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Development and characterization of smart indicator composed of gelatin/carboxymethyl cellulose/morning glory anthocyanin for red meat freshness monitoring

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ABSTRACT

This study investigates the development and characterization of a new smart colorimetric indicator system based on anthocyanin-enhanced gelatin (Gel) and carboxymethyl cellulose (CMC) films for real-time monitoring of meat spoilage. This formulation combines natural anthocyanins with a Gel/CMC matrix, specifically designed to achieve broad pH responsiveness and clear visual discrimination of spoilage stages, addressing the need for effective natural indicators in intelligent food packaging. FTIR and SEM analyses confirm the successful integration of anthocyanin into the matrix, revealing minor spectral shifts and surface morphology changes that suggest enhanced intermolecular interactions. The indicator exhibits distinct pH-dependent color changes, transitioning from reddish at pH 3 to greenish-yellow at pH 12, as demonstrated by anthocyanin extract and film tests. In a practical application, smart indicator color packaged with meat shifts from red to yellow over 48 h, correlating with a pH increase from 5.8 to 8, indicating spoilage. Color parameter changes (L: 20.3 to 46.3, a: 21.3 to 10.6, b: 10 to 11.6) further support its sensitivity to freshness. These findings highlight the potential of this indicator as an effective natural tool for intelligent food packaging applications.

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1. Introduction

The need for safe and high-quality food, along with the increasing demand for fresh and healthy food that can last longer, has resulted in the creation of new food packaging technologies. One of these innovative approaches is intelligent packaging, which monitors the food's environmental conditions and quality factors

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to ensure its safety and quality, meeting the consumers' demands (1, 2). Food quality indicators (FQIs) are smart devices that are affixed to food packaging as a label or tag, and they keep track of the quality status of the packaged food products by displaying a visual color change. As food spoils, the concentration of organic acids, volatile nitrogen compounds, carbon dioxide, and biogenic amines increases, which alters the pH of the food, which can be monitored using an FQI (3). Typically, FQIs consist of a polymeric substrate and a pH-sensitive pigment.

Currently, the primary focus of research for solid substrate matrices of FQIs is on materials that are obtained from renewable natural resources. These materials are preferred due to their biodegradability, biocompatibility, abundance, and low cost. Biopolymers derived from natural sources, such as gelatin, chitosan, and cellulose derivatives, offer eco-friendly substitutes (4-6). Among them, gelatin (Gel), a polypeptide obtained from collagen hydrolysis, is valued for its biodegradability, biocompatibility, excellent film-forming ability, and capacity to absorb UV light due to aromatic amino acids (6, 7). Despite these benefits, pure gelatin films exhibit poor mechanical, thermal, and barrier properties, along with weak antimicrobial performance, which limits their direct application in food packaging. Therefore, recent studies have focused on modifying or blending gelatin with other polymers to enhance its functional properties for smart and sustainable packaging systems (7). One such polymer is Carboxymethyl cellulose (CMC), derived from cellulose, which is widely recognized for its abundance, biodegradability, and non-toxic nature, making it suitable for sustainable packaging (8, 9). As a water-soluble polymer, CMC forms transparent and flexible films but suffers from high hydrophilicity and limited mechanical strength. To overcome these drawbacks, blending CMC with other polymers like Gel has proven effective. The CMC/Gel combination has attracted increasing attention in active and intelligent packaging due to its biocompatibility and functional behavior (6, 10, 11).

