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Smart and biodegradable labeling system using Butterfly Pea flower (*Clitoria ternatea* L.) Anthocyanin extract for monitoring of chicken meat freshness during chilled storage

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ABSTRACT

Chicken meat is highly perishable due to its high water content, which fosters microbial growth and spoilage, reflected by increased pH levels. Smart packaging, particularly colorimetric indicator films, offers a solution by visually monitoring meat freshness. Fresh chicken breast fillets were packaged with biodegradable films made from chitosan–PVA and infused with butterfly pea flower anthocyanin extract, which was prepared using ultrasonic-assisted ethanol extraction. The films were applied as freshness indicators and evaluated over 7 days of chilled storage through colorimetric analysis (Lab* values), total anthocyanin content, and pH sensitivity. Meat spoilage was assessed using Total Plate Count (TPC), Total Volatile Base Nitrogen (TVB-N), H_2S production, and the Postma test. The results showed that the anthocyanin content in the films decreased from 7.71 mg/L to 5.63 mg/L after seven days of storage. The pH, TPC, and TVB-N of chicken meat increased consistently during storage, reflecting spoilage and protein decomposition. The H_2S and Postma test further validated the transition of meat freshness to spoilage. The meat remained fresh for 3 days, whereas a noticeable purple-to-green change of the smart label occurred on day 4 to 5. Colorimetric evaluation shows that freshness threshold pH (6.1) corresponded to L* < 9.98, a* > -0.22, and b* < 0.43. These results affirm the potential of butterfly pea flower anthocyanin-based films as effective, biodegradable indicators for monitoring chicken meat freshness.

Introduction

Chicken meat is a highly perishable commodity with a relatively short shelf life if not handled properly (Smadi *et al*., 2012). Its high-water content makes it particularly susceptible to microbial contamination, which can rapidly degrade product quality. Bacterial contamination affects the pH of the meat, leading to spoilage and posing safety concerns.

Packaging plays a crucial role in preserving food quality and extending shelf life. Smart or intelligent packaging, an innovative system in the food industry, offers advanced functionalities such as monitoring, recording, and detecting changes in product conditions (e.g., temperature or pH), thereby providing real-time information on product freshness and safety (Chiu *et al*., 2024). Among the most commonly used smart packaging technologies are colorimetric indicator films, which incorporate natural or synthetic pigments that change color in response to environmental shifts (Luo *et al*., 2022). These films provide early warnings about changes in product temperature, pH, or quality, ensuring consumer safety.

In the current study, chitosan and polyvinyl alcohol (PVA) are employed as the primary materials for developing smart packaging films. Chitosan, a biodegradable biopolymer, possesses excellent film-forming properties (Pratama *et al*., 2019), while PVA, a synthetic polymer that is water-soluble and environmentally degradable, enhances the film's tensile strength and adhesive properties (Silva *et al*., 2008). These materials are well-suited for use in smart packaging applications due to their biodegradability and functional versatility.

To enhance the functionality of the indicator films, butterfly pea flower (*Clitoria ternatea* L) extract, which is rich in anthocyanins can be employed to give colorimetric characteristics to the film (Fu *et al*., 2021). Anthocyanins are natural pigments capable of undergoing color changes in response to pH variations, making them effective for detecting food spoilage. Butterfly pea flower, which thrives in tropical and subtropical regions like Indonesia (Filio *et al*., 2023), produces vibrant blue and purple flowers due to its high anthocyanin content. Numerous studies have demonstrated the potential of anthocyanins as pH indicators in smart packaging (Khezerlou *et al*., 2023; Qin *et al*., 2021; Yong *et al*., 2019). Anthocyanins immobilized within chitosan films during the drying process can monitor pH changes in food products (Khezerlou *et al*., 2023; Yoshida *et al*., 2014). These colorimetric films, derived from natural pigments and biopolymers, are non-toxic, safe, and biodegradable. In our previous study, we successfully developed biodegradable smart packaging using chitosan, PVA, and anthocyanin extract from butterfly pea flowers, with the films showing clear color changes in response to different pH levels (Hidayati *et al*., 2021).

