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Halochromic smart film: A gelatin-based pH-sensitive film embedded with anthocyanin from roselle (*Hibiscus sabdariffa*) extracts for potential food spoilage indicator application

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Abstract

This study developed and characterized a pH-sensitive gelatin-based film incorporated with anthocyanin from roselle extracts (*Hibiscus sabdariffa*) at varying concentrations (0%–15%). The addition of anthocyanins significantly influenced the film's properties, reducing tensile strength from 1.5 to 1.1 MPa while increasing elongation at break from 312% to 416%. Water vapor permeability also increased with anthocyanin concentration, ranging from 1.02 to 1.47 × 10^{-8} g·m^{-1}·s^{-1}·Pa^{-1}. The film displayed a distinct color change from red to yellow-green across pH 3–10 and when exposed to ammonia gas, highlighting its potential for real-time detection of volatile basic compounds. Fourier transform infrared analysis revealed molecular interactions between gelatin and anthocyanins, with increasing roselle extract concentrations as it showed enhanced amide A, I, II, and III functional groups. Scanning electron micrographs revealed increased surface roughness and microstructural changes with anthocyanin addition. These findings demonstrate the potential of 15% roselle anthocyanin–gelatin films as eco-friendly, pH-sensitive smart packaging materials for food applications.

KEYWORDS
anthocyanin, chicken skin gelatin, pH-sensitive film, smart film

1 | INTRODUCTION

Smart film packaging is an eco-friendly alternative to plastic films, which have caused significant environmental issues. Environmental issues caused by plastic film encompass alterations to the carbon dioxide cycle, challenges in the composting process, and elevated levels of toxic emissions. Consequently, driven by environmental apprehensions, numerous scientists have now focused on methods to create biodegradable packaging, which

garners substantial interest in the industry due to its sustainability, reliance on renewable resources, abundance, eco-friendliness, and potential to serve as a substitute for petrochemical materials (Shaik et al., 2022). Hence, novel concepts such as active and smart biodegradable film packaging have surfaced, attributed to their crucial role in providing numerous inventive solutions for enhancing the shelf-life or improving the quality and safety of food (Loo & Sarbon, 2020). In recent years, a smart film with the addition of natural colorant has drawn much interest because it gives consumers real-time, qualitative, and quantitative information on the freshness of food products, such as meat products (Ezati et al., 2020). This natural colorant is often incorporated into the biodegradable polymer as a pH-sensitive smart film.

pH-sensitive smart films integrated with natural colorants can enable color changes in response to pH variations, making them valuable tools for monitoring food freshness status. These smart films can serve as sensors for meat freshness monitoring, offering a reliable and efficient means of assessing food quality (Shaik et al., 2022). This pH-sensitive natural colorant is often used as a smart film in biodegradable polymers, such as polysaccharides and polypeptides. Among the biodegradable polymers, polypeptides are the best matrix to immobilize natural colorants. This is because proteins are heteropolymers encompassing a diverse range of functional groups. Hence, it is important to highlight that protein-based films can serve as efficient carriers for integrating various additives, such as colorants (Purewal et al., 2024).

Gelatin film, one of the protein-based films, is gaining interest among researchers since it exhibits remarkable film-forming properties, abundance, and exceptional barrier properties against O_2, CO_2, and lipids (Said & Sarbon, 2021). Gelatin films are often derived from mammalian sources; however, due to foot and mouth illness and bovine spongiform encephalopathy in cows, substitute gelatin sources, such as chicken skin, used in the film's manufacturing have offered a safer choice. Additionally, the economic implications of utilizing chicken skin for gelatin production are significant. The poultry industry generates substantial amounts of by-products, including chicken skin, which can be repurposed to produce gelatin; hence, the utilization of chicken skin for gelatin extraction not only utilizes a by-product of the poultry industry and reduces additional costs but additionally provides a sustainable alternative to traditional sources such as bovine or porcine gelatin, which may raise dietary and ethical concerns among consumers. Besides, the chicken skin gelatin film showed better mechanical properties than mammalian sources (Said & Sarbon, 2021), where this property is crucial for film packaging applications.

Nonetheless, the single gelatin film has some significant weaknesses such as poor flexibility and ductility because the molecular chains of gelatin are aligned in a way that restricts their movement, leading to limited flexibility (Lou & Chen, 2023). As a result, these films lack the ability to stretch significantly before breaking. Hence, one of the approaches is to blend gelatin with tapioca starch. The incorporation of tapioca starch into gelatin films enhances the ductility of the films because of the formation of a three-dimensional network during gelatinization. When tapioca starch undergoes heating in the presence of water, it gelatinizes, leading to swelling and the formation of a gel-like structure. This process facilitates the creation of intergranular binding forces among starch molecules via hydrogen bonding (Stephen et al., 2021). Therefore, the resulting hydrogen bonding might extend the flexibility and elongation at break (EAB) (Stephen et al., 2021).

In addition, both gelatin and tapioca starch have high water affinity properties. Gelatin can form a gel network with some water-holding capacity, while tapioca starch assists in the formation of film but swells and disperses in water. Nevertheless, the primary aim of this work was to obtain an edible/biodegradable pH-sensitive film rather than high water resistance films. Furthermore, the films in this research are not intended for use in water-containing environments and are not in direct contact with perishable food. Instead, they are targeted to be used where direct exposure to high amounts of moisture is minimal such as smart films that detect pH changes for food freshness monitoring. On top of that, one of the primary advantages of utilization of high water affinity polymers in the development of films is their ability to regulate moisture levels within the packaging environment. Such films can absorb excess moisture from the food or the surrounding environment, thereby preventing the accumulation of water that can lead to spoilage, mold growth, and bacterial contamination (Lai et al., 2021). Besides, films with high water affinity can mitigate the risk of condensation forming inside the package, which is particularly important for perishable items like meat products that are sensitive to moisture fluctuations (Chen et al., 2024). Hence, blending gelatin/ tapioca starch with a natural colorant, which is anthocyanin, in the development of smart films is a very relevant approach to monitoring meat freshness. This relevant approach is due to the halochromic properties of anthocyanin, which is the capability to change color at different pHs. Hence, studying these halochromic properties is crucial as they are very useful in assessing the food's freshness status (Li et al., 2024).

Besides the halochromic properties, the characterization of the physical, mechanical, and water vapor permeability (WVP) of gelatin films is crucial in determining their qualities (Said & Sarbon, 2022). The physical

properties of gelatin films are measured including tensile strength (TS), EAB, secondary structure by Fourier transform infrared (FTIR), WVP, and microstructure. While colorimetric properties of composite gelatin smart films incorporated with natural colorants are evaluated based on their color response to pH, pH changes that occur in food packaging often indicate food spoilage, hence causing changes in the color of smart films (Rawdkuen et al., 2020). Thus, the film's colorimetric analysis is crucial to developing effective gelatin films for smart film applications. Although pH-sensitive films have been widely studied, the integration of roselle anthocyanins into chicken skin gelatin for dual pH and ammonia gas sensitivity remains largely unexplored.

Therefore, in this study, the integration of roselle anthocyanin into a gelatin matrix, particularly from underutilized chicken skin, represents a novel approach to developing a smart film with simultaneous pH and ammonia gas sensing. This study aims to develop and characterize gelatin-based films incorporated with anthocyanins extracted from roselle (*Hibiscus sabdariffa*) to gain insights into the pH-responsiveness of smart films toward ammonia gas. Leveraging the excellent film-forming properties of gelatin and tapioca starch, and the high pH sensitivity of anthocyanins, films were prepared with varying anthocyanin concentrations (0%–15%) and analyzed for their physical, mechanical, and barrier properties. The findings revealed that increasing anthocyanin concentration improved elongation properties and pH sensitivity and introduced distinct ammonia gas responsiveness. These results demonstrate the potential of roselle anthocyanin–gelatin films as eco-friendly, smart packaging materials for food applications.

2 | MATERIALS AND METHODS

2.1 | Materials and chemicals

The roselle was acquired in Kuala Terengganu from Halwa Roselle, and freshly bought chicken skins were acquired from TNJ Frozen Food, Kuala Terengganu. Fresh chicken skins were thoroughly cleaned by eliminating signs of blood and feathers and after that retained in a freezer at −18°C for subsequent application. All the chemicals and reagents used in this study were of analytical grade and purchased from Sigma Aldrich Company (M) Sdn. Bhd.

