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Research Article

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Effect of Soy Protein Isolate (SPI) Concentration on Anthocyanin-Based Smart Indicator Labels from Purple Sweet Potato Waste

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Abstract

In Indonesia, a high level of food waste is experienced, with up to 30% of the total fruit production being discarded. Smart packaging, such as anthocyanin-based freshness indicator labels and soy protein isolate (SPI), can help reduce waste by providing visual information about the product's condition within the packaging. This research aims to determine the effect of SPI concentrations on the characteristics of anthocyanin-based smart packaging indicator labels extracted from purple sweet potato waste (Ipomoea batatas (L.) Lam). The indicator labels were fabricated using cornstarch (2.5%) as the matrix, glycerol as the plasticizer, and SPI as the crosslinking agent. A completely randomized design was employed with SPI concentrations of 0%, 1%, 2%, 3%, and 4%. Several parameters on the indicator labels were tested, including pH sensitivity, color stability against storage temperature (4 and 25°C), water absorption capacity, color reversibility, and biodegradability. The results indicated that the addition of SPI affected the label characteristics, with a 1% SPI concentration yielding the best performance. The 1% SPI concentration obtained the lowest color stability value at cold temperatures (ΔE^*ab = 5.15), the highest biodegradation rate (97.33% on day 4), the most varied color response to pH, and good physical durability of the label. Therefore, a 1% SPI concentration is recommended as the most effective formulation for an anthocyanin-based freshness indicator label.

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1. Introduction

Fruit consumption is essential for health (Kompas, 2022), with the World Health Organization (WHO; 2023) recommending 400 g of fruit consumption per capita per day. However, fruit consumption in Indonesia remains low, at 88.56 g per capita per day. Bappenas (2021) stated that during the period 2000-2019, food waste in Indonesia reached 23-48 million tons/year, with fruit being the largest contributor (20%) (Databoks, 2021). The National Research and Innovation Agency (BRIN) revealed that 30% of fruit production is wasted. Indonesia's low fruit consumption is partly attributed to the perception that fruit preparation is time-consuming, combined with increasingly fast-paced lifestyles that favor convenience.

To address this issue, supermarkets now offer pre-cut and ready-to-eat fruits. In addition to practicality, consumers value the safety and freshness of the cut fruit. Cut fruits are generally packaged in plain polypropylene (PP) or polyethylene (PE) containers without freshness indicators, limiting consumer assessment to external appearance alone. (Frasiska et al., 2022).

Smart packaging can be used to monitor fruit freshness in real time. Smart packaging features pH-sensitive indicator labels that detect volatile compounds that affect the acidity/alkalinity of the environment within the packaging. The resulting color change is an indicator of the quality of packaged food. Many previous studies have used synthetic materials such as low-density polyethylene (LDPE), polypropylene (PP), and polyethylene (PE) to create freshness indicator films. Zia et al. (2019) used LDPE-based media as freshness indicator labels. Although LDPE is often used because it is inexpensive and has good mechanical properties, it can take decades to hundreds of years to completely degrade (Singh et al., 2020).

Recent studies have focused on creating polysaccharide-based freshness indicator films. Starch is an alternative to these films because it is biodegradable, inexpensive, environmentally friendly, forms good films, and has high oxygen resistance (Li et al., 2022). However, starch has drawbacks, including low mechanical strength, high water solubility, and poor moisture resistance; therefore, a cross-linking agent is needed to improve its mechanical properties (Wang et al., 2024).

Anthocyanins serve as effective colorimetric sensors and are often utilized in active packaging to indicate the freshness of food products. They change color in response to variations in pH, reflecting the underlying biochemical changes in food related to freshness and spoilage (Chen et al., 2023; Remedio & Parada Quinayá, 2024; Yue et al., 2022). For instance, recent innovations have produced pH-sensitive films from natural sources, such as Clitoria ternatea and red cabbage, which exhibit robust color changes across a wide pH range, facilitating real-time monitoring of food quality (Abedi-Firoozjah et al., 2022; Remedio & Parada Quinayá, 2024).

Soy protein isolate (SPI) has emerged as a promising candidate for enhancing anthocyanin-based indicators. Its biocompatibility, film-forming ability, and inherent properties as a natural biomaterial make SPI an attractive choice for packaging applications. When used as a matrix, SPI has been shown

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to improve the mechanical properties and stability of biopolymer films (Chiu & Yang, 2024; Hasan et al., 2024; Koshy et al., 2022). For instance, combining SPI with anthocyanins in a film matrix can stabilize pigments against degradation by environmental factors.

To the best of our knowledge, no previous studies have determined the optimal concentration of SPI as a binding agent for freshness indicator labels on cut fruit. Therefore, this study aimed to determine the optimal concentration of SPI for freshness indicator labels.

