varying concentrations for detecting pH and ammonia vapor.

2. METHODOLOGY

2.1 Materials

Commercial pectin from citrus peel (purity ≥74.0%; Sigma-Aldrich) and corn starch were used to prepare the bioplastics. Choline Chloride (ChCl; <98% purity; Sigma-Aldrich) and Acetic Acid (AA; >99 %, reagent grade, Fisher) were used for the preparation of DES. Purple cabbage (*Brassica oleracea*), was used as a source of anthocyanins from a local market (Arau, Perlis, Malaysia).

2.2 Anthocyanin extraction

Purple cabbage was washed, dried, and cut into smaller pieces before being fully immersed in methanol (purity >99 %). The mixture was subsequently filtered to remove the solid components and the liquid was concentrated by evaporation at 65 °C using a rotary evaporator. The extracted substance was preserved in a vial, sealed with aluminum foil, and stored in the dark [2]. The anthocyanin content is analysed with UV-visible [15] and determined as the equation (1).

$$\left[(A_{515} - A_{700})_{pH\ 1.0} - (A_{515} - A_{700})_{pH\ 4.5}\right] \text{ x Mw x } \frac{1000}{\varepsilon \ell} \quad (1)$$

where A = absorbance, Mw = molecular weight of anthocyanin (449.2 g/mol), ε = molar extinction coefficient for anthocyanin (26.900 L/mole/cm) and ℓ = path length (cm).

2.3 Preparation of DES

The DES was prepared according to the method described by Zhou et al. [16], with some modifications. ChCl and AA (1:2 mole ratio) were heated and stirred until they appeared as colorless liquids. The synthesized DES was then cooled to room temperature.

2.4 Preliminary testing for development of pH indicator films

Preliminary testing was conducted to determine the optimal starch-to-pectin blend ratio for the development of pH indicator films (Table 1). Starch and pectin were dissolved in distilled water (150 mL) and mixed with a constant volume of anthocyanin (1.5 mL) and DES (4.5 mL). Subsequently, 10 mL of the solution was poured into a petri dish and dried in an oven at 35 °C for 24 h.

Table 1. Ratios of Starch to Pectin (S/P) used for Development of pH Indicator Films

Starch (g)	Pectin (g)	Starch to pectin ratio (S/P)
3.0	3.0	1:1
3.0	6.0	1:2
6.0	3.0	2:1

2.5 Development of pH indicator films with purple cabbage extract

The solution casting method for the development of pH indicator starch/pectin films was employed as described by do Nascimento et al. [17], with certain modifications. Preliminary testing was conducted using three starch-to-pectin ratios (1:1, 1:2, and 2:1 w/w); and based on superior surface smoothness and mechanical flexibility, the 1:2 (w/w) formulation was selected for further development.

Starch (3g) and pectin (6g) were dissolved in distilled water (150 mL) at room temperature and continuously stirred until the mixture was completely dissolved. DES (8.25g) was added into 150 mL of the starch-pectin solution under continuous stirring. This solution was then partitioned into four flasks (10 mL each). Anthocyanin extract was added to three flasks to achieve final concentrations of 3%, 6%, and 9% (v/v), corresponding to volume of 4.5 mL, 9.0 mL, and 13.5 mL respectively. The fourth flask served as a control without anthocyanin. The resulting formulation (Table 2) were cast into Petri dishes and oven dried at 35 °C for 24 h. Finally, the dried biofilms were peeled, cut to specific dimensions and subjected to further characterizations.

Table 2. Starch/Pectin/DES/Anthocyanin (S/P/DES/A) Films with Various Concentrations of Anthocyanin

Concentration Anthocyanin in Film [a]	Abbreviations
0%	S/P/DES (control)
3%	S/P/DES/3A
6%	S/P/DES/6A
9%	S/P/DES/9A

[a] *starch (3g), Pectin (6g) and DES (8.25mL)*

2.6 Characterisation of bioplastic

2.6.1 Fourier Transform Infrared spectrometer (FTIR) Analysis

FTIR (Fortier, PerkinElmer, USA) was carried out to observe the structural interactions of the anthocyanin extract, bioplastic samples with different anthocyanin concentrations, control, and DES mixtures. The film samples were cut into squares (1 cm × 1 cm) and placed on an FTIR plate. A few drops of DES and anthocyanins were placed on an FTIR plate. Each sample was scanned 16 times in the working range 4000–600 cm^{-1}. A background scan was performed before each analysis. Each sample was analysed in triplicates.

2.6.2 Thickness

The thickness of the bioplastic samples was examined using a digimatic thickness gauge (Model 547-301, Mitutoyo, Japan). The film samples were then cut into squares (1 cm × 1 cm). Six measurements were performed at different points for each sample.

2.6.3 Tensile properties

The tensile strength (TS), elongation at break (EAB), and Young modulus (YM) of the bioplastics were determined according to ASTM D638. Tensile and elongation analyses were conducted using a texture analyzer, the Instron Universal Testing Instrument (Instron 3365, Instron, USA) fitted with a 1 kN load cell. Strips of films measuring (1 cm × 3 cm), was placed between tensile grip fixtures (A/TG) mounted on the texture analyzer. The initial separation distance of the grip was set to 45 mm and the crosshead velocity was set to 15 mm/min. The force required to deform the bioplastic film samples was measured. The samples were run in triplicates. The mathematical expressions used to determine the TS, YM, and EAB are as equation (2), (3) and (4).

$$TS = \frac{F_{max}}{\phi} \quad (2)$$

$$EAB = \frac{\Delta l}{l_0} \times 100 \quad (3)$$

$$\text{Young's modulus (MPa)} = \frac{\text{Tensile strength}}{\text{Elongation at break}} \quad (4)$$

where F_{max} is the maximum load, ϕ is the cross-sectional area, Δl is the film extension at the sample rupture point, and l_0 is the initial length.

