Development of Starch-based Packaging with Corn Starch and Banana Flour (*Musa paradisiaca*), incorporating Butterfly Pea Flower (*Clitoria ternatea L.*) Extract as pH-sensitive Smart Packaging

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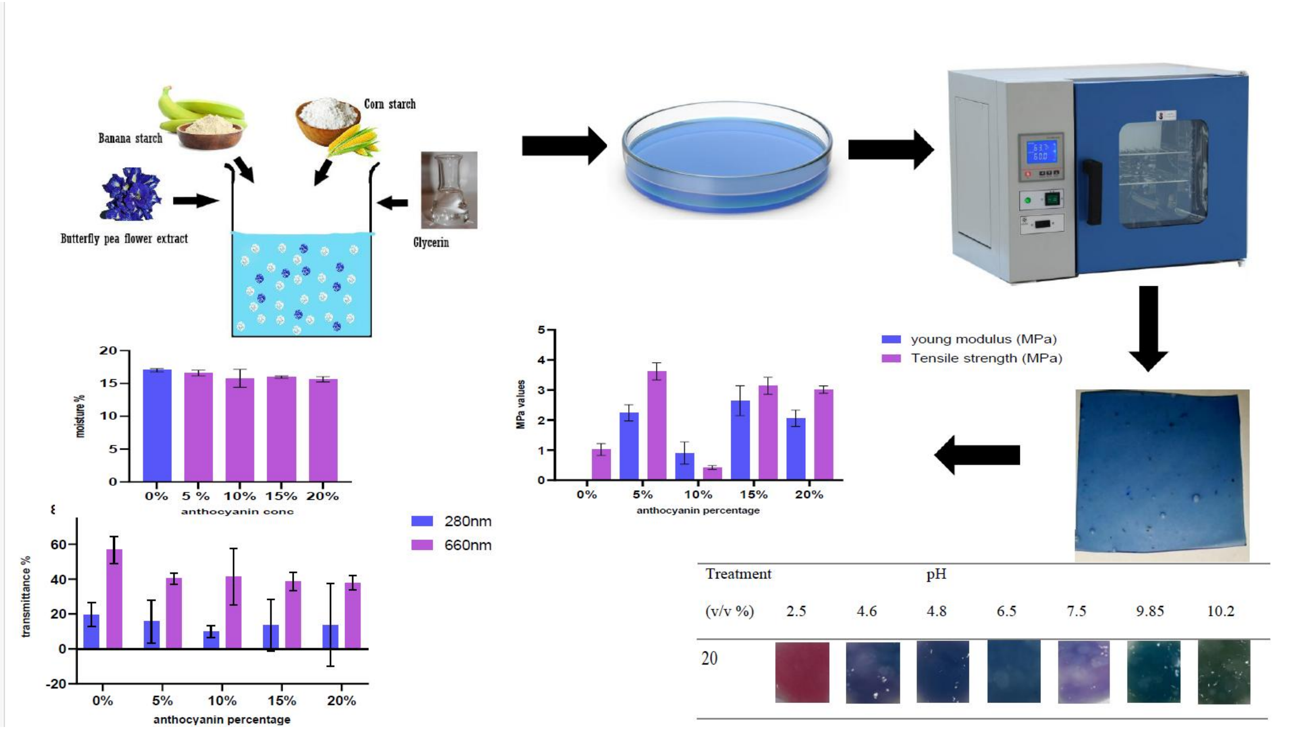

Abstract

The accumulation of petrochemical packaging materials has led to environmental pollution. It is vital to address this issue by meeting the high demand for bio-based food packaging materials in the global market, thereby promoting sustainability within the food system. Starch has become an ideal raw material for the manufacturing of biodegradable packaging. The main objective of the study was to develop a pH sensitive intelligent packaging material with banana flour, corn starch, glycerin (10:2.5:2) in to different ratios of 0%, 5%, 10%, 15%, and 20% (v/v) Butterfly pea flower extract and observe any detectable hue alteration in the pH range of one to twelve**.** According to the colorimeter readings, 0% (v/v), 5% (v/v), and 10% (v/v) showed red and yellow color tone, while 15% (v/v) and 20% (v/v) showed green color and blue color tone with respective a* and b* values. Moisture percentage, density, light transmittance, and water resistance decreased while thickness, light absorbance, and water vapour permeability increased with increasing Butterfly pea flower extract (BPFE) ratio. Films with 15% and 20% BPFE ratio showed visually perceptible color changes with pH range of one to twelve inclusive, and 20% (v/v) film showed high potential to be used as intelligent packaging.

