

Synthesis, Characterization and Application of Biofilm Incorporated with Anthocyanin extracted from Red Cabbage

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Abstract

The growing environmental challenges posed by petroleum-based plastic materials have driven significant interest in the advancement of biodegradable and stimulus-responsive materials for eco-friendly packaging solutions. In the present work, a pH-sensitive biodegradable biofilm was developed using a polymer matrix of chitosan (CS) and polyvinyl alcohol (PVA), enriched with anthocyanin (ATH) derived from red cabbage (*Brassica oleracea*) as a naturally sourced colorimetric sensing agent. Anthocyanin pigment was isolated through an acid-assisted ultrasonication extraction technique and uniformly incorporated into the CS/PVA blend via a solution casting process, yielding homogeneous and mechanically flexible biofilms. The physicochemical, structural, thermal, and surface morphological properties of the resulting CS/PVA/ATH biofilm were comprehensively evaluated using UV-Diffuse Reflectance Spectroscopy (UVDRS), X-ray Diffraction (XRD), Thermogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC), and Scanning Electron Microscopy (SEM). UV-DRS analysis confirmed the effective immobilization of anthocyanin within the polymer network while preserving its characteristic optical absorption and pH sensitivity. XRD results revealed a semi-crystalline structure with a dominant amorphous phase, arising from strong intermolecular hydrogen bonding among CS, PVA, and ATH molecules. Thermal analyses demonstrated enhanced thermal stability and well-defined degradation stages, indicating improved polymer-pigment interactions. SEM observations showed a dense, smooth, and crack-free surface morphology, reflecting excellent film-forming ability and homogeneous anthocyanin distribution. Complementary data from a 5-day gravimetric food packaging trial using Biowrap-style treatment on four produce varieties was also assessed. Overall, the integration of a renewable polymer blend with a naturally derived pH-sensitive pigment resulted in a stable, eco-friendly, and functional biofilm. The fabricated material demonstrates considerable promise for application in smart food packaging and continuous pH monitoring applications, presenting an eco-friendly substitute for traditional petroleum-derived synthetic indicators.

1. INTRODUCTION

The increasing environmental concerns related to non-biodegradable petroleum-based plastics have driven extensive research into creating sustainable and eco-friendly polymer materials for uses such as packaging, biomedical devices, and sensors [1]. In this regard, films made from biopolymers sourced from renewable materials have gained attention as promising alternatives due to their biodegradability, non-toxicity, and compatibility with natural functional additives [2]. Among the various biopolymers studied, chitosan (CS) and polyvinyl alcohol (PVA) have received significant interest because of their excellent ability to form films, strong mechanical properties, and chemical stability [3].

Chitosan, a natural polysaccharide derived from the deacetylation of chitin, has distinctive characteristics including biodegradability, biocompatibility, antimicrobial properties, and reactive amino and hydroxyl groups that facilitate strong intermolecular bonding [4]. However, films made solely from chitosan tend to be brittle and have limited mechanical flexibility, which limits their practical applications. To address these limitations, combining chitosan with compatible synthetic but biodegradable polymers like PVA has been extensively studied [5]. PVA is a water-dispersible, biocompatible

synthetic polymer that exhibits excellent tensile strength and resistance to thermal degradation. Its backbone, rich in hydroxyl groups, enables extensive hydrogen bonding with chitosan, which enhances miscibility, flexibility, and the overall quality of the film [6].

In recent years, there has been growing interest in the development of "smart" or "intelligent" packaging systems, especially for real-time monitoring of food quality and safety [7]. Among the different freshness indicators, changes in pH are particularly important, as they are closely linked to microbial activity and the production of acidic or basic volatile compounds during food spoilage. As a result, integrating pH-sensitive indicators into biodegradable polymer matrices has become an effective method for creating colorimetric sensing materials. Natural pigments are preferred over synthetic dyes due to their non-toxic nature, environmental friendliness, and renewable sources [8].

Anthocyanins are water-miscible flavonoid-based pigments responsible for imparting red, violet, and blue hues to a wide variety of fruits and vegetables. These compounds undergo reversible molecular transformations in response to varying pH conditions, leading to distinct and perceptible chromatic transitions across a wide pH range. Owing to this characteristic behavior,

anthocyanins have attracted considerable research interest as naturally derived pH-sensing agents for incorporation in active packaging systems, biosensing platforms, and ecological monitoring applications. Red cabbage (*Brassica oleracea*) is among the top sources of anthocyanins, featuring a wide variety of acylated derivatives that have strong light absorption and greater colour stability than many other plants [9].