2.6.4 Water Vapour Transmission Rate (WVTR)

In the WVTR study, the film was placed over the opening of a test tube (2 cm × 2 cm) with 5 g of silica gel. It was then placed in a desiccator containing a saturated sodium chloride (NaCl) solution with a relative humidity of 75%. The sample was weighed every 24 hours for 3 days, and the WVTR was computed using the equation (5).

$$WVTR = \frac{\Delta W}{\Delta t} \div A \quad (5)$$

where $\frac{\Delta w}{\Delta t}$ is the amount of water transferred (mg) per unit time (s), and A is the exposed area (m^2).

2.7 Sensor Function Testing

2.7.1 Sensitivity to pH

This test was conducted as previous study with some modifications [18]. Buffer solutions with the initial of pH 3, 5, 7, 9, and 10 were adjusted using 1M of HCl to prepare buffer solution with pH 2, 4, 6, 8, respectively. The biofilms were cut (1 cm × 1 cm), and the buffer was dripped onto the film for 15 min. Changes in the color of the biofilms were observed by taking photographs of the film before and after dripping.

2.7.2 Sensitivity to Ammonia vapour

This test was performed in accordance with a previous study [19]. The sensitivity of the biofilms to volatile ammonia was measured by placing them on a conical flask contained 10mL of ammonia water (20% v/v). Changes in the color of the biofilms were observed and captured by a camera for 1-hour at 10 min interval.

3. RESULTS AND DISCUSSION

3.1 Extraction of anthocyanin from purple cabbage

Figure 1 shows the anthocyanins extracted from purple cabbage. The anthocyanin content measured was 20.49 mg/L, lower than that reported in previous studies [15] which may be attributed to plant maturity. Nevertheless, this concentration was adequate to induce observable color changes. The extracted anthocyanin solution exhibited a deep purplish-red colour with high intensity and clarity, indicating successful pigment extraction. The colour arises from the flavylium cation structure, which is highly sensitive to pH changes.

Fig. 1. Anthocyanin Extracted from Purple Cabbage

3.2 DES appearance

The mixture of DES appeared as a colorless and homogenous liquid, indicating successful formation of the eutectic (Figure 2). The resulting DES exhibited good solubility and remained stable at room temperature. The mixture was kept in a vial at room temperature before proceed for further characterizations.

Fig. 2. Prepared DES (ChCl:AA ; 1:2 mole ratio)

3.3 Biofilm appearance

3.3.1 Preliminary Testing

Preliminary testing was conducted to determine the optimal starch-to-pectin ratio for biofilm development. The films made from pure starch did not perform well, necessitating further testing with the addition of pectin. Therefore, different starch-to-pectin ratios (1:1, 1:2, and 2:1) were evaluated to identify the most suitable composition. Figure 3 shows the appearance of the films with different starch-to-pectin ratios. Visual inspection revealed that the 1:2 ratio yielded superior surface uniformity and enhanced flexibility compared to the more brittle 1:1 and 2:1 formulation. At this ratio, the film appeared with fewer visible air bubbles and higher clarity. This

supports the selection of the 1:2 ratio for further characterization, as it suggests a better interfacial interaction between the biopolymer chains and the anthocyanins.

Fig. 3. The Appearance of pH Indicator Films at Different Ratios of Starch to Pectin (1:1, 1:2 and 2:1)

3.3.2 Development of pH indicator films with purple cabbage extract

Anthocyanins extracted from purple cabbage were incorporated into the development of starch/pectin (1:2 ratio) and DES at concentrations of 1, 3, 6, and 9% (v/v) of anthocyanin. A control film was prepared using 0% anthocyanins. Each film was cast into separate petri dishes under identical conditions to ensure consistency in the film formation. After drying, the films that contained anthocyanins took on a magenta shade, whereas those without anthocyanins remained clear. Table 3 shows the developed films with different anthocyanin concentrations (S/P/DES, S/P/DES/1A, S/P/DES/3A, S/P/DES/6A, and S/P/DES/9A). The intensity of the purple color was directly proportional to the anthocyanin concentration, with the 9% (v/v) concentration producing the darkest magenta color. The increase in color intensity with higher concentrations of anthocyanins is due to the greater presence of pigment molecules within the biopolymer matrix, which enhances the visual contrast and indicator functionality. The film containing 1% anthocyanin (v/v) was colorless, making it unsuitable as a color indicator. Consequently, higher concentrations (3, 6, and 9%) were selected for further evaluation to ensure adequate visibility and performance of the pH indicator properties in the subsequent characterization stages.

3.4 Characterisation of bioplastic

3.4.1 FTIR Analysis

FTIR analysis was conducted on the DES and its components, extracted anthocyanins, and films (Figure 4). The hydroxyl (O-H) stretching in AA and ChCl, which were 3043 cm^{-1} and 3220 cm^{-1}, respectively, shifted to 3365 cm^{-1} in the prepared DES. The presence of OH indicated a potential interaction between DES and anthocyanins in the development of biofilms. The carbonyl (C = O) stretching from 1705.38 cm^{-1} in AA spectrum to 1710.00 cm^{-1} in prepared DES spectrum. The peaks of the individual components were shifted compared to their peaks in DES, suggesting the formation of hydrogen bonds between AA and ChCl [20, 21]. Figure 5 shows the spectrum of anthocyanins extracted from purple cabbage. The presence of aromatic groups was confirmed by the peak at 1639 cm^{-1} (aromatic C = C). In addition, a few peaks were identified at 1065 cm^{-1} for C-O bonds and 3265 cm^{-1} for O-H stretching. The extracted anthocyanin peak was confirmed as aligned with a previous study by Maylinda et al. [22]. Figure 6 shows the spectrum of biofilms with different anthocyanin concentrations. The peaks attributed to the formation of hydrogen bonds between starch, pectin, DES, and AA were located in the ranges of 3270 – 3350 cm^{-1}, 1740 – 1741 cm^{-1}, 1600 – 1635 cm^{-1}, and 1400 – 1480 cm^{-1}, indicating the presence of O - H stretching, C =O stretching, C - H bending, and C – O, respectively.