Currently, there is an increasing trend towards using natural indicator dyes instead of synthetic colorants, and anthocyanin are a popular choice for developing pH-sensitive colorimetric FQIs (12). However, the effectiveness of anthocyanin as pH-responsive indicators for monitoring food freshness and spoilage depends on their source (13). The type of plant used to obtain anthocyanin has a significant impact on the performance of FQI. Ipomoea purpurea, which is commonly known as Morning Glory, is the largest genus in the Convolvulaceae family (14). This genus is often considered a weed and is found in tropical and certain temperate regions. Morning Glory is known for its trumpet-shaped flowers, which are visually appealing and are popularly cultivated for ornamental purposes (14, 15). The Morning Glory anthocyanins (MGA) have already been studied for their biological properties, including hypoglycemic, anti-inflammatory, antioxidant, and antimicrobial abilities, as well as their anti-arthritic activity (16). To the best of our knowledge, morning glory anthocyanin has not previously been used as a pH-responsive indicator than other anthocyanins. Therefore, this study reports on the fabrication of Gel/CMC-based color sensitivity FQI incorporated with MGA (MGA@Gel/CMC). Initially, the colorimetry and microstructural properties of the

designed FQI were examined. The halochromic ability of MGA@Gel/CMC FQI in response to changes in pH and ammonia levels was then tested. Eventually, the MGA@Gel/CMC performance for tracking of red meat spoilage during storage at room temperature (2 days) was evaluated.

2. Materials and Methods

2.1. Materials

Food-grade gelatin (Gel; type A, fish source, 300 g bloom, CAS: 9000-70-8), chemical-grade carboxymethyl cellulose (CMC; viscosity: 800-1200 cp, 250,000 of average MW, purity > 99.5 %, CAS NO: 9004-32-4) were acquired from Sinopharm Co., Ltd. (Beijing, China). The following reagents were also utilized: hydrochloric acid (HCl, CAS; 7647-01-0), glycerol ($C_3H_8O_3$, CAS; 56-81-5, purity ≥ 99%), and ethanol (CAS: 64-17-5, Merck (Darmstadt, Germany)). Fresh raw red meat was bought from a local market in Tabriz, Iran, and transported to the laboratory under aseptic refrigerated conditions (below 4±1°C) within 45 min. *Ipomoea purpurea* (morning glory) flowers were purchased from Mazandaran Province, Iran.

2.2.Anthocyanin extraction

To extract MGA, a modified version of the method proposed by Chen et al. was utilized (11). Here, the samples, after rinsing with water, were peeled, and weighed. These fragments were then immersed in 25% ethanol at a 1:10 solid-to-liquid ratio, stirred gently, and kept in darkness at 25 ± 2 °C for 3 h. Then, the extract was filtered through Whatman No. 1 filter paper and then centrifuged twice at 5000 × g and 15 °C for 8 min each to maximize MGA extraction. The supernatant was subsequently subjected to rotary evaporation at 39 ± 2 °C to remove the solvent. The concentrated extract was then transferred to a dark glass container and stored in a refrigerator for future use. The total anthocyanin content, expressed as cyanidin-3-glucoside equivalents in mg, was determined (17). The extracts were dissolved in buffer solutions at pH 1.0 and pH 4.5, then scanned at 520 nm and 700 nm using a UV-Vis spectrophotometer. The anthocyanin levels were calculated based on the absorbance differences observed at these two pH values. Finally, to verify MGA's effectiveness as a pH-responsive dye, its color change was evaluated within a wide pH range from 3 to 12 at room temperature.

2.3.Fabrication of MGA@Gel/CMC smart indicator

Biodegradable FQI based on Gel/CMC were prepared using the casting method. Here, mixture A was prepared by dissolving 3% w/v Gel in 100 mL of distilled water and stirring at 45 °C for 3 h. In parallel, 1.5 g of CMC was dissolved in 100 mL of water with continuous stirring to form mixture B. Finally, mixture B and 1.5 g of glycerol (acting as a plasticizer, 30 wt% of the polymer) were gradually added to mixture A, and the combined solution was stirred gently for 2 h to ensure thorough mixing. After that, different concentrations of MGA (4%, 6%, and 8%, v/v) were incorporated into the solution and stirred magnetically for 2 h at 25 °C. To remove bubbles, the mixture was sonicated for 15 min. The solution was then poured onto uniform plates at a rate of 15 g and left at 25 °C for 48 h to dry and facilitate separation. The resulting pH-sensitive FQI were designated as Gel/CMC, 4%MGA@Gel/CMC, 6%MGA@Gel/CMC, and 8%MGA@Gel/CMC.