Although free anthocyanins have appealing qualities, they are highly vulnerable to external factors like light, temperature, oxygen, and moisture, which can cause them to degrade quickly and lose their colour. Therefore, embedding anthocyanins within an appropriate polymer matrix is crucial to improve their stability and maintain their functional properties [10]. Various biopolymer matrices such as starch, gelatin, cellulose, and chitosan have been used for this purpose. The strong hydrogen bonding and electrostatic interactions between the hydroxyl and amino groups of these polymers and the phenolic groups of anthocyanins are important for enhancing pigment retention and ensuring a consistent colour response [11].

The CS/PVA blend system offers an ideal framework for incorporating anthocyanin due to its strong compatibility, clarity, and mechanical strength [12]. The cooperative interaction between CS and PVA results in a semi-crystalline structure that improves thermal and structural stability, which is advantageous for film fabrication and practical uses. Additionally, adding anthocyanin can further modify the blend's microstructure and physicochemical characteristics by forming extra hydrogen bonds and potentially creating complexes with the polymer chains [13].

In this study, the goal is to create a biodegradable and pH-sensitive biofilm made from chitosan and polyvinyl alcohol, which incorporates anthocyanin extracted from red cabbage as a natural indicator. Anthocyanin is extracted using an acid-assisted ultrasonic method and then embedded into the CS/PVA matrix through a solution casting process. The resulting CS/PVA/ATH biofilm is thoroughly analyzed using UV-DRS, XRD, TGA, DSC, and SEM techniques to understand its optical properties, structural arrangement, thermal stability, and surface features. This research aims to show that combining renewable polymers with naturally sourced pigments can produce a stable, environmentally friendly, and functional material with significant potential for smart packaging and pH detection uses.

2. MATERIALS AND METHODS

The following ingredients were bought from Sigma-Aldrich: Chitosan (CS, 75%-85% deacetylated), Poly vinyl alcohol (PVA), Hydrochloric acid (37%), Sodium hydroxide (NaOH) and glacial acetic acid were supplied by Merck. Red cabbages were purchased fresh from local market. Chemicals were used exactly as received and were all of analytical quality. All the chemicals used in the present were of analytical grade.

Preparation of red cabbage extract

Red cabbage (*Brassica oleracea*) purchased from the local market was used to extract anthocyanin (ATH) for this study, mainly following the solvent extraction method described by Fuleki and Francis [14]. The extraction procedure for ATH from red cabbage was slightly modified as follows. Generally, 300 mL of solvent, made up of 3 mL concentrated HCl in 150 mL deionized water, was added to 150 g of chopped red cabbage and soaked for 60 minutes in an ultrasonic bath [15]. After extraction, the anthocyanin-rich solution was separated from

the solid-liquid mixture by filtering through a nylon bag and collected into a sample bottle for further analysis.

Preparation of biofilm

Chitosan and polyvinyl alcohol were used as polymer bases, with anthocyanin (ATH) extracted from red cabbage serving as a natural pH-sensitive indicator. A 2% (w/v) chitosan solution was prepared by dissolving the required amount of chitosan in 1% (v/v) acetic acid while stirring continuously at room temperature until a clear, uniform solution was achieved. Separately, a 5% (w/v) polyvinyl alcohol solution was made by dissolving PVA in deionized water at 80-90 °C with constant stirring until fully dissolved. The chitosan and PVA solutions were then combined in a 1:1 volume ratio and stirred for 2 hours to ensure thorough mixing. Next, a measured amount of red cabbage anthocyanin extract was gradually added to the polymer mixture under continuous stirring to create a uniform CS/PVA/ATH film-forming solution. This solution was degassed to eliminate air bubbles and poured onto clean, level glass Petri dishes. The films were dried in a hot-air oven at 40 °C for 48 hours until all solvent had evaporated. Once dried, the biofilms were carefully removed and stored in a desiccator for future use [16].