Table 3. The Developed of pH Indicator Films at Different Purple Cabbage Anthocyanins Concentrations

Type of film	Abbreviation	Appearance
Starch/pectin/DES (Control)	S/P/DES	
Starch/pectin/DES/1% Anthocyanin	S/P/DES/1A	
Starch/pectin/DES/3% Anthocyanin	S/P/DES/3A	
Starch/pectin/DES/6% Anthocyanin	S/P/DES/6A	
Starch/pectin/DES/9% Anthocyanin	S/P/DES/9A	

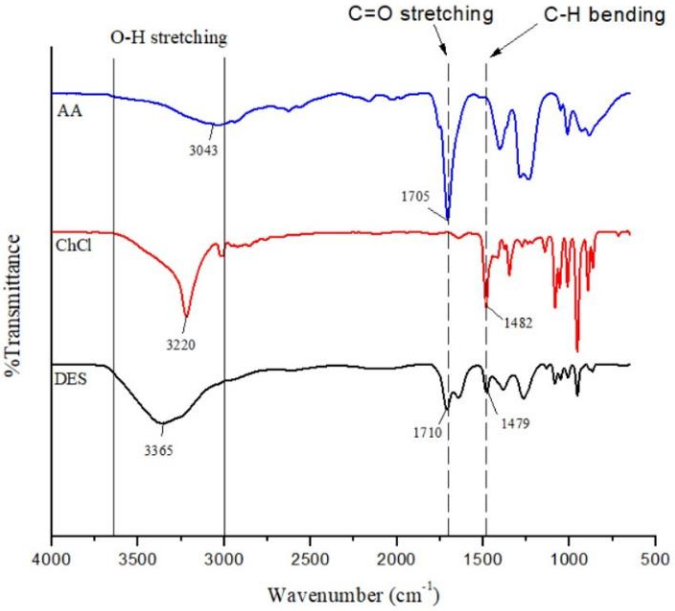


Fig. 4. FTIR spectra of prepared DES (ChCl:AA)

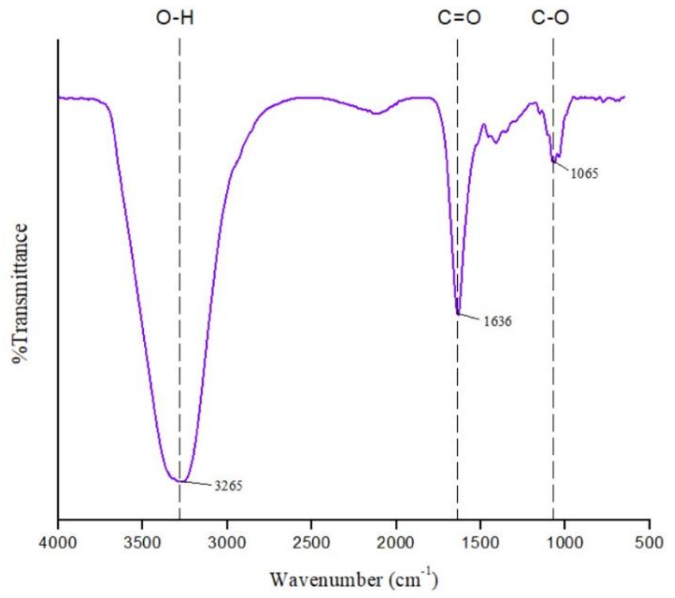


Fig. 5. FTIR spectra of extracted anthocyanin

The O-H stretching in the control film without anthocyanin (S/P/DES) was red-shifted from 3338 cm^{-1} to 3273 cm^{-1} in S/P/DES9A. The higher the anthocyanin concentration, the higher the wavenumber of O-H stretching in the films, indicating an interaction between starch, pectin, and DES in the films [23]. The small shift observed between 1634 and 1630 cm^{-1} was attributed to C=O stretching. Similarly, the C–H bending and C-O stretching showed shifts from 1481 to 1412 cm^{-1} and 1014 to 1009 cm^{-1}, respectively. These observations contradict the findings of Erna et al. [24], who reported that the incorporation of anthocyanins causes FTIR peaks to shift toward a higher wavenumber owing to the formation of hydrogen bonds between the anthocyanins and the film matrix. However, the FTIR spectra showed a red shift to a lower wavenumber with increasing anthocyanin concentration due to hydrogen bond formation between the anthocyanins and the film matrix (starch, pectin, and DES), which reduced the bond vibration energy, resulting in absorption at lower frequencies [25].

