

# Characterization of Intelligent Packaging from Chitosan-Cassava Starch with Butterfly Pea Flower Extract and Gambier Catechin as Freshness Sensor

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## Abstract

The increasing environmental concerns associated with conventional plastic packaging have accelerated research into biopolymer-based alternatives for sustainable food packaging. Starch and chitosan have emerged as promising biodegradable materials, while the incorporation of bioactive and natural pigments enables the development of intelligent packaging systems. Anthocyanins from butterfly pea flower extract, stabilized through co-pigmentation with gambier catechins, provide pH-sensitive functionality for monitoring food quality and safety. This study contributes to the formulation of intelligent biopolymer films as pH sensors for assessing chicken freshness under varying storage conditions. The production of intelligent film used various concentrations of butterfly pea flower extract and gambier (BPG) (5%, 10% and 15%) incorporated into chitosan-cassava starch solution. The physical properties of the film were observed following the analysis of the color response of the BPG extract and intelligent film. Then, the BPG intelligent film was applied to monitoring chicken breast freshness for 72 h at 7 and 25 °C. The color and pH changes were observed every 24 h. The BPG intelligent film significantly increased film thickness, moisture content, solubility, water vapor transmission rate (WVTR), and water vapor permeability (WVP). The color of the BPG extract and BPG film shifted from purple to green at pH levels between 2 and 11. When applied to chicken breast, the color of the BPG film will change from purple to green due to the decay in the chicken breast which is indicated by an increase in pH. Based on the research results, it can be concluded that BPG extract mixed with the film matrix can act as a freshness indicator in chicken breast stored at cold or room temperature. The color change that occurs is clearly visible (does not fade) due to the co-pigmentation of gambier.

**Keywords:** Bio-sensor, Butterfly pea anthocyanins, Cassava starch, Chitosan, Co-pigmentation, Food freshness, Food packaging

## Introduction

Food packaging is crucial for preserving the safety of food products and extending their shelf life. The use of plastic packaging is associated with environmental contamination concerns [1]. This motivates researchers to develop renewable, eco-friendly, and adaptable packaging. Numerous investigations indicate that bioplastics possess the capability to replace conventional plastics in food packaging applications. Ongoing research focuses on the creation of bioplastics to provide packaging with physical qualities close to

conventional plastic while being biodegradable. Bioplastics are being developed as an alternative to sustainable packaging for food, health, and agriculture due to their renewable qualities, compatibility with biological processes, and environmental benefits. The utilization of biopolymers derived from agricultural by-products diminishes environmental pollution, enhances the value of these by-products, and lowers the production costs of bioplastics. It can be said that biopolymers have the potential to be used as raw

materials for bioplastics. This can reduce environmental pollution and produce eco-friendly food packaging [2,3].

Currently, the advancement of bioplastics is transitioning towards active and intelligent packaging solutions. Active packaging integrates bioactive ingredients into the matrix to enhance quality and prolong shelf life. Intelligent packaging incorporates components into the matrix to assess food quality during storage, hence ensuring safety. Intelligent packaging may provide consumers with real-time data regarding the quality of packaged food. Intelligent packaging combines the interactions between the polymer matrix, the packaged food, and the environment inside the package [4,5]. There are six fundamental roles involved in intelligent packaging, which are inspection, detection, reporting, sensing, tracking, and transmission. Observable factors include pH, volatile compounds, temperature, oxygen concentrations, and microbial proliferation [6,7]. A natural pH indicator integrated into bioplastics may emerge as an innovative intelligent packaging solution, functioning as a food sensor that allows for visible changes without requiring advanced technology. The pH indicator facilitates consumer food selection, diminishes food waste, and mitigates foodborne illness caused by spoilage [8,9].

Natural dyes obtained from agricultural or plant waste can serve as reliable indicators for detecting pH changes food that signify deterioration [10]. Natural dyes are regarded as safer for use as sensors in intelligent packaging. Anthocyanins are naturally found, water-soluble pigments prevalent in nature, making them suitable for application in pH-sensitive materials [11,12]. Anthocyanin hues range from red to blue, orange, and purple, contingent upon the source. Butterfly pea flowers (*Clitoria ternatea*) are rich in anthocyanins, imparting a vivid blue hue. Butterfly pea flowers are extensively utilized for their antioxidant properties and as a natural food dye. Moreover, many studies have investigated their application as pH indicators for food degradation [13-15].

The stability of color pigments in butterfly pea flowers can be increased by the co-pigmentation method using catechins. Co-pigmentation is the phenomenon wherein a co-pigment (such as metal ions, phenolic compounds, and other flavonoids) forms supramolecular assemblies with anthocyanins, resulting

in more vibrant and intense colors than those produced by anthocyanins alone. In this case, catechin is a flavonoid compound that is also found in gambier. The gambier plant (*Uncaria gambir*), discovered in Indonesia, possesses a significant catechin concentration ranging from 40.94% - 80.71% [16,17]. Gambier catechins in chitosan-starch biofilms improve packaging material physical and functional qualities. Hydrogen bonding between catechins' hydroxyl groups and chitosan's amine ( $-NH_2$ ) and starch's hydroxyl ( $-OH$ ) groups is a key interaction with the biofilm matrix. This interaction strengthens and stretches the biofilm, making it more stress-resistant and retaining its elongation at break [18,19]. The antioxidant properties of gambier catechins play a crucial role in preventing oxidative degradation, both of the biofilm itself and the encapsulated product, thereby extending shelf life [20]. By increasing biofilm UV resistance, catechins reduce nutritional degradation and color fading in packaged food. Finally, gambier catechins' natural, biodegradable nature corresponds with growing environmental concerns, as their presence in the biofilm increases package biodegradability [21,22]. These improvements make gambier catechins a versatile and useful ingredient in functional packaging materials.