Characterization

The UV-DRS spectrum of the prepared alginate beads was recorded using a Shimadzu UV 5600 plus spectrophotometer (Japan). The crystal structure of the biofilm was analyzed using an X-ray diffractometer (Rigaku Smart Lab, Japan). Thermal stability and phase transitions were assessed by TGA and DSC (TA Instruments, USA) under a nitrogen atmosphere with a heating rate of 10 °C per minute. Surface morphology was examined through FE-SEM (ZEISS SIGMA 300, Germany).

3. RESULT AND DISCUSSION

UV-DRS analysis

The UV-DRS of the prepared biofilm was measured across the wavelength range of 200-800 nm, as illustrated in Figure 1. The spectrum shows three distinct absorption peaks at 224.26 nm, 244.95 nm, and 312.12 nm, which suggest various electronic transitions related to the polymer matrix and the anthocyanin molecules embedded within it. The intense absorption peak seen at 224.26 nm is due to the $\pi \rightarrow \pi^*$ electronic transitions occurring in the conjugated C=C bonds found in the chitosan and PVA structures, as well as in the aromatic rings of anthocyanin. The absorption peak observed at 244.95 nm is primarily attributed to $n \rightarrow \pi^*$ transitions involving the C=O and amide groups present in chitosan and PVA, as well as the phenolic and heterocyclic groups found in anthocyanin. This peak suggests potential intermolecular interactions, such as hydrogen bonding, between the -OH and -NH groups of chitosan/PVA and the hydroxyl groups of anthocyanin, indicating that the natural dye has been successfully integrated into the polymer matrix. The absorption peak at 312.12 nm is typical of anthocyanin chromophores and relates to the extended delocalization of π -electrons in the flavylium ion structure. This peak's presence confirms that the biofilm maintains the light-absorbing and pH-responsive characteristics of anthocyanin. The slight broadening of this band, compared to that of free anthocyanin, suggests a strong interaction and potential complex formation between the polymer chains and the anthocyanin molecules [17].

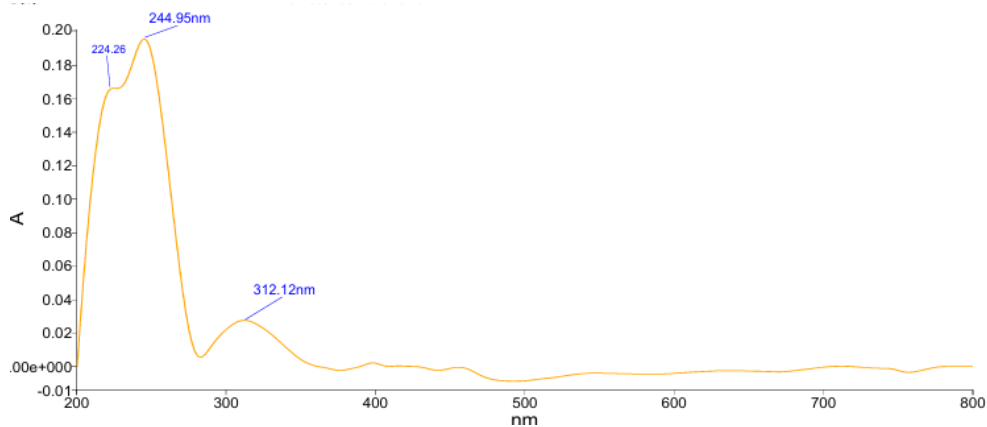


Figure 1. UV-DRS spectrum of biofilm

XRD analysis

The XRD pattern of the synthesized biofilm was measured over a 2θ range of $5-90^\circ$. As shown in Figure 2, the pattern displays a mix of broad halos along with some low-intensity diffraction peaks, suggesting that the polymeric biofilm is semi-crystalline, with the amorphous phase being predominant. A broad diffraction peak appearing in the 2θ range of $20-25^\circ$ is attributed to the amorphous regions of chitosan and PVA, resulting from the irregular arrangement of polymer chains and extensive hydrogen bonding. This indicates that the film matrix is predominantly amorphous. Overlaid on this broad peak, several weak yet sharp reflections are detected at 2θ angles of 21.08° , 25.86° , 27.35° , 28.64° , 29.94° , 33.03° , 34.71° , 37.62° , 39.36° , 41.11° , 44.21° , 46.60° , 48.34° , 50.34° , 54.41° , 56.29° , 58.87° , 62.04° , 66.56° , 71.79° , and 74.05° . The strongest reflections