2.4. Morphological and structural properties

The analysis of potential intra- and intermolecular interactions between the biopolymers and MGA was conducted using a Fourier transform infrared (FT-IR) spectrophotometer (Irprestige-21, Shimadzu, Japan). Spectral data were collected within the range of 4000 to 650 cm^{-1} at a resolution of 4 cm^{-1}, with each sample undergoing 16 scans. To examine the morphological characteristics, the samples were first sputter-coated with a gold layer under vacuum conditions (sputtering time of 2 min, applied voltage of 15 kV, and a working distance of approximately 5 mm). The surface features of the prepared indicators were then observed using a scanning electron microscope (SEM; Quanta 450, USA).

2.5. pH and ammonia sensitivity of smart indicator

The pH sensitivity of MGA solution and the fabricated MGA@Gel/CMC indicator was examined by immersing the samples in phosphate buffer solutions with varying pH levels ranging from 3 to 12, and the resulting color changes were documented through digital photography. The pH of each buffer solution was meticulously adjusted using 0.1 M hydrochloric acid (HCl) and 0.1 M sodium hydroxide (NaOH), and subsequently verified with a calibrated pH meter. More so, the FQIs' capacity to detect ammonia vapors was assessed following the methodology previously described by Tan et al. (18). In brief, the indicators were cut into 25 mm diameter discs and positioned 1 cm above a dish containing an 8 mM ammonia solution at room temperature for a duration of 20 min. During this exposure, the indicators' color transformation was captured every 5 min using photographic documentation. The sensitivity of the indicators to ammonia vapor was then quantified based on the extent of color change observed, with calculations performed accordingly.

$$S_{RGB} = \frac{(R_a - R_b) + (G_a - G_b) + (B_a - B_b)}{R_a + G_a + B_a}$$

The initial and final values of red, green, and blue, denoted as Ri, Gi, Bi, Rf, Gf, and Bf, correspond to the respective color components measured before and after the process. These measurements were obtained using the Pixie program.

2.6. Determination of meat quality

To assess the FQI's performance, the meat samples were placed inside polyethylene plastic bags, each containing a label made from MGA@Gel/CMC indicators. These sealed bags were then stored at 25 °C for a period of up to 2 days, during which the color changes of the FQI were monitored. The progression of color transformation was documented using a digital camera, providing visual data on the film's response over the storage duration.

The pH and color changes of meat sample were measured at different storage times: 0, and 2 days at 25 °C. 10 g of red meat was crushed, homogenized, and stirred in 90 mL distilled water for 5 min, with vigorous shaking every 5 min. The mixture was then filtered, and the filtrate's pH was recorded at specific intervals using a digital pH meter (Thermo Scientific, Indonesia). The color difference, ΔE, was calculated using the CIELAB color space formula: $\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$, to quantify color changes between the initial (0 h) and spoiled (48 h) states for each measured area (19).

2.7. Statistical analysis

All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation

(SD). Statistical analyses were conducted using GraphPad Prism (version 8, GraphPad Software, USA). Differences among groups were evaluated using one-way analysis of variance (ANOVA). Statistical significance was considered at $P<0.05$. Different letters in the tables/ or figures indicate statistically significant differences between groups.

3. Results

3.1. pH-responsive color change of anthocyanin solution

Fig. 1 displays the color changes of MGA extract within a pH range of 3 to 12. At pH 3, the solution appears a red color, altering to a pinkish-red at pH 4 and maintaining a reddish color through pH 5 and 6. At pH 7, the color stabilizes as a peach color, which persists through pH 8 and 9 with slight darkening. As the pH increases to 10, the color shifts to a brownish-yellow, becoming more pronouncedly yellow at pH 11 and 12.

Figure 1. Color changes of anthocyanin solution at various pH.