The freshness and safety of chicken meat are essential due to its common consumption and significant implications for human health. Chicken meat is a food ingredient characterized by high amounts of protein, fat, and moisture, rendering it very susceptible to degradation by endogenous enzymes and microbes during storage and transportation, resulting in product spoiling [23]. The decomposition of chicken meat generates volatile alkaline organic amines, such as trimethylamine, which elevate the pH in the packaging headspace and impart a bad odor and flavor to the meat, consequently diminishing the product's shelf life. Therefore, the development of indicators for chicken meat freshness is a necessity to guarantee its safety [24,25].

Biodegradable polymers are increasingly being favored in food packaging as substitutes for conventional plastic packaging. Starch-based films represent a more economical alternative for food packaging among other natural polymers [26,27]. Starch is plentiful, cost-effective, biodegradable, and safe for consumption, rendering it an appropriate material for

film production [28]. Starch-based and composite packaging exhibit considerable promise as sustainable packaging solutions [29]. Nevertheless, starch-based bioplastics exhibit worse mechanical characteristics and are susceptible to moisture. Consequently, this issue must be addressed by utilizing alternative polymers, such as chitosan, to enhance their mechanical properties. The combination of starch and chitosan can enhance their solubility and permeability [30,31].

The chitosan–starch biopolymer matrix is widely regarded as an excellent host material for anthocyanin–catechin sensor systems due to its chemical compatibility, strong intermolecular bonding potential, and stabilizing effects on polyphenolic compounds. Chitosan contains primary amino groups ( $-NH_2$ ) that can be protonated in acidic environments ( $-NH_3^+$ ), enabling electrostatic attraction and hydrogen bonding with hydroxyl groups of anthocyanins and catechins, thus improving their immobilization within the polymer network [32,33]. Meanwhile, starch contributes multiple hydroxyl groups ( $-OH$ ) that enhance intermolecular hydrogen bonding, improving film-forming ability and mechanical stability when blended with chitosan [34]. These interactions not only prevent the rapid diffusion and degradation of anthocyanins (commonly unstable to pH, heat, and light) but also facilitate the copigmentation effect with catechins, which promotes color stability essential for intelligent pH-sensitive sensor systems [32]. Additionally, the semi-crystalline structure of starch and the polycationic nature of chitosan create a controlled microenvironment that supports responsive colorimetric behavior of anthocyanins when exposed to volatile amines or pH changes in food spoilage monitoring [35]. Therefore, the chitosan–starch combination provides a synergistic matrix that is not only biodegradable and safe for food applications but also chemically favorable for anthocyanin–catechin interactions, enhancing the stability, sensitivity, and functionality of natural optical sensors.

This research will develop intelligent packaging utilizing chitosan and cassava starch as eco-friendly biopolymers. The active ingredient integrated into the packaging matrix is butterfly pea flower extract, supplemented with gambier catechin to enhance the color of its anthocyanin pigment. This research contributes to the development of environmentally

friendly and consumer-safe intelligent packaging. This intelligent packaging will be applied as a pH sensor to assess the freshness of chicken at both room and refrigeration temperatures.

## Materials and methods

### Materials

The materials used in this research were chitosan (98.03%) from Phy Edumedia, cassava starch (Rose Brand), dried butterfly pea flower, and catechin gambier from PDH herbal. The chicken breast used for simulation was obtained from a supermarket in Yogyakarta, Indonesia. All chemical reagents used for preparation and analysis are analytical grade.

### Butterfly pea flower extract production

The production of butterfly pea flower extract was conducted based on the research by Rawdkuen *et al.* [36] with modifications [36]. Dried butterfly pea flowers were ground and sieved with an 80-mesh sieve to homogenize the particle size. Then, the butterfly pea flower powder was macerated using 70% ethanol (1:5 w/v) for 72 h at room temperature (25 °C). The maceration process was stirred every 24 h. After that, it was filtered to obtain a filtrate which was then concentrated using a rotary evaporator at 50 °C, 60 rpm and pressure condition 130 mbar. After obtaining the concentrated extract from the butterfly pea flowers, it was then added to gambier catechins (concentration 20%). The gambier catechin solution was made by dissolving gambier with distilled water and then filtering to obtain the filtrate. The filtrate was then added to the butterfly pea flower extract in a 2:3 v/v ratio. Next, the mixture of butterfly pea flower extract and gambier catechins (BPG) was stirred until homogeneous.

### Intelligent packaging production

A total of 2% w/v chitosan was dissolved in 1% acetic acid 100 mL and agitated until a homogeneous solution was achieved. Subsequently, 1% v/v glycerol was included in the solution. The 4% w/v cassava starch was dissolved separately in distilled water and heated to 80 °C until fully dissolved. The chitosan solution was subsequently combined with the cassava starch solution (1:1 v/v), followed by the addition of BPG. The mixture was stirred until it became homogeneous using a magnetic stirrer. The uniform film solution was poured

into a glass mold and dried at 30 °C for 12 h [18,37]. This study demonstrates the intelligent packaging formulation in **Table 1**.

