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## Betacyanin–curcumin smart films for detecting fresh chicken quality in real time

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pH-based indicators play an important role in ensuring food safety by providing a real-time visual indication. The objective of this study was to evaluate the potential of the natural colors of turmeric (curcumin) and beetroot peel (betacyanin) as colorimetric pH indicators for the determination of chicken freshness. For pH changes, curcumin (CR), betacyanin (BPE), and a 1:1 mixture of CR and BPE were tested with different buffer systems. The sensitometric films were prepared by dispersing these dyes at 1, 2, 5 and 10% concentrations into a biopolymeric film matrix of sodium alginate and almond gum. Formulation with a 10% concentration exhibited superior color retention and visual sensitivity. Film characterization was performed, including moisture content, solubility, and FTIR analysis. The response of the film was evaluated in fresh chicken stored at room temperature and refrigerated temperature (4 °C). The obtained results, including stability, color change correlation with total volatile basic nitrogen (TVB-N) levels, and 20-day film durability, confirm the effectiveness of these films as a non-destructive food quality monitoring system. The combination of curcumin and betacyanin provided enhanced pH sensitivity, a broader detection range, and improved visual clarity, making it more effective than single-indicator systems for real-time spoilage monitoring. This study presents a novel, affordable, biodegradable smart packaging solution for meat freshness monitoring in real-time, which has the potential to increase food safety and reduce food waste.

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### Sustainability spotlight

This study presents a biodegradable food packaging film developed using natural biopolymers and agro products. By utilizing natural, renewable biopolymers, the film offers an eco-friendly alternative to conventional plastic packaging. The incorporation of food waste-derived anthocyanins enhances both functionality and environmental value. This packaging responds to pH changes, enabling real-time monitoring of food freshness. It reduces food waste by signaling spoilage visually, supporting a circular economy. The film's biodegradability ensures minimal environmental impact post-use. Water, light, and storage stability were evaluated to support practical application. No synthetic additives were used, promoting consumer and environmental safety. This innovation contributes to sustainable food systems through smart, clean-label packaging. It aligns with global goals for waste reduction, biodegradability, and food quality monitoring.

## 1 Introduction

Food spoilage is a significant global concern, leading to massive food waste and potential health risks. Consumers mainly rely on visual cues such as changes in color, texture, or odor to determine whether food is safe to consume. However, these methods can be unreliable, especially in the early stages of

spoilage when harmful bacteria may already be present. Food packaging plays a crucial role in protecting food from external contaminants, maintaining its freshness and extending its shelf-life.<sup>1</sup> Technological advancements offer numerous innovative solutions, including electrospinning, layer-by-layer assembly, sol-gel techniques, foam/pad development, 3D printing, and pH indicator films.<sup>2,3</sup> Among these, pH-based indicators are particularly cost-effective. These films act as simple, colorimetric sensors that visually indicate food spoilage by detecting changes in pH levels, allowing for real-time detection and monitoring and providing consumers with immediate information about the condition of the food. A pH-based indicator film is composed of two main components: a pH-sensitive natural dye and a solid matrix, typically made of biopolymers or polysaccharides. The significance of the pH-sensitive dye lies in its ability to change color in response to

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volatile compounds such as ammonia that increase as spoilage progresses in the headspace of the packaging material.<sup>4-7</sup>

The use of natural indicators such as beetroot peel betacyanin and curcumin offers a promising approach to real-time quality monitoring in food packaging. Beetroot (*Beta vulgaris*) peel is a source of natural colorants belonging to the betalain group, and it has the notable characteristic of changing color under various pH conditions.<sup>8-10</sup> Betalains are water-soluble pigments found in prickly pear, red pitaya, red beetroot, and amaranth. Structurally, they are divided into two compounds: betacyanins, which provide a red to violet hue, and betaxanthins, which give a yellow to orange hue.<sup>11</sup> Betacyanins contain the chromophore betalamic acid and cyclo-3,4-dihydroxyphenylalanine, while the condensation product of betalamic acid and amines forms betaxanthins.<sup>12</sup> These compounds undergo structural changes under different conditions, producing various colors. They are stable in the pH range from 3 to 7 but, under alkaline conditions, show structural and color changes from bright red to yellow. For example, the freshness of chilled beef was monitored in real time using betacyanin as a pH indicator, with carboxymethyl cellulose (CMC) and flaxseed gum as biopolymers.<sup>13</sup>

Similarly, *Curcuma longa* L., commonly known as turmeric, is a good source of the bioactive compound curcumin, also known as diferuloylmethane.<sup>14</sup> Curcumin exhibits structural and color changes under different pH conditions, making it a promising candidate for food quality monitoring. The color change from yellow to red is due to the degradation of the curcumin structure, releasing primary products such as *trans*-6-(2,4-dioxo-5-hexenal), while secondary products include feruloylmethane, vanillin, and ferulic acid. In simple terms, the decomposition of curcumin occurs due to the loss of H<sup>+</sup> from its phenolic groups.<sup>15,16</sup> The antimicrobial and antioxidant properties of curcumin even make it an effective candidate for detecting and preventing food degradation.<sup>17</sup>

To achieve pH-based indicator films, biopolymer-based components can be used due to their ease of preparation and modification.<sup>18</sup> For example, the structural components of indicator films depend on the biopolymers used. Polysaccharides are commonly combined with natural gums to form an integral structure. Alginates, a source of polysaccharides obtained from brown algae species, are typically used in their salt forms. Sodium alginate, in particular, is used as a biopolymer. Natural gums serve as good sources of structural support, nutrients, water-binding components, and fiber. They are widely used in industry as stabilizers, emulsifiers, and thickening agents. Almond gum is a biopolymer naturally obtained from almond trees and is commonly used as a stabilizer and emulsifier.<sup>19,20</sup>

This study proposes a biodegradable colorimetric film for food packaging, developed by synergistically combining natural pigments such as curcumin from turmeric and betalains from beetroot peel into a dual biopolymer matrix. The film is designed to provide a visual indication of meat spoilage and allows for on-site monitoring of chicken freshness. The approach addresses some of the limitations associated with conventional packaging by offering a potential alternative that

is non-invasive, cost-effective, and environmentally friendly. This work contributes to the broader effort of integrating materials science with food safety applications.