appear at 2θ values of 28.64° , 29.94° , and 34.71° . These peaks are likely due to short-range ordered crystalline areas of PVA and chitosan, along with potential ordered stacking of anthocyanin molecules within the polymer matrix. The existence of these peaks suggests partial crystallization caused by intermolecular hydrogen bonding among the -OH and -NH groups of chitosan, the -OH groups of PVA, and the phenolic -OH groups of anthocyanin. Quantitative phase analysis of the diffractogram shows that the biofilm is made up of about 34% crystalline and 66% amorphous phases, indicating its semi-crystalline character. The predominance of the amorphous phase implies strong compatibility between CS and PVA, as well as an even distribution of anthocyanin, which interferes with the orderly arrangement of polymer chains and lowers the overall crystallinity [18].

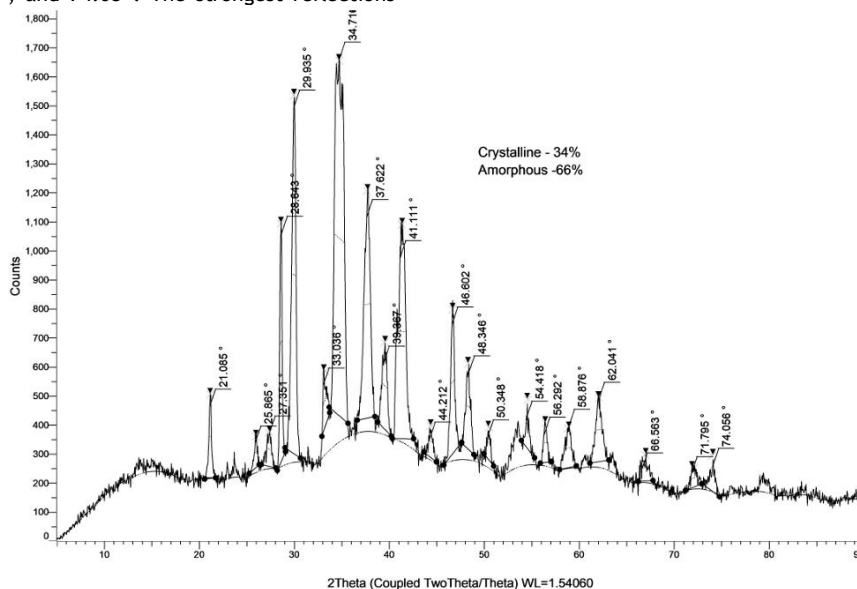


Figure 2. XRD pattern of the biofilm

TGA analysis

The thermal stability and decomposition characteristics of the prepared CS/PVA/anthocyanin biofilm were examined through thermogravimetric analysis (TGA) over a temperature range of 30 to 800°C , with a heating rate of 10°C per minute under an inert atmosphere. As shown in Figure 3, the TGA and derivative thermogravimetric (DTG) curves display a multi-stage weight loss pattern, reflecting various thermal processes including

moisture evaporation, polymer breakdown, and the ultimate carbonization of the biofilm components. The weight loss phase takes place from room temperature up to around 150°C , showing a DTG peak at 110.17°C and a mass reduction of approximately 9.30%. This initial decrease is due to the evaporation of physically adsorbed water and moisture bound within the hydrophilic chitosan and PVA matrix, along with the elimination of residual solvents and loosely attached hydroxyl

groups. The primary degradation phase occurs between 250 and 380 °C, featuring a distinct DTG peak at 343.77 °C and a notable weight loss of about 14.42%. This phase corresponds to the thermal breakdown of the polymer backbone, including the cleavage of glycosidic bonds in chitosan, the degradation of the PVA main chain, and the decomposition of anthocyanin chromophores. The significant mass loss in this temperature range reflects the breakdown of organic functional groups such as -OH, -NH, and -COOH, along with the emission of volatile substances like CO, CO₂, and small hydrocarbons. A small weight loss occurs at approximately 399.12 °C,

corresponding to about 2.21% of the mass. This phase is linked to the continued breakdown of the remaining carbon-based residues and the transformation of the polymer structure into a more stable char. Between 450 °C and 800 °C, the weight decreases slowly, indicating the creation of a thermally stable char residue. This remaining mass implies that the biofilm has good thermal stability, which is attributed to strong intermolecular forces and hydrogen bonds between chitosan, PVA, and anthocyanin molecules [19].