Keywords: *Intelligent packaging, pH sensitive, Anthocyanin, Butterfly pea flower, Banana flour*

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1. Introduction

In recent decades, the accumulation of petrochemical materials has become a significant environmental issue. The modern food industry is striving for sustainability, which primarily focuses on conserving resources for long-term use without negatively impacting the environment. Biodegradable packaging made from renewable sources has gained popularity as a replacement for conventional polymers, because they naturally decompose without affecting the environment (Avella et al., 2005). Natural sources like carbohydrates, lipids, and proteins are used to develop biodegradable packaging materials (Romruen et al., 2022), and the starch films have become abundant as an alternative for conventional packaging due to the low cost, film-forming ability, and renewability.

Banana is rich in starch content and is classified into the family *Musaceae,* and genus Musa with three common species: *Musa acuminata, Musa balbisiana, and Musa paradisiaca*. *Musa paradisiaca* is highly valued for its high starch content (Maseko et al., 2024). Banana flour is packed with starch granules that effectively alter the texture and consistency of food.

In the present time frame, there has been brisk progress in the awareness of designing intelligent packaging, which enhances food security through real-time observation and communication regarding the quality, freshness, safety, and environment inside the package (Alizadeh Sani et al., 2024; Kuswandi and Jumina, 2020). Researchers are now focusing their attention towards formulating anthocyanin-incorporated material as pH-sensitive packaging, as they are nontoxic and safe. This formulation process is called the sol-gel method, where pigment particles are immobilized vertically within the starch layer during the evaporation and drying process (Yong et al., 2019). Anthocyanin is a phenolic compound that can be dissolved in water.

Delphinidin is responsible for the dark blue color in Butterfly pea flower (*Clitoria ternatea L.*), which undergoes configuration changes with varying pH conditions indicated by color variations (Mehmood et al., 2019; Lakshan et al., 2019). The butterfly pea flower is packed with health-boosting natural antioxidants like phenolic compounds, flavonoids, and anthocyanins in the extract (Vidana Gamage and Choo, 2023; Singh et al., 2022). Consequently, it boasts multiple health benefits, encompassing antioxidants (Gupta et al. 2010), antidiabetic (Daisy and Rajathi, 2009; Chu et al., 2017), anti-inflammatory (Morris, 2023), and anticancer properties.

The primary goal of this study is to formulate a pH sensitive packaging film utilizing corn starch, banana flour and water extract of Butterfly pea flowers. This could become a great solution to reduce petrochemical waste accumulation and enhance food safety in Sri Lanka, while increasing the industrial utilization of banana flour.

2. Materials and methods

2.1. Materials

Bananas of the initial ripening stage (green mature) and Butterfly pea flowers were collected from farms in the North Central Province of Sri Lanka to create banana flour and Butterfly pea flower extract. Corn flour, Glycerin USP 99.5%, Distilled water, buffer solutions (pH: 1-12).

2.2. Film preparation

2.2.1 Butterfly pea flower extract preparation

The conventional method of Butterfly pea flower extraction described by researchers (Thuy et al. 2021) was used to produce Butterfly Pea Flower Extract (BPFE). Cleaned Butterfly pea flower petals were diced into small segments and submerged in distilled water in a 1:5 proportion and heated at 80°C for 15 minutes. The solution was filtered and centrifuged (centrifuge-HERMLE Z 306) at 4000 rpm for 10 minutes. The clear violet colored suspension was obtained and filled into an amber bottle and kept in a freezer for future use.