Building on this prior research, the current study aims to apply the smart packaging (label) into a real food scenario. Specifically, we aim to evaluate the use of butterfly pea flower extract as a natural pH indicator in smart packaging films for monitoring the freshness of chicken fillets during storage. The objective was to investigate the effectiveness of these biodegradable films in detecting spoilage by providing real-time visual cues which indicated by pH increase due to meat spoilage. Thus, it gives practical benefits to consumers in the selection of high quality chicken and to avoid the consumption of spoiled or unsafe poultry products.

Materials and methods

Fresh chicken breast fillets were sourced from a local supermarket in Semarang, Indonesia, on the same day as the experiment and stored in a chiller until further processing. Dried butterfly pea flowers (BPF) were obtained from Besthoney, Malang, Indonesia. Plate count agar (PCA) was supplied by Merck. Chemicals including chitosan, polyvinyl alcohol (PVA), sodium chloride (NaCl), lead acetate (Pb acetate), glycerol, ethanol (96 %), pH universal indicator, acetic acid, and hydrochloric acid (HCl) were purchased from a local chemical supplier in Semarang, Indonesia. Styrofoam and plastic wrap were used as packaging materials for the application experiment.

Anthocyanins extraction from butterfly pea flower

Anthocyanin from BPF was extracted following the procedure described in our previous study (Hidayati *et al*., 2021). In brief, the BPF was

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cut into small pieces and mixed with 96% ethanol in an Erlenmeyer flask at a 1:10 ratio (w/v). The mixture underwent ultrasonic extraction for 15 minutes at a depth of 2 cm. The extract was then filtered through filter paper and concentrated using a vacuum rotary evaporator (Biobase, China) until equilibrium was reached. The resulting anthocyanin concentrate was stored in a brown glass bottle, covered with aluminum foil, and kept at a chilled temperature to preserve stability.

Film preparation

The biodegradable films were also prepared following methods on our previous work (Hidayati *et al*., 2021; Pratama *et al*., 2019). Chitosan powder was dissolved in a 1% acetic acid solution at room temperature to create chitosan solution of 2% (w/v). PVA 5% (w/v) solution was prepared by dissolving 5 g of PVA in 100 ml of distilled water. Chitosan and PVA solutions were then mixed in a ratio of 2:3. Glycerol 1% (v/v) was added to the solution as a plasticizer. All mixing procedures were performed using a magnetic stirrer set at 200 rpm for 15 minutes at room temperature. 10 ml of the BPF's anthocyanin extract was added and dissolved to make a total of 150 ml of film solution. Biodegradable films were obtained by casting technique, 150 ml of the solution were poured onto a 15 cm^2 teflon pan and dried at 40°C for 18 hours in a ventilated oven with 50% relative humidity (RH) to obtain films with uniform thickness.

Film application in smart packaging

Fresh broiler chicken meats were placed on Styrofoam tray. Biodegradable BPF anthocyanin film was cut into 1.5 cm^2 strip and then placed on top of the meat. Further, the meats were then covered with plastic wrap with the strip underneath to ensure contact with chicken meat. The packaged meats were placed in the refrigerator with a temperature of 4°C. Observations were conducted every 24 hours for 7 days.

Total anthocyanin content

Total anthocyanin content in the film was measured on the day 0 and 7 using the pH differential method according to Luchese *et al.* (2017). Two pieces of indicator film were cut into 4 cm2. Each piece of film was immersed in a blank solvent of 1 ml of KCl buffer pH 1 and 1 ml of sodium acetate buffer pH 4.5. Both treatments were left for 30 minutes. Total anthocyanin absorbance was measured using a UV-Vis spectrophotometer at 520 nm and 700 nm wavelengths. The absorbance of the samples was determined by the following equation:

$$A = (A520\text{-}A700)pH\ 1 - (A520\text{-}A700)pH\ 4.5 \quad (1)$$

Total anthocyanin content was calculated using the following formula:

$$\text{Anthocyanin (mg/g)} = (A \times MW \times DF \times V)/(\varepsilon \times L) \times 100 \quad (2)$$

Where A is the absorbance value, MW is the molecular weight of the predominant anthocyanin, cyanidin 3-glucoside, 449.2 (g/mol), DF for the dilution factor, "ε" for the molar absorbance of cyanidin 3-glucoside, which is 26900, and L for the cuvette's width.