2.2 | Preparation of chicken skin

At 4°C, the frozen chicken skins were defrosted overnight before the test. The chicken skins' visible fats were physically eliminated and then properly rinsed with plenty of water to eliminate impurities. The cleaned chicken skins were chopped into 4 × 4 cm and manually squeezed to remove the excess water. Then, the skins were dried for 24 h in a cabinet dryer (Protech FSD-380, Selangor, Malaysia) at 45°C until entirely dried and mashed by a commercial blender (BL 1012, Khind, Selangor, Malaysia). It then went through the defatting process using Soxtec (AOAC, 2006).

2.3 | Extraction of chicken skin gelatin

Chicken skin gelatin was extracted according to the technique proposed by Soo and Sarbon (2018). First, 15 g of defatted and dried chicken skin was soaked in 200 mL of a 0.15% sodium hydroxide solution (w/v). The mixture was then thoroughly homogenized and stirred for half an hour at ambient temperature using a magnetic stirrer. Following this, centrifugation at 35,000 rpm of the mixture was completed in 10 min using a centrifuge (1580R, Gyrozen Co., Ltd., Daejeon, Korea). This alkaline pre-treatment process was done three times to ensure the maximum removal of non-collagenous proteins and pigments from the sample. The pellets were subsequently cleansed with distilled water to eliminate any remaining salts and centrifuged once again at 3500 rpm for 10 min. The resulting pellets were then subjected to three consecutive rounds of acidic treatment, comprising 200 mL of a 0.15% (w/v) sulfuric acid solution and 200 mL of a 0.7% (w/v) citric acid solution. This treatment was employed to enhance the production of gelatin. The pellets obtained were washed using distilled water, and the hot water extraction process was carried out overnight at a temperature of 50°C. The extract was subsequently subjected to a thorough filtration process by Buchner funnel and Whatman No. 4 filter paper. To reduce the filtered solution containing gelatin to a tenth of its original volume, it was subjected to evaporation under vacuum conditions at a temperature of 45°C, utilizing a rotary evaporator (Rotavapour R25, Buchi, Switzerland). The solution, now concentrated with gelatin, remained in a −80°C freezer for 24 h before undergoing freeze-drying with the assistance of a vacuum pump freeze-dryer. Finally, the dried gelatin powder was crushed and preserved for future application. Proximate analysis according to AOAC (2006) was carried out to obtain the purity of gelatin.

2.4 | Extraction of roselle anthocyanins

The method proposed by Zhang et al. (2019) was used to produce roselle extract. First, 1 g of dried roselle petals were pollinated. Then, 80% ethanol (15 mL) was added to the 1 g of powdered roselle, and the pH was accustomed to 2.0

TABLE 1 Formulations of gelatin/tapioca starch films incorporated with roselle extract.

Formulation	Gelatin (g)	Tapioca starch (%)	Roselle extract (%)	Glycerol (%)
A	4	4	0	30
B	4	4	5	30
C	4	4	10	30
D	4	4	15	30

Abbreviations: A, film containing 0% of roselle extract concentration; B, film containing 5% of roselle extract concentration; C, film containing 10% of roselle extract concentration; D, film containing 15% of roselle extract concentration.

using 1 M HCl. The resulting extract was then heated to a temperature of 50°C for 1 h and subjected to centrifugation at a force of 3000 x *g* for 6 min, yielding the extract. The solvent was evaporated in the dark at 40°C to concentrate the sample solution using a rotary evaporator. Lastly, the concentrated anthocyanin solution was freeze-dried using a vacuum freezer dryer for 2 days.

2.5 | Preparation of pH-sensitive film

The casting procedure employed by Loo and Sarbon (2020) to create films of chicken skin gelatin with roselle extract was used with some modifications. The formulation of the gelatin/tapioca starch incorporated with varying concentrations of roselle extracts is presented in Table 1. Individually, the solution mixture was agitated for 1 h at a temperature of 60°C. In a separate process, 4 g of chicken skin gelatin powder and 4 g of tapioca starch were dissipated finely in distilled water at 45°C and 85°C, respectively. Subsequently, the tapioca starch solution was combined with the chicken skin gelatin solution (with a pH of 6.0) while being continually agitated, followed by adding the roselle extract. Finally, glycerol at a concentration of 30% (w/w) of the gelatin was mixed into the film-forming solution (FFS) at a temperature of 45°C with continuous stirring for 30 min. A total of 25 g of the solutions were poured onto a glass petri dish with a diameter of 90 mm and dried in an oven at a temperature of 45°C until complete dryness was achieved. Subsequently, the films were manually carefully peeled off and stored in an airtight container for further analysis.

2.6 | Physical properties of pH-sensitive film

2.6.1 | TS and EAB of pH-sensitive film

The TS and EAB of the films were measured using a texture analyzer (Stable Microsystem, TA XT2 Plus, Godalming, Surrey, UK) at a crosshead speed of 5 mm min^{-1}. Rectangular samples (1 × 7 cm) were cut from the films and pre-conditioned at 25°C and 50% relative humidity for 48 h before testing. The film strip was positioned on the texture analyzer, which was equipped with grip pairs of AT/G probes and a 30-kg load cell. The initial grip distance between the upper and lower sections of the grip was established as 50 mm. The film's strip was extended by relocating the upper grip at a crosshead speed of 120 mm min^{-1} until it fractured. The average thickness of the film strip was measured at five different points.

2.6.2 | Film morphology of pH-sensitive film

The films' microstructural study was conducted via scanning electron microscopy (SEM) on the JEOL model JSM-6610LV (Japan). The observation focused on the film samples' surface and cross-sections initially cracked under liquid nitrogen. These samples were mounted perpendicular to the surface on copper stubs and sputtered with gold. This enables the film to be sufficiently conductive to allow for the direct flow of 10 kV of accelerated voltage at magnifications ranging from 1500 to 3000×.

2.6.3 | Secondary structure of pH-sensitive film by FTIR

The functional group of pH-sensitive films was determined by using an FT-IR spectrometer (Nicolet, Thermo Electron, USA) following the method proposed by Li et al. (2024). To perform the analysis, the film was carefully cut and scanned on the surface of the reduced total reflection (ATR) crystal. The FTIR spectrum is configured in the range of 4000–400 cm^{-1}, using 32 scans and 4 cm^{-1} resolution. The peaks of amides A, I, II, and III were observed.

2.6.4 | WVP of the pH-sensitive film

WVP was used to characterize the properties of water barriers. The film's WVP was determined using the

modified ASTM E96 (ASTM, 1991) method. The average value was obtained by measuring triplicates for each film formulation The film was put in closed glass permeating bottles with 10 g of silica gel (0.0% RH). The initial weight of the bottle was measured before it was placed in a dessicator with a distilled water at ambient temperature. Hourly, the weight of each film cup was recorded for 6 h. The thickness measurement of each film strip was taken at five distinct locations using a micrometer (Mitigo 406-350) to measure the WVP according to thickness. The calculation of WVP was carried out in the following manner:

$$\mathrm{WVP}(\mathrm{g}\cdot\mathrm{m}^{-1}\cdot\mathrm{s}^{-1}\cdot\mathrm{Pa}^{-1}) = w\times x/At\times(P_2-P_1) \quad (1)$$

The weight of the cup (w), the average thickness of the film (x), the surface area of penetration (A), the time (t), and the difference in the partial pressure ($P_2 - P_1$) are all variables in the equation.

2.6.5 | Opacity value

The transmittance of the film was measured using a UV–visible spectrophotometer (Shimadzu UV-1800, Shimadzu Corp, Japan). The opacity value was calculated as follows:

$$\mathrm{Opacity} = A/x \quad (2)$$

where A represents the film absorbance at 600 nm and x indicates the thickness (mm) of the films. Opacity is represented as A mm^{-1}.

2.6.6 | Water contact angle

The water contact angle (WCA) of the films was measured according to Li et al. (2024) using a WCA analyzer (Lauda LSA 60, Nexus Analytics, China). The film samples ($1\times1\ \mathrm{cm}^2$) were positioned and secured on a horizontally adjustable plate of the contact angle device. The contact angle was subsequently measured right after a water drop (approximately 5 μL) was placed on the surface of the film samples with a micro-syringe.