3.2. pH-responsive color change of smart indicator

The color changes of a smart indicator containing anthocyanin between pH of 3 to 12 has been illustrated in Fig. 2. At pH 3, the indicator exhibits a reddish-brown color, changing to brown at pH 4 and 5, and maintaining a brownish tone through pH 6 and 7. As the pH increases to 8, the color shifts to a lighter brown with a yellowish color, becoming more pronouncedly yellow at pH 9 and 10. At pH 11 and 12, the indicator turns a greenish-yellow, indicating a significant color shift.

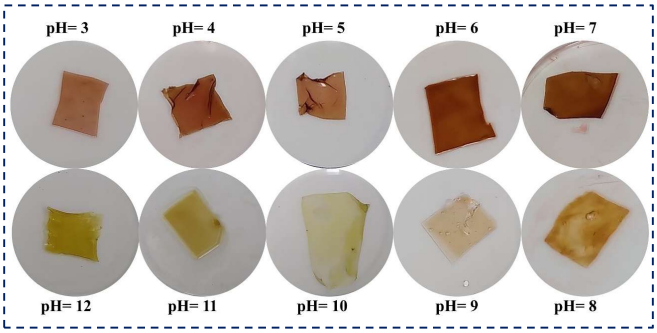

Figure 2. Color changes of smart indicator containing 8% anthocyanin at various pH

3.3. Ammonia sensitivity of smart indicator

The ammonia sensitivity was measured using SRGB analysis, revealing that the SRGB score of the colorimetric films reached 11.45 ± 0.8 % at 10 min and steadily increased over the next 30 min. Sensitivity of the colorimetric films to ammonia vapor markedly increased over time ($p<0.05$), with color change percentages reaching 10.25 ± 0.74 % at 30 min. This color shift likely results from interactions involving MGAs' phenolic hydroxyl groups, hydroxide ions, hydrated ammonia, and phenolic oxygen anions. The films' quick and clear color change from rosy brown to olive green, shown in Fig. 3, highlights their potential for intelligent packaging.

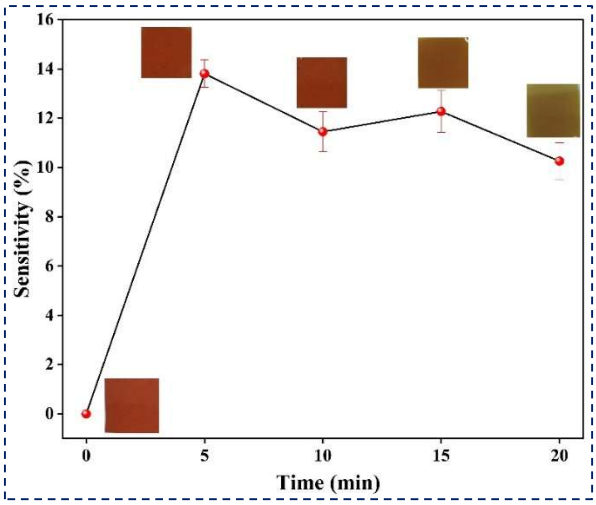

Figure 3. Ammonia sensitivity assay of smart indicator containing 8% MGA

3.4. Characteristics of smart indicator

3.4.1. FTIR analysis

The FTIR spectra of Gel/CMC (Fig. 4A) and Gel/CMC enhanced with MGA (Fig. 4B) shows broadly similar absorption patterns dominated by the base polymer matrix. Minor differences are observed due to anthocyanin incorporation. In the O–H/N–H stretching region (3000–3500 cm^{-1}), Gel/CMC sample exhibits a more intense broad band, whereas MGA@Gel/CMC sample shows slight changes in intensity and/or peak broadening, indicating interactions between MGA and the Gel/CMC network (20, 21). The 1600–1700 cm^{-1} region (C=O and N–H related vibrations) and the 1200–1500 cm^{-1} region (C–H and O–H bending) show subtle variations consistent with anthocyanin–polymer interactions (20, 21). Notably, the bands at 1396, 1546, and 920 cm^{-1} observed in MGA@Gel/CMC are better interpreted as shifted bands rather than newly formed peaks compared with Gel/CMC (22). In contrast, MGA@Gel/CMC exhibits truly new/appearing peaks at 2835, 1425, and 1739 **cm^{-1},** which are attributed to anthocyanin-associated vibrational modes. Additionally, the disappearance of peaks at 810 and 2158 cm^{-1} in MGA@Gel/CMC further supports the change in vibrational environments upon MGA integration (23, 24). Overall, FTIR confirms that the base composition remains largely consistent, while MGA introduction induces specific spectral changes associated with molecular interactions and new functional contributions.