Color changes intensity of the indicator film

The color change of the indicator film was measured according to Romero *et al.* (2021) using a colorimeter. The measurements were carried out by attaching the sensor from the colorimeter to the sample and the color of the sample is expressed in L*a*b* scale. L* values characterize the brightness level, a* values show the red-green color dimension and b* values are for yellow-blue color dimension.

pH sensitivity

The pH of chicken meat was measured based on Liu *et al.* (2022) with slight modifications. A total of 10 grams of chicken fillet samples were crushed and homogenized with 90 ml of distilled water. The homogeneous meat was measured with a pH meter which had previously been calibrated with standard buffers pH 4 and 7.

Total Volatile Bases Nitrogen (TVB-N) in Chicken meat

The Total Volatile Bases Nitrogen (TVB-N) test was performed according to the method described by Huang *et al.* (2014) utilizing Kjeldahl distillation. A 10-gram sample of chicken meat was finely mashed and placed in a beaker containing 100 mL of distilled water. The mixture was left to stand for 30 minutes, with intermittent stirring every 10 minutes. The solution was then filtered through filter paper to remove solid residues.

For the distillation process, 5 mL of the filtrate was mixed with 5 mL of 10 g/L magnesium oxide (MgO) solution, and Kjeldahl distillation was carried out for 5 minutes. During this process, 10 mL of 20 g/L boric acid was prepared with 5–6 drops of methyl red–methyl blue (MMMB) indicator to absorb the distillate. The distillate was then titrated with 0.1 N hydrochloric acid (HCl) until the color changed to a purplish-blue hue. The TVB-N content was calculated using the following formula:

$$\text{TVB-N (mg/100 g meat)} = ((V\_1\text{-}V\_2) \times c \times 14)/(m \times 5/100) \times 100 \quad (3)$$

Where V1 is the titration volume for the sample tested (ml), V2 is the blank titration volume (ml), and c is the concentration of HCl (mol L-1), m is the weight of the chicken fillet sample.

Total Plate Count (TPC)

The Total Plate Count (TPC) analysis using the pour plate method was performed following Ryser & Schuman (2015). A solvent solution of 0.86 % NaCl was prepared and homogenized using a magnetic stirrer. For the Plate Count Agar (PCA) medium, 4.37 g of PCA powder was dissolved in 250 mL of distilled water, covered with aluminium foil, and homogenized with a magnetic stirrer. Both the solvent and PCA media were sterilized by autoclaving at 121°C for 15 minutes. Petri dishes and sample spoons were dry sterilized in an oven at 110°C for 1 hour.

For sample preparation, 1 g of chicken meat was weighed and ground using a mortar and pestle. Serial dilutions were performed by adding 1 g of the sample to a screw cap tube containing 9 mL of distilled water (resulting in a 10^{-1} dilution). Subsequently, 1 mL of the 10^{-1} dilution was transferred to a new tube containing 9 mL of distilled water to achieve a 10^{-2} dilution. This process was repeated up to a 10^{-7} dilution.

For plating, 1 mL of the dilutions 10^{-5}, 10^{-6}, and 10^{-7} were inoculated into petri dishes in duplicate. Approximately 13 mL of liquid PCA medium was poured into each dish, close to a Bunsen burner flame to maintain sterility. The mixture was gently swirled in a figure-eight motion to ensure even distribution. The plates were incubated at 37°C for 20 hours, after which microbial colonies were counted. The total microbial count was calculated using the following formula:

$$\text{Colony Forming Unit (CFU)/ml} = \text{number of colonies per plate} \times 1/(\text{dilution factor}) \quad (4)$$

H_2S and Postma Test

The hydrogen sulfide (H_2S) test was adapted from the procedure described by Zhang *et al.* (2023), with several modifications. A sample of chicken meat was placed in a sterile petri dish, and a filter paper was positioned on top of the sample. A 10 % lead(II) acetate ($Pb(CH_3COO)_2$) solution was carefully applied to the filter paper. The petri dish was then

covered by lid. After an incubation period of 1 hour, the filter paper was inspected for any visible color change. The appearance of brownish spots on the filter paper indicated the production of H_2S by the meat, suggesting spoilage or the onset of the decomposition process.

