Research Article

Development of Betalains Incorporated Starch Films as Ph Sensor Smart Packaging

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Abstract | Smart packaging technologies are rapidly evolving to meet consumer demand for improved food safety and quality monitoring. To avoid the environmental and health concerns posed by synthetic plastic, biopolymer based smart packaging films need to be synthesized. In this connection, biodegradable packaging films were developed using potato and sago starches as the polymer matrix, plasticized with glycerol and enriched with betalains extracted from *Beta vulgaris* (beetroot) to serve as natural pH-sensitive indicators. The structural interactions between starch, glycerol, and betalains were characterized using Fourier Transform Infrared Spectroscopy (FTIR), confirming successful incorporation of betalains into the film matrix. Biodegradability tests showed significant soil degradation, with Sago SB (betalain loaded) and Potato SB films achieving 40% and 42% biodegradation within one week, respectively. Water solubility and swelling analysis indicated that Sago SB films exhibited higher swelling and solubility compared to Potato SB films, while both films maintained lower moisture content, suggesting hydrogen bonding between betalains and starch. Colorimetric analysis revealed significant color differences ($p < 0.05$) in betalain-infused films, supporting their role as effective pH indicators. Films' suitability as smart labels were checked by monitoring their potential in indicating meat spoilage. Synthesized betalains loaded films showed color changes correlating with pH shifts during meat storage. These findings suggest that betalain-incorporated starch films can serve as sustainable, functional food packaging materials and can better replace synthetic smart labels.

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Introduction

Nowadays, most consumers primarily prefer to product freshness, extended shelf life and food quality. In this case, traditionally used materials like glass, aluminum foil, paper and plastic etc. can pose serious environmental hazards due to their non-biodegradability. Moreover, migration of film components to food is also possible which may lead to various food safety problems (Gupta *et al*., 2024). Therefore, the food packaging era is being shifted to the use of biopolymers such as starch, chitosan, gelatin etc. Bio polymer-based substances such as starch and cellulose have negative water vapor barrier characteristics because of their hydrophilic nature. These attributes make contributions to a reduction in the mechanical energy of biopolymer films and obstruct their long-time period stability, making them vulnerable to moisture content material. Poor film process ability, brittleness, and susceptibility to breakage are some drawbacks of biopolymer based packaging which are continuously being resolved by active packaging system (Sinha, 2024).

Active packaging refers to incorporation of particular functional components (e.g., medicinal plant extracts or polyphenols or essential oils) to films whereas smart packaging is also a kind of food packaging system which can monitor the quality of packaged food in real time and elongate the food shelf life (Kadirvel *et al*., 2025). Smart packaging is also known as a food packaging system that can monitor food quality in real-time with the help of some integrated pH indicators or pathogen detecting sensors (Said and Lee, 2025). Spoilage of packaged food products either by microbial or chemical means may lead to release of various byproducts in the system thus causing a pH change which is detected by in packaging indicator. Various synthetic and natural colors can serve as pH indicators to provide a visual signal in food quality monitoring. However, synthetic colors may prove carcinogenic or mutagenic therefore natural colors like anthocyanin, curcumin etc. are being used widely in packaging films (Liu *et al*., 2022).

Beetroot (*Beta vulgaris* L.) extract has been well known to possess tremendous biological properties (e.g., antimicrobial, antioxidant, anti-inflammatory, anti-cancer etc. In general beet root is taken as a vegetable rich in flavonoids, carotenoids, vitamins and minerals (Lazăr *et al*., 2021). Beet roots are also one of the rich source of betalains which are water-soluble nitrogenous pigments consisting on betacyanins (red-violet) and betaxanthins (yellow-orange.). Betalains are used as food colorants owing to their prominent colors at pH 3–7. Moreover, betalains show antioxidant and antimicrobial characteristics and are shown to turn yellow under alkaline conditions (Abedi-Firoozjah *et al*., 2023).