**Table 1** The formulation of intelligent packaging with butterfly pea flower extract and gambier catechins.

Materials	F0	F1	F2	F3
Chitosan (% w/v)	2	2	2	2
Cassava starch (% w/v)	4	4	4	4
BPG (% v/v)	0	5	10	15
Glycerol (% v/v)	1	1	1	1
Acetic acid (ml)	100	100	100	100
Aquadest (ml)	100	100	100	100

#### Mechanical properties analysis of intelligent packaging

Film thickness was analyzed using a micrometer screw gauge with an accuracy of 0.01 mm. Films were analyzed at five different points on each sample [38,39]. Solubility analysis on the film using a sample measuring 3×3 cm. The film was dried in an oven at 105 °C for 6 h ( $W_i$ ). The dried film was then soaked in 50 mL of distilled water for 24 h and dried again for 2 h at 105 °C ( $W_f$ ). The solubility value on the film was calculated as the percentage of weight differences of the film [40]. The solubility percentage can be calculated using Eq. (1).

$$\text{Solubility (\%)} = \frac{W_i - W_f}{W_i} \times 100 \quad (1)$$

where  $W_i$  is the initial weight of the sample (g) and  $W_f$  is the final weight of the sample (g).

#### Moisture content analysis of intelligent packaging

Moisture content was analyzed using gravimetric method. A total of 2 g of sample was analyzed in triplicate [41].

#### Water vapor permeability (WVP) analysis of intelligent packaging

The WVP analysis of the sample began by cutting the sample into 3×3 cm, and it was placed above glass cup containing 3 g of silica gel. The glass cups were placed into a desiccator at 28 °C and 80% RH with saturated NaCl solution. The weight changes of the film were observed every 12 h until 72 h [40]. The WVTR and WVP can be calculated using Eqs. (2) - (3).

$$WVTR = \frac{m}{A \times t} \quad (2)$$

$$WVP = \frac{WVTR \times \text{thickness}}{\Delta P} \quad (3)$$

where  $m$  is mass change of the film,  $A$  is surface area,  $t$  is time, and  $\Delta P$  is partial WVP pressure across the film (Pa).

#### Antioxidant analysis of intelligent packaging

The sample films were dissolved in aquadest and homogenized using a vortex. Then, the solutions were shaken using orbital shaker at 200 rpm for 24 h. Subsequently, the film extracts (4 μL) were mixed with 196 μL DPPH solution 0.06 mM and homogenized using a vortex. The absorbances were measured at the wavelength 490 nm with a spectrophotometer UV-Vis [42,43]. The antioxidant of the film was calculated using Eqs. (4) - (5).

$$\text{Antioxidant activity (\%)} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100 \quad (4)$$

$$IC_{50} = \frac{y-b}{a} \quad (5)$$

#### Anthocyanin analysis of intelligent packaging

The total anthocyanin content was determined using the difference technique, employing pH 1 and pH 4.5. The absorbance of total anthocyanin was quantified using a UV-Vis spectrophotometer at wavelengths of 520 nm and 700 nm [44]. Total anthocyanin content of the sample was calculated using Eqs. (6) - (7).

$$\text{Absorbance} = (Abs_{520} - Abs_{700})_{pH1} - (Abs_{520} - Abs_{700})_{pH4.5} \quad (6)$$

$$\text{Anthocyanin} = \frac{A \times MW \times df \times 1000}{\epsilon \times \text{sample weight}} \quad (7)$$

where  $A$  is absorbance,  $MW$  is molecular weight (449.2 g/mol),  $df$  is dilution factor, and  $\epsilon$  is molarity coefficient (26,900 L/mol.cm).

#### Intelligent packaging application on chicken breast

The chicken breasts were weighed at 10 g each and were placed in the petri dishes. The film indicators were cut into 2.5×2.5 cm and were patched on the lid of the petri dishes that contained chicken breast inside them. The distance between the intelligent packaging and chicken breast surface was 1 cm [37]. The packed chicken breasts were stored at approximately 7 °C and 25 °C. The application of intelligent packaging is illustrated in the **Figure 1**.



**Figure 1** Intelligent packaging film application on the chicken breast.

#### pH sensitivity and color responses of intelligent packaging

pH and color changes were measured every 24-hours for three days. The picture was taken using Samsung A55 camera. pH was quantified on the chicken breast using pH meter and the  $L^*$ ,  $a^*$ , and  $b^*$  color was measured on the intelligent film using chromameter [37], [45]. The 72-hour (three days) testing period represents the normal shelf life of raw chicken breast under refrigerated and ambient circumstances, when

microbiological decomposition and quality decline occur. According to chicken storage studies, pH increase, TVB-N production, and color change occur within 48 - 72 hours at 4 - 8 °C, making this the important timeframe for freshness monitoring. Therefore, the 72-hour period permits enough observation of the film's color response to spoilage without exceeding the product's practical consumption window.