The Postma test was adapted from the method described by Patriani & Apsari (2022), with slight modifications. Five grams of chicken fillet were finely mashed using a mortar and pestle and combined with 50 mL of distilled water in an Erlenmeyer flask. The mixture was left to stand for 15 minutes and then filtered to obtain a clear meat extract. A plastic tube containing 100 mg of magnesium oxide (MgO) was filled with 10 mL of the filtered meat extract and homogenized. A universal pH indicator strip was attached to the inner surface of the tube cap. The tube was then submerged in a beaker of water and heated in a water bath at 50°C for 5 minutes. A positive result was indicated by a pH reading above 7 (alkaline) on the universal indicator, suggesting ammonia formation due to protein breakdown.

Results

Anthocyanin Content

The anthocyanin content of the smart label was measured before and after storage treatment (day 0 and day 7, respectively). Anthocyanin decreased from an initial concentration of 7.71 mg/L to 5.63 mg/L after 7 days of storage. This shows that the longer the storage duration, the lower the anthocyanin value.

pH Value and Freshness

As shown in Figure 1, the pH of chicken fillet during storage follows the linear equation y= 0.2901x + 5.4877 with a strong correlation (R^2= 0.9595). Figure 1 shows that chicken meat on storage day 0 to day 2 had a pH of 5.41 to 6.01, thus it can still be categorized as fresh. However, on the 3rd day the pH was 6.24 with a relatively high standard deviation of 0.14. We therefore considered this as borderline fresh. Starting day 4, the pH of the meat was significantly higher than 6.1, thus chicken breast meat stored for 4 days or more exceeded the standard limit for fresh meat and was deemed no longer in best quality for consumption.

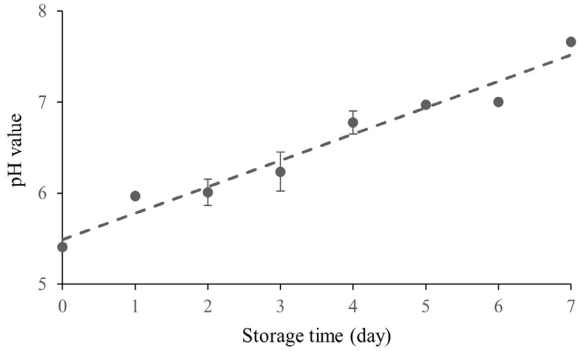

Fig. 1. pH value of chicken meat during storage.

Total Plate Count (TPC)

During storage, the Total Plate Count (TPC) of chicken fillet increased steadily over time. The regression equation for the TPC is y= 0.6146x+5.2006, with a strong correlation coefficient (R^2= 0.9513), indicating a significant relationship between storage duration and microbial growth. The positive slope of the regression model confirms that TPC rises as storage time increases. This trend, evident in the regression curve from day 0 to day 7 (Figure 2), demonstrates that extended storage facilitates the proliferation of microorganisms on the chicken meat.

According to Indonesian Standard (SNI 7388:2009), the maximum acceptable TPC for fresh chicken meat is 6 log CFU/g. In Figure 2, the TPC of chicken meat on the 3rd day of storage was 6.98 log CFU/g, which exceeded the standard limit for fresh meat. This finding aligns with the pH observation (Figure 1) where meat is considered not fresh on day 3. In this study, the meat was semi-fresh on day 3 and became unacceptable by day 4, when TPC reached 8.08 log CFU/g.

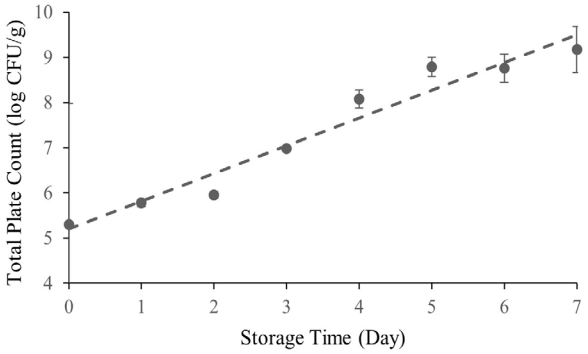

Fig. 2. Total Plate Count of chicken meat during chilled storage.