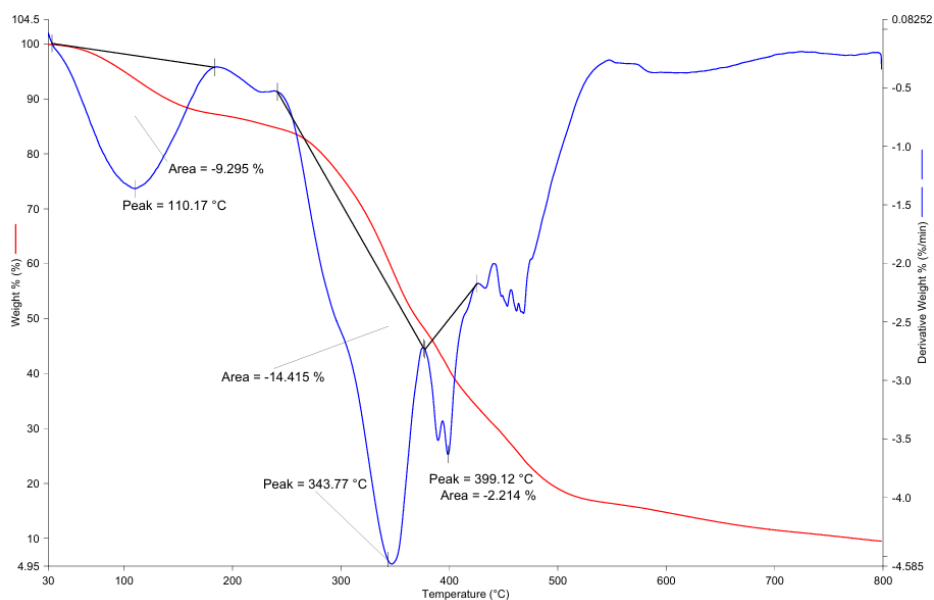


Figure 3. TGA analysis of biofilm

DSC analysis

The thermal transitions and phase behaviour of the biofilm were examined using Differential Scanning Calorimetry (DSC) within a temperature range of 30 to 450 °C, with a heating rate of 10 °C per minute. The DSC thermogram shown in Figure 4 displays several clear endothermic peaks, reflecting multiple thermal events related to moisture evaporation, polymer relaxation, and the thermal degradation of the biofilm's components. A wide endothermic peak is observed starting at 34.93 °C, reaching its peak at 102.44 °C, and ending at 165.13 °C, with an enthalpy change of 213.10 J g⁻¹. This transition is attributed to the evaporation of both physically adsorbed and bound water molecules from the hydrophilic chitosan and PVA matrix, along with the breaking of hydrogen bonds between the polymer chains and anthocyanin molecules. An endothermic event occurs between 195.10 and 219.05 °C, peaking at 211.51 °C with an enthalpy change of 21.91 J/g. This transition represents the glass transition or softening of the CS/PVA blend, along with the

relaxation of the polymer chains. The inclusion of anthocyanin can cause a shift in this transition because of strong intermolecular interactions, especially hydrogen bonding between the -OH and -NH groups of the polymers and the phenolic groups of anthocyanin. A significant endothermic peak appears between 276.90 and 356.44 °C, reaching its highest point at 324.01 °C, accompanied by a substantial enthalpy change of 149.24 J g⁻¹. This peak corresponds to the thermal decomposition of the primary polymer backbone, which involves the cleavage of glycosidic bonds in chitosan, degradation of PVA chains, and the structural breakdown of the anthocyanin chromophore. An additional endothermic transition is observed at a higher temperature, beginning at 393.53 °C, reaching a peak at 397.55 °C, and ending at 413.92 °C, with an enthalpy change of 14.06 J g⁻¹. This process represents the last phase of thermal degradation and carbonization of the remaining organic material, signifying the development of a stable char structure [20].