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2.2.2 Development of banana flour

Banana flour was prepared as described in Salazar et al. (2021). Bananas were peeled, sliced, and blanched at 80 °C for five minutes. The sample was oven-dried (Faithful 101- 3AB) at 60 °C for about 6 hours. They were then pulverized using a hammer mill until a fine powder was obtained. The powder was passed through a 40-mesh size, standard sieve to obtain a uniform consistency and stored in an air-sealed plastic container. Moisture (Moisture meter- KERN, DBS60-3) percentage was 8.77± 0.16 %, and banana flour yield was 18.4%.

2.2.3 Development of the film

To prepare the banana flour solution, banana flour was dissolved in distilled water 1:10 ratio (Ramirez Brewer et al., 2024). The solution was then filtered through a muslin cloth. Corn flour 25% (w/w) and 2% (v/v) of glycerin were incorporated with the filtrate.

To build the films with different Buterfly pea flower Extract (BPFE) concentrations 5, 10, 15, and 20% (v/v) of the BPFE was incorporated to the film mixture. A control sample was prepared with out adding BPFE.

The solutions were heated to 85°C and poured onto porcelain plates (13cm diameter) and kept for ten minutes to reach room temperature before oven-dried (Faithful 101- 3AB) at 60 °C for 8 hours (Salazar et al. 2021).

2.3 Physicochemical characterization of the film

2.3.1 Film forming ability and visual transparency

The method was followed as previously described (Salazar et al., 2021). The film forming ability and visual transparency were determined using the parameters noted in Table 1.

Table 1: Properties of the film-forming ability and visual transparency of the packaging material

Film-forming ability	Detachment from Petri plates	Manageability and texture	Visual transparency
No ability (-)	Does not peel off (-)	Brittle and discontinuous (-)	Very Transparent (+++)
Moderate (+)	Does not peel off easily (+)	Flexible, manageable, and Rough surface (+)	Transparent (++)
Good (++)	Peel off easily (++)	Flexible, manageable, and Smooth surface (++)	Translucent (+)
			Opaque (-)

2.3.2 Color properties

The chromatic properties were estimated using a handheld colorimeter (Lovibond, Tintometer Ltd, UK) against a white background. Readings were illustrated using the CIELAB color scale, highlighting the L*, a*, and b* values. Each sample underwent at least 15 measurements (Salazar et al., 2021). The color variance between each formula was estimated using the formula by Luchase et al. (Luchase et al., 2018).

$$\Delta E_a = \sqrt{(\Delta L *)^2 + (\Delta a *)^2 + (\Delta b *)^2} \quad (1)$$

$\Delta L^* = L^*_x - L^*_y$, $\Delta a^* = a^*_x - a^*_y$, $\Delta b^* = b^*_x - b^*_y$

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

$\Delta L^* = L^*_C - L^*_S$, $\Delta a^* = a^*_C - a^*_S$, $\Delta b^* = b^*_C - b^*_S$

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2.3.3 Moisture

Pre-weighted packaging samples (4cm × 4cm) were oven-dried at 105 °C for 24 hours and subsequently placed in a desiccator until they cooled to room temperature. Moisture values were analyzed in triplicate, using the Equation. 3 (Salazar et al., 2021)

$$Moisture\ (\%) = \frac{initial\ weight\ of\ the\ smaple - (final\ weight\ of\ the\ sample - cricible\ weight)}{initial\ weight\ of\ the\ sample} \times 100 \quad (3)$$

2.3.4 Thickness and Density

Using a digital micrometer, sample thickness (4 cm × 4 cm) was recorded in 15 average measurements, and 10 measurements of film weight with an analytical balance (IG03458, precision 0.0001g) were measured (Salasar et al., 2021). The density was measured by using Eq. 4.

$$Density = \frac{weight\ of\ the\ film(g)}{volume\ (v)} \quad (4)$$

2.3.5 Light barrier properties

UV-Vis spectrometer (UV-1900i / A12535780396) at 280 nm and 660 nm wavelengths was used to measure the UV absorption capacity and transparency. Sample strips of 1cm x 4 cm were placed vertically in the cell to measure the light absorption, and an empty cell was used as a blank. (Salazar et al., 2021).

$$Absorbance = 2 - log_{10}\,(Transmittance)\% \quad (5)$$

2.3.6 Water Resistance (WR)

Distilled water (2.5 ml) was added onto the surface of the packaging film with an area of 16 cm^2, and the quantity of water that passed through the film was recorded for 25 hours. All determinations were performed three times per sample (Arancibia et al., 2014).