Taking above considerations, in order to provide an alternative to non-biodegradable synthetic indicator films, starch based smart food packaging films were synthesized by adding pH-sensitive betalains from beetroot as a biosensor. Starch matrices were made from potatoes and sago starch and glycerol was used as a plasticizer. These films can act as pH sensors, changing color in reaction to changes brought on by spoilage due to microorganisms. The produced films' water solubility, biodegradability, and food preservation efficacy were assessed. Through the integration of intelligent functionality and sustainability, this study offers an environmentally friendly packaging solution that may find use in food safety monitoring.

Materials and Methods

Three kilograms of beetroot were thoroughly washed under running tap water to remove surface impurities. Uniform, undamaged roots were selected, peeled, and sliced to a thickness of 1–3 mm. The slices were dried in a hot air oven at 60–70 °C for 8–10 hours, ground to a fine powder, sieved, and stored in airtight containers. Extractions were carried out at intervals of 20, 60, and 100 minutes, followed by centrifugation for 15–20 minutes. Supernatants were filtered using Whatman No. 1 filter paper (Zheng *et al*., 2022).

Starch sources and extraction methods

Sago starch was extracted from sago pith purchased locally in Hyderabad, following a traditional water-based slurry method (Zhu, 2019). The slurry was sieved to remove fibers, and the resulting starch cake was dried, ground to a fine powder, sieved, and stored in airtight bags. Tapioca starch was commercially procured from an online store (Daraz.pk) and stored in airtight packaging. Potato starch was extracted manually at home from 1 kg of locally sourced potatoes. The potatoes were washed, peeled, crushed, and soaked in water to release the starch. The mixture was strained using a colander and cheesecloth, and the collected starch was air-dried, ground, and stored in airtight bags for long-term use.

Film preparation

Biodegradable films were prepared using the solvent casting method with sago and potato starches. For each, 1.5 g of starch was dissolved in 50 ml distilled water and stirred (20–30 min depending on starch type), followed by the addition of 0.5 g glycerol and 5 minutes stirring. The solutions were heated at 70 °C for 20–40 min to achieve gelatinization. Afterwards, one drop of acetic acid and 4 ml of beetroot-derived betalain extract were added and stirred for

15 min without heating. The mixtures were cast into aluminum molds and dried at 45–50 °C for 24 hours. Dried films were peeled off and stored in polythene bags (Khan *et al*., 2024).

FTIR analysis

Films prepared from sago and potato starch with incorporated beetroot extract (betalains) were directly placed on the FTIR spectrometer. FTIR spectra were recorded over 4000–400 cm^{-1} (16 scans, 2 cm^{-1} resolution) to examine functional groups and molecular interactions.

Color analysis

Color parameters (L*, a*, b*) of the films were measured using a Minolta CM-600D spectrophotometer (Konica Minolta, Tokyo, Japan), following the method described by Otálora *et al*. The total color difference (ΔE*) was calculated relative to the control film using the formula:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Where ΔL*, Δa*, and Δb* represent the differences in lightness and chromaticity values between the sample and the control film. A ΔE* a value greater than 2.3 indicates a perceivable color difference, whereas values below 2.3 suggest no significant color change (Otálora González *et al*., 2022).

Water solubility analysis

Solubility analysis of films was carried out using a gravimetric method (Khanzada *et al*., 2023). Film samples (2×3 cm^2) were initially weighed (m_1), then oven-dried at 90 °C for 1 hour to obtain a constant dry weight (m_2). The dried samples were immersed in 20 ml of distilled water at 40 °C for 24 hours (static conditions) and reweighed after drying (m_4). Moisture content, water solubility, and swelling properties were calculated using the following equations:

$$\text{Moisture content (\%)} = (m_1 - m_2) / m_2 \times 100$$
$$\text{Water solubility (\%)} = (m_2 - m_4) / m_4 \times 100$$
$$\text{Swelling ratio (\%)} = (m_3 - m_4) / m_2 \times 100$$

Here, m_3 represents the weight of the wet films after immersion.