Total Volatile Base Nitrogen (TVB-N)

Figure 3 shows the TVB-N values of chicken breast during storage. TVB-N increased following a second-order polynomial equation of y = $0.0813x^2$-0.1355x+0.7587, with a strong correlation coefficient (R^2= 0.943). This indicates a significant relationship between storage time and volatile compound release.

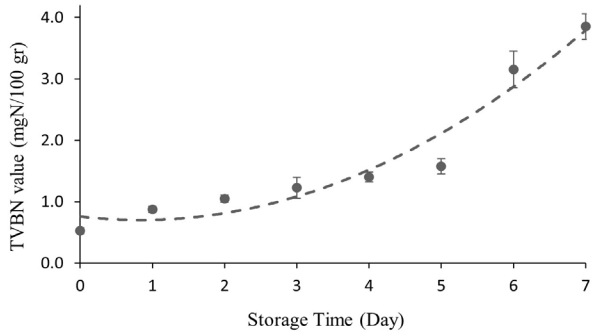

Fig. 3. TVB-N value of chicken meat during storage.

H_2S and Postma Test

As shown in Table 1, the H_2S and Postma tests on days 0 to 3 gave negative results, indicating the freshness of the chicken breast samples. In contrast, positive results were obtained from day 4 onwards. This shows that the chicken meat samples from day 4 to day 7 underwent spoilage. These results are consistent with the observations of pH (Figure 1) and TPC (Figure 2).

Film Color Changes

Figure 4 shows the color changes of the smart label film made from butterfly pea anthocyanins. At day 0, the film was very dark purple. Over time, the intensity of the purple hue decreased. By day 5, the color turned pine green, and by day 7, it became leaf green. CIELAB measurements (Figure 5) confirmed these visual changes, showing increases in L* (brightness), shifts of a* from positive (red) to negative (green), and b* from negative (blue) to positive (yellow).

Visual Color versus Chemical/Microbiological Evaluation

Table 2 compares the visual changes of the smart label with chemical

Table 1. Postma and H_2S test results during storage at 4°C for 7 days

Storage time (Day)	H_2S Test	Postma Test
0	-	-
1	-	-
2	-	-
3	-	-
4	+	+
5	+	+
6	+	+
7	+	+

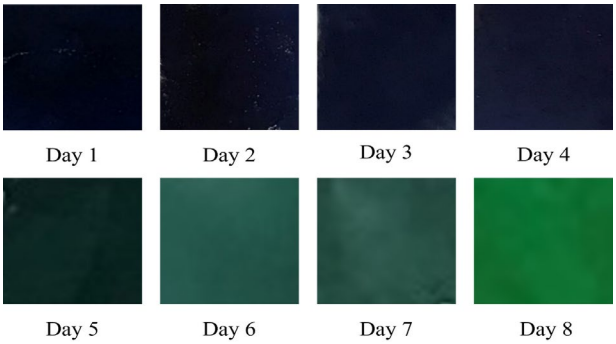

Fig. 4. The color changes of smart label contacting the surface of chicken breast during chilled storage.

and microbiological freshness indicators. pH, TPC, and H_2S/Postma tests consistently showed freshness until day 3 and spoilage from day 4 onwards. In contrast, TVB-N values remained below the threshold throughout 7 days, leading to discrepancies with other indicators.

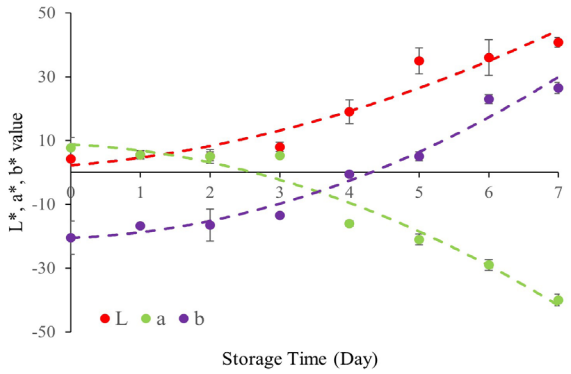

Fig. 5. Evolution of L*, a*, b* values of smart label during chilled storage.