2.6.7 | Water solubility

The water solubility (WS) of the films was determined using the method described by Shakouri et al. (2023). Initially, $3\times3\ \mathrm{cm}^2$ film samples were dried at a temperature of 110°C for 24 h. The films were measured to find out the original solid content, then submerged in 40 mL of water and mixed for 24 h at room temperature. Insoluble films were removed and dried at 110°C, and their final weights were recorded. Their WS was subsequently determined using the following formula:

$$\mathrm{WS}\,(\%) = (W_\mathrm{i} - W_\mathrm{f}/W_\mathrm{i})\times100 \quad (3)$$

Here, W_i is the initial sample weight and W_f is the insoluble sample weight.

2.7 | Antioxidant radical scavenging assay

The antioxidant characteristics of the film samples were determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay described by Shakouri et al. (2023), with minor modifications. To prepare the samples for analysis, 50 mg of each film was mixed with 10 mL of distilled water for 2 h and then centrifuged at 4000 ×g for 5 min. The supernatant was then collected using three distinct in vitro procedures to determine antioxidant activity. The DPPH solution (0.1 mM) was produced in ethanol. A volume of 400 μL of film extract or control sample (400 μL distilled water) was mixed with 1.0 mL of the DPPH solution, and after 30 min of incubation at room temperature in the dark, the absorbance was measured at 517 nm. The DPPH radical scavenging was then computed using the equation below:

$$\mathrm{DPPHradicalscavenging}\,(\%) = (1-(A_\mathrm{s}/A_\mathrm{C}))\times100 \quad (4)$$

Here, A_C is the absorbance of the control at 517 nm, and A_s is the absorbance of the sample at 514 nm (4).

2.8 | Colorimetric properties of pH-sensitive film

2.8.1 | Color sensitivity of pH-sensitive film in response to different pH

The pH response of the film was analyzed following Peralta et al. (2019), with a slight modification. The 4-cm diameter film was immersed in a plastic Petri dish with a buffer solution. Subsequently, the film was submerged in varied pH buffer solutions (3, 7, and 10). The photographs were taken before and after contact with media with different pH levels.

2.8.2 | Color sensitivity of pH-sensitive film in response to NH_3 at different response times

The color sensitivity reaction of gelatin composite films to volatile ammonia was determined at different response times following Ma et al. (2017). The film was cut (1 × 4 cm) into pieces and hung on glass bottles at room temperature. The glass weighing container was filled with 5 mL of water ammonia to evaluate the ability to detect at varying reaction times (0, 5, 10, and 15 min). A colorimeter (CR-400 Chromameter, Konika Minolta Sensing, Inc., Osaka, Japan) was used to measure film color variations in different NH_3 response times. The white standard plate was calibrated to the color meter, and the values of light (L*), red (a*), and yellow (b*) were recorded. The tests were carried out three times for each film formulation.

2.9 | In vitro anthocyanin release study

The release rate of roselle extract anthocyanin into the phosphate-buffered saline (PBS) solution was evaluated based on the method by Hematian et al. (2022) with slight adjustments. The film sample (2.5 × 2.5 cm) was placed in a measuring cylinder filled with 50 mL of PBS. The pH and temperature were adjusted to 6.5 and 37°C, respectively. From the solution, 2 mL was taken at specified time intervals (every 10 min for 200 min) and replaced with an equal volume of fresh PBS. The absorbance was measured at 515.2 nm using a UV–vis spectrophotometer (Shimadzu 1800, Shimadzu Corp, Japan). A standard curve was employed to quantify the anthocyanin concentration in each sample. The cumulative release of anthocyanin from the films was calculated using the following equation:

$$\text{Release}\,(\%) = M_t / M_0 \times 100 \quad (5)$$

where M_t and M_0 show the released anthocyanin at time t and the total anthocyanin loaded to the films, respectively.

2.10 | Statistical analysis

The experiment was carried out in triplicate using three replicates ($n = 3$). The means of ± standard deviations are displayed for all analyses. Minitab Version 14.0 was the statistical analysis software used in this study. The pH-sensitive film was evaluated, and its application was assessed using a one-way analysis of variance (ANOVA), while Fisher's test was used to calculate the differences between mean pairings based on confidence intervals. A significant threshold of $p < 0.05$ was established.

3 | RESULTS AND DISCUSSIONS

3.1 | Purity of gelatin extracted from chicken skin

In this research, the yield of gelatin produced was 17.48%. Additionally, the protein content in freeze-dried chicken skin gelatin was found to be 80.76%, with moisture and ash contents measuring 9.81% and 0.37%, respectively. The bloom value of the obtained gelatin was 355 g. The obtained protein content of 80.76% indicates a relatively high level of purity, which is essential for ensuring the functional properties of gelatin in various applications. A bloom value of 355 g is considered high, suggesting that the gelatin extracted from chicken skin has excellent gelling properties, making it suitable for applications in film packaging (Wang et al., 2025). In this case, the moisture content of 9.81% and ash content of 0.37% further corroborate the high purity of the gelatin, as lower moisture and ash levels are associated with higher protein concentrations and fewer impurities. The extraction process significantly influences the purity of gelatin. The method employed in this study typically involves the hydrolysis of collagen, which is abundant in chicken skin. Hydrolysis by applying hot water extraction at 50°C overnight can be considered a good approach, obtaining high purity of the gelatin. The values of protein, ash, moisture content, and bloom were similar to a previous study by Işık et al. (2023). The chicken skin gelatin obtained from the previous study showed the values of protein, ash, moisture content, and bloom ranging from 80.30% to 86.11%, 0.36% to 0.95%, 7.08% to 13.35%, and 103.54 to 349.01 g, respectively. Similar findings between the previous and current studies are due to the high collagen content in chicken skin, and the collagen is converted into gelatin by using heat treatment as well.

The purity of chicken skin gelatin is quantitatively assessed through several key parameters, including protein content, ash content, moisture content, and bloom value. The protein content of gelatin is a primary indicator of its purity. High protein content is generally associated with lower levels of impurities, which enhances the quality of the gelatin (He et al., 2022). According to the Chinese National Standard GB6783-2013, gelatin used as a food additive should have a protein content exceeding 80% to be considered of high quality (He et al., 2022). Furthermore, the ash content reflects the mineral and inorganic residue present in the gelatin, where the acceptable limit is 2% for food standards (He et al., 2022). Low ash content is indicative of a purer gelatin product, as it suggests minimal contamination from non-proteinaceous materials. Besides, the moisture content of extracted gelatin determines the purity level of gelatin as well. According to industry standards, moisture content should ideally be

TABLE 2 Tensile strength (TS), elongation at break (EAB), and water vapor permeability (WVP) of chicken skin gelatin with different concentrations of roselle extract.

Film formulation	Tensile strength (MPa)	Elongation at break (%)	Water vapor permeability ($\times 10^{-8}$ g m^{-1}s^{-1} Pa^{-1})
A	1.5 ± 0.0^{a}	312 ± 12.0^{d}	1.02 ± 0.0^{a}
B	1.4 ± 0.0^{b}	371 ± 2.1^{c}	1.24 ± 0.0^{b}
C	1.3 ± 0.0^{c}	387 ± 6.2^{b}	1.31 ± 0.0^{c}
D	1.1 ± 0.0^{d}	416 ± 5.6^{a}	1.47 ± 0.0^{d}

Abbreviations: A, film containing 0% of roselle extract concentration; B, film containing 5% of roselle extract concentration; C, film containing 10% of roselle extract concentration; D, film containing 15% of roselle extract concentration.

The different superscript letters $^{(a–d)}$ within the column at each data demonstrate significant differences ($p < 0.05$). Data reported are mean values ± standard deviations.

below 15% to prevent microbial growth and maintain the stability of the gelatin (Usman et al., 2024). The relatively low moisture content in this gelatin sample indicates effective drying processes and contributes to its shelf stability. The bloom value of gelatin is a critical measure of its gelling strength, which is associated with its functional performance (Mohammadnezhad & Farmani, 2022).