Biodegradability

The biodegradation of the prepared films was evaluated using soil collected from the Institute of Nutrition and Food Science, University of Sindh, Jamshoro, Pakistan. Film samples (2×2 inches) were buried at a depth of 2 inches in terracotta pots for 7 days (Khanzada *et al*., 2024). The soil was moistened regularly by spraying water twice daily. After 7 days, the films were retrieved, and weight loss was calculated using the formula:

$$\text{Weight loss (\%)} = [(\text{Initial weight} - \text{Final weight}) / \text{Initial weight}] \times 100$$

Food quality assay

Biodegradable smart films were evaluated for their effect on food quality using fresh chicken slices (Akbari and Nikoo, 2025). Pre-weighed chicken slices were placed in indirect contact with the films, while unwrapped slices served as controls. After 7 days of storage, all samples were reweighed. The variance between the initial and final weights indicated the weight loss.

Results and Discussion

Synthesis of biodegradable smart films

To confirm the purity of the extracted betalains, we conducted a spectrophotometric analysis. This analysis revealed characteristic absorption peaks that are specific to betalains, with betacyanins typically absorbing around 535 nm. In terms of quantitative analysis, we can determine the concentration of betalains by using a standard curve method that correlates the absorbance with known concentrations (Sokolova *et al*., 2024). The physical appearance of the extracted betalains, as depicted in Figure 1, shows that the method we employed was effective, as evidenced by the high yield and purity. These natural plant pigments (such as betalains) can serve as best alternatives to synthetic pigments due to their minimized environmental impact and sustainability. However, they face the challenge of stability under heat, light and pH changes which can be overcome by encapsulation techniques (Perera *et al*., 2025).

Figure 2 shows the prepared betalains incorporated sago and potato smart films. Beetroot extract contains betalains pigments that are sensitive to high temperatures, degrading rapidly under heat. This required cooling the starch solution after gelatinization before incorporating the extract to preserve its color and stability (Zin and Bánvölgyi, 2023). Additionally, the natural acidity of beetroot extract presented another challenge. Since starch is inherently basic, mixing the two directly often led to film instability and spoilage. To address this, 2-3 drops of acetic acid were added to the starch solution to create a slightly acidic environment, which stabilized the beetroot extract and improved film integrity.

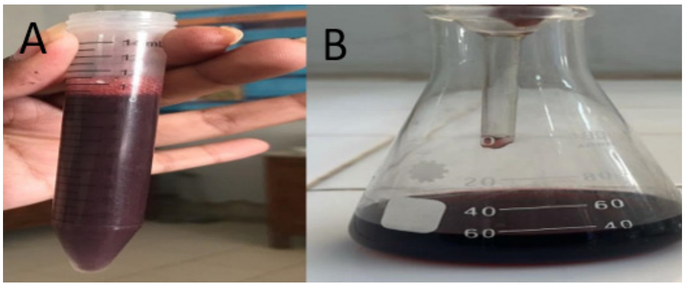

Figure 1: *(a) Solution of beetroot powder after centrifuge (b) Betalains extract.*

FTIR analysis

The FTIR spectra of the control starch films (without betalains) were compared with the spectra of the biodegradable starch films enriched with betalains pigments (of potato and sago starch) as shown in Figure 3.