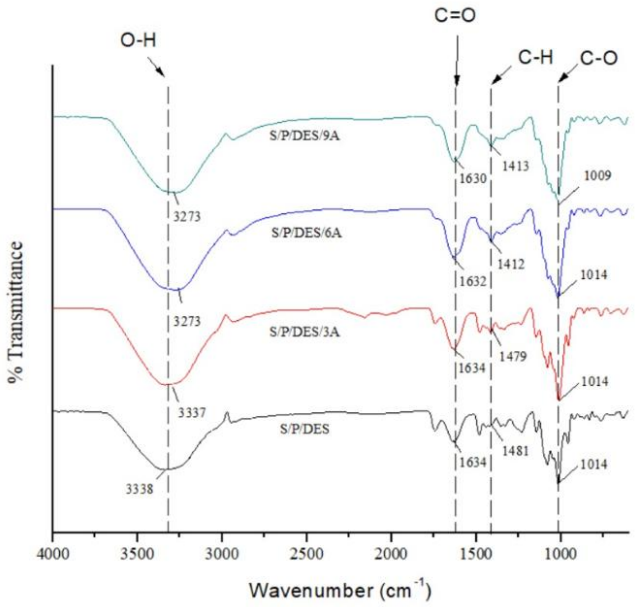


Fig. 6. FTIR spectra of biofilms incorporated with anthocyanin at different concentrations

3.4.2 Thickness and Tensile properties

The thicknesses of all biofilms were measured and compared (Figure 7). The thickness increases with amount of anthocyanin concentrations. The film containing 3% formulation (S/P/DES/3A) was the thinnest at 0.25±0.03 mm, whereas 9% anthocyanin (S/P/DES/9A) exhibited the highest thickness at 0.38±0.05 mm. The films with 6 and 9% anthocyanin were thicker than the control.

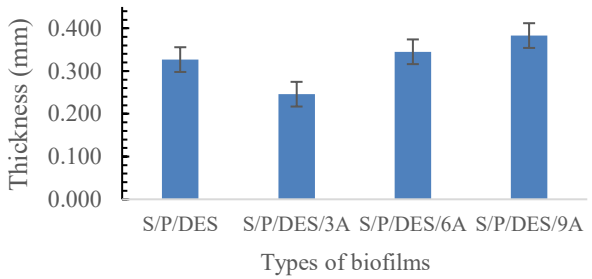


Fig. 7. Thickness of biofilms

This trend corroborates the findings of Erna et al. [24], who indicated that films incorporated with anthocyanin were thicker than films without anthocyanin. Adding anthocyanin increases the total solids in the film-forming solution, resulting in a thicker deposition when the film dries. However, the reduced thickness in the 3% formulation suggests that at lower concentrations, the anthocyanins may have facilitated a more compact intermolecular arrangement within the starch-pectin-DES matrix, leading to a denser and thinner film structure.

The tensile properties of the films, including tensile strength (TS) and elongation at break (EAB), and Young's modulus (YM), are shown in Figure 8 and 9, respectively. The control biofilm without anthocyanin (S/P/DES) exhibited higher TS (4.98 ± 2.11 MPa) and YM (305.05 ± 113.83 MPa).

However, the films incorporated with the anthocyanin extract significantly altered these mechanical properties. Both TS and YM progressively decreased with increasing anthocyanin concentrations. To be specific, the film with highest anthocyanin (S/P/DES/9A) exhibited a reduced values of 1.25 ± 0.14 MPa for TS and 15.59±5.45 MPa for YM. In contrast, EAB increased significantly from 6.13 ± 1.87% for S/P/DES to 20.66 ± 3.19% for S/P/DES/9A.

These results show that the incorporation of hydroxyl groups (–OH) and aromatic ring anthocyanins affects the mechanical properties of the films, which significantly increases EAB and decreases TS [26]. This enhancement is likely due to the interactions between the anthocyanin molecules and the starch/pectin polymer chains within the DES-plasticized matrix, where anthocyanins may function as secondary plasticizers by increasing chain mobility and reducing intermolecular rigidity. Hence, the elongation at the break of the polymers increased. However, TS and YM levels decreased, suggesting that the mechanical properties of the biofilms were compromised.

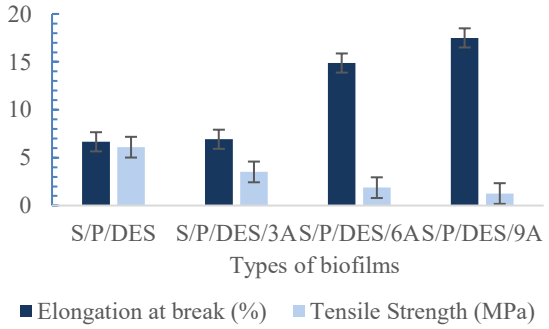


Fig. 8. Effect of different concentration of anthocyanin on maximum tensile strength and elongation at break of biofilms

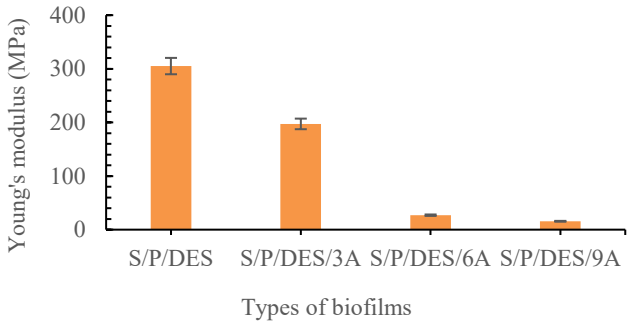


Fig. 9. Effect of different concentration of anthocyanin on Young's modulus of biofilms

3.4.3 Water Vapour Transmission Rate (WVTR

The WVTR describes how easily water vapor passes through a film, which is essential for determining the ability of packaging materials to preserve the packed food. In food packaging, a low WVTR is desirable because it limits moisture transfer, helps to preserve food quality, and prolongs shelf life. Figure 10 shows the WVTR of the prepared biofilms. The highest value of WVTR was obtained for S/P/DES/9A (86.22 g/m^2. days, while the lowest was from S/P/DES/3A, with a value of 78.11 g/m^2. day. Increasing the anthocyanin concentration led to higher WVTR values, as excessive anthocyanin disrupted the polymer matrix, increased the free volume within the film, and enhanced its hydrophilicity, thereby facilitating greater water vapor permeability. A similar finding was observed by Bouftou et al. [15], where an increase in anthocyanin concentration led to higher WVTR values. Hence, the S/P/DES/3A film is the best food packaging because it has the lowest WVTR (value among the rest.