3.2 | Physical properties of pH-sensitive film

3.2.1 | TS and EAB of pH-sensitive film

Table 2 presents the TS of chicken skin gelatin–based composite films with different roselle extract concentrations. The addition of roselle extract to the chicken skin gelatin film at different concentrations (0%–15%) shows a significant decrease ($p < 0.05$) in TS value from 1.5 to 1.1 MPa. The TS value obtained in this study was categorized as low, and the results showed that as the anthocyanin extract increased from 0% to 15% in the films, the TS reduced. The TS values of the films below 5 MPa are considered low, while medium TS values range from 5 to 15 MPa, and TS values exceeding 15 MPa are considered as high (Jati et al., 2024). This might be due to the formation of hydrogen bonds and molecular interactions between anthocyanins and the gelatin matrix. Hence, as the concentration of roselle extract anthocyanin increased in the films, the formation of the hydrogen bonding and molecular interaction increased, decreasing the TS. Anthocyanins, being polyphenolic compounds, can interact with the protein matrix of gelatin through hydrogen bonding and other molecular interactions as it is being incorporated into the gelatin films. The interference of anthocyanin in the films could form hydrogen bonds between the hydroxyl groups present in anthocyanins and the amino groups in gelatin. This interaction may alter the protein–protein interactions hence reducing the film's TS (Lou & Chen, 2023). Additionally, the presence of anthocyanins can interfere with the gelatin's ability to form a cohesive and strong film matrix, as the anthocyanins may compete with the gelatin for binding sites, thereby reducing the effective cross-linking density of the protein network. This competition can lead to a decrease in the TS (Tosif et al., 2021). The results drawn from this study were corroborated by Nazmi and Sarbon (2019), who demonstrated a noteworthy decline in the strength of the gelatin-based film, specifically from 5.0 to 4.5 MPa, upon the inclusion of *Centella asiatica* extract (0.3%–0.7%). The outcomes uncovered a decline in the film's strength caused by the polyphenolic compounds derived from *Centella asiatica* extract, which interact with the amino acid found in gelatin. This interaction may compromise the protein interaction, leading to a decrease in TS (Suderman & Sarbon, 2019).

The EAB of the chicken skin gelatin film was incorporated with varied concentrations of roselle extract (0%–15%), which is displayed in Table 2. The values were categorized as high and were significantly increased ($p < 0.05$) for film formulations A to D from 312% to 416% as the concentration of roselle extract in the films increased. The interactions between anthocyanin and amino groups of gelatin can disrupt the protein–protein interactions that typically stabilize the gelatin network, leading to a more flexible and less brittle film structure. As the concentration of anthocyanins increases, these interactions become more pronounced, resulting in a film that can stretch more before breaking, thereby increasing the EAB (Azlim et al., 2021). A similar trend of increasing EAB value of pH-sensitive film with the increasing anthocyanin content from dragon fruit was reported by Azlim et al. (2021). The incorporation of dragon fruit anthocyanin extract into the gelatin-based film caused an increase in the EAB value from 91.19% to 107.86%, mainly owing to the OH-group's interaction from anthocyanins with the gelatin in the film, thus causing the elasticity to increase. The relationship of TS and EAB is often inversely related in most gelatin composite films (Lou & Chen, 2023). The incorporation of anthocyanins can disrupt the gelatin protein matrix, leading to a less uniform and more heterogeneous

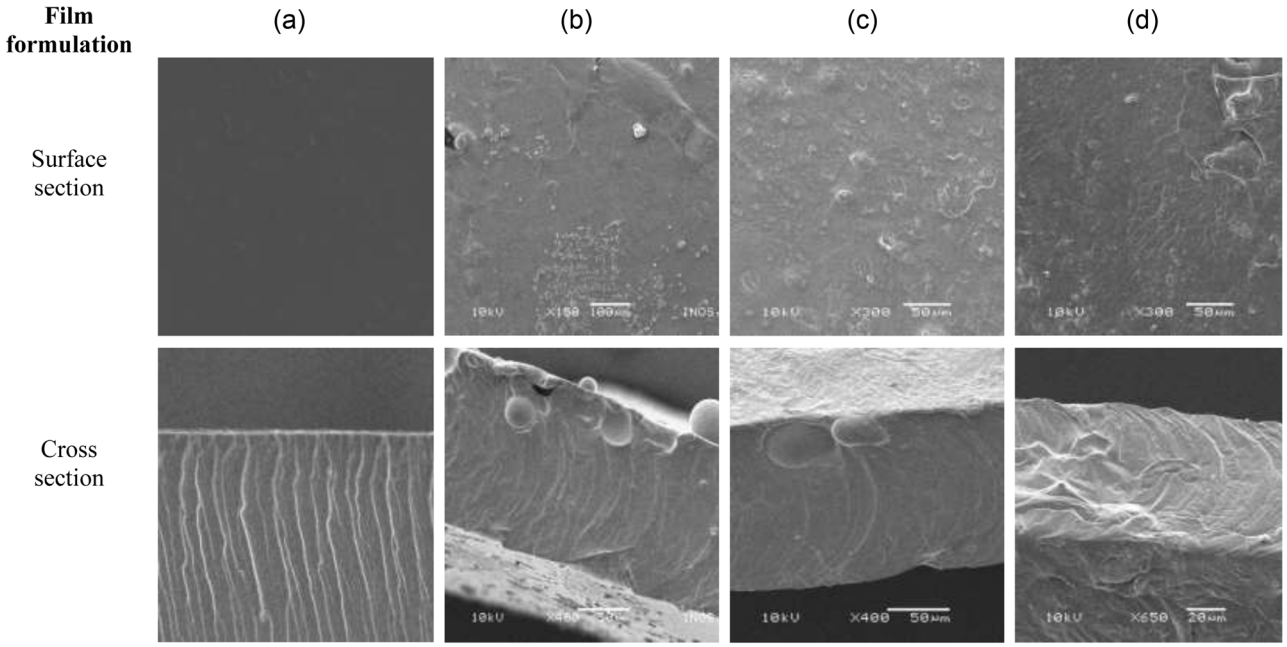

FIGURE 1 Surface morphology and cross section of chicken skin gelatin films at various concentrations of roselle extract. A, film containing 0% of roselle extract concentration; B, film containing 5% of roselle extract concentration; C, film containing 10% of roselle extract concentration; D, film containing 15% of roselle extract concentration.

structure. This disruption weakens the cohesive forces within the matrix, resulting in a decrease in TS. Simultaneously, the presence of anthocyanins enhances chain mobility and flexibility, contributing to an increase in EAB. This interplay explains the observed inverse relationship between TS and EAB (Jati et al., 2024; Azlim et al., 2021).

3.2.2 | Film morphology of the pH-sensitive film

Figure 1 presents the micrographs associated with the surface and cross-sections of chicken skin gelatin with varying concentrations of roselle extract (0%–15%). Regarding the surface section, it was reported that the micrograph of formulation A (0%) film appeared to be smoother and more homogeneous, with a bump-free surface and a denser and more compact structure. As the roselle extract concentration increased, it could be observed that the films for formulations B (5%), C (10%), and D (15%) had rougher, uneven surfaces and less compact and dense structures. The interplay between film constituents and drying conditions greatly influences films' microstructure, influencing the films' physical characteristics. However, the surface and cross-sectional roughness did not influence the film color alteration concerning the pH indicator (Luchese et al., 2021).

From the film's microscopy images, the 0% roselle anthocyanins film appeared smoother, more homogeneous, bump-free, denser, and more compact. Since potato starch increases inter- and intramolecular hydrogen bonding, chicken skin gelatin, including potato starch in films, can improve the smoothness of the film surface and cross-section (Alias & Sarbon, 2019). On the other hand, the chicken skin gelatin film becomes less compact and dense in structure, has an uneven surface, and becomes rougher when roselle anthocyanins are added. This might be because the extract failed to be entirely solubilized in the starch solution, as the segregation size decreases with the decrease in anthocyanin concentration in films (Che et al., 2022). Adding phenolic compounds like anthocyanin promotes the generation of hydrogen bonds with the starch matrix and may destroy the orderly arrangement of starch (Zhang et al., 2019).

This result was reinforced by a study by Prietto et al. (2017), which demonstrated non-homogeneous cross-section morphologies and a rough surface with corn starch/red cabbage anthocyanin film. This phenomenon was ascribed to the diminished interaction of anthocyanin compounds with substances like starch and glycerol. According to Sohany et al. (2021), including anthocyanin derived from purple sweet potatoes in the film resulted in a slightly uneven structure due to the limited interaction between the anthocyanin compound and starch and glycerol.