2.3.7 Water Vapour Permeability (WVP)

The gravimetric method was executed to evaluate the WVP as stated by Sorbal et al. (2001). Oven-dried desiccant (calcium chloride, 5g) at 105 °C for 24 hours was placed in a 2.6 cm diameter cell. Film cuts were affixed on the cell openings and placed in a desiccator with a 100% relative humidity level at 25 °C. Readings were taken of each cell every six hours for three days. Determinations were made in triplicate, and readings were used to calculate WVP using Equation 6.

$$WVP = \frac{W}{tA}\frac{X}{\Delta P} \quad (6)$$

W/t- linear regression from the point of weight gain and time in the constant rate period

A – Film area (cm^2)

x - Average film thickness

ΔP – partial vapor pressure difference between the atmosphere with desiccant and pure water

2.3.8 Mechanical properties

Mechanical properties were analysed according to the method stated by Liu et al. (2018). A micrometer (micrometer, YMT, China, with 0.001cm accuracy) was used to measure the thickness of film cuts (2 cm × 7 cm). ASTM standard (D882-12) method was applied to determine the Young's modulus, elongation, and Tensile strength (TS) (Eq. 8). Samples testing was carried out in triplicate using a texture analyser (traction jaw at an initial distance of 43 mm, speed of 2 mms^{-1}, and firing load of 100kgf).

$$Tensile\ Strength = \frac{force\ (N)}{width \times mean\ thickness} \quad (7)$$

2.3.9 Color response with different pH

A buffer solution series of pH 2.5, 4.6, 4.8, 6.5, 7.5, 9.85, and 10.2 was prepared and poured into Petri dishes. Samples with BPFAE of 0%, 5%, 10%, 15%, and 20% were cut into squares and dipped

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in each buffer solution separately. Samples were kept for five to ten minutes, and color variations were observed.

2.3.10 Food applications of the edible intelligent film

Susages were firmly wrapped with 0%, 5%, 10%,15%, and 20% (v/v%) packaging samples and stored in different temperature conditions (-18 ºC frozen conditions, 4 ºC refrigerated conditions, and room temperature (27 °C) to observe color deviations.

2.3.11 Statistical analysis

One-way ANOVA with Tukey’s test in GraphPad Prism 9.5.0 (730) for multiple group comparisons at a 95% certainty level; sensory evaluations were analyzed with Excel 2019 was used for data interpretation.

3. Results and Discussion

3.1 Film preparation

Figure 1 shows the appearance of BPFE incorporated films, where the intensity of the blue color increases with higher anthocyanin concentrations. This enhancement occurs because the film matrix becomes more complex due to the formation of hydrogen bonds between starch and anthocyanin. The increased color intensity is attributed to the presence of high BPFE concentration, while all other factors remain constant.