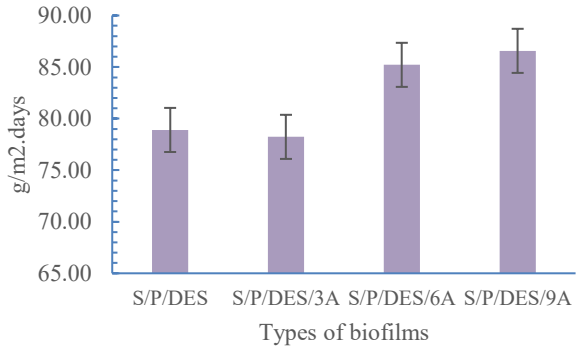


Fig. 10. Effect of different concentration of anthocyanin on WVTR of biofilms

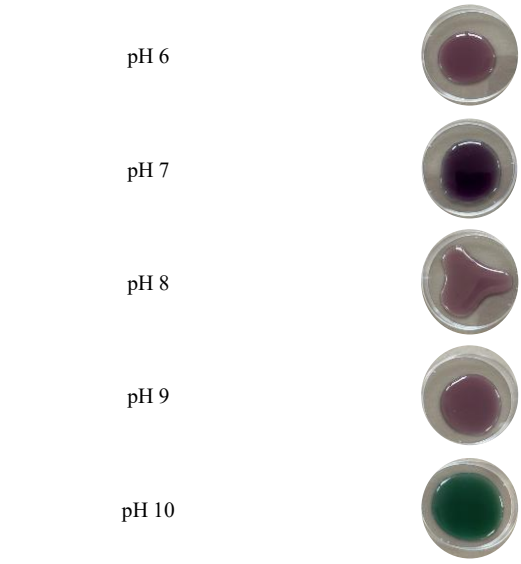
3.5 Sensor Function Testing

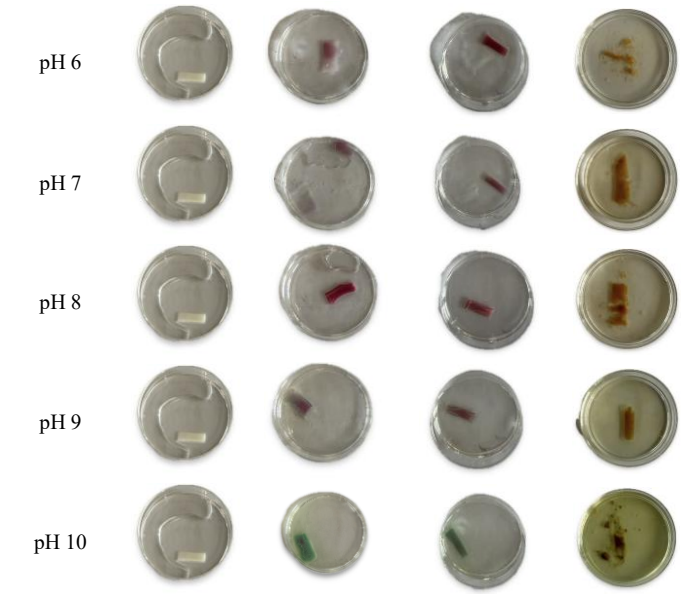
3.5.1 The colour response of anthocyanin and film to pH changes

Purple cabbage anthocyanin extract was observed in pH 2–10 buffer solutions to characterize its pH-dependent color changes before film integration. As shown in Table 4, purple cabbage anthocyanin extracts exhibited different shades of color under different pH conditions. The anthocyanin solution in bright pink from pH 2-4, become dark purple at pH 5-7 and turn light purple at pH 8-9 and changes to greenish blue at pH 10. The clear pH-responsiveness of anthocyanin in solution supports its potential as a natural dye for indicator films. The sensitivity of the prepared films was tested at different pH values to determine the ability of anthocyanins to act as indicators in the film.

Table 4. The colour response of pure anthocyanin solution in buffer pH 2-10

pH level	Pure anthocyanin solution
pH 2	
pH 3	
pH 4	
pH 5	





The anthocyanin solution in bright pink from pH 2-4, become dark purple at pH 5-7 and turn light purple at pH 8-9 and changes to greenish blue at pH 10. The clear pH-responsiveness of anthocyanin in solution supports its potential as a natural dye for indicator films. The sensitivity of the prepared films was tested at different pH values to determine the ability of anthocyanins to act as indicators in the film. As shown in Table 5, the anthocyanin film showed color changes after immersion in a buffer solution with pH values ranging from 2 to 10. The S/P/DES/3A film, which was initially bright pink, changed to dark pink under acidic conditions (pH 2–5), and shifted to bright green under alkaline conditions (pH 10). Meanwhile, the S/P/DES/6A dark magenta film became dark pink at pH 2–3 and displayed a bright green color at pH 10. In contrast, the S/P/DES/9A dark magenta film turned brownish-orange at pH 2-6 and change from dark brown to blackish at pH 10.