## 2 Materials and methods

### 2.1 Materials

Beetroot (*Beta vulgaris*) and dried *Curcuma longa* R. were purchased from a local market in Mettupalayam, Coimbatore, India. Almond gum was obtained from Amazon (India, ASIN: B07JFCH58J). Sodium alginate was sourced from Sigma-Aldrich (India, CAS: 9005-38-3). Glycerol and sorbitol were acquired from Nice Chemicals (India), with product codes G89129 and S23429, respectively.

### 2.2 Preparation of beetroot peel extract (BPE) and curcumin extract (CE)

Fresh beetroot was washed under running water to remove surface impurities and dried with tissue paper to eliminate moisture. The beetroot skin was peeled to a thickness of 2 mm and minced. Extraction was performed according to the method described by Guo *et al.*<sup>17</sup> Briefly, minced beetroot peel (100 g) was combined with 100 mL of a 70:30 (v/v) ethanol–water mixture, and the pH was adjusted to 2 using acetic acid. The mixture was incubated in an orbital shaker at 150 rpm for 4 hours, followed by centrifugation at 5000 rpm for 5 minutes. The supernatant was stored at 4 °C. Dried *Curcuma longa* R. was ground into a powder (R1) and commercially available turmeric powder (R2) was subjected to extraction following the method by Shrisath *et al.*<sup>21</sup> The powder was dissolved in ethanol at a 1:25 (solid) ratio, and ultrasonication was performed at 22 kHz and 35 °C for 2 hours. The resulting solution was stored in a dark environment.

### 2.3 Total betalain and curcumin content

The UV spectrophotometric method was used to determine the total betalain and curcumin content. For betalain, the extract was diluted and measured at a wavelength of 535 nm, with calculations based on the equation provided by Singh A.<sup>22</sup>

$$\text{Total betalain content} \left( \frac{\text{mg}}{\text{g}} \right) = A \times Df \times MW \times 1000 / \epsilon L$$

where A: absorption value at 535 nm, Df: dilution volume, L: path length of the cuvette (1 cm), MW: molecular weight of betalain (550 g mol<sup>-1</sup>), and  $\epsilon$ : the extinction coefficient for betalain 60 000 l mol<sup>-1</sup>.

For curcumin, the extract was diluted and measured at a wavelength of 425 nm. Absorbance was noted and calculated according to the equation given below.

$$\text{Curcumin content} \left( \frac{\text{mg}}{100 \text{ g}} \right) =$$

$$\frac{0.0025 \times A \times \text{volume made up} \times Df \times 100}{0.42 \times \text{weight of the sample} \times 1000}$$



## 2.4 UV spectra of BPE and CE at different pH

1 mL of dye solution was dissolved in 10 mL of pH buffer solution. The buffer solutions, with pH values ranging from 3 to 13, were prepared according to the method outlined in the European Pharmacopoeia. Color changes at different pH levels (3–13) were studied using spectra obtained with a Shimadzu UV spectrophotometer over a wavelength range of 300 to 700 nm.

## 2.5 Preparation of colorimetric films

Colorimetric films were developed based on the method described by Suresh S. N. *et al.*<sup>20</sup> Almond gum was powdered using a mixer grinder and dried at 60 °C to remove moisture. A 0.3% concentration of almond gum and 0.67% of sodium alginate were dissolved in 150 mL of water. The solution was stirred using a magnetic stirrer at 500 rpm for about 30 minutes at 70 °C to ensure homogenization. Next, a 0.33% concentration of plasticizers (glycerol and sorbitol, in equal parts) was added to the solution, which was then stirred for an additional 45 minutes at 500 rpm to facilitate interaction with the biopolymer. Finally, a 10% concentration of dye solution with various compositions, as shown in Table 1, was added to the film solution and stirred for 5 minutes.

Using the solution casting approach, 80 mL of the pre-made film-forming solution was poured onto a 90 × 15 mm Petri plate. To help the volatile solvents evaporate, the film was initially allowed to air dry for 24 hours at ambient temperature. The semi-dried material was carefully removed after being cured for two hours at 45 °C to create an edible film. Until it was used again, the colorimetric film was kept at room temperature (23 ± 2 °C).