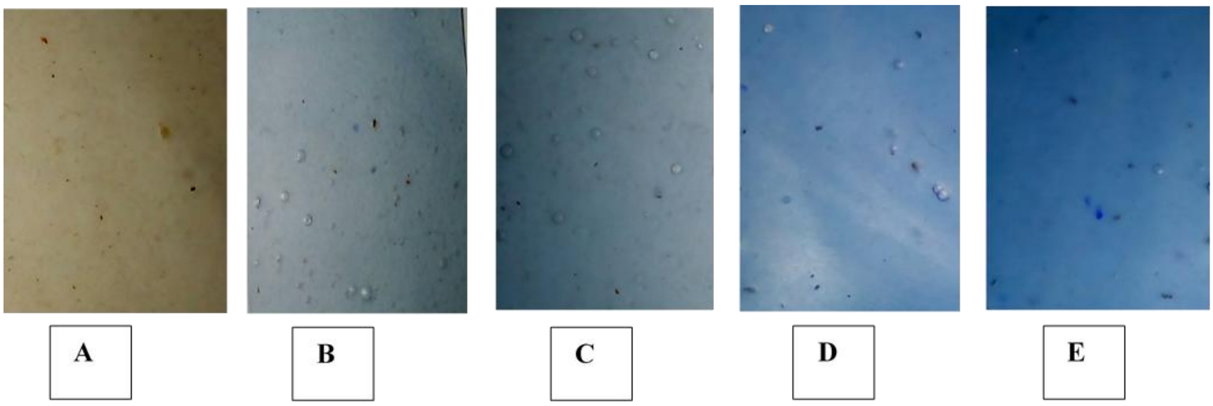

Figure 1: BPFE incorporated film: A-0%, B- 5%, C-10%, D- 15%, E- 20%

3.2 Film forming ability and visual transparency

All the film samples showed good film-forming ability and detached from the plate easily. They were also soft textured, easily manageable, and flexible (Table 2). Anthocyanin pigment particles are immobilized in the banana starch film structure to provide color to the film (EI Nagar et al., 2021).

Table 2: Film forming ability and visual transference of films

Anthocyanin (v/v %)	Film-forming ability	Detached from the plate	Manageability and texture	Visual transparency
0	++	++	++	+++
5	++	++	++	++
10	++	++	++	+
15	++	++	++	_
20	++	++	++	_

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3.3 Color Attributes

According to the experimental results, the amount of L* values decreased with an increase in anthocyanin percentage. The developed films with 0%, 5%, and 10% (v/v) BPFE showed red and yellow color tones, while those with 15% and 20% (v/v) showed green and blue color tones with respective a* and b* values. To determine if the differences are noticeable to the human eye, the color variances (ΔEa) were calculated. If the ΔEa value is higher than 3.0, it means the color difference of two samples can be detected by the human eye; values above 6.0 signify a notable color difference (Golasz et al., 2013). In Table 3, the color attributes of the films corresponding to various BPFE percentages are presented.

3.4 Moisture content

The results show that moisture percentages decreased with an increase in BPFE ratios. The free hydroxyl groups in starch interact less with moisture because they form intermolecular hydrogen bonds with the hydrophilic hydroxyl groups found in both starch and anthocyanin. This results in a reduced moisture content (Qin et al., 2019; Zhai et al., 2017; Erna et al., 2022). A similar outcome was observed in intelligent packaging developed with anthocyanin by Mary et al. (2020).

3.5 Thickness and density

The results of thickness (Table 3) has increased with increased BPFE ratio and density, exhibiting an inverse correlation with increasing BPFE percentage. With the addition of BPFE, the film matrix becomes more complex and forms a thicker film (Nogueira et al., 2019) because the dry matter content is increased.

3.6 Light barrier properties

Observations of Light barrier properties (Table 3) indicates that transmittance decreased with increasing BPFE percentage, while absorbance increased with increasing BPFE percentage. UV-Vis light-blocking capacity of the film is largely determined by the aromatic structure of anthocyanin. These aromatic rings can absorb UV-Vis light (Ashrafi et al., 2018; Hematin et al., 2022). Therefore, UV-Vis light-blocking capability of films is increased with elevated anthocyanin concentration (Yun et al., 2019).

3.7 Water resistance

The film's resistance against water without rupturing shows their capacity to tolerate deformation caused by the load of water without rupturing (Zhao et al., 2022). According to the results (Table 3) it has been observed that WR decreases with increasing BPFE ratio because the volume of water passing through the film increases as the anthocyanin content increases. Anthocyanins infiltrate the spaces between starch polymers and disrupt polymer-polymer interactions (Bianca et al., 2021; Sothornvit and Krochta, 2005; Wu et al., 2021) and make bonds with starch polymers. This, in turn, could disrupt the polymer network and increase the permeability of water.