Table 5. The colour response of biofilms in buffer pH 2-10

pH level	S/P/DES	S/P/DES/3A	S/P/DES/6A	S/P/DES/9A
pH 2				
pH 3				
pH 4				
pH 5				

Generally, the color transitions of anthocyanins in the prepared indicator films followed the general pH-dependent mechanism described by Halasz et al. [27], shifting from red (flavylium) at acidic pH to purple (quinonoid base) under near-neutral conditions and bluish at mildly alkaline pH. In contrast to pure solutions, the integration of polymers and DES alters the visual intensity and clarity of these transitions. At low concentrations, anthocyanins were more uniformly dispersed in the starch–pectin matrix, producing brighter and clearer color transitions. At higher concentrations, anthocyanins tended to self-associate within the polymeric matrix, leading to aggregation and a darker shade in S/P/DES/9A. DES improved the stabilization of the protonated flavylium form, thereby enhancing the red coloration and delaying the shift to a bluish hue. These color variations suggest that the anthocyanin concentration not only enhanced the pH sensitivity of the films but also influenced the structural stability of anthocyanins within the polymer-DES matrix, resulting in modified colorimetric behavior. Notably, the control film without anthocyanins (S/P/DES) showed no visible color change across the tested pH range, confirming the role of anthocyanins as the primary pH-sensitive component.

The pH-dependent color change observed in biofilms is consistent with the findings of Qin et al. [28], who developed a film incorporating chitosan/gelatin with anthocyanins derived from Zingiber striolatum Diels. Additionally, Wu et al. [29] observed a similar color change in their konjac glucomannan/zein (KZ) composite film, which was enhanced by anthocyanins from various sources. Overall, the indicator films in this study could potentially be used to screen the deterioration levels of food products in real-time.

3.5.2 The colour response of films to volatile compounds

Ammonia vapor was used to evaluate the responsiveness of the film to volatile alkaline compounds, with the aim of assessing its potential application as an active food packaging material. The biofilms were exposed to ammonia vapor for 1 h at 10 min intervals. The color changes in the biofilms are shown in Table 6.

The control film (S/P/DES), which did not contain anthocyanins, showed no visible color changes. Meanwhile,

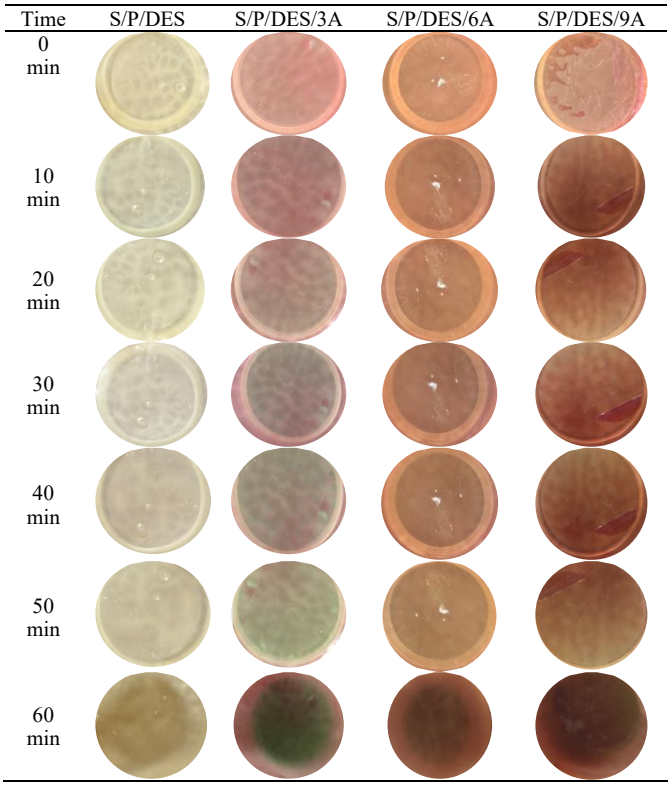
S/P/DES/3A, S/P/DES/6A, and S/P/DES/9A showed color changes. Specifically, the S/P/DES/3A film changed from bright pink to slightly greenish in 30 minutes, and finally became dark green after 1 h of exposure to ammonia vapor. By contrast, the S/P/DES/6A and S/P/DES/9A films exhibited minimal differences when subjected to pH changes, and these variations were not distinctly observable. However, both the films became dark brown after 1 h. Hence, S/P/DES/3A exhibited the most observable color change to the naked eye and seemed to have a greater potential for food spoilage. The color changes were attributed to the phenolic compounds in the anthocyanin extracted from purple cabbage. When ammonia vapor undergoes hydration and hydrolysis, it produces ammonium ions (NH_4^+), resulting in an alkaline environment on the biofilm surface. The change in pH alters the anthocyanin structure from the red flavylium cation to the quinoidal base or chalcone forms, leading to distinct color changes [28]. This finding is aligned with a study by Ren et al. [30], who developed a film that incorporated anthocyanin from purple cabbage within the κ-carrageenan/carboxymethyl cellulose (CA/CMC) matrix and observed a color change from purple to green when the films were exposed to ammonia vapor. This finding suggests that S/P/DES/3A could be a valuable tool for real-time screening of food product deterioration.

4. Conclusion

This study successfully extracted and quantified anthocyanins from purple cabbage, which later being incorporated into pH-sensitive bioplastic films composed of starch, pectin, and DES. The results demonstrated that the physical and mechanical properties of the films were significantly influenced by the anthocyanin concentration. Specifically, the S/P/DES/3A (3% anthocyanin) formulation emerged as the optimal indicator, providing a superior balance of mechanical strength, flexibility, and a functional WVTR of 78.11 g/m²·day.