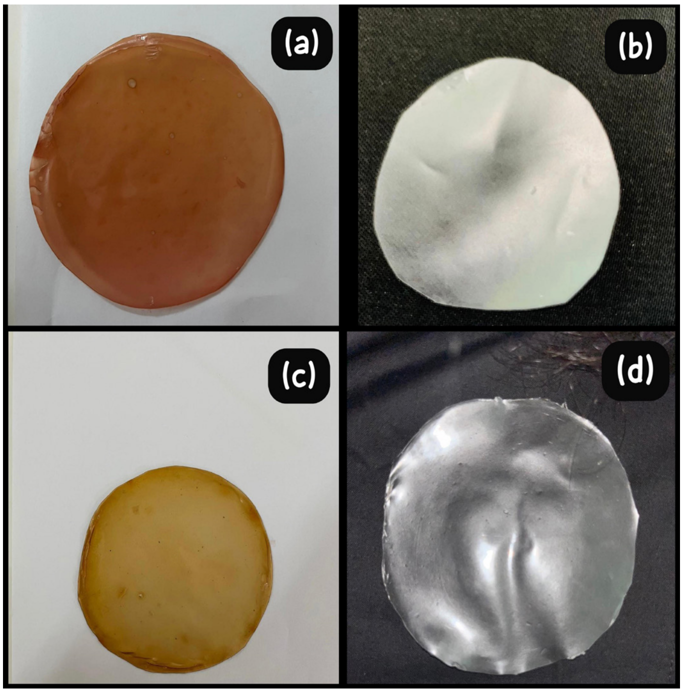

Figure 2: *(a) Potato SB, (b) Potato S, (c) Sago SB, (d) Sago S.*

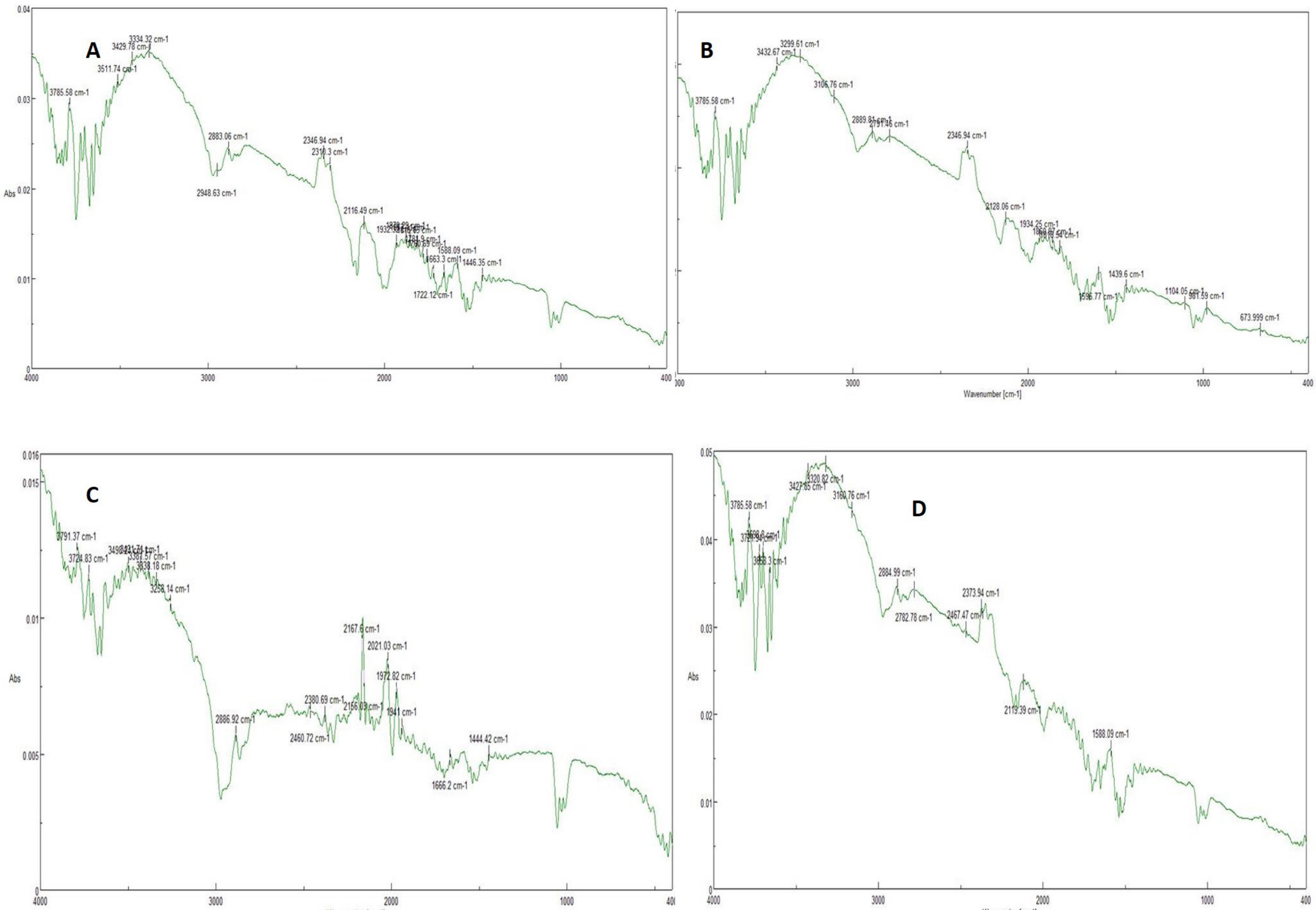

Figure 3: *FTIR spectra of (A) potato film with betalain, (B) sago starch film with betalain, and (C) and (D) control starch for both potato and sago starch film.*

Figure 3C and D show control films made from potato and sago starch, displaying characteristic starch peaks like C-H and C=O stretching vibrations. However, they lack significant betalains peaks, such as 673.999 cm^{-1}, indicating betalains are absent. The analysis of Potato Starch-loaded Betalain film (Figure 3A) shows the characteristic FTIR peaks shifting such as the O-H stretch at 3498.2. cm^{-1} in potato starch blank film was seen to be shifted to 3334.32 in case of potato starch betalains loaded film. The starch's alkyl groups and maybe plasticizers are responsible for the C-H stretching vibrations which were shifted to 2833.06 cm^{-1} and 2888.91 cm^{-1}in potato and sago betalains loaded films (Figure 3A, B). This indicates specific molecular interactions between starch, glycerol, and betalains (Abdolmaleki *et al*., 2024).

In case of potato film, the C-O stretch at 1722.12 cm^{-1} may come from carbonyl groups in betalains. In case of sago betalains loaded film, a peak at 673.999 cm^{-1} confirm the presence of betalain pigments. The more pronounced C-O stretch at 1104.05 cm^{-1} suggests differing bonding with sago starch. Therefore, Fourier transform infrared analysis revealed that betacyanins in the beet root extract might have interacted with sago starch, potato starch and glycerol through hydrogen bonds. This suggests successful integration of betalains into the starch matrix, potentially altering the films' physical properties (Akter *et al*., 2024; Qin *et al*., 2020).

