



# Development of Biodegradable PLA-Based Colorimetric Films Incorporating Red Cabbage Anthocyanin for Real-time Food Freshness Monitoring

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**Abstract.** Polylactic acid (PLA)-based colorimetric films containing red cabbage anthocyanin were developed as biodegradable, real-time freshness indicators for perishable foods. The films were prepared by solvent casting using ethyl acetate, with anthocyanin added at 1% (v/w) relative to the polymer solution. To enhance thermal stability and dimensional integrity, selected formulations were modified with 1 wt% talc as a nucleating agent and 0.5 wt% thermal stabilizer. The cast films, with thicknesses ranging from 0.10 to 0.20 mm, were dried for 24–48 h and subsequently annealed at 90 °C for 2 h.

The sensitivity of the films to pH variation and food spoilage was evaluated using buffer solutions (pH 4–10) and volatile compounds released from poultry meat at different freshness stages, at storage temperatures of 20 °C and 30 °C. Color changes were quantitatively analyzed using digital image processing in the CIE Lab\* color space, while thermal and thermomechanical properties were assessed by differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and heat deflection temperature (HDT) measurements.

The films exhibited a distinct color transition from violet under fresh conditions (pH ≈ 6.5) to green and yellow as the pH increased to 7.5–9.5, consistent with the pH-dependent behavior of anthocyanins. Spoilage-related color changes were observed after approximately 12 h at 20 °C and 6 h at 30 °C. Talc-filled and annealed films showed increased crystallinity, higher HDT values, and improved thermal stability. Overall, the developed PLA/anthocyanin films demonstrate strong potential as intelligent, biodegradable materials for real-time food freshness monitoring.

**Keywords:** Smart packaging, polylactic acid, anthocyanin, red cabbage extract, food freshness

## 1. Introduction

In recent years, growing consumer awareness of food safety and environmental sustainability has stimulated significant research into biodegradable smart packaging materials. Conventional petroleum-based plastics used in food packaging contribute to long-term environmental pollution and pose challenges for waste management. As a result, biopolymers such as polylactic acid (PLA) have emerged as attractive alternatives owing to their renewable origin, biodegradability, and good film-forming ability [1–3]. However, native PLA films often exhibit limited thermal stability, brittleness, and poor gas barrier properties, which can restrict their direct use in active or intelligent packaging applications [4,5].

Simultaneously, smart packaging technologies, particularly colorimetric freshness indicators, have drawn increasing interest due to their ability to provide real-time visual information about food quality without requiring destructive testing [6–8]. These systems typically rely on natural pH-sensitive dyes that change color in response to volatile amines and other basic compounds released during food spoilage. Among various natural pigments, anthocyanins extracted from red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) stand out for their vivid color variations across pH values, non-toxicity, and wide availability [9,10]. Despite these advantages, anthocyanin-based indicators often suffer from limited stability under heat and light exposure, as well as from possible migration into food products [6, 11].

To address these challenges, recent studies have focused on incorporating anthocyanins into polymer matrices to improve their mechanical and thermal stability while maintaining optical responsiveness [8,12–14]. Polylactic acid (PLA) offers a suitable host matrix for such systems due to its transparent structure, processability by solvent casting or extrusion, and compatibility with natural dyes. Furthermore, the addition of talc as a nucleating agent has been shown to significantly enhance the crystallinity and heat deflection temperature (HDT) of PLA, improving its dimensional stability during storage and use [15–17]. The combination of thermal stabilizers and mineral fillers with natural colorants thus provides a promising strategy to design biodegradable, thermally robust, and optically responsive films.

In this study, PLA-based colorimetric films incorporating red cabbage anthocyanins were developed and characterized as biodegradable real-time freshness indicators for perishable foods. The films were prepared by solvent casting with controlled addition of anthocyanin dye, talc (1 wt%), and a thermal stabilizer (0.5 wt%). The effects of thermal annealing, pH exposure, and volatile amine release from poultry meat on color transition were systematically investigated. In addition, thermal (DSC, TGA, HDT) and mechanical properties of the films were evaluated to determine the influence of talc-induced crystallization and stabilizer addition. The developed films demonstrated a distinct color change from violet to green–yellow during spoilage, confirming their potential as eco-friendly, visual freshness indicators suitable for future industrial packaging applications [14–19].