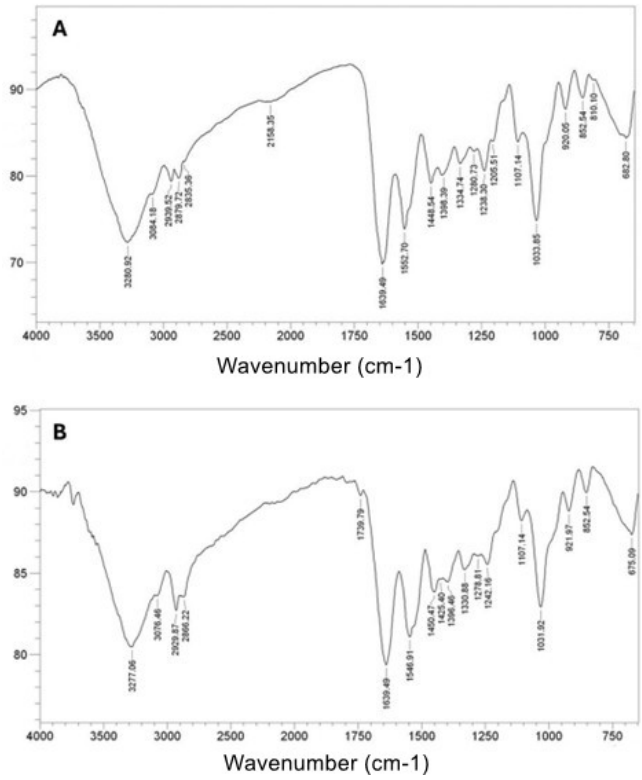

Figure 4. FTIR spectra of Gel/CMC sample (A) and 8%MGA@Gel/CMC sample (B).

3.4.2. SEM analysis

The SEM images depict the surface morphology of two samples at a 10 µm scale (Fig. 5). Figure 5a, representing the control sample (gelatin and carboxymethyl cellulose), shows a relatively smooth surface with a visible scratch or feature, suggesting minor surface

Table 1. Color properties of smart indicators applied for meat freshness monitoring

	Time (h)						
	0			48			
Indicators	L	a	b	L	a	b	ΔE
8%MGA	20.3±1.12^{Aa}	21.3±0.47^{Aa}	10.0±0.81^{Aab}	46.3±.94^{Ba}	10.6±0.47^{Ba}	11.6±1.24^{Aa}	17.84±0.44^{a}
6%MGA	32.0±0.81^{Ab}	18.6±1.24^{Ab}	12.3±0.47^{Aa}	41.6±0.47^{Bb}	8.0±0.81^{Ba}	10.6±0.94^{Aa}	15.88±0.75^{b}
4%MGA	46.6±0.47^{Ac}	12.0±0.81^{Ac}	8.3±1.24^{Ab}	34.3±0.94^{Bc}	9.6±0.94^{Ba}	7.3±0.47^{Ab}	3.45±0.30^{c}

Lowercase letters in the columns and uppercase letters in the rows indicate significant differences among treatment

irregularities or mechanical damage. Figure 5b, which includes the addition of anthocyanin, displays a more uniform and granular texture with small, scattered particles, indicating the incorporation of anthocyanin has altered the surface structure, possibly due to new intermolecular interactions or particle distribution (22-25). The differences suggest that anthocyanin enhances surface complexity, which may influence other properties (26, 27). Both FTIR and SEM tests confirm a good interaction between anthocyanin and the matrix, suggesting its potential use as a smart indicator.