## Results and discussion

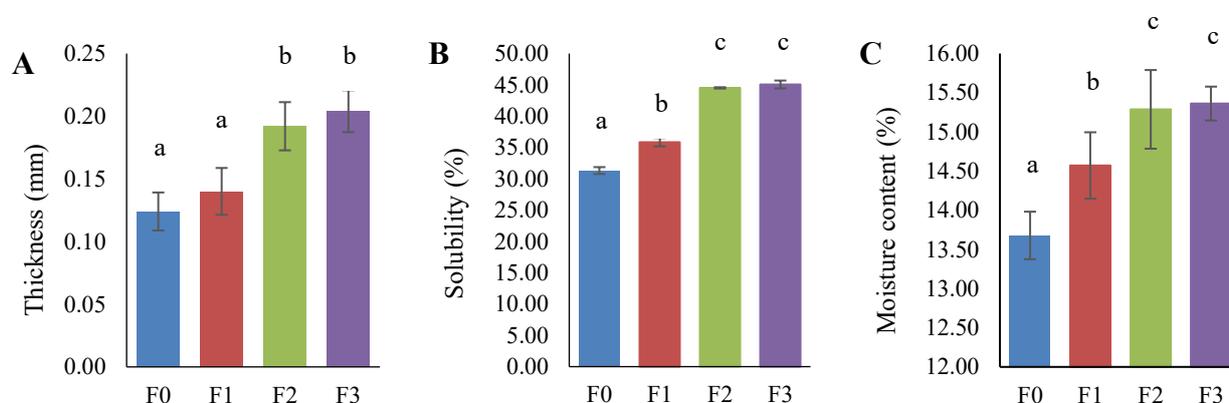
### Mechanical properties of intelligent packaging film

The thickness of the film correlates with the mass of solids used in its development. The increase in film thickness coincides with the incorporation of BPG (**Figure 2**). The film thickness increased markedly with the incorporation of BPG mass (5% - 15%) compared to the control formulation. The film thickness in this investigation varied from 0.12 - 0.20 mm. The thickness of the film correlates with its functional features, including water content, solubility, water vapor permeability, and its utilization as a film sensor [2,45]. A similar trend was observed in intelligent packaging with sugarcane wax/agar with the incorporation of butterfly pea extract. The film packaging exhibited a thickness increase of 0.123 - 0.171 mm [1].

The use of BPG markedly enhanced the solubility of the films (**Table 2**). The control film exhibited a solubility of 31.31%, but the BPG-added film had a solubility ranging from 35.83% - 45.06%. The solubility

value was affected by the increasing thickness and water content of the films. The enhancement in solubility has a positive influence on deterioration; nonetheless, it constitutes a disadvantage when used as packing material [46]. The solubility of double-layered films increased with the incorporation of butterfly pea flower extract above 5%, although films containing 5% extract did not exhibit enhanced solubility relative to those without extract [2].

**Figure 2** indicates that the moisture content of the film markedly increased with the incorporation of BPG. The moisture content in the films varied from 13.67% - 15.36%, with the maximum value seen in F3. The incorporation of BPG increased film thickness, thereby enhancing its moisture content. The augmentation of water content is associated with weak intermolecular interactions between the polymer matrix constituents and the extract [47]. This effect enhances the availability of -OH groups in the polymer structure, resulting in a reduction in hydrophobicity [42].



**Figure 2** Mechanical properties of intelligent packaging film (A) thickness, (B) solubility, and (C) moisture content. Letter symbols indicate significant differences between formulations ( $p < 0.05$ ).

### WVP number of the intelligent packaging film

The Water Vapor Transmission Rate (WVTR) and Water Vapor Permeability (WVP) are essential evaluations in the packaging industry, as they quantify the permeability of packaging materials to water vapor. This characteristic is crucial for smart packaging films, as it prevents product degradation within the container and enables the film to withstand high humidity conditions, including immersion or contact with liquid water or air moisture [37]. **Table 2** indicates a substantial rise in the film's WVTR and WVP with the

increase of BPG incorporation. The upward trend in WVTR and WVP values correlates with a reduction in the film's hydrophobicity. This number is also affected by the film's mechanical characteristics and moisture content, both of which exhibit a rising trend.

A high WVTR is suitable for food items that require respiration and humidity regulation, such as fresh produce. Elevated humidity levels in the environment can impact the sensor's reaction, precision, and longevity. The halochromic response in intelligent packaging is associated with variations in pH levels.

Consequently, monitoring WVTR is crucial for evaluating the efficacy of pH-sensitive dyes, ensuring their stability, enhancing their performance, and addressing potential application issues. In film biosensor applications, ambient humidity is inevitable [48,49].

In films based on carboxymethyl cellulose and agar with added butterfly pea flower extract, the WVTR value is  $0.0583 \times 10^{-3} \text{ g/m}^2 \text{ s}$  [49]. These levels are significantly lower in comparison to the WVTR values in this investigation. The elevated WVTR and WVP

values in this film may also be affected by the quantity of extract integrated into the polymer matrix. The lowest values of WVTR and WVP were observed in F1 compared to the film without extract. The film containing 5% BPG exhibited WVTR and WVP values that were nearly comparable to those of the chitosan-cassava starch film, despite a noticeable increase. Conversely, the incorporation of over 5% extract resulted in significantly elevated WVTR and WVP levels [50].

**Table 2** Water vapor transmission rate and permeability of intelligent packaging.

Sample	WVTR (g/m <sup>2</sup> s)	WVP ( $\times 10^{-6} \text{ g/m}^2 \text{ s Pa}$ )
F0	$0.0341 \pm 0.0001^a$	$1.567 \pm 0.005^a$
F1	$0.0348 \pm 0.0002^b$	$1.804 \pm 0.008^b$
F2	$0.0364 \pm 0.0002^c$	$2.591 \pm 0.012^c$
F3	$0.0380 \pm 0.0001^d$	$2.869 \pm 0.012^d$

Note: Superscript letter symbols indicate significant differences between formulations ( $p < 0.05$ ).