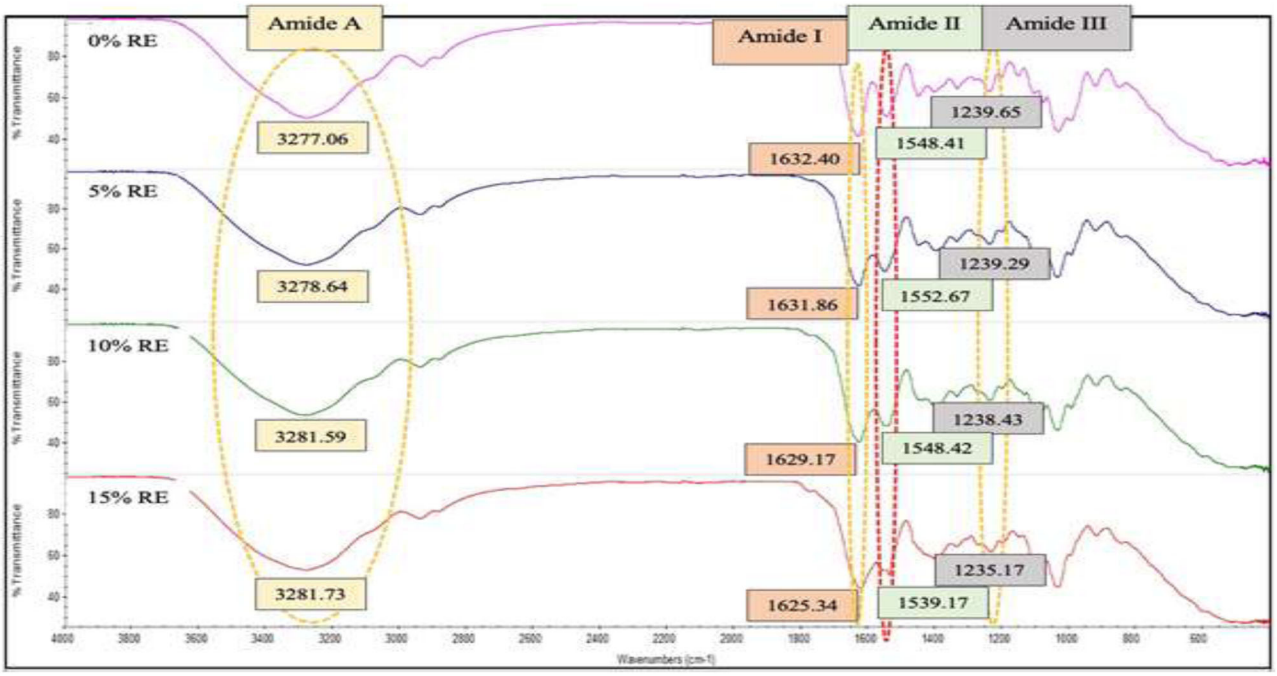

FIGURE 2 Fourier transform infrared (FTIR) spectra of chicken skin gelatin film with different concentrations of roselle extracts.

3.2.3 | Secondary structure of pH-sensitive film by FTIR

Figure 2 demonstrates the FTIR spectrum of chicken skin gelatin film, which is incorporated with different concentrations of roselle extracts. The FTIR spectrum pattern represents the functional groups of each film formulation: amide A (3377.07–3281.73 cm^{-1}), amide I (1625.25–1632.40 cm^{-1}), amide II (1539.17–552.67 cm^{-1}), and amide III (1235.17–1239.65 cm^{-1}). Amide A represents the N-H stretching vibration of the pH-sensitive film (Bitencourt et al., 2014). The FTIR spectra value obtained for the gelatin film shows a broad peak of 3277.06 cm^{-1}, slightly increased to 3281.73 cm^{-1} with the increase in roselle anthocyanins. Incorporating anthocyanin in pH-sensitive films increases the wave number more than in films without anthocyanin. The trend in FTIR spectrums showed the presence of O-H extending vibrations due to the formation of hydrogen bonds as anthocyanin was present in the film (Zhu et al., 2024). The results presented here were validated by Azlim et al. (2021), which showed that the spectra of amide A increased from 3211.48 to 3448.72 cm^{-1} as a result of the chemical interaction between the aromatic rings of anthocyanin and the elements of the film matrix. Consequently, O-H stretching vibrations were discerned and influenced by hydrogen bonding within or among molecules in chemical interactions involving the aromatic rings of anthocyanins and the constituents of the film matrix.

Amide I demonstrated the stretching vibration of C = O and the hydrogen bond associated with COO in the pH-sensitive film. The amide I band is the most reactive spectral region to the secondary structure of proteins. The maximum intensity decreased the amide I in chicken gelatin film containing different concentrations of roselle anthocyanin from 1632.40 to 1625.34 cm^{-1}, indicating some disruptions of the hydrogen bonds between starch and gelatin, resulting in a reduction in absorbance intensity (Huang et al., 2020). It was observed that after extracting the anthocyanin from purple sweet potato, the band shown by the Agar/potato starch mixture film was 1645 cm^{-1} and decreased to 1617 cm^{-1} because of the formation of hydrogen bonds between the film matrix and extract (Choi et al., 2017). The peak detected at 1645–1735 cm^{-1} is an aromatic ring with a C–H contraction vibration and a flavone with a C = O contraction vibration. The study's conclusions validate the presence of aromatic substances within the anthocyanin extract derived from red cabbage.

Additionally, the generation of amide II can be attributed to the bending vibrations of the N-H group and the extending vibrations of the C-N group. The observations of amide II indicate that the secondary structure of gelatin polypeptide chains has undergone modifications due to the integration of anthocyanins. This integration has led to a decrease in the value from 1548.41 (0% of roselle extract) to 1552.67 cm^{-1} (5.5% of roselle extract), attributed to anthocyanins. Consequently, this decrease has caused an increase in the value from 1548.42 (10.0%

of roselle extract) to 1539.17 cm^{-1} (15.5% of roselle extract) due to the addition of anthocyanins. This study was supported by Sohany et al. (2021), where, it was shown that the absorption band at 1645 cm^{-1} was transferred to the lower wave number at 1617 cm^{-1}, and the addition of anthocyanin from purple sweet potatoes led to changes in the absorption band, while amide III represented the vibrations of the N-H and C-N binding groups or the vibrations of the CH_2 of glycine group in the gelatin.

3.2.4 | Water vapor permeability of the pH-sensitive film

Table 2 displays the chicken skin gelatin film's WVP at various roselle extract concentrations (0%–15%). The WVP values for the pH-sensitive film for formulations A to D have significantly increased ($p < 0.05$) from 1.02×10^{-8} to 1.47×10^{-8} g m^{-1} s^{-1} Pa^{-1} as roselle extract concentration increased. The increase in WVP of gelatin/tapioca starch films with increasing anthocyanin concentration can be attributed to the hydrophilic nature of anthocyanins, which further enhance the film's hydrophilicity. The presence of polar groups in anthocyanins can interact with water molecules, hence leaving an intermolecular free volume within the gelatin matrix, thereby facilitating water vapor migration to the films (Guo et al., 2023). Moreover, the incorporation of anthocyanin into the films leads to the formation of hydrogen bonds between the hydroxyl groups of anthocyanins and the amine groups in gelatin, creating a more flexible network, allowing the penetration of water molecules to the films, hence increasing the WVP values (Bakhshizadeh et al., 2023). High WVP allows for greater moisture exchange within the gelatin matrix, leading to a more favorable for volatile amines detection, because higher humidity encourages the contact between volatile amines and water, generating more hydroxyl groups and creating an alkaline environment on the film surface, which hastens the color change of the films (Li et al., 2024).

The WVP value in this study coincides with the investigation carried out by Li et al. (2024). They discovered that the WVP of the chitosan/gelatin film was increased from 6.9×10^{-13} to 8.2×10^{-13} g m^{-1} s^{-1} Pa^{-1} as the concentration of butterfly pea anthocyanin increased in the films. Both studies yielded similar findings, as anthocyanin was incorporated into the films. Anthocyanins are inherently hydrophilic due to their polar hydroxyl group. When incorporated into films, they increase the affinity of the film matrix for water molecules. This hydrophilicity enhances water vapor transmission through the film, thus increasing the WVP value (Li et al., 2024).