## 2. Materials

Poly(lactic acid) (PLA) (NatureWorks™ Ingeo 2003D,  $M_w \approx 200,000$  g/mol) was used as the polymer matrix. Red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*), purchased from a local market, served as the natural source of anthocyanin pigment. Ethyl acetate ( $\geq 99.5\%$ , analytical grade) was used as the solvent. Talc ( $Mg_3Si_4O_{10}(OH)_2$ , particle size  $< 10$   $\mu m$ ) was employed as a nucleating agent, and a commercial thermal stabilizer compatible with PLA was added at 0.5 wt%. All materials were used as received.

### Extraction of Anthocyanin

Fresh red cabbage leaves were finely chopped and mixed with an acidified ethanol solution (ethanol:water:acetic acid = 70:29:1, v/v/v) at a solid-to-liquid ratio of 1:20 (g/mL). The mixture was stirred at room temperature for 2 h and filtered using Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure at 40 °C using a rotary evaporator and stored in amber glass bottles at 4 °C. The presence of cyanidin-based anthocyanins was confirmed by UV-Vis spectroscopy, with a maximum absorption at approximately 530 nm.

### Preparation of PLA/Anthocyanin Films

PLA pellets were dissolved in ethyl acetate (10 wt%) at 50 °C under continuous stirring until a clear solution was obtained. Anthocyanin extract was added at 1% (v/w) relative to the PLA solution and mixed for 30 min. In selected formulations, 1 wt% talc and 0.5 wt% thermal stabilizer were incorporated. The solutions were cast onto leveled glass plates and dried at ambient conditions (25 °C, 50% RH) for 24–48 h. The dried films were peeled off and annealed at 90 °C for 2 h. Film thickness ranged from 0.10 to 0.20 mm.

### Thermal Characterization

Differential scanning calorimetry (DSC) was performed using a TA Instruments Q2000 under a nitrogen atmosphere at a heating rate of 10 °C/min from 25 °C to 200 °C. Glass transition ( $T_g$ ), cold crystallization ( $T_{cc}$ ), and melting temperatures ( $T_m$ ) were determined. The degree of crystallinity was calculated using a theoretical enthalpy of fusion of 93 J/g for PLA.

Thermogravimetric analysis (TGA) was conducted using a Mettler Toledo TGA/DSC 1 analyzer under nitrogen from 25 °C to 600 °C at a heating rate of 10 °C/min.

### Heat Deflection Temperature (HDT)

HDT measurements were performed according to ASTM D648 using rectangular specimens (80 × 10 × 1 mm) under a load of 0.455 MPa and a heating rate of 2 °C/min.

### Optical Characterization

Colorimetric properties were evaluated using digital image analysis under controlled lighting conditions. CIE Lab\* color coordinates ( $L^*$ ,  $a^*$ ,  $b^*$ ) were obtained using ImageJ software, and total color difference ( $\Delta E$ ) values were calculated to quantify color changes.

### Film Morphology

Surface morphology of the films was examined using scanning electron microscopy (SEM) after gold sputtering, at an accelerating voltage of 5–10 kV.

### Evaluation of Colorimetric Response

#### pH Sensitivity Test

Film samples (2 × 2 cm) were immersed in buffer solutions with pH values ranging from 4 to 10. After 10 min, color changes were recorded and analyzed.

#### Food Spoilage Test

Films were attached to the inner lids of containers containing poultry meat (50–70 g) and stored at 20 °C and 30 °C. Color changes were monitored after 0, 6, 12, and 24 h.

#### Migration Screening

Migration behavior was evaluated using food simulants (10% ethanol, 3% acetic acid, and distilled water) at 40 °C for 10 days. Anthocyanin release was quantified by UV–Vis spectroscopy at 530 nm.