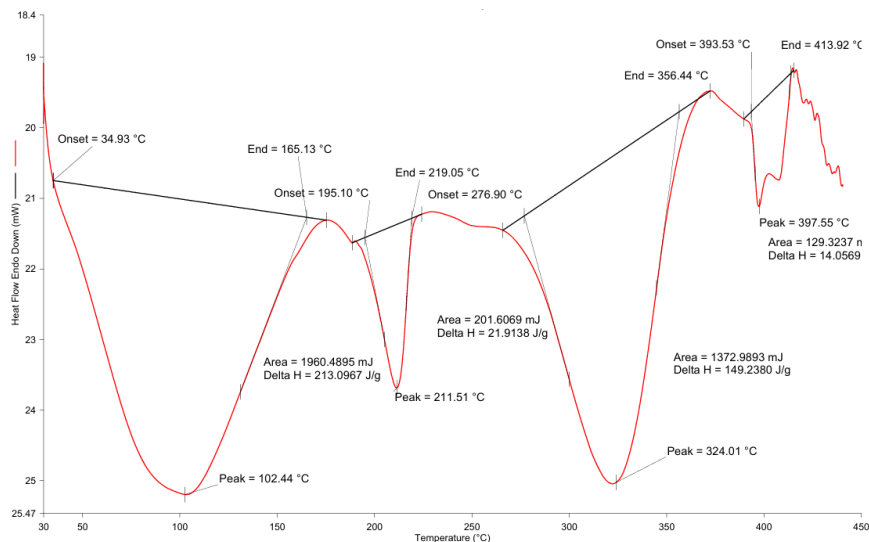


Figure 4. DSC analysis of biofilm

SEM analysis

The morphological characteristics of the fabricated biofilm were examined through Scanning Electron Microscopy (SEM) analysis. The SEM image (Figure 5) shows a continuous, dense, and uniform texture with a compact and fairly smooth surface, without any visible cracks or phase separation. This indicates excellent film-forming capability and strong interfacial compatibility between chitosan and PVA when anthocyanin is present. The consistent morphological texture confirms that anthocyanin molecules are evenly distributed throughout the polymer matrix, demonstrating their successful integration into

the blended biopolymer network. At higher magnification, the biofilm shows a slightly uneven and micro-heterogeneous texture, featuring fine granular and fibrous structures. This surface roughness is due to intermolecular hydrogen bonds and physical entanglement between CS and PVA chains, along with interactions involving anthocyanin molecules. The lack of large pores, cracks, or gaps suggests a tightly packed and well-integrated polymer structure, leading to a mechanically stable and cohesive film. This micro-rough texture increases the effective surface area, which enhances interaction with the surrounding environment, making it especially useful for pH-responsive and sensing applications [21].

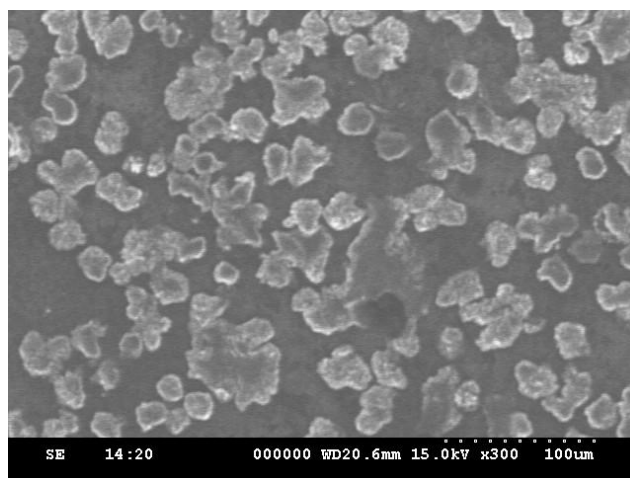


Figure 5. SEM analysis of biofilm

Technique	Key Result	Significance
UV-DRS	Peaks at 224, 245, 312 nm	Confirmed anthocyanin integration; $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions; pH-sensitive flavylum ion structure preserved
XRD	Semi-crystalline (34% crystalline / 66% amorphous)	Intermolecular H-bonding among CS, PVA, and ATH disrupts chain ordering; dominant amorphous phase
TGA	3-stage degradation; residue stable >450 °C	Main decomposition at 343.77 °C; strong thermal stability from H-bond network
DSC	Key peaks at 102, 211, 324 °C	Confirms moisture release, polymer softening, and backbone degradation; enthalpy data validates pigment-polymer interactions
SEM	Dense, smooth, crack-free surface	Uniform ATH distribution; micro-roughness enhances surface area for pH sensing; no phase separation

Table 1 Key findings from UV-DRS, XRD, TGA, DSC, and SEM characterisation of the CS/PVA/ATH biofilm.