## 2.6 Film characterization

The moisture content of the film was tested for the developed colorimetric film. The hot air oven method was used for moisture content determination according to Siva Nandhini *et al.*<sup>20</sup> The film samples were cut into 2 × 2 cm pieces and dried in a hot air oven at 105 °C. At one-hour intervals, the weight of the film was noted and calculated using the equation:

$$\text{Moisture content}(\%) = \frac{m - M}{m} \times 100$$

where  $m$  is the weight of the film before drying and  $M$  is the constant weight after drying. The solubility of the film is an important factor for the stability of the film under various conditions. The prepared films were cut into 2 × 2 cm pieces,

immersed in 50 mL of distilled water, and placed in an orbital shaker at room temperature for 24 hours. Then, they were filtered using filter paper, and the undissolved particle residues retained in the filter paper were weighed and dried at 103 °C for 1 hour using the hot air oven method. The soluble matter was then calculated using the initial and final weights on a dry weight basis using the equation:

$$\text{Solubility}(\%) = \frac{M - W}{M} \times 100$$

where  $M$  is the dry weight of the initial film and  $W$  is the dry weight of the remaining film.

## 2.7 FT-IR analysis

Fourier transform infrared (FTIR) spectra were measured using an FTIR Affinity spectrometer (MIRacle 10, SHIMADZU), which is used to analyze the chemical composition of different colorimetric films. FTIR was measured in the range of 4000–400 cm<sup>−1</sup> at a resolution of 4 cm<sup>−1</sup>. The film without any dye solution was taken as the control.

## 2.8 pH response

The colorimetric film was cut and immersed in the 5 mL buffer solution of pH ranging from 3 (acidic) to 13 (alkaline) for a period of 10 min.

## 2.9 Live subject statement

To check the colorimetric pH response, fresh chicken was purchased from Uma Chicken Centre, Masakalipalayam Road, Peelamedu, Coimbatore, Tamil Nadu, 641004, and the experiment was performed as described below.

## 2.10 Application of the colorimetric film on fresh chicken

The Petri plates (9 cm in diameter) were sanitized and sterilized. Colorimetric films (control, BPE, CE, and CE:BPE) were cut into 2 × 2 cm strips and affixed to the inner side of the Petri dish lids using food-safe adhesive tape. Fresh chicken meat (approximately 10 g) was placed inside the Petri plates, which were then sealed with parafilm to simulate a closed-packaging environment. The sealed plates were stored under two environmental conditions: room temperature ( $R_T$ ) at 26 ± 2 °C and refrigerated temperature ( $R_fT$ ) at 4 ± 2 °C, each maintained at a relative humidity (RH) of approximately 75%. The samples were stored without vacuum or a modified atmosphere to mimic realistic retail storage conditions. Color changes in the films were

**Table 1** Composite films incorporated with natural dyes at different concentrations

	Matrix		Sensor dye	
	Bio polymer	Plasticizer	CE%	BPE%
Control	0.3% almond gum + 0.67% alginate	0.33% sorbitol + 0.33% glycerol	—	—
BPE	0.3% almond gum + 0.67% alginate	0.33% sorbitol + 0.33% glycerol	—	10
CE	0.3% almond gum + 0.67% alginate	0.33% sorbitol + 0.33% glycerol	10	—
CE:BPE	0.3% almond gum + 0.67% alginate	0.33% sorbitol + 0.33% glycerol	5	5



observed and recorded daily to assess spoilage progression visually.

### 2.11 Total volatile basic nitrogen (TVB-N)

5 g of chicken sample was placed in a distillation condensing tube and dispersed in 50 mL of deionized water. It was then stirred for 30 minutes before filtering. Next, 10 mL of boric acid and 5 mL of mixed indicator were added to the tube and distilled for 10 minutes. The distilled solution was titrated against  $0.01 \text{ mol L}^{-1}$  HCl until the solution turned blue-purple. The titration value was noted and calculated using formula 2.6:

$$\text{TVB-N (\%)} = (V_1 - V_2) \times 0.01 \times 2800$$

where  $V_1$  and  $V_2$  are the titration volumes of HCl in the sample and the blank (in the absence of meat), respectively.

### 2.12 Statistical analysis

All analyses were repeated three times, and their mean values and standard deviations were obtained. A completely

randomized design with the least squares technique using VetStat was used for the evaluation.

## 3 Results and discussion

### 3.1 Evaluation of BPE and CR extracts

The betacyanin content (%) in the extracts at room temperature and under refrigerated conditions was measured to assess the stability of betalain. At room temperature, the betacyanin content decreased from  $55.89 \pm 4.3$  to  $47.8 \pm 2.1$  by day 4. In contrast, under refrigerated conditions, the decrease was minimal, with values dropping from  $55.89 \pm 4.3$  to  $54.24 \pm 3.7$  on day 4. The refrigerated extracts remained more stable compared to those stored at room temperature. These results align with the findings of Lombardelli *et al.*<sup>23</sup> regarding the effect of storage temperature on beetroot extracts. Similarly, the total curcumin content of the powders was also measured. The curcumin content of R1 and R2 was  $2.533 \pm 0.16\%$  and  $1.77 \pm 0.01\%$ , respectively. R1 was observed to have a higher curcumin content compared to R2. These values fall within the range reported in the review study by El-Saadony *et al.*<sup>24</sup> The variation in