3.2.5 | Opacity value

Table 3 shows the opacity value of the gelatin smart films for formulations A–D. The opacity value showed an increasing trend as anthocyanin concentration increased in the gelatin-based film ($p < 0.05$). The gelatin-based films exhibited higher opacity values, ranging from 3.17 to 4.93 A mm^{-1}, with the addition of anthocyanins (5%–15%), compared to the control gelatin film, which had an opacity value of 2.20 A mm^{-1}. Higher opacity values indicate that the films are more opaque, allowing less light to pass through. The highest opacity values were shown by film D with a 15% concentration of anthocyanin incorporated into the films, which indicated the opaquest film.

The incorporation of anthocyanins into gelatin films significantly improves the opacity of these films due to several interrelated mechanisms. As anthocyanins are natural colorants with inherent color and light-absorbing properties, incorporating these colorants into gelatin films could alter the light transmission properties, leading to increased opacity. As the concentration of anthocyanins increases in the gelatin films, the films exhibit a darker color, which directly correlates with a decrease in light transmittance (Aowpitaya & Sasanatayart, 2024). Additionally, the incorporation of anthocyanins into the gelatin films contributes to the formation of hydrogen bonds and electrostatic interactions, as hydroxyl groups of anthocyanins could engage in hydrogen bonding with the amine and carboxyl groups present in gelatin. This interaction enhances the cross-linking density within the gelatin matrix, resulting in a more compact and denser film structure (Wen et al., 2024). Therefore, the resulting compact and denser films can further contribute to light scattering and, consequently, increased opacity (Bakhshizadeh et al., 2023).

The findings in this study agree with Pang et al. (2023) whose study on the incorporation of purple cabbage anthocyanin (0.07%–0.35%) into gelatin films showed an increasing opacity value of the gelatin films (−0.077–7.087). In previous studies, the integration of purple cabbage anthocyanin could enhance the interactions between gelatin and anthocyanin, leading to strong cross-linking. Hence this compact structure would improve the light-blocking role of the films (Pang et al., 2023).

3.2.6 | Water contact angle

Table 3 displays the chicken skin gelatin film's WCA at various roselle extract concentrations (0%–15%). The WCA values for the pH-sensitive film for formulations A to D have significantly decreased ($p < 0.05$) from 122.57° to 77.33° as roselle extract concentration increased.

TABLE 3 Opacity, water contact angle (WCA), water solubility (WS), and antioxidant properties of chicken skin gelatin with different concentrations of roselle extract.

Film formulation	Opacity (A mm^{-1})	WCA (°)	WS (%)	DPPH (%)
A	2.20 ± 0.01^{a}	122.57 ± 0.17^{a}	52.30 ± 0.16^{a}	14.08 ± 0.38^{a}
B	3.17 ± 0.01^{b}	89.85 ± 0.05^{b}	46.41 ± 0.23^{b}	37.39 ± 0.08^{b}
C	3.50 ± 0.02^{c}	85.35 ± 0.25^{c}	43.44 ± 0.32^{c}	45.41 ± 0.19^{c}
D	4.04 ± 0.04^{d}	82.40 ± 0.15^{d}	37.42 ± 0.29^{d}	53.55 ± 0.13^{d}

Abbreviations: A, film containing 0% of roselle extract concentration; B, film containing 5% of roselle extract concentration; C, film containing 10% of roselle extract concentration; D, film containing 15% of roselle extract concentration.
The different superscript letters ($^{a–d}$) within the column at each data demonstrate significant differences ($p < 0.05$). Data reported are mean values ± standard deviation.

The incorporation of anthocyanins into gelatin films leads to a decrease in the WCA, which is indicative of increased hydrophilicity. This phenomenon was due to the hydrophilic nature of anthocyanins as it contains multiple hydroxyl groups. As anthocyanin was added to gelatin films, they interact with the gelatin matrix through hydrogen bonding and other polar interactions. This interaction enhances the surface hydrophilicity of the films, as the anthocyanins occupy space within the gelatin matrix, they effectively increase the number of hydrophilic sites available on the film surface, which decreases the WCA values (Guo et al., 2023). Moreover, the interaction of anthocyanins with gelatin amine groups can disrupt the dense network of gelatin's polypeptide chains, which typically contain hydrophobic regions of the amino acid side chains. This disruption allows more water molecules to interact with the hydrophilic groups of gelatin, further decreasing the WCA (Wen et al., 2024).

The finding in this study agrees with Li et al. (2024), who found that the addition of butterfly pea anthocyanin to gelatin films decreased the WCA from 103.62° to 63.72°. The decreased trend of WCA in the previous study was due to the hydrophilic hydroxyl group of butterfly pea anthocyanin, where this group facilitates water molecules to interact with the gelatin films, hence decreasing the WCA.

3.2.7 | Water solubility

Table 3 displays the chicken skin gelatin film's WS at various roselle extract concentrations (0%–15%). The WS values for the pH-sensitive film for formulations A to D have significantly decreased ($p < 0.05$) from 52.30% to 31.32% as roselle extract concentration increased. The results showed that the addition of anthocyanin has increased the hydrophilicity of the films proved by the decreasing trend of WCA values. However, the addition of anthocyanin into gelatin smart films has shown to decrease the WS. This effect can be explained through the interplay of molecular interactions, structural changes, and the inherent properties of anthocyanins.

Anthocyanins, as polyphenolic compounds, can form hydrogen bonds with the amino groups present in the gelatin structure. This interaction enhances the stability of the gelatin matrix, leading to a more compact and less permeable film structure which in turn decreases the film's affinity for water, thereby reducing its solubility (Bakhshizadeh et al., 2023; Hematian et al., 2022). While the compact structure formed by the interactions with anthocyanins may reduce solubility, it can simultaneously create a surface that is more favorable for water interaction, leading to an increase in hydrophilicity. Moreover, the structural integrity of gelatin is enhanced through the formation of cross-links facilitated by anthocyanins, which contributes to their stability and resistance to water (Aowpitaya & Sasanatayart, 2024). In addition to these interactions, the presence of anthocyanins can also influence the moisture retention properties of the gelatin films. The hydrophilic nature of gelatin allows it to absorb water; however, the incorporation of anthocyanins can alter this property. Furthermore, the structural changes induced by anthocyanins can also enhance the films' overall hydrophilicity (Wen et al., 2024).

The results of this study are consistent with the findings of Shakouri et al. (2023), who reported that incorporating saffron petal anthocyanin extract (5%–20%) into gelatin films reduced their WS from 34.89% to 30.05%. Previously, a study reported that the incorporation of anthocyanins into gelatin films might interact with the amino and hydroxyl groups present in the gelatin, leading to a more stable network that resists water penetration (Tavassoli et al., 2022).

3.3 | Antioxidant radical scavenging activity

The DPPH radical scavenging activity of the films is shown in Table 3. The DPPH scavenging activity for the

pH-sensitive films for formulations A to D has significantly increased ($p < 0.05$) from 14.08% to 55.98% as the roselle extract concentration increased (0%–15%). In this study, the total anthocyanin content of roselle extract was 100.63 ± 0.48 mg/L.

The increase in DPPH radical scavenging activity observed in gelatin films with higher concentrations of anthocyanins can be attributed to the enhanced phenolic content as the concentration of anthocyanin in films increased, hence leading to greater availability of active sites for radical scavenging (Tavassoli et al., 2024). The incorporation of anthocyanins alters the film's morphology and density by introducing a more heterogeneous structure within the gelatin matrix, which increases the surface area available for interactions with free radicals (Hematian et al., 2022). The antioxidant activity of anthocyanins is primarily attributed to their molecular structure, which features multiple hydroxyl groups capable of donating hydrogen atoms. This ability is critical for the radical scavenging mechanism, as the hydroxyl groups neutralize free radicals by donating hydrogen atoms, effectively stabilizing them (Enaru et al., 2021). As the concentration of anthocyanins increases in the gelatin films, the number of active hydroxyl sites also rises, thereby enhancing the films' DPPH scavenging activity.