3.8 Water vapour permeability

WVP is another measure that reflects the barrier properties of water in the film. WVP is a critical factor in determining the potential of the packaging film in preserving food (Koosha and Hamedi, 2019). The results of WVP (Table 3) reveal that the control sample showed the lowest WVP, while the film with 20% (v/v) BFPE film exhibited the highest value, being aligns with the observations of Geo et al. (2023), which states the WVP is proportional to the anthocyanin ratio (Guo et al., 2013). With the addition of anthocyanin extract, the uniformity and compactness of the polymer matrix are disturbed. (Nogueira et al., 2019; Luchese et al., 2018; Zhang et al., 2020). Previous studies have shown that excessive extract accumulation causes clumping and creates additional open spaces within

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the film, facilitating moisture transfer (Bertuzzi et al., 2007; Hematian et al., 2022; Qin et al., 2021). A similar observation was found in an intelligent packaging film developed with blackberry pulp and starch by Thakur et al. (2019).

3.9 Mechanical properties

TS is a significant parameter that estimates the degree of stress a matter can endure before fracturing. According to the data presented in Table 3, TS values of the developed films showed no discernible pattern with different BPFE percentages.Eventhough the previous studies showed that TS decrease as the amount of extract increased (Yong et al., 2019). Natural extracts were thought to weaken the interactions between starch polymer molecules (Koosha and Hamedi, 2019) and reduce the compactness of the film matrix. The hydrogen bond formation between anthocyanin and the hydroxyl groups of starch is possibly responsible for the TS value, because it leads to enhanced surface adhesion between starch and anthocyanin (Chen et al., 2017; Zhai et al., 2017). The readings might vary from previous findings due to the errors occurring at sample preparation for the Tensile strength analysis.

Young's modulus values also did not follow a clear trend and varied with increasing BPFE. A disproportionate link is present between the elasticity of the film and elongation at break. Film flexibility is decreased because anthocyanin makes clusters and limits the motion of the starch polymer chain (Mollah et al. 2016).

Table 3: Mechanical properties of developed films with banana flour, corn starch and BPFE.

Physical properties	**0 (v/v %)**	**5(v/v %)**	**10(v/v %)**	**15(v/v %)**	**20(v/v %)**
Colour properties					
L*	81.81 ± 0.44^{a}	80.85 ± 0.59^{a}	74.81 ± 0.46^{a}	74.63 ± 1.31^{b}	65.22 ± 4.49^{c}
a*	3.19 ± 0.28^{a}	2.19 ± 0.09^{b}	0.52 ± 0.08^{c}	-1.28 ± 0.43^{d}	-3.76 ± 1.21^{e}
b*	5.38 ± 0.71^{a}	4.05 ± 0.52^{ab}	2.80 ± 0.60^{b}	-14.02 ± 0.55^{c}	-6.30 ± 3.13^{d}
ΔE_a	-	1.92	6.39	16.91	12.42
ΔE_b	-	1.92	7.93	21.16	21.44
Moisture	17.01 ± 0.10^{a}	16.59 ± 0.17^{ac}	15.73 ± 0.54^{bc}	15.96 ± 0.07^{c}	15.63 ± 0.16^{c}
Thickness (mm)	0.14 ± 0.04^{a}	0.17 ± 0.01^{b}	0.26 ± 0.01^{c}	0.25 ± 0.01^{c}	0.26 ± 0.01^{c}
Density (gm^{-3})	2.34 ± 0.13^{a}	1.98 ± 0.00^{b}	1.38 ± 0.08^{cd}	1.47 ± 0.06^{c}	1.35 ± 0.01^{d}
WL-280nm					
Absorbance	0.72±0.17	0.89±0.35	1.02±0.17	1.82±1.89	2.79±2.08
Transmittance (%)	19.68±6.79	15.78±12.31	9.85±3.39	13.73±14.81	13.78±23.84
WL-660nm					
Absorbance	0.24±0.06	0.39±0.03	0.41±0.20	0.41±0.06	0.42±0.04
Transmittance	56.70±7.80	40.33±3.20	41.27±16.22	38.56±5.28	37.95±4.01
Breakup time (hr.)	24.71 ± 0.50^{a}	17.38 ± 1.84^{b}	22.30 ± 0.15^{ac}	21.67 ± 0.51^{c}	22.08 ± 0.48^{c}
Water resistance (ml)	0.52 ± 0.03^{a}	0.12 ± 0.00^{b}	0.13 ± 0.02^{b}	1.67 ± 0.02^{c}	1.74 ± 0.22^{c}