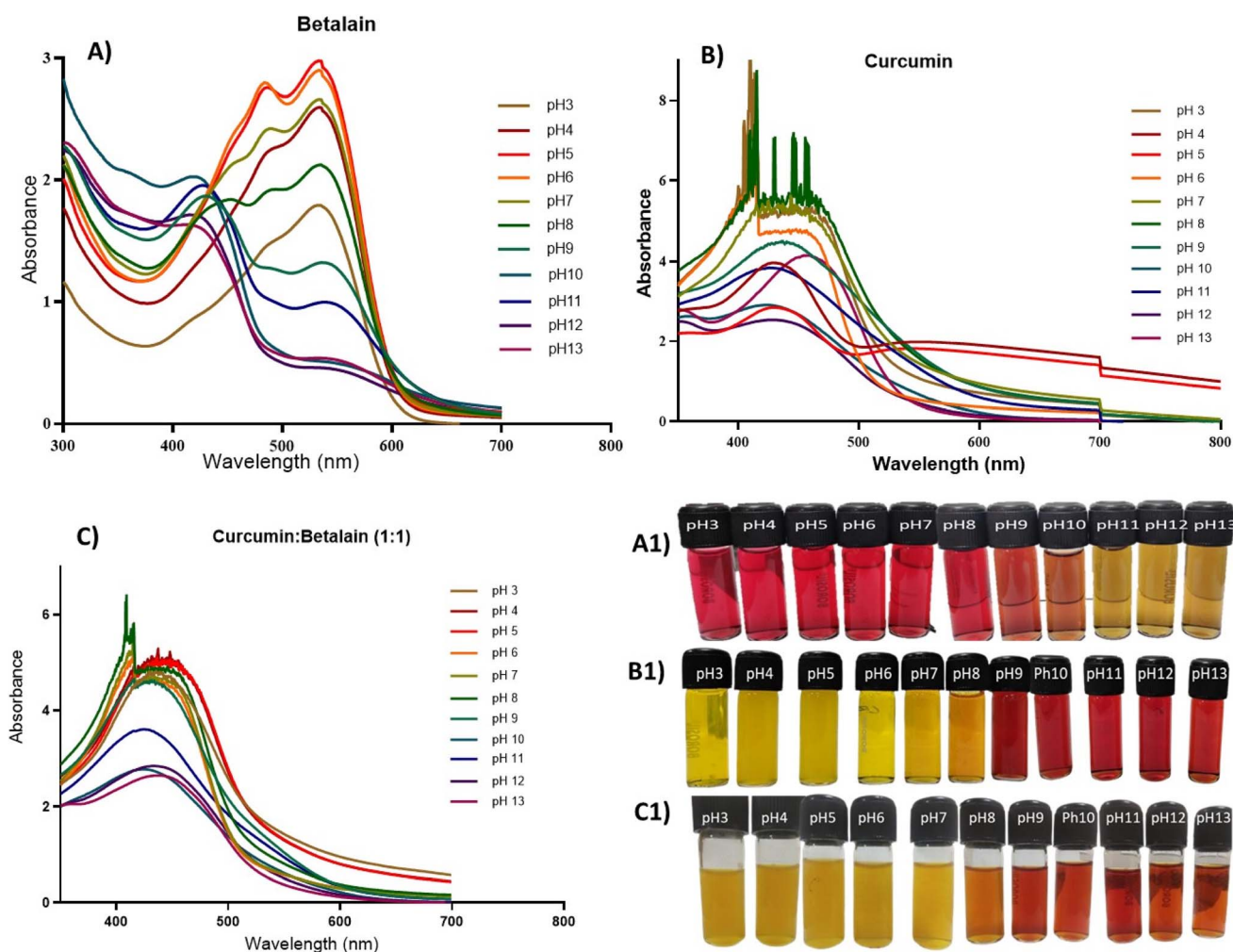


Fig. 1 UV-visible spectra and corresponding color changes of natural dye extracts at pH 3–13: (A) Beetroot peel extract (BPE); (A1) corresponding visual representation of the color change. (B) Curcumin (CR); (B1) corresponding visual representation of the color change. (C) CR:BPE mixture (1 : 1); (C1) corresponding visual representation of the color change.



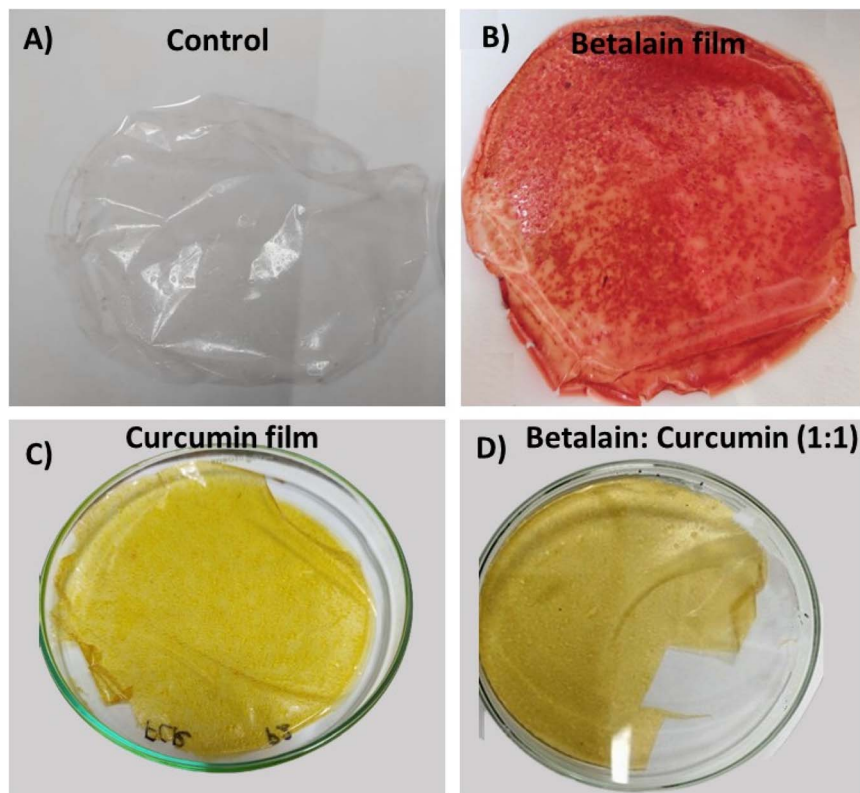


Fig. 2 Visual appearance of biopolymer films incorporated with natural colorants. (A) Control film without any pigment, showing transparency. (B) Betalain film exhibiting a deep red coloration derived from beetroot peel extract. (C) Curcumin film showing a bright yellow hue attributed to turmeric extract. (D) Composite film (betalain : curcumin, 1 : 1) displaying an orange–brown coloration, reflecting pigment blending.