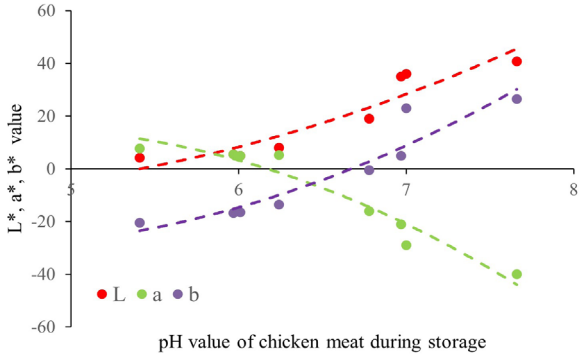

Fig. 6. Correlation between the color change of smart label film and pH of chicken meat.

Discussion

The decrease in anthocyanin content from 7.71 mg/L to 5.63 mg/L after 7 days (Section 3.1) confirms the low stability of anthocyanins. Their degradation is strongly influenced by environmental parameters such as temperature, humidity, pH, oxygen, and the presence of enzymes or metal ions (Sharma *et al*., 2016). The concurrent rise in chicken meat pH during storage (Figure 1) provides an explanation, as higher pH accelerates anthocyanin degradation (Sui *et al*., 2014). Furthermore, the reactive unsaturated structure of anthocyanins makes them vulnerable to oxidative degradation, both directly and via enzymatic pathways mediated by peroxidases and polyphenol oxidases (Liu *et al*., 2018). This mechanistic understanding supports the observed decline in film anthocyanin content.

The amount of anthocyanin absorbed during the immobilization process impacts the quantity of anthocyanin contained in the indicator film. Some parts of the anthocyanin of the butterfly pea are bound to chitosan and PVA due to the interaction of the phenol groups with the hydroxyl groups of chitosan, whereas PVA also interacts with a small part of the water contained in the anthocyanin (Hasanah *et al*., 2023). The amount of anthocyanin adsorbed is also influenced by the morphology of the polymer film because the film pores are potential spaces to be filled by anthocyanin extracts (Pourjavaher *et al*., 2017).

The steady increase in pH values, reaching 6.24 by day 3 and exceeding 6.1 from day 4 onwards (Figure 1), signals a transition from fresh to spoiled meat. The positive slope indicates that longer storage increases the pH, as observed in the regression curve from day 0 to day 7. According to Beauclercq *et al*. (2022), fresh chicken meat has a pH value ranging from 5.7–6.1. This rise can be attributed to glycogen depletion and reduced lactic acid formation in postmortem muscle (Puolanne *et al*., 2002). In addition, microbial metabolism of amino acids produces alkaline compounds such as ammonia, which further elevates pH and promotes spoilage (Zhao *et al*., 2019). This dual effect of biochemical and microbiological processes explains why pH is a reliable early freshness indicator, aligning with the TPC and H_2S results.

The regression model for TPC ($y = 0.6146x + 5.2006$, $R^2 = 0.9513$) demonstrates strong correlation with storage time (Figure 2). The microbial counts exceeded 6 log CFU/g by day 3 and reached 8.08 log CFU/g on day 4, coinciding with pH values above the freshness threshold. This close correspondence highlights the role of microbial proliferation in

Table 2. Smart label visual color versus chemical/microbiological parameters.

Storage time (Day)	Film color	pH	TPC (log CFU/g)	H_2S and Postma Test
0	Dark purple (+++)	5.41 (fresh)	5.30 (fresh)	- (fresh)
1	Dark purple (+++)	5.97 (fresh)	5.78 (fresh)	- (fresh)
2	Dark purple (+++)	6.01 (fresh)	5.85 (fresh)	- (fresh)
3	Dark purple (++)	6.24 (borderline fresh)	6.98 (semi fresh)	- (fresh)
4	Dark purple (+)	6.78 (not fresh)	8.08 (not fresh)	+ (not fresh)
5	Pine green	6.97 (not fresh)	8.79 (not fresh)	+ (not fresh)
6	Pine/blue green	7.00 (not fresh)	8.76 (not fresh)	+ (not fresh)
7	Leaf green	7.66 (not fresh)	9.18 (not fresh)	+ (not fresh)

spoilage progression. Following the categorization by Vasconcelos *et al*. (2014), meat with microbial counts between 7–8 log CFU/g can be considered semi-fresh, while counts exceeding 8 log CFU/g indicate spoilage. As reported previously, Pseudomonas spp. dominate the spoilage flora of aerobically stored chilled chicken (Wickramasinghe *et al*., 2019). Their growth is facilitated by elevated pH and nutrient availability, with lactic acid serving as a secondary carbon source once glucose is depleted (Nychas & Drosinos, 2014). The alignment between TPC and pH underscores their combined diagnostic value.