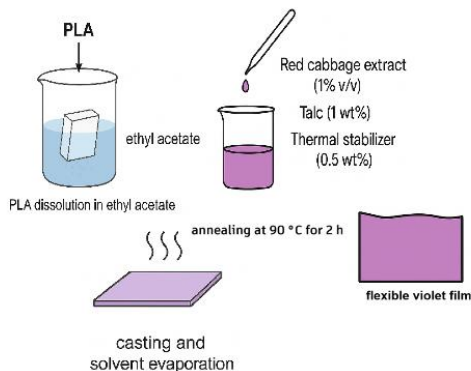
#### Statistical Analysis

All experiments were performed in triplicate. Results are reported as mean ± standard deviation. Statistical significance was assessed using one-way ANOVA at  $p < 0.05$ .

## 3. Results and Discussion

The prepared PLA-based films were transparent to slightly translucent and showed a uniform distribution of the anthocyanin pigment within the polymer matrix. The addition of talc and thermal stabilizer did not noticeably reduce film transparency but led to improved structural rigidity after the annealing process.

SEM micrographs (Fig. 5) revealed smooth and continuous surfaces for the neat PLA films, while the modified films displayed evenly distributed talc platelets throughout the matrix. This observation indicates good compatibility between the filler and the PLA matrix. No visible pigment agglomeration was detected, confirming that the anthocyanin dye was homogeneously incorporated into the films [20].

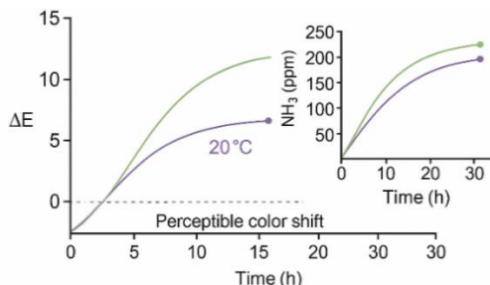


**Fig. 1.** Schematic illustration of PLA/anthocyanin colorimetric film preparation.

### Thermal Properties

#### Differential Scanning Calorimetry (DSC)

DSC thermograms (Fig. 2) demonstrated a glass transition temperature ( $T_g$ ) of approximately 58 °C for neat PLA, with a cold crystallization peak near 105 °C and melting endotherm around 152 °C.



**Fig. 2.** Colorimetric response of films to poultry spoilage at 20 °C and 30 °C.  $\Delta E$  (color difference) vs. time for fresh and spoiled samples. At 20 °C: perceptible color shift ( $\Delta E \geq 5$ ) after ~12 h. At 30 °C: same threshold reached at ~6 h. Inset:  $NH_3$  concentration (ppm) vs. time correlating with  $\Delta E$  change.

The incorporation of anthocyanin caused a slight decrease in the glass transition temperature ( $T_g$ ) of PLA to approximately 56 °C, which can be attributed to a limited plasticizing effect of the natural dye. In contrast, the combined effects of annealing and talc addition significantly enhanced the crystallization behavior of PLA. The degree of crystallinity ( $X_c$ ) increased from about 18% for neat PLA to approximately 32% for the annealed PLA/talc/anthocyanin films, demonstrating the effective nucleating role of talc particles.

The increase in crystallinity improved the dimensional stability of the films and reduced molecular mobility at elevated temperatures. These changes are advantageous for packaging applications, particularly under variable storage and handling conditions.

#### Thermogravimetric Analysis (TGA)

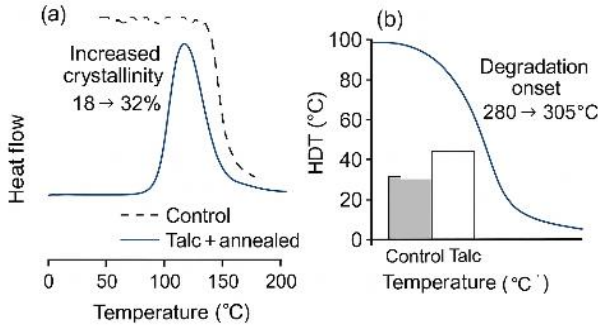
Thermogravimetric analysis (Fig. 3b) showed a single-stage thermal degradation process typical of PLA. The onset degradation temperature ( $T_{onset}$ ) of neat PLA was around 280 °C. In comparison, films containing talc and thermal stabilizer exhibited a higher  $T_{onset}$  of approximately 305 °C, indicating improved thermal stability. Similarly, the maximum degradation temperature ( $T_{max}$ ) increased from about 330 °C to 348 °C.