curcumin content may be attributed to differences in the source, quality, or processing methods. Therefore, R1 was selected for the preparation of the colorimetric film due to its higher curcumin content.

### 3.2 Visible spectra of natural dyes at different pH

These natural dye extracts were analyzed for color variation at different pH levels to validate their use in colorimetric indicator films. The UV-vis spectra of the dye containing betalain (BPE) at pH 3–13 and the corresponding color change are shown in Fig. 1A and A1. The solution color changed from bright red to bright yellow when transitioning from an acidic to a basic medium. This change is due to structural modifications, leading to the degradation of betacyanin (red-purple) and the predominance of betaxanthin (yellow) at higher pH levels.<sup>25</sup> The dye solution appeared red at pH 3–8, with a maximum absorption peak between 527–543 nm, confirming that betalains are stable within this pH range. At higher pH, the maximum absorption wavelength shifted to 480 nm due to structural changes. Similarly, the UV-vis spectra of the dye containing curcumin (CR) at pH 3–13 and the corresponding color change are shown in Fig. 1B and B1. The solution color changed from bright yellow to bright red with increasing pH. This color variation is attributed to structural changes. The dye solution appeared yellow at pH 3–7, orange-yellow at pH 8–9, and reddish-brown at pH 10–13, as shown in Fig. 1B. The keto

form of curcumin dominates at low pH (acidic and neutral conditions), while the enol form predominates at higher pH. The maximum absorption was observed at different pH levels, with the absorption peak shifting from 429 nm to 535 nm.<sup>6</sup> In the case of the dye containing a 1 : 1 mixture of CR:BPE at pH 3–13, the UV-vis spectra and corresponding color change are shown in Fig. 1C and C1.

The CR:BPE (1 : 1) mixture displayed a broader and more nuanced color transition, from yellow to orange-red, with distinct intermediate hues. The color profiles were distinct, and the absorption spectra exhibited different hues in response to pH changes. Below pH 8, the maximum absorption peak was observed at around 437 nm. With increasing pH, structural changes caused a shift in the maximum absorption wavelength from 429 nm to 415 nm. These gradual shifts and overlapping spectral responses suggest complementary structural transformations of both dyes in the mixture. The mixture CR:BPE (1 : 1), based on its enhanced pH sensitivity, distinct visual color changes, and the broader spectral response compared to the individual dyes, was chosen.

Fig. 2A shows the developed biopolymer film (a combination of almond gum and sodium alginate), which is transparent and colorless, serving as the baseline for comparison. Fig. 2B shows the film infused with beetroot peel extract (betalain), exhibiting a deep red hue that indicates the presence of betacyanins. Fig. 2C shows the film incorporating curcumin, demonstrating



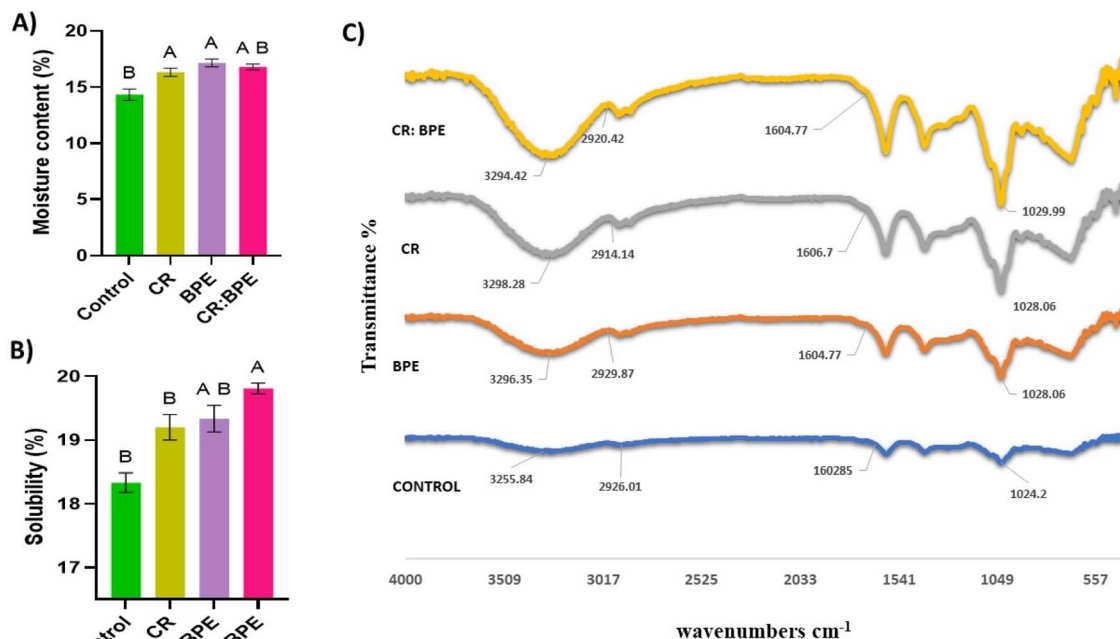


Fig. 3 Film characterization: (A) moisture content (one way ANOVA (multiple comparison test using the Turkey method)), (B) solubility (one way ANOVA (multiple comparison test using the Turkey method)), and (C) FTIR analysis.