In films with sugar palm starch/chitosan nanoparticles enriched with 5% and 15% butterfly pea anthocyanins, the WVP value exceeded that of films with 10% extract. The increase is likely due to high anthocyanin, which compromises structural integrity via aggregation, enhances water molecule transit, and elevates WVP [51]. The incorporation of anthocyanins reduces WVTR and WVP by strengthening intermolecular hydrogen bonding, resulting in denser and more compact film formations. This study found that the film containing 5% anthocyanin demonstrated the lowest WVTR and WVP, attributable to enhanced hydrogen bonding.

The incorporation of butterfly pea flower extract and gambier catechin into chitosan-cassava starch films improves sensing capabilities, but at the expense of heightened solubility and water vapor permeability (WVP) values, potentially diminishing the film's protective properties. These modifications cause difficulties in the practical application of the film for food packaging, particularly in conditions requiring moisture and oxygen regulation. The improved freshness sensing may prove beneficial for real-time

deterioration detection, and other optimization methodologies can be investigated to manage these trade-offs for particular packaging requirements.

#### Antioxidant of the intelligent packaging film

The antioxidant capacity of the intelligent film included with BPG is indicated by a half-maximal inhibitory concentration (IC<sub>50</sub>) value between 22.15 and 41.73  $\mu\text{g/mL}$  (Table 3). The IC<sub>50</sub> value is categorized into three groups: strong (10 - 50  $\mu\text{g/mL}$ ), medium (50 - 100  $\mu\text{g/mL}$ ), and weak (> 100  $\mu\text{g/mL}$ ). Consequently, it can be claimed that the film containing BPG has a potent antioxidant capacity, as indicated by a value of < 50  $\mu\text{g/mL}$ . Simultaneously, the chitosan-cassava starch film exhibits moderate antioxidant properties. Numerous studies have indicated that the presence of anthocyanins has potent antioxidant effects [52].

The incorporation of anthocyanins into the film matrix enhances its antioxidant capacity owing to the presence of phenolic groups in the anthocyanin molecular configuration [53]. The film's raw material, chitosan, enhances its antioxidant effect alongside the anthocyanins derived from butterfly pea flowers. Chitin

and chitosan are significant bioactive substances, exhibiting numerous powerful actions including immunological modulation, hemostatic properties,

wound healing, antioxidant effects, antibacterial efficacy, and the elimination of heavy metals and other pollutants [54].

**Table 3** Antioxidant and anthocyanin content of intelligent packaging.

Sample	Antioxidant ( $\mu\text{g/mL}$ )	Anthocyanin ( $\text{mg/L}$ )
F0	$62.66 \pm 0.20^{\text{a}}$	$0.00 \pm 0.00^{\text{a}}$
F1	$41.73 \pm 0.11^{\text{b}}$	$35.94 \pm 0.01^{\text{b}}$
F2	$35.95 \pm 0.58^{\text{c}}$	$78.09 \pm 0.27^{\text{c}}$
F3	$22.15 \pm 0.89^{\text{d}}$	$93.38 \pm 0.01^{\text{d}}$

Note: Superscript letter symbols indicate significant differences between formulations ( $p < 0.05$ ).

#### Anthocyanin content of the intelligent packaging film

The anthocyanin concentration in intelligent packaging films increased with the addition of BPG extract to the film matrix (**Table 3**). The control film lacked anthocyanin. The anthocyanin concentration in BPG extract may increase the anthocyanin levels in intelligent packaging materials. Intelligent packaging films containing 15% BPG extract had the greatest anthocyanin concentration at 93.38 mg/L.

Anthocyanins are polyphenolic pigments within the flavonoid category and constitute the predominant group of water-soluble pigments in nature. They can exhibit a spectrum of colors from red-orange to blue-violet depending on variations in pH levels. The blue hue of butterfly pea flowers is recognized as a rich source of anthocyanin. This study demonstrates that an increased anthocyanin content in intelligent films correlates with a more intense blue hue in the film's coloration [45,55]. The use of anthocyanins derived from the aqueous extract of clitoria flowers in biopolymer films facilitates the development of intelligent packaging with active properties. These anthocyanins are not solely restricted to a colorimetric effect; they also exhibit antibacterial and antioxidant characteristics [56,57].

#### pH sensitivity of the BPG extract and intelligent packaging film

Anthocyanins are compounds that are unstable to environmental changes. pH is one of the most influential factors affecting the color changes of anthocyanin pigments. The visible color is determined by the specific molecules that dominate at various pH levels. This occurs because anthocyanins undergo a conversion between various structural forms due to changes in pH [58]. **Figure 3** shows the variations in color of the BPG extract at different pH levels. The higher the pH, the greener the BPG extract appears. Due to the high concentration of the BPG extract produced in this study, the color is more intense.

After the BPG extract was applied to the intelligent packaging film, the color change due to the pH difference was still visible (**Figure 4**). This demonstrates the effectiveness of incorporating BPG extract into chitosan-cassava starch-based films. The color change trend in the intelligent packaging film was similar to that in the BPG extract, except that the resulting color was more pronounced due to the volume of extract used.