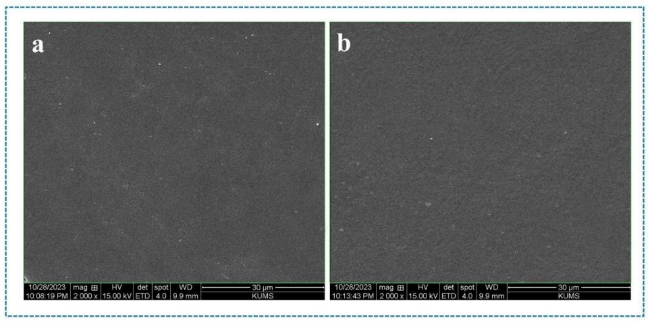

Figure 5. SEM image of Gel/CMC sample (a) and 8%MGA@Gel/CMC sample (b).

3.5. Meat freshness monitoring

Fig. 6 shows meat samples packaged with a smart indicator, monitored over 48 h to assess spoilage. To accelerate product spoilage and quickly observe indicator color changes, the samples were stored and examined at room temperature. At first, the meat is fresh, with the indicator displaying a red color, and color parameters are L: 20.3, a: 21.3, b: 10 for smart indicator containing 8% anthocyanin (Table 1), while the meat's pH is approximately 5.8. After 48 h, the meat is spoiled, with the indicator shifting to a yellowish color, and color parameters change to L: 46.3, a: 10.6, b: 11.6, reflecting increased lightness (L) and reduced redness (a), alongside a rise in yellowness (b).

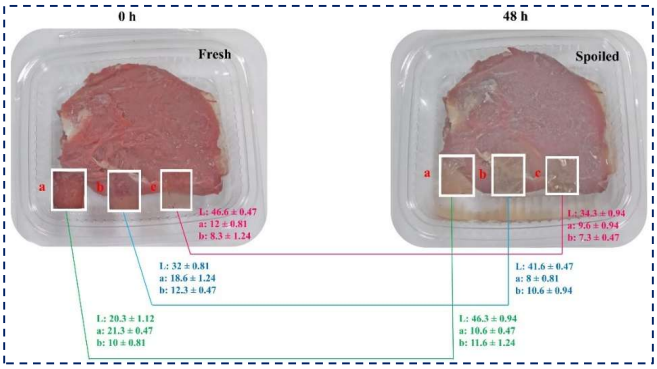

Figure 6. Monitoring freshness of meat using smart indicator containing 8% anthocyanin (a), 6% anthocyanin (b), 4% anthocyanin (c).

4. Discussion

4.1. pH-responsive color change of anthocyanin solution

Fig. 1 displays the color changes of MGA extract within a pH range of 3 to 12. These changes occur due to changes in the structure of anthocyanins at different pH (28, 29). These variations highlight anthocyanin's pH-sensitive nature, demonstrating its potential as a natural pH indicator in a wide range, with distinct color

changes that could be utilized for applications such as food quality monitoring or chemical sensing (30). The pH-dependent color transitions observed for our anthocyanin extract, shifting from reddish-brown at pH 3 to greenish-yellow at pH 12, are consistent with the known behavior of anthocyanins as natural pH indicators. For instance, research by Alizadeh Sani et al. on anthocyanins extracted from barberry demonstrated similar color changes across a pH range of 1-14 (24). Likewise, our study extends this observation to a broader pH range and specifically correlates these spectral shifts with the performance of the developed indicator film.

4.2. pH-responsive color change of smart indicator

The color changes of a smart indicator containing anthocyanin between pH of 3 to 12 has been illustrated in Fig. 2. These changes demonstrate the pH-sensitive nature of anthocyanin, making the indicator effective to detect a wide range of pH values, which is valuable for applications such as food spoilage monitoring or environmental sensing (31, 32). Accordingly, smart indicator exhibited distinct and visually discriminative pH-dependent color changes, mirroring the solution's behavior but offering the advantage of a solid-state format. Studies on anthocyanin-based indicators, such as the work by Alizadeh Sani et al. using barberry anthocyanin incorporated into methylcellulose/chitosan nanofiber films, have reported color shifts from red to yellow in the alkaline range (24). Similarly, our film shows a transition from red to yellow in the spoilage-indicating range (pH 5.8-8), the specific matrix (Gel/CMC) and the resulting color transitions (reddish-brown to greenish-yellow across the full pH range tested) offer a potentially different visual output and stability profile.