its inherent yellow color and potential pH sensitivity. Fig. 2D shows the composite film with a yellow-orange tone, resulting from the combination of curcumin and betalain extracts. The variation in coloration reflects the nature of each pigment and its potential response to environmental changes.<sup>26–28</sup>

### 3.3 Film characterization

**3.3.1 Moisture content.** The moisture content of the colorimetric films was evaluated to determine their water-binding capacity. It was observed that moisture content increased upon the addition of natural dyes compared to the control film. A significant difference was noted between the control and dye-incorporated films. The control film had a moisture content of  $14.3 \pm 0.22\%$ , while the BPE film showed the lowest moisture content among the dyed films at  $16.3 \pm 0.20\%$ . The films containing CR and CR:BPE had slightly higher moisture contents of  $17.1 \pm 0.20\%$  and  $16.7 \pm 0.14\%$ , respectively (Fig. 3A).

**3.3.2 Solubility.** Film solubility is an important parameter for assessing the stability of the film in various media. The solubility values for the films containing BPE, CR, and CR:BPE were  $18.3 \pm 0.08\%$ ,  $19.2 \pm 0.11\%$ ,  $19.3 \pm 0.12\%$ , and  $19.8 \pm 0.04\%$ , respectively (Fig. 3B). The control film exhibited the lowest solubility at approximately  $18.3\%$ . The increase in solubility with dye incorporation is likely due to the enhanced hydrophilic nature of the films, which promotes greater interaction with water.

**3.3.3 FT-IR spectra of films.** FTIR analysis was performed to investigate the effect of natural dyes on the structural properties of the alginate-almond gum film matrix (Fig. 3C). All film samples exhibited a broad absorption band in the range of  $3255\text{--}$

$3298\text{ cm}^{-1}$ , corresponding to the stretching vibration of  $\text{--OH}$  groups from intermolecular hydrogen bonding, which originates from the biopolymer and plasticizers (glycerol and sorbitol). In the control film (alginate/almond gum without the dye), the peak appeared at  $3255\text{ cm}^{-1}$ , while in the dyed films (FD1, FD2, and FD5), this peak shifted to  $3296$ ,  $3298$ , and  $3294\text{ cm}^{-1}$ , respectively, indicating enhanced hydrogen bonding due to the incorporation of natural dyes. A peak in the range of  $1606\text{--}1602\text{ cm}^{-1}$  was associated with the stretching vibration of the  $\text{C}=\text{N}$  bond. This peak was most intense in the CR film and least in the control, suggesting the presence of aromatic compounds. A symmetric stretching vibration of the  $\text{COO}^-$  group was observed at  $1406\text{ cm}^{-1}$  in all films, regardless of dye addition.<sup>29</sup> Additionally, an absorption band near  $1024\text{ cm}^{-1}$  was attributed to the symmetric stretching of  $\text{C--O--C}$  bonds. When comparing the spectra of the control film and those with BPE, CR, and CR: BPE, it was evident that the natural dyes slightly increased the wavenumbers. Both FD1 (BPE) and FD2 (CR) exhibited similar peaks at  $1028\text{ cm}^{-1}$ , while the composite dye film (CR:BPE) showed a new peak at  $1029\text{ cm}^{-1}$ , suggesting the formation of new hydrogen bonds. Overall, the FTIR results indicate that the addition of natural dyes slightly modified the molecular structure of the films compared to the control.

### 3.4 pH responsive color change of colorimetric films

Fig. 4 shows the pH-responsive color changes and underlying structural mechanisms of the three types of dye-loaded films: BPE (Beetroot Peel Extract), CR (curcumin), and a composite CR:BPE blend (1 : 1), across a pH range from 3 to 13. In the colorimetric analysis (Fig. 4A), BPE films exhibit a transition from bright red under acidic conditions (pH 3–4) to reddish-orange near neutral