At a pH below 2, anthocyanins mostly occur as red-hued flavylium cations. As the pH rises, these cations progressively transform into colorless carbinol pseudo-bases and chalcones, leading to a diminished red hue. Within the pH range of 6 - 9, anthocyanins undergo a conversion into quinonoidal base structures, exhibiting a blue-violet coloration. Subsequent elevations in pH result in the generation of quinonoidal anions, which exhibit a green coloration [58-60].

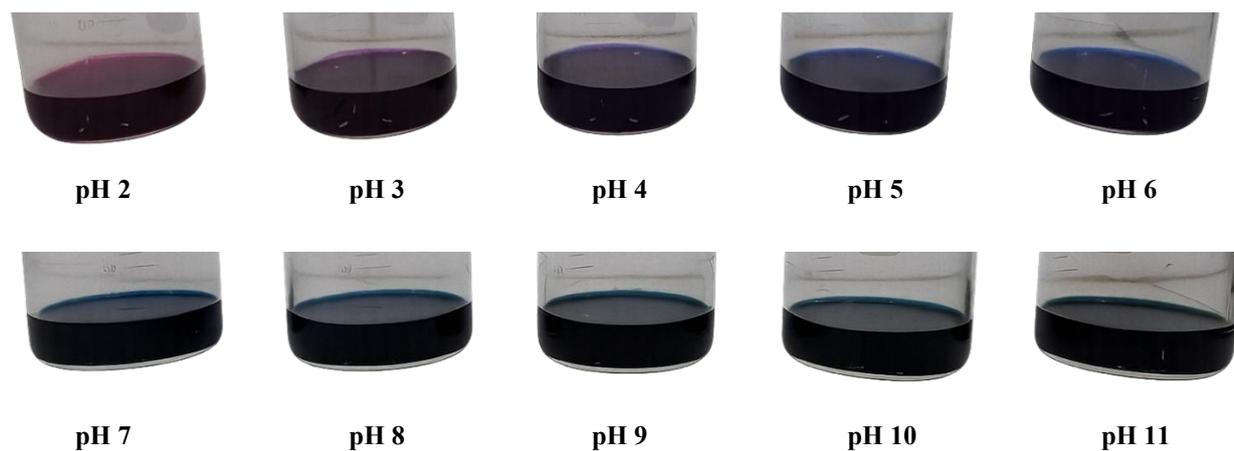


Figure 3 pH sensitivity of the BPG extract.

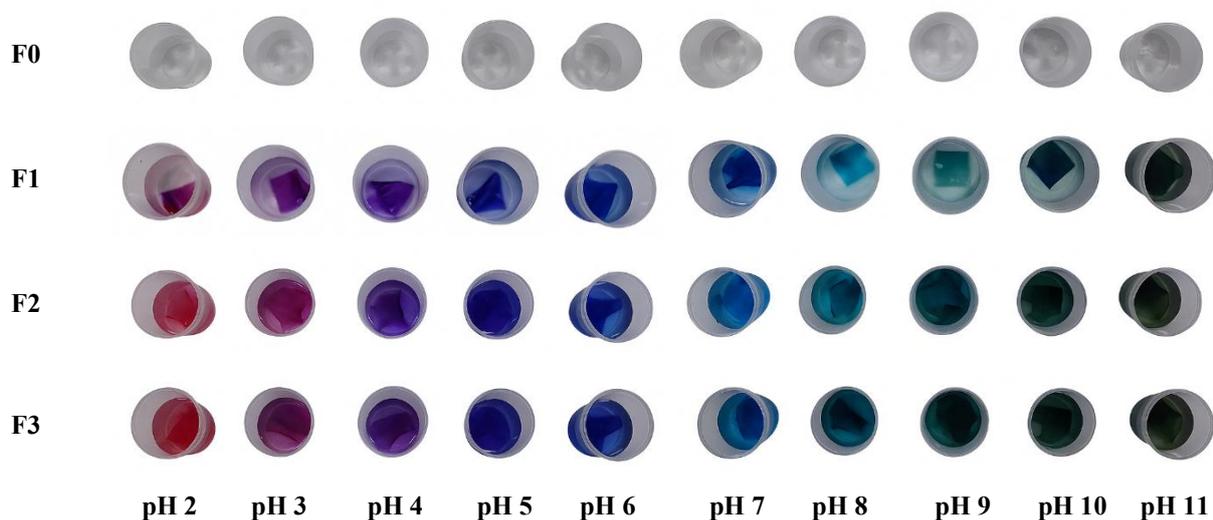


Figure 4 pH sensitivity of the intelligent packaging film.

### The response of the film indicator on the color and pH

Chickens subjected to intelligent film as a spoiling sensor were monitored for pH variations over time. Tables 4 and 5 demonstrate a notable elevation in pH correlated with prolonged storage duration. Chicken breasts maintained at 7 and 25 °C exhibited a rise in pH levels. A color shift on the intelligent packaging film signified the pH alteration. The hue of the film put on chicken breasts maintained at 25 °C exhibited a more significant alteration than those preserved at 7 °C. This signifies that chicken breasts maintained at 25 °C deteriorated more rapidly than those preserved at 7 °C. The elevated pH in the sample was attributed to an increased in volatile basic chemicals, particularly NH<sub>3</sub>, resulting from protein breakdown caused by microbial

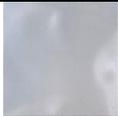
activity [45,61]. Research on freshness monitoring using intelligent packaging film also showed a similar trend to this study regarding pH changes. The pH of *S. scombrus* increased to 7.42 on the 15<sup>th</sup> day of storage [45].