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WVP(g m$^-$ s$^-$ pa$^-$)	$7.08\times10^{-8}\pm 5.20\times10^{-8}$	$3.30\times10^{-7}\pm 7.74\times10^{-8}$	$3.85\times10^{-7}\pm 9.17\times10^{-8}$	$4.41\times10^{-7}\pm 4.98\times10^{-7}$	$8.96\times10^{-7}\pm 5.36\times10^{-8}$
Elongation at Break (%) Young's Modulus(MPa) TS (MPa)	0.00 ± 0.00^{a} 0.00 ± 0.00^{a} 1.02 ± 0.19^{a}	29.07 ± 9.98^{b} 2.24 ± 0.26^{b} 3.62 ± 0.28	9.24 ± 6.87^{ac} 2.24 ± 0.26^{b} 0.42 ± 0.06^{c}	23.69 ± 5.76^{bc} 2.64 ± 0.49^{b} 3.13 ± 0.28^{bd}	23.69 ± 5.76^{c} 2.05 ± 0.27^{b} 3.01 ± 0.12^{d}

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Various superscripts of mean value in the same row are notably distinct (Tukey's test: $p<0.05$) from each other.

Data given in the table are the mean ± standard deviation (color properties and Thickness (n=15), Moisture, Light barrier properties, WVP, film solubility, and water resistance (n=3).

3.10 Color response analysis of the film with different pH

The 15% and 20% (v/v) BPFE incorporated film exhibited color alteration according to pH variations. BPFE, exhibits red at pH lower than 3.2, violet to blue color at pH 3.2-5.2; pH 5.2-8.2 light blue color and dark green color at pH 8.2-10 (Escher et al., 2020). Protonation in the hydroxyl group of Red flavylium cation leads formation of a blue neutral quinoidal at pH 3-5 (Liu et al., 2014). Further deprotonation of quinoidal base at pH 6-8 forms anionic quinoidal in purple-blue color. The green color at pH 8.5 is due to the formation of chalcone, after hydrolysis of the acyl bond in anionic quinoidal. Stability of anthocyanin is reduced with elavated pH conditions and isomerized into chalcone through hydro catalysed-tautomerisation. This leads into formation of yellow-green color of the system (Liu etal., 2014; Hasanah et al., 2023). Similar observations were observed in developed15% and 20% (v/v) BFPE incorporated films (Figure 2).