Although TVB-N values increased consistently during storage (Section 3.4), they remained below the accepted spoilage threshold of 25.5 mg/100 g (Mohsenzadeh *et al*., 2016). Despite the increase, TVB-N values remained below the 25.5 mg/100 g threshold proposed by Mohsenzadeh *et al*. (2016) throughout 7 days of storage, suggesting that the meat still met TVB-N standards for freshness. This finding contradicts the results of pH, TPC, and H_2S/Postma tests, which indicated spoilage from day 4 onwards. The discrepancy likely stems from limitations of the Kjeldahl-based distillation method, where volatile compounds may evaporate during measurement (Urmila *et al*., 2015). This reinforces the need to complement TVB-N with other indicators or adopt alternative non-destructive methods (Li *et al*., 2019).

The H_2S and Postma tests provided consistent results with pH and TPC measurements, showing spoilage onset at day 4 (Table 1). Since hydrogen sulfide originates from microbial degradation of sulfur-containing amino acids, its detection is a direct indication of microbial spoilage activity (McMeekin & Patterson, 1975). The concordance of this simple test with other chemical and microbiological indicators strengthens its applicability as a freshness detection method.

The visual color change of the anthocyanin-based smart label, from dark purple to green (Figure 4), correlated well with the declining freshness of chicken meat. CIELAB data confirmed shifts in brightness (L*), hue (a*), and chromaticity (b*), with fresh meat (pH ≤ 6.1) corresponding to L* < 9.98, a* > 1.40, and b* < -12.76 (Beauclercq *et al*., 2022). However, anthocyanin's limited color expression within the narrow pH range of chicken meat (5–7) restricts visible transitions to purple and green (Sai-Ut *et al*., 2021; Wulandari *et al*., 2020). While chemical interactions between anthocyanins and volatile bases such as ammonia explain the green shift (Rawdkuen *et al*., 2019), the distinct visual change occurs only after spoilage has advanced.

The final comparison between visual smart label changes and chemical/microbiological evaluations (Table 2) shows that freshness was lost at day 4 according to pH, TPC, and H_2S/Postma tests. However, the most noticeable color change on the smart label, from purple to green, appeared between day 4 and 5. This indicates a one-day delay between actual spoilage and clear visual detection. Although subtle purple fading could theoretically be quantified using instruments, such approaches are impractical for consumers. Therefore, the butterfly pea anthocyanin smart label cannot pinpoint the exact freshness threshold but is effective as a consumer-friendly visual indicator once the meat is no longer acceptable for consumption.

Conclusion

The current study demonstrates the feasibility of using butterfly pea flower anthocyanin-based smart labels as freshness indicators for chicken meat stored under chilled conditions. Chemical and microbiological analyses revealed that chicken meat retained freshness until day 3, as indicated by pH values below 6.1, TPC levels under 6 log CFU/g, and negative results from H_2S and Postma tests. From day 4 onwards, the meat exceeded these freshness thresholds. Correspondingly, the smart label exhibited noticeable color changes, particularly the transition from purple to green, which provided a clear visual cue for the deterioration of meat quality.

The study highlights the potential of anthocyanin-based smart labels to serve as real-time, non-destructive freshness indicators. However, the narrow pH range of chicken meat (5–7) poses a limitation, as it confines the observable color changes to specific hues. Further optimization of the indicator formulation or integration with complementary detection methods may enhance the sensitivity and accuracy of such systems. This work underlines the utility of colorimetric smart labels in addressing consumer demands for simple and effective food freshness monitoring tools, while contributing to improved food safety and reduced waste in the supply chain.

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Conflict of interest

The authors have no conflict of interest to declare.

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