While higher concentrations (6% and 9%) enhanced film flexibility, they negatively impacted the WVTR and produced darker hues that limited the clarity of color changes, reducing their suitability as indicators. In contrast, the 3% formulation provided distinct, high-contrast color transitions shifting from bright pink to bright green at pH 10. Furthermore, the film proved its potential for food spoilage monitoring by detecting ammonia vapor through a visible shift to dark green within one hour of exposure. FTIR analysis confirmed stable intermolecular interactions between the starch, pectin, DES, and anthocyanins, validating the S/P/DES/3A film as a robust, bio-based tool for real-time detection of alkaline spoilage vapors.

Table 6. The colour response of biofilms after exposed to ammonia vapour for 1 hour



References

[1] Chandra, D. K., Kumar, A., & Mahapatra, C. (2024). Ecofriendly bioplastics from biowaste: Antimicrobial and functional enhancements for sustainable packaging. *European Polymer Journal,* 113557. doi: https://doi.org/10.1016/j.eurpolymj.2024.113557

[2] Poudel, R., Dutta, N., & Karak, N. (2023). A mechanically robust biodegradable bioplastic of citric acid modified plasticized yam starch with anthocyanin as a fish spoilage auto-detecting smart film. *International journal of biological macromolecules*, 242, 125020.. doi: https://doi.org/10.1016/j.ijbiomac.2023.125020

[3] Xiao, Z., Han, L., Gu, M., Zhu, Y., Zhang, Y., Li, Z., Xie, T., & Lu, F. (2023). Performance comparison of anthocyanin-based smart indicator films. *Food Packaging and Shelf Life*, 40, 101187. doi: https://doi.org/10.1016/j.fpsl.2023.101187

[4] Noiwan, D., Suppakul, P., & Rachtanapun, P. (2022). Preparation of methylcellulose film-based CO2 indicator for monitoring the ripeness quality of mango fruit cv. Nam Dok Mai Si Thong. *Polymers*, 14(17), 3616. doi: https://doi.org/10.3390/polym14173616

[5] Sharma, K., Pandit, S., Mathuriya, A. S., Gupta, P. K., Pant, K., & Jadhav, D. A. (2022). Microbial electrochemical treatment of methyl red dye degradation using co-culture method. *Water*, 15(1), 56. doi: https://doi.org/10.3390/w15010056

[6] Xiong, G., Zhou, X., Zhang, C., & Xu, X. (2024). A comprehensive review of intelligent packaging materials based on biopolymers: Role of anthocyanins, type and properties of materials, and their application in monitoring meat freshness. *International Journal of Biological Macromolecules*, 137462. doi: https://doi.org/10.1016/j.ijbiomac.2024.137462

[7] Gamarra-Castillo, O., Hernández-Carrión, M., & del Pilar Sánchez-Camargo, A. (2025). Revalorization of purple passion fruit peel: Compositional analysis, anthocyanin microwave-assisted extraction, and beverage application *Future Foods*, 11, 100536. doi: https://doi.org/10.1016/j.fufo.2024.100536

[8] Faisal, M., Bevilacqua, M., Bro, R., Bordallo, H. N., Kirkensgaard, J. J. K., Hebelstrup, K. H., and Blennow, A. (2023). Colorimetric pH

indicators based on well-defined amylose and amylopectin matrices enriched with anthocyanins from red cabbage. International Journal of Biological Macromolecules, 250. doi: https://doi.org/10.1016/j.ijbiomac.2023.126250

[9] Smith, E. L., Abbott, A. P., and Ryder, K. S. (2014). Deep Eutectic Solvents (DESs) and Their Applications. In Chemical Reviews (Vol. 114, Issue 21, pp. 11060– 11082). American Chemical Society. doi: https://doi.org/10.1021/cr300162p

[10] Rolińska, K., Jakubowska, E., Żmieńko, M., & Łęczycka-Wilk, K. (2024). Choline chloride-based deep eutectic solvents as plasticizer and active agent in chitosan films. *Food Chemistry*, 444, 138375. doi: https://doi.org/10.1016/j.foodchem.2024.138375

[11] Hikmawanti, N. P. E., Ramadon, D., Jantan, I., and Mun'im, A. (2021). Natural Deep Eutectic Solvents (NADES): Phytochemical Extraction Performance Enhancer for Pharmaceutical and Nutraceutical Product Development. Plants, 10(10), 2091. doi: https://doi.org/10.3390/plants10102091

[12] Kurek, M. A., Custodio-Mendoza, J. A., Aktaş, H., and Pokorski, P. (2024). Exploring deep eutectic solvent extraction's impact on anthocyanin degradation kinetics in various conditions. LWT, 198. doi: https://doi.org/10.1016/j.lwt.2024.115994

[13] Thakur, R., Gupta, V., Ghosh, T., and Das, A. B. (2022). Effect of anthocyanin- natural deep eutectic solvent (lactic acid/fructose) on mechanical, thermal, barrier, and pH-sensitive properties of polyvinyl alcohol based edible films. Food Packaging and Shelf Life, 33. doi: https://doi.org/10.1016/j.fpsl.2022.100914

[14] Thakur, D., Kumar, Y., Sharanagat, V. S., Srivastava, T., & Saxena, D. (2023). Development of pH-sensitive films based on buckwheat starch, critic acid and rose petal extract for active food packaging. Sustainable Chemistry and Pharmacy, 36, 101236 doi: https://doi.org/10.1016/j.scp.2023.101236

[15] Bouftou, A., Aghmih, K., Belfadil, D., Lakhdar, F., Gmouh, S., & Majid, S. (2025). Intelligent and active films with thymol and red cabbage anthocyanin for advanced fish packaging. *Food Science and Biotechnology*, 34(2), 337-348. doi: https://doi.org/10.1007/s10068-024-01662-5