Biowrap packaging Trial on fresh produce

A complementary experimental trial assessed the efficacy of Biowrap biodegradable packaging in reducing gravimetric moisture loss across four fresh produce varieties – avocado,

potato, kiwi, and strawberry – over a 5-day observation period. Each variety was tested under two conditions: an unwrapped control and a Biowrapped treatment group. Sample weights were recorded at baseline, Day 1, and Day 3, and visual appearance was assessed at Day 5.

Sample / Treatment	Before (g)	Day 1 (g)	Day 3 (g)	Loss % Day 3	Day 5 Appearance
Avocado – Control	1.54	1.50	1.47	4.5%	Slight shrinkage
Avocado – Biowrapped	1.70	1.61	1.54	9.4%	Firm, minimal change
Potato – Control	2.50	2.24	1.84	26.4%	Visible wilting
Potato – Biowrapped	2.15	1.94	1.08	49.8%	Significant loss
Kiwi – Control	2.84	2.54	1.08	61.9%	Shrivelled
Kiwi – Biowrapped	2.72	2.14	1.07	60.7%	Similar decline

Sample / Treatment	Before (g)	Day 1 (g)	Day 3 (g)	Loss % Day 3	Day 5 Appearance
Strawberry – Control	3.14	3.01	2.95	6.1%	Slight softening
Strawberry – Biowrapped	3.17	2.97	2.86	9.8%	Retained colour

Table2. Sample weight measurements and percentage weight loss for Biowrapped and control produce over a 5-day observation period.