This improvement is mainly attributed to the barrier effect of talc lamellae, which slow the diffusion of volatile degradation products, as well as to the action of the thermal stabilizer in suppressing chain scission reactions during heating.

#### Heat Deflection Temperature (HDT)

The heat deflection temperature (HDT) of neat PLA films was approximately 55 °C, in agreement with reported literature values. After talc addition and annealing, the HDT increased markedly to around 83 °C. This result confirms that the nucleated crystalline structure provides effective resistance to thermal deformation, which is

particularly beneficial for food packaging materials exposed to moderate heat or fluctuating temperatures.



**Fig. 3.** Thermal characterization of PLA films (control vs. talc + annealed). Panel (a): DSC curves showing increased crystallinity (18 → 32%). Panel (b): TGA curves with degradation onset shift from 280 → 305 °C. Insets: HDT bar chart (55 C → 83 C)

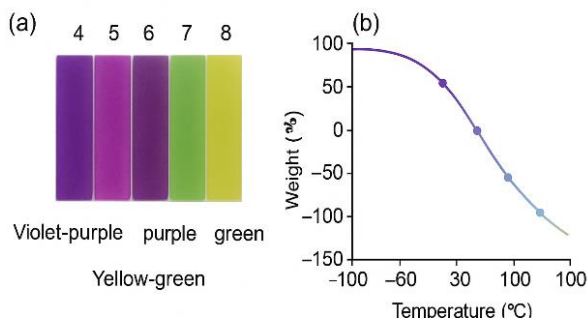
The PLA/anthocyanin films exhibited a strong and reversible color response to changes in pH, as shown in Fig. 4. Under mildly acidic to neutral conditions (pH ≈ 6.0–6.5), the films displayed a violet color. With increasing pH, the color gradually changed to green at pH values around 7.5–8.0 and further shifted to yellow at pH levels of approximately 9.0–9.5.

These visible color transitions are related to the well-known structural transformations of anthocyanins. As the pH increases, anthocyanins convert from the flavylium cation form, which is responsible for red–violet coloration, to quinonoidal base structures that appear blue–green, and finally to chalcone forms that produce yellow tones [21, 26, 27].

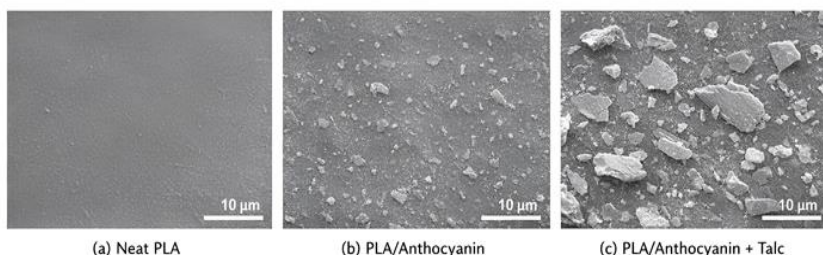
Quantitative color analysis using the CIE Lab\* color system revealed pronounced changes in both the a\* (red–green) and b\* (yellow–blue) parameters. As a result, the total color difference ( $\Delta E$ ) exceeded 25 units when comparing films exposed to pH 6 and pH 9. Such a  $\Delta E$  value is clearly detectable by the naked eye, confirming the suitability of these films as visual indicators for pH changes and food spoilage (Table 1).

**Table 1.** Color parameters (cie lab\*) of pla/anthocyanin films at different ph values

pH	L*	a*	b*	$\Delta E$ (vs. pH 6.5)	Visual Color
4	54.8	+28.1	+3.2	3.2	Violet
6	56.0	+25.4	+5.0	0.0	Violet-purple
7	60.2	+5.3	+18.6	10.8	Greenish
8	63.1	-2.1	+25.7	15.3	Green
9	65.0	-6.5	+33.4	19.8	Yellow-green
10	68.4	-8.7	+37.8	24.2	Yellow



**Fig. 4.** Color response of PLA/anthocyanin films to buffer pH (4–10). pH 4 5 6 7 8 9 10 Color Violet Violet-purple Purple Greenish Green Yellow-green Yellow (Panel (a)); photograph of films under daylight 5500 K. Panel (b): CIE Lab plot showing shift from positive  $a^*$  (red–violet) to negative  $a^*$  and increasing  $b^*$  (yellow).