[16] Zhou, H., Guan, Y., Yan, X., Pan, Z., Xu, J., Dai, L., Zhang, M., & Si, C. (2023). All-lignocellulose-based hard bioplastic. *Industrial Crops and Products*, 193, 116164. doi: https://doi.org/10.1016/j.indcrop.2022.116164

[17] do Nascimento, R. A., Uchida, D. T., Balbinot, R. B., Nakamura, C. V., Uesu, N. Y., Reis, A. V., & Bruschi, M. L. (2025). Effect of glycerol diglycidyl ether crosslinker on pectin-starch blended pharmaceutical films. *International journal of biological macromolecules*, 307, 142257. doi: https://doi.org/10.1016/j.ijbiomac.2025.142257

[18] Zhou, H., Guan, Y., Yan, X., Pan, Z., Xu, J., Dai, L., Zhang, M., & Si, C. (2023). All-lignocellulose-based hard bioplastic. Industrial Crops and Products, 193, 116164 doi: https://doi.org/10.1016/j.indcrop.2022.116164

[19] Bao, Y., Cui, H., Tian, J., Ding, Y., Tian, Q., Zhang, W., Wang, M., Zang, Z., Sun, X., & Li, D. (2022). Novel pH sensitivity and colorimetry-enhanced anthocyanin indicator films by chondroitin sulfate co-pigmentation for shrimp freshness monitoring. *Food Control,* 131, 108441. doi: https://doi.org/10.1016/j.foodcont.2021.108441

[20] Sun, L., Han, J., Tang, C., Wu, J., Fang, S., Li, Y., Mao, Y., Wang, L., & Wang, Y. (2022). Choline chloride-based deep eutectic solvent system as a pretreatment for microcrystalline cellulose. *Cellulose*, 29(15), 8133-8150. doi: https://doi.org/10.1007/s10570-022-04771-8

[21] Matchim Kamdem, M. C., Tamafo Fouegue, A. D., & Lai, N. (2023). A comprehensive study on DES pretreatment application to microalgae for enhanced lipid recovery suitable for biodiesel production: Combined experimental and theoretical investigations. *Energies*, 16(9), 3806. doi: https://doi.org/10.3390/en16093806

[22] Maylinda, E. V., Rinadi, A., Putri, E. A., Fadillah, G., & Wayuningsih, S. (2019). Color Stability of Anthocyanins Copigmentation from Red Rice (Oryza sativa L.) Bran by Spectrophotometry UV-Vis. IOP Conference Series: Materials Science and Engineering, 578(1). doi: https://doi.org/10.1088/1757-899x/578/1/012001

[23] Zacheski, J. B. A., Rojas, C. E. B., Gerhardt, R., Cappellari, N. P., De Farias, B. S., Ribeiro, A. C., Cadaval, T. R. S., Junior, & De Almeida Pinto, L. A. (2025). Chitosan-based films enriched with anthocyanin compounds for potential use as food packaging. *International Journal of Biological Macromolecules*, 317, 144867. doi: https://doi.org/10.1016/j.ijbiomac.2025.144867

[24] Erna, K. H., Felicia, W. X. L., Vonnie, J. M., Rovina, K., Yin, K. W., & Nur'Aqilah, M. N. (2022). Synthesis and physicochemical characterization of polymer film-based anthocyanin and starch. *Biosensors*, 12(4), 211. doi: https://doi.org/10.3390/bios12040211

[25] Hasan, M., Tasya, G., Khaldun, I., Rusman, R., & Hasanah, U. (2025). Eco-friendly bio-nanocomposite films for sustainable packaging. *Journal of Ecological Engineering*, 26(4), 301-312. doi: https://doi.org/10.12911/22998993/200335

[26] Li, Y., Xie, L., Jiang, X., Cai, G., Zhu, G., Zheng, Z., & Liu, F. (2023). Effect of anthocyanins on mechanical and physicochemical properties of wheat dough. *Journal of Cereal Science*, 110, 103652. doi: https://doi.org/10.1016/j.jcs.2023.103652

[27] Halász, K., Kóczán, Z. & Joóbné Preklet, E. pH-dependent color response of cellulose-based time-temperature indicators impregnated with red cabbage extract. Food Measure 17, 2555–2565 (2023). doi: https://doi.org/10.1007/s11694-023-01805-y

[28] Qin, Y., Wang, Y., Tang, Z., Chen, K., Wang, Z., Cheng, G., Chi, H., & Soteyome, T. (2024). A pH-sensitive film based on chitosan/gelatin and anthocyanin from Zingiber striolatum Diels for monitoring fish freshness. *Food Chemistry: X*, 23, 101639. doi: https://doi.org/10.1016/j.fochx.2024.101639

[29] Wu, K., Zhu, D., Zeng, Y., Cheng, J., Wang, R., Peng, B., Chen, K., Deng, P., Jiang, F., & Zhao, X. (2024). Impact of anthocyanin extract sources on the physical properties and pH sensitivity of konjac glucomannan/zein composite film. *Food and Bioprocess Technology*, 17(11), 3926-3943. doi: https://doi.org/10.1007/s11947-024-03358-4

[30] Ren, M., Wang, N., Lu, Y., & Wang, C. (2025). Preparation and Characterization of Antioxidative and pH-Sensitive Films Based on κ-Carrageenan/Carboxymethyl Cellulose Blended with Purple Cabbage Anthocyanin for Monitoring Hairtail Freshness. Foods, 14(4), 694. doi: https://doi.org/10.3390/foods14040694