Biodegradability

The biodegradation of these films in soil was examined and documented, with pictures shown in (Figure 4) illustrating the film's appearance before and after degradation. The biodegradability results of the film samples are presented in Figure 5, which indicates that the sago SB samples have a high biodegradability rate of 42.85% after one week. This characteristic of biodegradability may be attributed to the incorporation of natural pigments, such as betalains, into the biopolymer films, which influences and increases the rate at which these biodegradable films break down into environmentally friendly products in soil environments (Ingale *et al*., 2025).

Color parameters

The color of composite films significantly affects the appearance of food, influenced by parameters L, a, and b. The addition of Betalain alters these color coordinates and the total color difference (ΔE). The color analysis (Table 1) reveals that Sago S and Potato S films are lighter and less saturated, while Sago SB and Potato SB films are darker with more intense red and yellow hues. Notable ΔE values (21.05 for Sago SB and 19.83 for Potato SB) indicate substantial color differences, suggesting that changes in processing or formulation lead to distinct visual properties with implications for their application (Che Hamzah *et al*., 2022; Sathianathan *et al*., 2025).

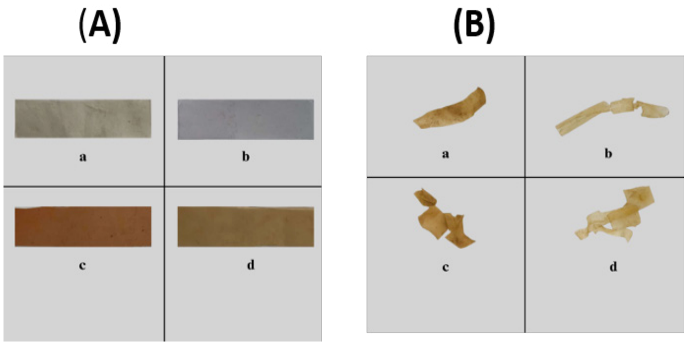

Figure 4: *Visual appearance of the films (A) before and (B) after incubation in soil.*