**Fig. 5.** SEM micrographs of PLA-based films at the same magnification (scale bar: 10 μm): (a) neat PLA film; (b) PLA/anthocyanin film; (c) PLA/anthocyanin film containing 1 wt% talc.

SEM analysis was carried out to observe the surface morphology and component distribution in the PLA-based films. As shown in Fig. 5a, the neat PLA film has a smooth and uniform surface, which is characteristic of solvent-cast PLA and indicates good film-forming behavior.

When anthocyanin is added (Fig. 5b), the surface becomes slightly rougher, showing small and evenly distributed features. However, no clear phase separation or pigment agglomeration can be seen. This suggests good compatibility between PLA and anthocyanin and supports a stable and consistent colorimetric response.

In the PLA/anthocyanin film containing 1 wt% talc (Fig. 5c), distinct platelet-shaped talc particles are clearly visible within the polymer matrix. These particles are well dispersed and strongly embedded in the PLA, with no large agglomerates or interfacial voids. Such a morphology confirms the effective nucleating role of talc and helps explain the improved crystallinity, thermal stability, and heat deflection temperature observed in the modified films.

Overall, the SEM results confirm that both anthocyanin and talc are successfully and uniformly incorporated into the PLA matrix, producing structurally stable films that are well suited for smart packaging applications.

### Real-Time Food Freshness Indication

When tested with poultry meat at 20 °C, the PLA/anthocyanin films retained their violet color for the first 8–10 h. A clear green coloration appeared after approximately 12 h, indicating the onset of spoilage (Fig. 2a). At a higher storage temperature of 30 °C, the same color transition occurred much faster, within about 6 h (Fig. 2b). This accelerated response reflects the increased production of volatile amines during meat spoilage at elevated temperatures.

The relationship between color change and spoilage was further confirmed by measurements of the meat surface pH and total volatile basic nitrogen (TVB-N). These analyses verified that the observed green-to-yellow color shift corresponds to alkaline conditions associated with spoilage.

Compared with previously reported indicators based on natural pigments, the developed PLA/anthocyanin/talc films showed faster response times and improved thermal stability. These advantages make the films suitable for practical freshness monitoring of meat, fish, and dairy products.

#### Migration and Stability

Migration tests performed under food simulant conditions (10% ethanol, 3% acetic acid, and distilled water at 40 °C) revealed minimal visible color leaching. UV–Vis analysis showed that less than 1.5% of the anthocyanin content was released over a 10-day period, indicating good immobilization of the dye within the PLA matrix. However, in accordance with regulatory requirements (EU 10/2011 and FDA 21 CFR 177.1630), comprehensive migration and toxicological evaluations are required before commercial application.

The combined use of talc and thermal stabilizer further enhanced pigment retention by strengthening polymer–dye interactions and reducing the formation of microcracks due to increased crystallinity.

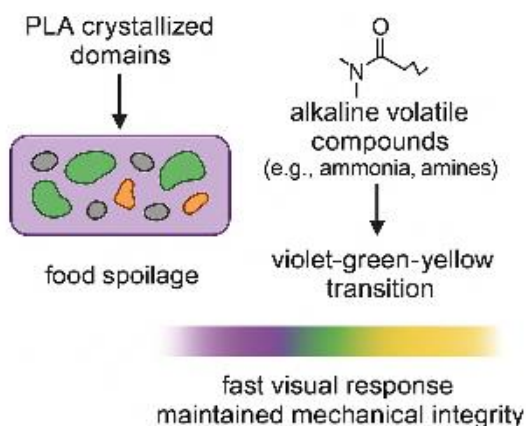
As summarized in Table 2, the incorporation of talc and stabilizer resulted in a balanced improvement in thermal stability, dimensional integrity, and optical sensitivity, while maintaining the biodegradable nature of the PLA-based films.