The results of this study align with those reported by Shakouri et al. (2023), where DPPH scavenging activity exhibited an increasing trend (15.2%–45.2%) as the concentration of saffron petal anthocyanins in gelatin films was raised from 0% to 20%. Higher anthocyanin concentration contributes to the increase of hydroxyl group side chains, which are capable of donating hydrogen atoms to the free radicals. This is very applicable in the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity, which transforms it to a temporary free radical and can be neutralized by anthocyanin through hydrogen donation.

3.4 | Colorimetric properties of pH-sensitive film

3.4.1 | Color sensitivity of pH-sensitive film in response to different pHs

Figure 3 shows the color changes of chicken skin gelatin film integrated with roselle extract treated with different pHs (3, 7, and 10). The color of the films changed clearly with the increase in roselle extracts (0%–15%). However, the control films without roselle extracts showed no color changes. The color of the films incorporated with roselle extracts (5%–15%) changed from red to pink when treated with acidic conditions (pH 3).

Different pH values can cause anthocyanins to change in structure and color, clarifying that plant extracts rich in anthocyanins are typically pH-sensitive (Guo et al., 2023). The development of bright red color when the pH-sensitive film was placed in an acidic environment in this present study was due to the structural change in the film by anthocyanins in roselle extract that convert flavylium ions to carbinol pseudo base (Guo et al., 2024). Then, the film color shifted from red to blue-green when treated with a neutral condition (pH 7). As the film was submerged in neutral pH, a blue-green color was formed because of the rearrangement of the carbinol pseudo-base structure. It formed a resonance-stabilized quinoidal base (Celik et al., 2021).

Lastly, the film color shifted from red to yellow-green when treated with an alkaline condition (pH 10). In this study, the film formed a yellow-green color when submerged in alkaline conditions. The generation of chalcones resulted from the deprotonation of the quinoidal bases in the film, which caused them to transform into anionic quinoidal bases and subsequently modify their structure. The results obtained in this investigation exhibited similarities to those published by Sohany et al. (2021) in examining starch film containing purple sweet potato anthocyanin. The film underwent a color transition, changing from red to purple at pH 5, then to blue at pH 8.0, and finally to green at pH 10. This transformation was ascribed to transforming chemical structures from flavylium anion to quinoidal base at pH 5 and chalcone at pH 10. The color changes are very useful in monitoring food freshness, especially meat products. As the freshness of meat products deteriorates, ammonia gas is released from the meat product due to protein degradation. The release of ammonia gas causes the increment of pH in an alkaline environment, causing the smart film with anthocyanin to change color from red to yellow-green.

3.4.2 | Color sensitivity of pH-sensitive film in response to NH_3 at different response times

The color sensitivity of a pH-sensitive film toward NH_3 was assessed through changes in L^* (indicating lightness), a^* (representing red-greenness), and b^* (referring to yellow-blueness) values, as illustrated in Table 4. The values of L^*, a^*, and b^* for the different film formulations showed significant differences ($p < 0.05$) in response times of 0, 5, 10, and 15 min. The pH-sensitive film revealed a lighter color of L^* value (84.33 to 44.71) and more redness of a^* value (−0.24 to 23.36) at 0 min of response time with a comparison of L^* value (83.35 to 42.04) and redness of a^* value (−0.59 to 4.97) at 5 min of response time, L^* value (84.64 to 44.53) and redness of a^* value (−0.62 to 6.91) at 10 min

TABLE 4 Color sensitivity of pH-sensitive films in response to NH_3 at different response times.

Gelatin-based film	0 min			5 min			10 min			15 min		
	*L**	*a**	*b**	*L**	*a**	*b**	*L**	*a**	*b**	*L**	a*	b*
0% roselle extract	84.33 ± 0.08[a]	−0.24 ± 0.04[d]	1.65 ± 0.25[d]	83.35 ± 0.04[a]	−0.59 ± 0.02[a]	7.77 ± 0.02[a]	84.64 ± 0.12[a]	−0.62 ± 0.05[a]	8.31 ± 0.02[a]	85.80 ± 0.03[a]	−0.83 ± 0.02[a]	9.33 ± 0.02[a]
												
5% roselle extract	71.57 ± 0.08[b]	2.32 ± 0.27[c]	13.43 ± 0.11[a]	54.94 ± 0.04[b]	3.49 ± 0.02[b]	26.23 ± 0.02[b]	57.09 ± 0.02[b]	5.11 ± 0.02[b]	27.74 ± 0.01[b]	57.09 ± 0.02[b]	6.16 ± 0.02[a]	28.41 ± 0.04[b]
												
10% roselle extract	63.28 ± 0.18[c]	8.63 ± 0.18[b]	8.63 ± 0.02[a]	49.44 ± 0.15[c]	3.90 ± 0.03[c]	26.82 ± 0.06[c]	51.11 ± 0.22[c]	5.79 ± 0.09[c]	28.14 ± 0.02[c]	51.11 ± 0.22[c]	7.29 ± 0.04[c]	30.39 ± 0.46[c]
												
15% roselle extract	44.71 ± 0.32[d]	23.36 ± 0.30[a]	10.51 ± 0.35[b]	42.04 ± 0.14[d]	4.97 ± 0.04[d]	23.42 ± 0.15[d]	44.53 ± 0.18[d]	6.91 ± 0.03[d]	25.26 ± 0.06[d]	44.53 ± 0.18[d]	9.23 ± 0.15[d]	27.57 ± 0.22[d]
												

Abbreviations: A, film containing 0% of roselle extract concentration; B, film containing 5% of roselle extract concentration; C, film containing 10% of roselle extract concentration; D, film containing 15% of roselle extract concentration.

The different superscript letters ([a–d]) within the column at each data demonstrate significant differences ($p < 0.05$). Data reported are mean values ± standard deviation.

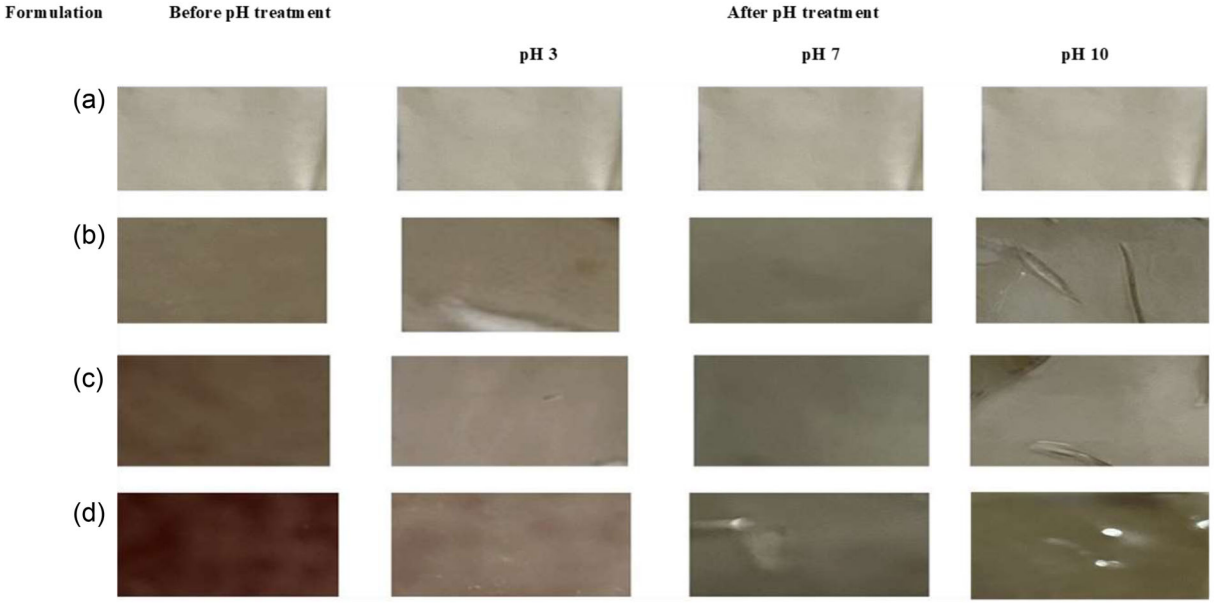

FIGURE 3 The variations of film color during contact with varied pH on the chicken skin gelatin film incorporated with roselle extract. A, film containing 0% of roselle extract concentration; B, film containing 5% of roselle extract concentration; C, film containing 10% of roselle extract concentration; D, film containing 15% of roselle extract concentration.

of response time, and L^* value (85.80 to 44.53) and redness of a^* value (−0.83 to 9.23) at 15 min of response time.