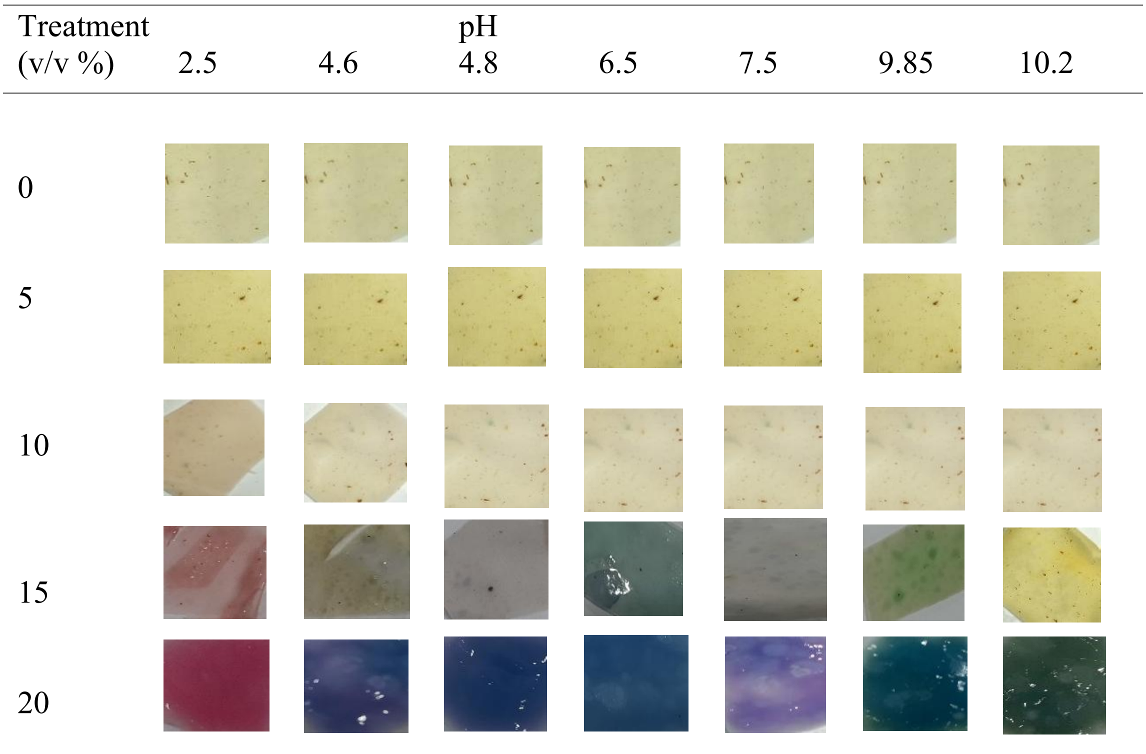

Figure 2: Color response analysis of the film with different pH

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3.11 Food applications of the intelligent film

All samples of 0%,5%,10%, 15%, 20% (v/v) showed no colour change at freezing conditions (-18 °C), and in refrigerated conditions for one month. The sample stored at room temperature, wrapped with 20% (v/v) BFPE incorporated film, showed colour change from the second day after storage, indicating the spoilage of sausages. On the first day, 20% BPFE film was dark blue, and on the second day it became light blue, green, and on the fourth day it showed a pale pink color (Figure 3). With the spoilage of sausages, the medium could become acidic, and as a result of that, easily distinguishable color changes occurred. This indicates that 20% (v/v) BPFE incorporated film is a good and safe indicator to use as eco-friendly intelligent packaging.

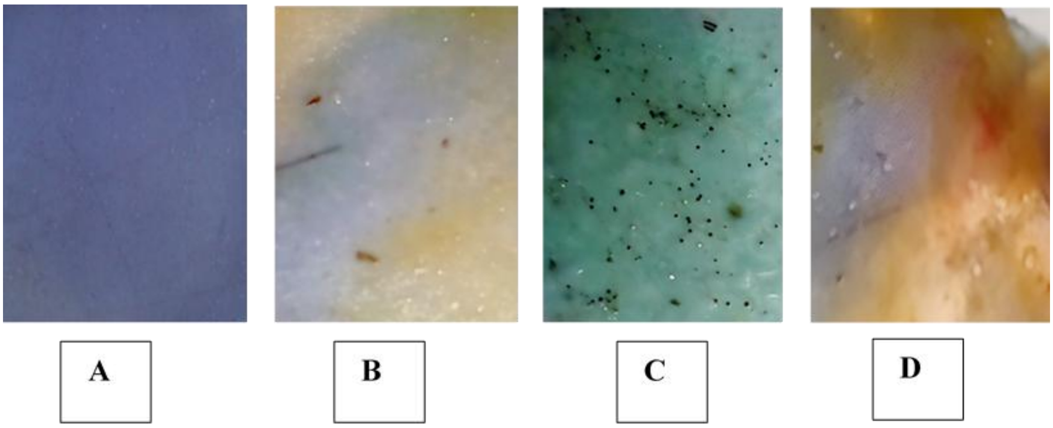

Figure 3: Color changes of 20% BPFE incorporated films, with spoilage of sausages **(A)** day 1, **(B)** day 2, **(C)** day 3, **(4)** day 4

Conclusions

Addition of BPFE enhances the thickness, light absorption, WVP, and decreases the moisture, density, transmittance, and WR in the developed films. BPFE-incorporated films with 15% and 20% concentrations showed significant color changes with various pH buffers, and the 20% BPFE-incorporated film showed color changes with the spoilage of sausages, indicating its potential to be used as intelligent packaging. This concludes that 20% (v/v) BPFE-incorporated film is a good and safe indicator to use as eco-friendly intelligent packaging.

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