Storage of chicken breast at 7 and 25 °C results in pH changes due to microbial and enzymatic activities. Initially, microbial fermentation generates organic acids such as lactic acid, resulting in a decrease in pH. During spoiling, proteolytic and lipolytic enzymes decompose proteins and lipids, producing alkaline byproducts including ammonia and amines. These chemicals elevate the pH, especially when acidogenesis decreases due to reduced microbial activity [62,63]. At 7 °C, the process is less rapid than at 25 °C, where accelerated microbial activity results in a more rapid initial reduction in pH, subsequently followed by an increase

when acid production diminishes. Consequently, the pH rises with time as alkaline byproducts from protein and

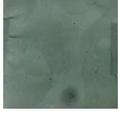
fat decomposition accumulate, while acid synthesis diminishes.

**Table 4** Response changes and pH of intelligent packaging at 7 °C.

Sample	Time			
	0 h	24 h	48 h	72 h
F0	 4.96 ± 0.09 <sup>1a</sup>	 4.99 ± 0.19 <sup>1b</sup>	 6.35 ± 0.02 <sup>1c</sup>	 7.56 ± 0.17 <sup>1d</sup>
F1	 5.05 ± 0.39 <sup>12a</sup>	 5.17 ± 0.11 <sup>12b</sup>	 6.36 ± 0.03 <sup>12c</sup>	 7.71 ± 0.05 <sup>12d</sup>
F2	 5.07 ± 0.23 <sup>12a</sup>	 5.26 ± 0.28 <sup>12b</sup>	 6.41 ± 0.06 <sup>12c</sup>	 7.73 ± 0.25 <sup>12d</sup>
F3	 5.08 ± 0.16 <sup>2a</sup>	 5.38 ± 0.26 <sup>2b</sup>	 6.64 ± 0.05 <sup>2c</sup>	 7.77 ± 0.19 <sup>2d</sup>

Note: Superscript letter symbols indicate significant differences between storage times, while superscript number symbols indicate significant differences between formulations ( $p < 0.05$ ).

**Table 5** Response changes and pH of intelligent packaging at 25 °C.

Sample	Time			
	0 h	24 h	48 h	72 h
F0	 5.07 ± 0.06 <sup>1a</sup>	 7.29 ± 0.13 <sup>1ab</sup>	 9.33 ± 0.27 <sup>1bc</sup>	 10.31 ± 0.15 <sup>1c</sup>
F1	 5.18 ± 0.07 <sup>2a</sup>	 7.41 ± 0.33 <sup>2ab</sup>	 9.42 ± 0.13 <sup>2bc</sup>	 10.44 ± 0.23 <sup>2c</sup>
F2	 5.29 ± 0.29 <sup>3a</sup>	 7.66 ± 0.34 <sup>3ab</sup>	 9.64 ± 0.03 <sup>3bc</sup>	 10.49 ± 0.24 <sup>3c</sup>
F3	 5.38 ± 0.17 <sup>4a</sup>	 7.75 ± 0.12 <sup>4ab</sup>	 9.86 ± 0.05 <sup>4bc</sup>	 10.55 ± 0.21 <sup>4c</sup>

Note: Superscript letter symbols indicate significant differences between storage times, while superscript number symbols indicate significant differences between formulations ( $p < 0.05$ ).

Color alterations in intelligent packaging films utilized for chicken breasts were monitored over 72 h at various storage temperatures (Figure 5). Films kept at 7 and 25 °C exhibited the same color change pattern; however, at 25 °C, the color progressively shifted towards green. L\* denotes lightness (ranging from black to white), a\* signifies the red (positive) to green

(negative) axis, and b\* indicates the blue (negative) to yellow (positive) axis [37]. The L\* readings for all formulations exhibited a variable trend, depending on the resultant color generated. The a\* value at a storage temperature of 7 °C exhibited a reddish hue due to its positive value, whilst the b\* value indicated a tendency towards blue.