All films were observed to show decreased brightness and change to denser brownish color as response time increased. The denser brown color shown by the films as the time of immersion increased might result from structural changes undergone by anthocyanin in response to variations in pH. When gelatin films containing anthocyanins are exposed to ammonia, which is alkaline, the pH of the environment increases. This increase in pH can lead to the deprotonation of anthocyanins, resulting in a shift from their colored, flavylium cation form to a colorless or less intensely colored form, which can subsequently react with ammonia to form denser brown–colored complexes (Al-Qahtani et al., 2023). Besides, the interaction between anthocyanins and ammonia may lead to the formation of new chemical structures that exhibit different color properties, resulting in a denser brown color (Wen et al., 2024). This reaction can result in a darker brown coloration as the anthocyanins interact with ammonia and possibly form new chromophores. Furthermore, it was noted that films B–D underwent color changes from light brown to a denser brown color within 5, 10, and 15 min of response time. In this work, the pH-sensitive film's color changes become more noticeable as response time increases and might be prompted by alterations in the roselle molecule brought on by its reactivity with an exposed NH_3 environment. The alteration has disrupted the amount of aromatic amino acids present in the original state of roselle compounds. Hence, the formation of disruption compounds reflected why the films could change their color quickly when exposed to an alkaline environment.

Higher anthocyanin concentrations in colorimetric films exhibit more remarkable color transitions with increased response time, indicating a more robust alkaline environment due to the increase in the generation of NH^+_4 and OH from NH_3 and H_2O molecules (Remedio & Parada, 2024). Hydroxide ions generated an alkaline environment from ammonia, and the anthocyanins' structure was transformed into chlorides, changing the film's color. This showed that higher response times expose more alkaline environments at the outermost layers of experimental films, thereby promoting the transformation of hydrogen atoms into films. The current study is like that conducted by Said and Sarbon (2019), who found that adding curcumin to films can also cause color changes when exposed to ammonia gas. The initial degradation products are ferulic acid and feruloylmethane, rapidly changing color from yellow to brown-yellow.

3.5 | In vitro anthocyanin release study

The release rate study of roselle extracts anthocyanin from the gelatin films is presented in Figure 4. The findings indicate that the initial burst release was followed by a sustained release until a steady state was achieved, demonstrating the controlled release of anthocyanins from roselle extract in gelatin films. Between 0 and 100 min, the release rate for formulation B increased sharply from 0% to 30.83%,

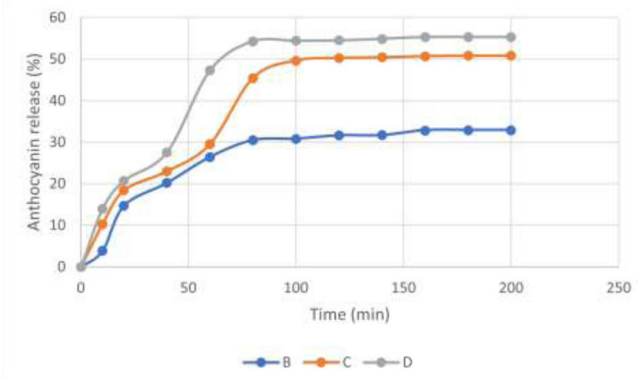

FIGURE 4 In vitro control release profile of chicken skin gelatin film at various concentrations of roselle extract. B, film containing 5% of roselle extract concentration; C, film containing 10% of roselle extract concentration; D, film containing 15% of roselle extract concentration.

for formulation C from 0% to 49.67%, and for formulation D from 0% to 54.41%. From 100 to 200 min, the release rates stabilized, reaching 32.95% for formulation B, 50.79% for formulation C, and 55.33% for formulation D. Furthermore, the data reveal that as the concentration of roselle extract in the gelatin films increased, the overall release rate over 200 min also increased: formulation B (0%–32.95%), formulation C (0%–50.79%), and formulation D (0%–55.33%). The release rate study of roselle extract anthocyanin from the gelatin films is crucial for ensuring that the roselle extracts remain effective over time, providing continuous monitoring of food quality.

Based on Figure 4, as the concentration of anthocyanins in gelatin films is elevated, the concentration gradient between the gelatin film and the surrounding medium increases, leading to a more substantial diffusion force that drives the anthocyanins out of the film. Higher anthocyanin content can lead to increased surface roughness of the films, which enhances the interaction between the gelatin film and the release medium (Pang et al., 2023). This increased surface area facilitates the release process. The rougher surface can create more pathways for diffusion, thereby accelerating the release rate (Cheng et al., 2022). Additionally, the interaction between anthocyanins and the gelatin matrix can influence the release kinetics. At higher concentrations, the presence of anthocyanins may increase the hydrogen bonds and other interactions formed with the gelatin, which can increase the disruption of the protein–protein interactions within the gelatin network. Therefore, these interactions can weaken the structural integrity of the gelatin matrix, allowing for easier release of anthocyanins (Zhai et al., 2017). Moreover, the hydrophilic nature of both gelatin and anthocyanins plays a significant role in the release mechanism. In this study, it shows that a higher concentration of roselle extracts in gelatin films increased the hydrophilic compounds. Hence, the increase of hydrophilic compound not only increased the WVP (Table 1) but also increased the release rate of roselle extract.

The findings of this study align with those of Hematian et al. (2022), which demonstrated that elevating the concentration of *Coleus scutellarioides* anthocyanin (CSA) (10–30 mL) in gelatin films enhanced the release rate of anthocyanin. Specifically, the release rates observed were 0%–22.8% for CSA 10 mL, 0%–23.41% for CSA 20 mL, and 0%–24.15% for CSA 30 mL (Hematian et al., 2022). The previous study attributed this effect to the increased surface roughness of the gelatin films at higher CSA concentrations, which facilitated better interaction with the release medium and improved the release of CSA from the films.

4 | CONCLUSIONS

In this study, pH-responsive films were successfully developed using gelatin, tapioca starch, and roselle extract, demonstrating promising potential as biodegradable smart films. In summary, increasing the concentration of roselle extract in the film formulation resulted in a decrease in physical and mechanical properties, while enhancing certain functional characteristics. The addition of roselle extract led to an increase in EAB and WVP, contributing to greater film flexibility but reducing its water vapor barrier properties. Furthermore, the intensity of functional amide groups was enhanced with higher concentrations of roselle extract, indicating potential interactions between gelatin and anthocyanin compounds. Film morphology by SEM revealed rougher internal cross-sections and surfaces in films containing roselle extract, suggesting modifications in the film's microstructure. Although the films contain water-soluble components, they are not intended for direct contact with perishable food or use in water-containing environments. Instead, they are designed for food packaging applications where direct exposure to moisture is limited, such as freshness monitoring. Future studies may focus on improving water resistance by blending with biopolymers that have good water barrier properties such as chitosan. Overall, these films exhibit remarkable pH-sensitivity and responsiveness, making them suitable as pH indicators for monitoring food freshness through visible color changes.

AUTHOR CONTRIBUTIONS

Najma Farhaten Latiff: Writing—original draft; methodology; visualization; formal analysis; investigation; data curation. **Nur Fazlin Sulaiman**: Writing—original draft; visualization; investigation; methodology; formal analysis; data curation. **Mannur Ismail Shaik**: Concep-

tualization; methodology; writing—review and editing; supervision. **Nizaha Juhaida Mohamad**: Conceptualization; methodology; writing—review and editing. **Wan Mohd Khairul**: Conceptualization; methodology; writing—review and editing. **Adibah Izzati Daud**: Conceptualization; methodology; writing—review and editing. **Norizah Mhd Sarbon**: Conceptualization; methodology; visualization; investigation; supervision; funding acquisition; project administration.

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CONFLICT OF INTEREST STATEMENT

The authors assert that they have no identifiable conflicting financial concerns or personal connections that might have impacted the research presented in this manuscript.

DATA AVAILABILITY STATEMENT

Data provided in this research are accessible upon request from the corresponding author.

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