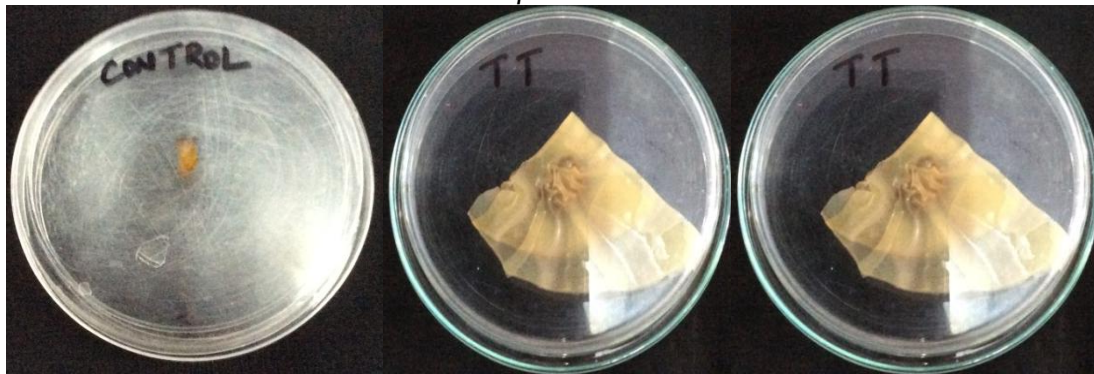


FIGURE 6A, 6B,6C - Avacado control, Day 1 and Day 3

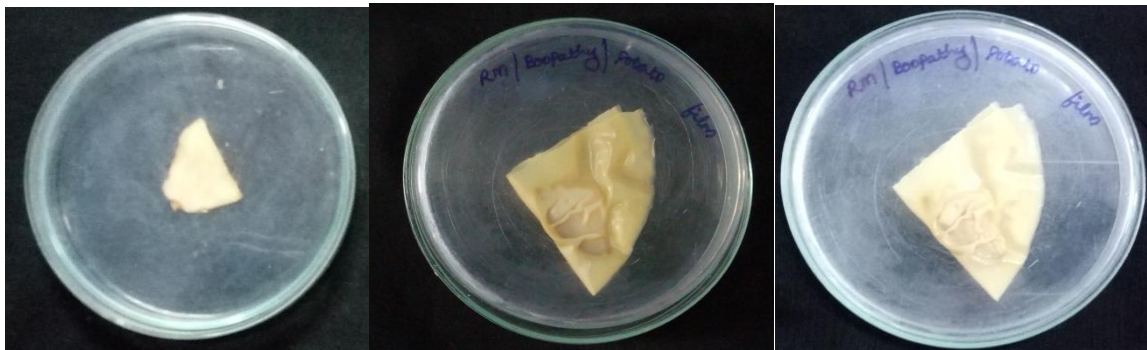


FIGURE 7A, 7B,7C - Potato control, Day 1 and Day 3



FIGURE 8A, 8B,8C - Kiwi control, Day 1 and Day 3

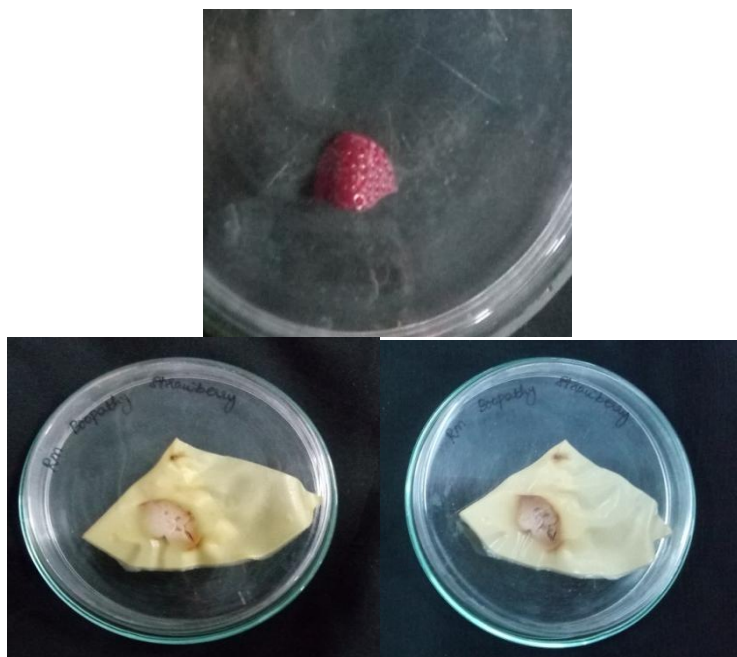


FIGURE 9A, 9B,9C - Strawberry control, Day 1 and Day 3

Avocado and strawberry demonstrated consistent moisture retention advantages in the Biowrapped group relative to controls, with the absolute weight at Day 3 remaining higher in the treated groups across both varieties. These results are consistent with the known moisture-barrier properties of polysaccharide-based coatings applied to smooth-skinned, lower-respiration fruits [22] Kiwi exhibited near-identical weight loss in both groups (control: 61.9%; Biowrapped: 60.7%), confirming that passive barrier coatings alone cannot overcome the high respiratory activity of climacteric fruits under ambient storage. The Potato data showed higher weight loss in the Biowrapped group (49.8%) compared to control (26.4%), a result confounded by unequal baseline sample weights (2.50 g vs 2.15 g respectively) and potentially attributable to metabolic acceleration under an occlusive coating. These findings reinforce the principle – well-established in the anthocyanin film literature – that packaging efficacy is highly produce- and context-specific, and that the selection and design of biodegradable coatings must account for the respiratory physiology of the target product.

4. CONCLUSION

A biofilm was effectively fabricated by incorporating red cabbage-derived extract as a naturally obtained pH-responsive colorimetric agent. UV-DRS analysis confirmed that anthocyanin was effectively incorporated and its optical properties were maintained within the polymer matrix. XRD results showed that the film has a semi-crystalline structure with predominant amorphous regions, attributed to strong intermolecular hydrogen bonding. TGA and DSC analyses demonstrated that the blended system possesses good thermal stability and distinct thermal transitions. SEM images revealed a uniform, compact, and crack-free surface, indicating excellent film-forming ability and compatibility among CS, PVA, and anthocyanin. The even distribution of anthocyanin improves the biofilm's functional performance. And furthermore, the biowrap packaging demonstrates produce-specific moisture retention benefits, with avocado and strawberry showing measurable advantages while kiwi and Potato require either supplementary preservation methods or improved experimental controls. Collectively, the results indicate that the developed biofilm is a

promising eco-friendly material suitable for smart packaging and pH-sensing applications.

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