4.3. Ammonia sensitivity of smart indicator

During muscle protein degradation, different volatile compounds such as ammonia, amines, and sulfides are generated. Among these, ammonia, a key volatile nitrogen compound, significantly increases the pH in muscle-based foods. Ammonia-sensitive films can serve as intelligent packaging tools, effectively monitoring the freshness or spoilage of protein-rich food products. A study simulating food spoilage was conducted to evaluate the fundamental gas-sensing capability by observing color changes in MGA-based indicator exposed to ammonia vapor (Fig. 3). This color shift likely results from interactions involving MGAs' phenolic hydroxyl groups, hydroxide ions, hydrated ammonia, and phenolic oxygen anions. The observed ammonia-responsive color change is consistent with previous reports on anthocyanin-based intelligent films. Alizadeh et al. reported similar sensitivity toward ammonia in indicator films containing anthocyanins extracted from barberry and saffron (19, 24), Eghbaljoo et al. also demonstrated comparable ammonia-induced color changes in films based on grape peel anthocyanins (23). Similarly, Riahi et al. reported that gelatin/PVA films with red cabbage anthocyanin@Cu-MOFs exhibited a marked color transition from pink to green within 15 min of ammonia exposure (33). These findings confirm that anthocyanin-rich natural extracts are effective pH- and ammonia-sensitive pigments for intelligent packaging applications.

4.4. Meat freshness monitoring

Fig. 6 shows meat samples packaged with a smart indicator, monitored over 48 h to assess spoilage. The meat's pH increases to 8, indicating spoilage due to microbial activity and pH elevation. This color alteration from red to yellow, correlated with pH changes from 5.8 to 8, demonstrates the indicator's effectiveness in visually tracking meat freshness over time. The results are consistent with previous studies on meat products (25, 34, 35). In practical application tests, our smart indicator demonstrated a clear color shift from red to yellow over 48 h when packaged with meat, correlating with an increase in pH from 5.8 to 8, indicative of spoilage. This is a significant advantage over many lab-based pH measurements or indicators that may not perform reliably under real food packaging conditions. For example, research by Alizadeh Sani et al. developed an anthocyanin-based indicator for meat spoilage that showed similar color changes (24). Our Gel/CMC matrix appeared to provide a stable platform that effectively captures the pH changes associated with meat spoilage, with quantifiable color parameter variations further validating its sensitivity and reliability.

5. Conclusion

The research successfully demonstrates that anthocyanin-enhanced indicators function as an effective smart indicator for meat spoilage detection. The integration of anthocyanin, validated by FTIR and SEM, enables a clear color transition from red to yellow over 48 h, aligning with a pH rise from 5.8 to 8, which indicates meat deterioration. The consistent color parameter shifts confirm the indicator's reliability in tracking freshness. This natural, pH-sensitive system offers a promising, eco-friendly solution for real-time food quality assessment, with potential applications in smart packaging to enhance food safety and reduce waste. While the developed smart indicator demonstrates good sensitivity for monitoring ammonia levels, future research should focus on optimizing its stability under varying environmental conditions, particularly concerning light-induced degradation, moisture sensitivity, and the potential impact of microbial growth on the indicator’s performance.

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Authorship contribution

Reza Abedi-Firoozjah and Farinaz Esmi: Conceptualization of idea, resources, visualization, data curation, writing of original draft.

Arezou Khezerlou: Conceptualization, software, data curation, formal analysis, validation, reviewing, and editing.

Mahmood Alizadeh Sani: Methodology, supervision, validation, data curation, review and editing.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Data availability

Data will be available on request.

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Not applicable

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