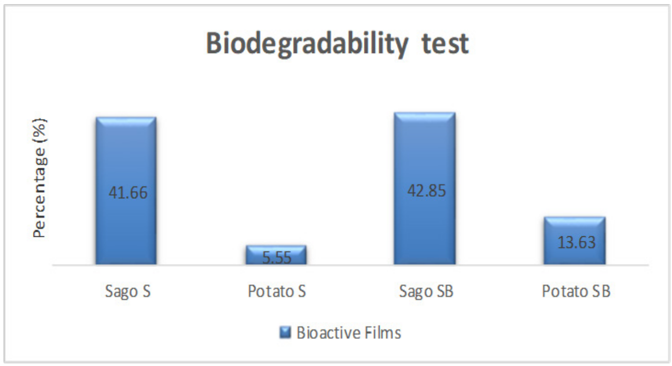

Figure 5: *Weight loss of films exposed to the soil environment.*

Table 1: *Color parameters of prepared films.*

Films	L	a	b	ΔE
Sago S	73.41±0.34	12.5±0.6	5.28±0.21	-
Sago SB	57.23±1.11	18.35±0.03	17.41±0.5	21.05
Potato S	63.68±1.75	4.08±0.36	6.12±0.14	
Potato SB	51.18±3.23	16.65±1.5	15.02±1.05	19.83

**Different letters (a-d) in the same column indicate significant differences ($p < 0.05$). The Sago S and Potato S film is used as the standard background.*

Water solubility, swelling and moisture content of films

Figure 6 depicts the swelling, water solubility and moisture properties of prepared films. The comparative analysis of Sago Betalain and Potato Betalain films demonstrates distinct differences in their physical properties. These findings support previous research regarding Sago-based films' higher solubility (Halimatul *et al*., 2019). However, Potato Betalain films demonstrate higher swelling capacity and moisture content compared to Sago Betalain films. This characteristic can be attributed to the microstructural properties of the composite films that enable solvent retention (Kong *et al*., 2023).

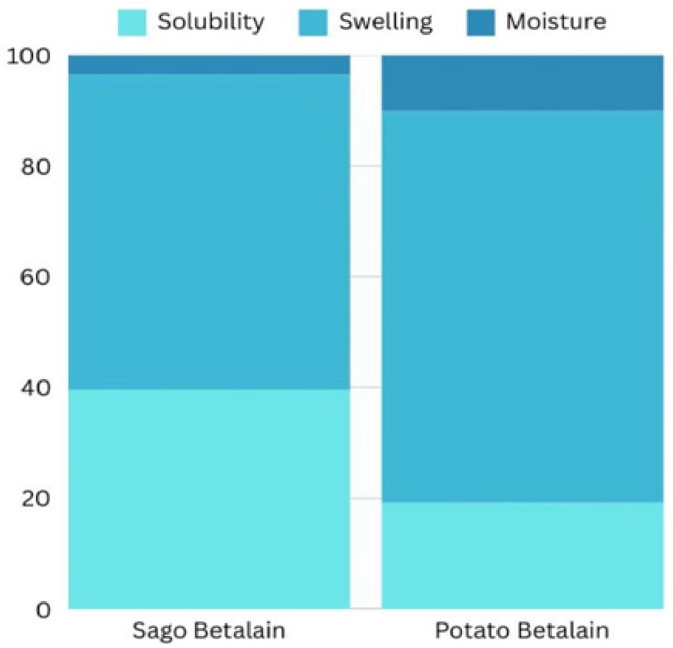

Figure 6: *Water solubility, swelling and moisture analysis.*

Food quality assay

Figure 7 displays the visual changes that occurred after cut chicken piece were indirectly exposed to biodegradable smart film for seven days, as opposed to keeping the chicken piece in the control. After 7 days of storage, the control chicken piece was dried completely, a biodegradable smart film sample showed a greater amount of moisture retaining property than that of unwrapped film. Also no fungal or bacterial growth was built up on the chicken piece. Synthesized smart films showed less weight loss of chicken as compared to control. In addition, the chicken piece wrapped in indirect contact in biodegradable smart film usually had a higher texture, color, and odor than those placed in control. As a result, the film protects food from external factors while residing safe and non-toxic (Akbari and Nikoo, 2025).