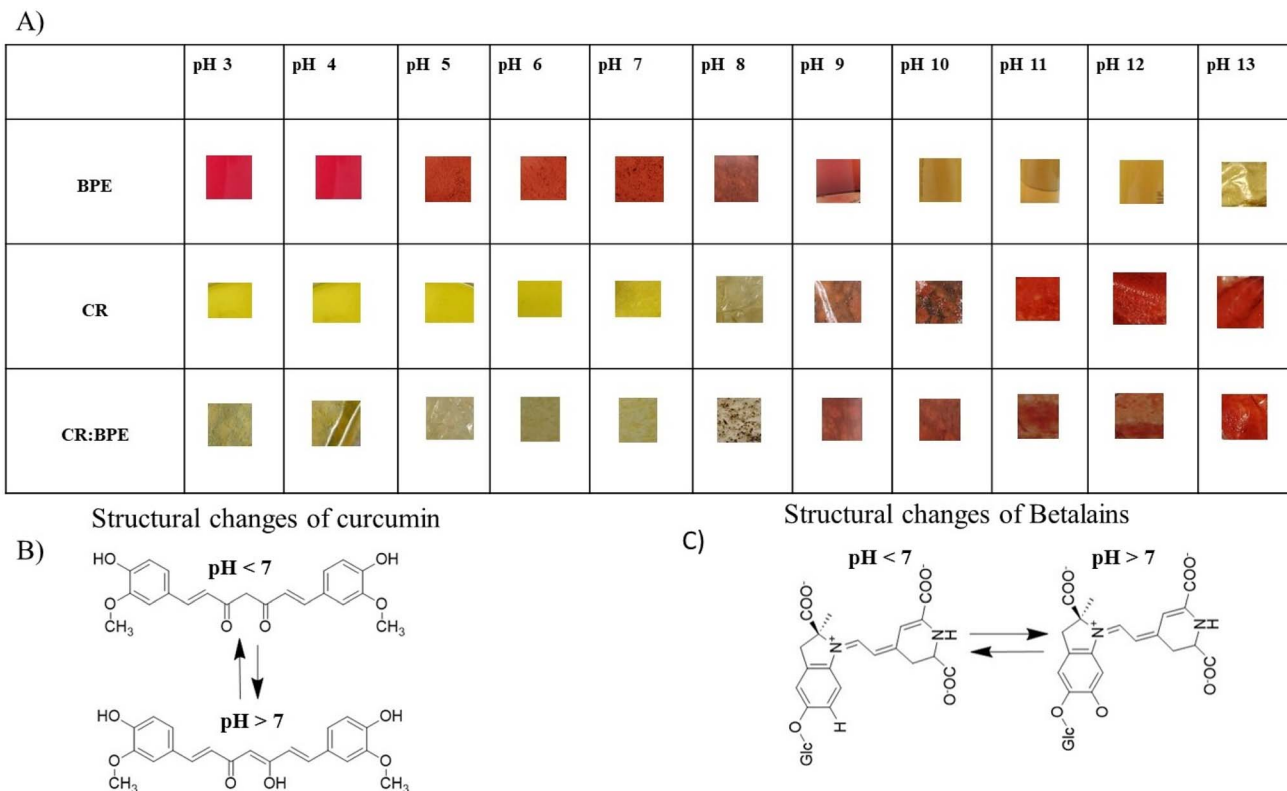


Fig. 4 (A) Color response of films to different buffer solutions. (B and C) Structural changes of curcumin and betalains.

pH (7) and shift to yellow under alkaline conditions (pH 10–13), which corresponds to the degradation of betacyanins and the increased presence of betaxanthins, as supported by UV-vis spectral data. CR films remain yellow between pH 3 and 7, reflecting curcumin's stable keto form; as the pH increases to 8–9, the films begin to brown, and at pH 10–13, they darken to reddish-brown due to keto-enol tautomerism and deprotonation of the curcumin molecule. The CR:BPE composite films show a yellowish hue at low to neutral pH, which progressively darkens to reddish-brown at higher pH, indicating an intermediate response that leverages the combined pH sensitivity of curcumin and betalains for an enhanced visual range. Fig. 4B shows curcumin existing predominantly in its keto form under acidic conditions ( $\text{pH} < 7$ ), while at alkaline pH ( $\text{pH} > 7$ ), it undergoes tautomeric conversion to the enol form, resulting in visible color changes. Fig. 4C illustrates the structural transformation of betalains: betacyanins are stable and red at acidic pH, but under basic conditions, structural rearrangements lead to their degradation and the dominance of betaxanthins, resulting in yellow coloration. Collectively, these pH-dependent structural and optical changes confirm the films' effectiveness as colorimetric pH indicators, supporting their application in intelligent food packaging systems.<sup>7,30,31</sup>

### 3.6 TVB-N content and color response of chicken over storage

The TVB-N content of the chicken during storage is shown in Fig. 5 at two different temperatures: room temperature ( $R_T$ ) and

refrigeration temperature ( $R_fT$ ). The TVB-N content was 7.15 mg/100 g on day 0, and it increased significantly during storage at room temperature compared to refrigeration. According to findings by Urmila Khulal (2017),<sup>32</sup> the baseline TVB-N content for fresh chicken is 15 mg/100 g, and values above this indicate spoilage. In the present study, samples stored at room temperature exceeded this spoilage threshold by day 2, whereas those kept under refrigeration remained below the limit until day 5. The visible color changes observed in the CR:BPE film corresponded closely with TVB-N values exceeding 15 mg/100 g. This strong correlation establishes a practical spoilage threshold, enabling the film's colorimetric response to serve as a standardized, real-time indicator of meat freshness.

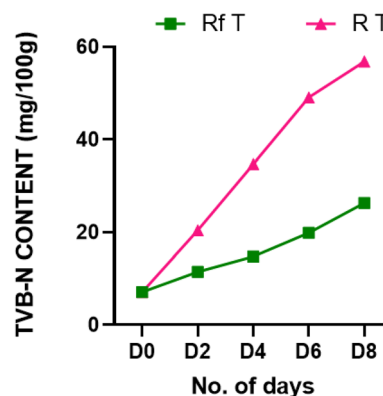
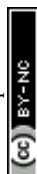


Fig. 5 TVB-N content of chicken over storage.