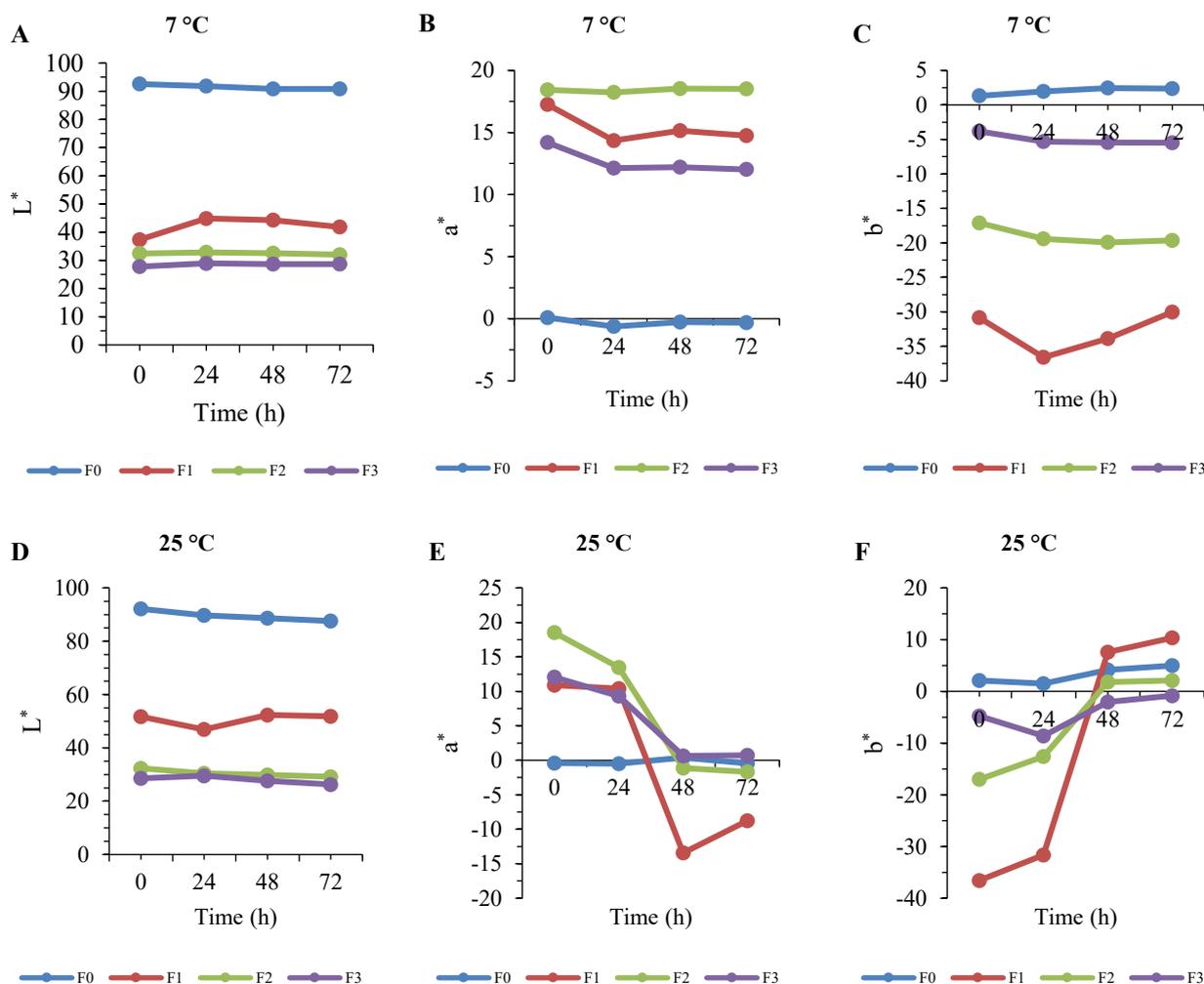


Figure 5 Color response of intelligent packaging at 7 and 25 °C. (A) L\* of BPG film at 7 °C; (B) a\* of BPG film at 7 °C; (C) b\* of BPG film at 7 °C; (D) L\* of BPG film at 25 °C; (E) a\* of BPG film at 25 °C; (F) b\* of BPG film at 25 °C.

Film stored at 25 °C exhibited a declining a\* value, signifying a progressive shift of the red hue towards green. The b\* value exhibited an upward trend, signifying a transition in color from bluish to yellowish. The color alteration results from the instability of anthocyanin pigments due to pH fluctuations in the preserved chicken breast.

The color changes in the film provide consumers with a visual cue to assess the freshness of packaged

food. A bluish-purple color signifies fresh food, whereas a greenish color denotes spoiled products. Anthocyanins, essential components, are recognized for their stability between 2 and 4 °C. Consequently, sustained low-temperature conditions are vital for good film performance [59,64]. The color of the chitosan-cassava starch film containing BPG in this study, which was kept at 7 °C, clearly demonstrated this. The color

alteration was not distinctly observable after 72 h of storage, compared to that maintained at 25 °C.

This study evaluates freshness using pH and optical color changes, which are important markers for perishable items like chicken breast. These approaches are fast and non-invasive for monitoring decomposition, but they don't show microbial activity, which is crucial to food deterioration. The pH change and color shift are indirect indicators for decomposition, but they may not indicate the presence or abundance of specific spoilage microbes, which might affect food safety and shelf life. Microbiological analysis, such as total viable count (TVC) and specific spoilage organisms' identification, can directly quantify bacterial growth and identify pathogens or spoilage microbes, making spoilage assessment more accurate. An integrated sensory and microbiological approach might improve the freshness indication.

### Conclusions

The BPG extract comprises anthocyanin chemicals that exhibit pH sensitivity when integrated into chitosan-cassava starch-based films. The BPG extract and film exhibited a color transition from bluish purple to greenish at pH levels ranging from 2 - 11. The incorporation of BPG extract markedly enhanced the thickness, solubility, water content, water vapor transmission rate (WVTR), and water vapor permeability (WVP). This intelligent packaging film exhibits significant antioxidant activity and an anthocyanin content that increases with the addition of extract. The intelligent packaging material exhibited a color change in response to pH alterations in the chicken breast, serving as a freshness sensor. The film's color alteration to greenish signified that the chicken breast was no longer fit for ingestion. This research resulted in the development of a food freshness sensor utilizing butterfly pea flower extract, which exhibited a distinct color change attributed to co-pigmentation with gambier.

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The authors acknowledge the use of generative AI tools (e.g., QuillBot and Grammarly) in the preparation of this manuscript, specifically for language editing and grammar correction. No content generation or data interpretation was performed by AI. The authors take full responsibility for the content and conclusions of this work.

### CRedit author statement

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