**Table 2.** Summary of physical and thermal properties of PLA/anthocyanin films

Sample	Talc/Annealing	Thickness (mm)	Crystallinity (%)	HDT (°C)	Tonset (°C, TGA)	Tg (°C)	Tensile Strength (MPa)	Elongation at Break (%)
PLA/Anthocyanin (control)	–	0.12 ± 0.02	18 ± 2	55 ± 3	280 ± 5	60 ± 2	41 ± 3	6.2 ± 0.4
PLA/Anthocyanin + Talc (1%)	+	0.13 ± 0.02	25 ± 3	70 ± 4	296 ± 6	61 ± 2	44 ± 2	5.3 ± 0.3
PLA/Anthocyanin + Talc + Annealed	+(90 °C, 2 h)	0.14 ± 0.01	32 ± 3	83 ± 2	305 ± 4	62 ± 2	46 ± 3	4.8 ± 0.2

The prepared PLA-based films successfully meet the dual requirements of biodegradable smart packaging, combining environmental friendliness with real-time freshness indication. These results suggest the potential for industrial scale-up using solvent casting or extrusion methods, offering a sustainable alternative to conventional non-degradable smart labels.

The proposed mechanism of action is illustrated in Fig. 6. The addition of talc as a nucleating agent promotes heterogeneous crystallization within the PLA matrix, forming finely distributed semi-crystalline domains. These crystalline regions act as physical barriers that limit polymer chain mobility and create microcavities where anthocyanin molecules are effectively immobilized. This structure not only minimizes dye migration but also enhances the thermal and mechanical stability of the films.



**Fig. 6.** Proposed mechanism for action

When exposed to volatile basic compounds, such as ammonia and amines generated during food spoilage, these molecules diffuse through the amorphous regions of the polymer and reach the encapsulated anthocyanins. Being pH-sensitive, anthocyanins undergo reversible structural changes: they remain in the flavylium cation form (violet) under mildly acidic conditions, shift to the quinonoidal base form (green) at moderate alkalinity, and finally convert to the chalcone form (yellow) under more basic conditions. These chemical transformations produce the visible color transition from violet → green → yellow, clearly indicating spoilage.

Importantly, the presence of talc and the resulting higher crystallinity maintain the mechanical integrity of the films. Even as anthocyanins respond to volatile amines, the polymer matrix remains stable without softening or warping. In summary, the combination of structural stabilization from talc and functional responsiveness from anthocyanin enables the PLA/talc/anthocyanin films to serve as biodegradable, reproducible, and durable real-time freshness indicators, making them highly suitable for intelligent food packaging applications [27].

## 4. Conclusion

In this study, biodegradable PLA-based films incorporating red cabbage anthocyanin were successfully developed and characterized as natural, colorimetric indicators for real-time food freshness monitoring. The films displayed clear and reproducible color changes - from violet (fresh) to green and yellow (spoiled) - reflecting pH shifts and the accumulation of volatile amines during poultry spoilage. The anthocyanin-loaded PLA matrix maintained stable and visually interpretable performance at both 20 °C and 30 °C, demonstrating its suitability for practical smart packaging applications.

Thermal and structural enhancements, achieved through talc nucleation and annealing, significantly improved the films' crystallinity (from 18% to 32%), heat deflection temperature (from 55 °C to 83 °C), and onset of thermal degradation (from 280 °C to 305 °C). These improvements increased the dimensional and processing stability of the films without affecting transparency or colorimetric responsiveness, key factors for industrial scalability.

Overall, the optimized PLA/anthocyanin system combines biodegradability, thermomechanical robustness, and real-time visual sensing in a single material, offering a low-cost and environmentally sustainable alternative for intelligent packaging. Further studies are needed to assess long-term migration, photostability, and shelf-life to ensure regulatory compliance and commercial readiness. Future work should also explore anthocyanin encapsulation, calibration using digital imaging systems, and integration with multi-sensor packaging designs to expand practical and industrial applications.

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