### 3.6 Real time monitoring of colorimetric films in chicken

Fig. 6 shows the visual progression of color changes in the three types of colorimetric films (CR, BPE, and CR:BPE) in response to the spoilage of meat samples under different storage conditions (room temperature and refrigeration) over an 8-day period. These films are designed to act as smart indicators of meat freshness by changing color in the presence of spoilage-related volatile compounds such as amines, which are produced as meat deteriorates. As shown in Fig. 6A and B (day 0), no visible color change was observed in any of the colorimetric films. This suggests that the meat samples were fresh and no significant spoilage had occurred. The CR (yellow film), BPE (red film), and CR:BPE (a blend) films all retained their original colors. As shown in Fig. 6C and D (day 2), a slight change in film color begins to appear, especially in the CR:BPE film, indicating the early stages of spoilage. This change is more pronounced at room temperature compared to refrigerated conditions. By day 8 (Fig. 6E and F), all films show clear and distinguishable color

changes. The yellow film (CR) appears to have faded or shifted, the red BPE film shows a darker shade, and the CR:BPE blend displays a mixed or intensified coloration, indicating significant spoilage. These results suggest that the developed colorimetric films, particularly the CR:BPE blend, are effective in visually indicating the progression of meat spoilage, with their sensitivity influenced by both temperature and storage duration.<sup>21,22</sup>

### 3.7 Scalability, cost-effectiveness, and regulatory aspects

The developed biopolymer films utilizing natural pigments offer clear potential for practical application in real food packaging systems. From a scalability perspective, the ingredients used (turmeric, beetroot peel, almond gum, and sodium alginate) are low-cost, biodegradable, and widely available, making large-scale production practically feasible. The film fabrication process is simple and presents a straightforward approach for industrial use. In addition, cost-effectiveness is enhanced by the use of food-grade and agricultural by-products, which reduce raw material costs and contribute to waste valorization. However, regulatory compliance will be critical, as these films come into direct or indirect contact with food products and must meet food safety standards set by relevant agencies. Hence, future work should focus on long-term stability, consumer acceptance, and alignment with existing food packaging regulations to support industrial adoption.

## 4 Conclusion

This study demonstrates the effective use of natural pigments, betacyanin and curcumin obtained from beetroot peel and turmeric, as pH-sensitive, intelligent food packaging that can track the freshness of chicken meat in real time. These extracted bioactive compounds were successfully integrated into sodium alginate and almond gum-based biodegradable films. With a strong link to total volatile basic nitrogen (TVB-N) values under both ambient and refrigerated storage, a 1:1 blend of curcumin (CR) and beetroot peel extract (BPE) at a 10% concentration demonstrated the most notable and consistent color changes to pH of all the formulations. Consistent pH responsiveness was also demonstrated by films containing only BPE or CR. After ten days of storage, the films notably retained their structural and functional integrity, demonstrating their potential for practical uses. While this study primarily focused on physicochemical indicators, incorporating microbial count data in future research will further validate and enhance the reliability of this smart packaging system. Nevertheless, the findings highlight the potential of CR, BPE, and their combination as environmentally friendly, non-destructive sensors for intelligent food packaging, contributing to improved food safety and reduced food waste through early spoilage detection.

## Author contributions

AT: data curation, methodology, validation, format analysis, writing – original draft, visualization; NS: data curation, methodology; VT: data curation, methodology; CP: validation, review

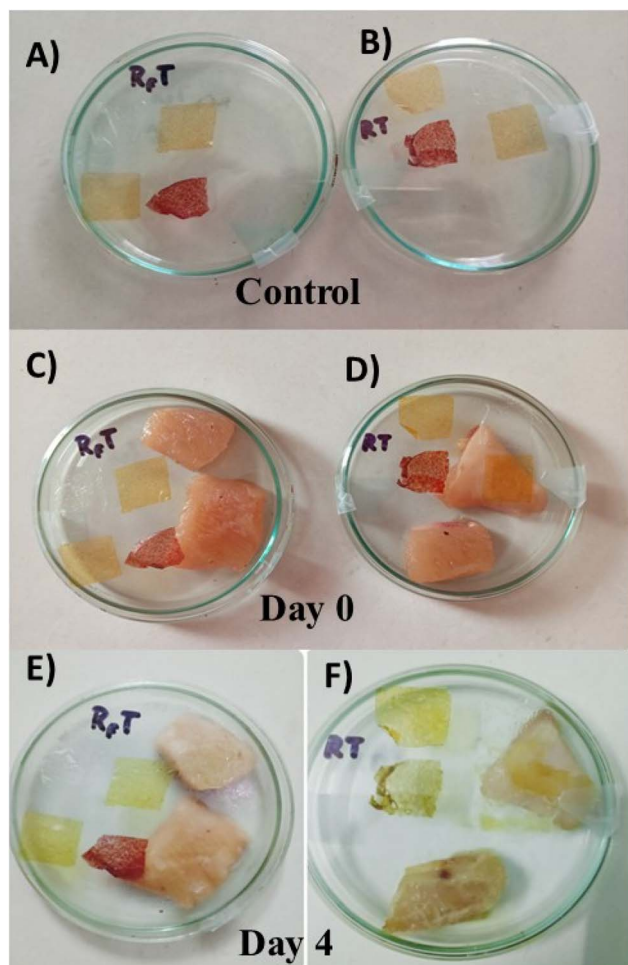


Fig. 6 Visual representation of pH-sensitive films in response to chicken spoilage over time at room temperature ( $R_T$ ): (A and B) control samples (no chicken) for curcumin and betalain films, respectively. (C and D) Films in contact with fresh chicken on day 0. (E and F) Films exposed to chicken on day 4.



and editing; SK: validation, review and editing; SR: validation, review and editing; RS: conceptualization, methodology, validation, format analysis, supervision, funding acquisition, writing – review & editing.

## Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

## Data availability

All the data generated or analyzed during this study are included in the manuscript.

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