Conclusion

This research highlights the exciting possibilities presented by the innovative biodegradable starch composite films. Betalains extracted from vibrant beetroot (*Beta vulgaris*) were utilized as a functional additive in sago starch and potato starch films. Sago betalains loaded films showed the highest levels of biodegradability, i.e. 41.66% after one week. Moreover, Sago starch betalains loaded films exhibited the highest water solubility and were seen to manage moisture under controlled drying conditions. Importantly, the betalains films served as PH smart labels in chicken preservation and detected meat spoilage by a change in color due to PH change. However, a correlation of this color change with total viable count is yet to be explored in our future studies. These advanced smart labels not only serve as effective sensors to ensure the safety of our food but also play a crucial role in safeguarding our environment.

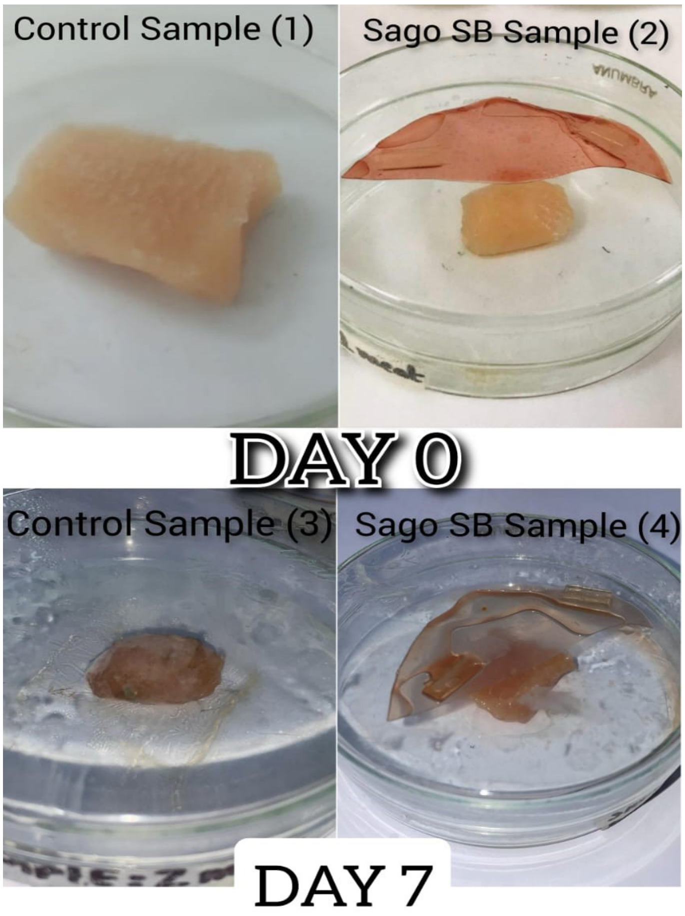

Figure 7: *The change in appearance of Chicken pieces after being in indirect contact with Betalains films.*

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Novelty Statement

Sago starch used in this study has not been used for incorporating beet root betalains in the production of smart packaging films which proves novelty of this project.

Author's Contribution

Beenish Khanzada: Supervision, writing, review and editing.
Marium Shaikh: Methodology, FTIR analysis, validation.
Zunaira shahid: Investigation, methodology
Mariam Aziz: Conceptualization, project administration.
Misbah Kanwal: Validation, visualization.
Mariam Aziz: Data curation.
Noman Cheema: Software, resources.

Data availability
Data requested will be provided as per request.

Funding statement
This study received no funding.

Generative AI and AI-assisted technology statement
This study did not use Generative AI or any AI assisted technology.

Conflict of interest
The authors have declared no conflict of interest.

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