2. Material and Methods

2.1 Research Design

This research was conducted at the Biomass Laboratory and Natural Products Analysis Instrumentation Laboratory, both located at Labtek IA, Bandung Institute of Technology, Jatinangor Campus. A completely randomized design (CRD) was used in this study because the environmental conditions were uniform. Variations in SPI concentration were the main treatments in this study, with five concentration levels: 0%, 1%, 2%, 3%, and 4%. Each treatment was performed four times. This study aimed to evaluate the effect of SPI concentration on the characteristics of anthocyanin-based indicator labels, including their physical and mechanical properties, and pH sensitivity. To determine the response to the SPI concentration treatment, color stability was assessed at different temperatures, pH sensitivity, color reversibility, water absorption, and biodegradability of the samples. The data obtained were analyzed using analysis of variance (ANOVA) at a 95% confidence level, followed by Duncan's multiple range test (DMRT). All analyses were performed using Minitab software.

2.2 Research Procedure

First, the tools and materials were prepared. All tools were calibrated, and the materials were prepared according to standard laboratory procedures. Freshness indicator films were prepared by dissolving a mixture of 2.5 g of cornstarch, 1 mL of glycerol, and SPI (0% (without SPI), 1% (1 g), 2% (2 g), and 3% (3 g)) in 100 mL of distilled water. The mixture was heated while stirring at 80°C for 1 h. The solution was then cooled to room temperature (~25°C). Next, 1 mL of anthocyanin from the purple sweet potato extract was added to the solution and stirred until homogeneous. The solution was then adjusted to pH 3.0 using HCl solution. The solution was sonicated for 5 min to remove any trapped air bubbles. Subsequently, 60 mL of the solution was poured into a 15 cm diameter Petri dish and leveled. The Petri dishes containing the solution were dried in an oven at 50°C for 24 h until a dry film was formed, and the resulting film was cut into 1 cm ⊚ 2 cm pieces. The film pieces were then stored at room temperature for 24 h before testing. Next, an indicator label was created and tested.

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2.3 Analysis Measurements

2.3.1 Color stability of indicator labels at different temperatures

This parameter was analyzed using the method described by Tan et al. (2024). Indicator labels can be used to assess food freshness by changing color according to pH and storage temperature. In this study, the initial color values of the indicator labels were measured using a CS-10 colorimeter, and each label was then placed in the packaging and stored at 4 and 25°C for 10 days. Color changes were recorded every 2 days (L*, a*, and b*) and the total color difference (ΔE) was calculated using Equation 1.

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

When *ΔL = L − L*; Δa = a − a*; Δb = b − b*

2.3.2 Sensitivity of indicator labels to various pH levels

The sensitivity test was conducted following the procedures described by Zhang et al. (2021) to evaluate the label's response to pH changes. The labels were immersed in pH 2–11 buffer solutions for 3 min, and the color values (L*, a*, b*) were measured using a CS-10 colorimeter. The total color difference (ΔE) was calculated using the following equation: 1, where ΔE > 5 indicates a visible color change.

2.3.3 Reversibility of freshness indicator labels color

Color reversibility was evaluated following Rusdianto et al. (2021) by exposing the labels to alternating acidic and basic pH conditions. The labels were treated with buffer solutions of different pH for 24 h, alternating between acidic and alkaline conditions. Color changes (L*, a*, and b*) were measured after each pH adjustment and the total color difference (ΔE) was calculated using Equation 1.

2.3.4 Water absorption capacity

Water absorption was measured according to Ke et al. (2024) and ASTM D570. The initial weights of the labels were recorded, and the labels were immersed in distilled water for 1 min, dried, and weighed again. The water absorption capacity of the labels was calculated using Equation 2.

$$\text{Water absorption capacity } (\%) = \frac{W_1 - W_0}{W_0} \times 100\% \quad (2)$$

Where: W_1 = Final sample weight W_0 = Initial sample weight

2.3.5 Biodegradability

Biodegradability testing was performed according to Ke et al. (2024). The initial label weight was measured, and the labels were buried in soil at a depth of ~10 cm for 5 days. Daily weight changes were recorded, and biodegradability was calculated using Equation 3.

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$$\text{Biodegradability (\%)} = \frac{W_1 - W_0}{W_0} \times 100\% \quad (3)$$

Where: W1 = Final sample weight W0 = Initial sample weight

2.3.6 Stastical analysis

Data were analyzed using analysis of variance (ANOVA; $\alpha = 0.05$) to evaluate the effect of SPI concentration (0–4%) on the pH sensitivity, reversibility, stability, water absorption, and biodegradability of the measured indicator label characteristics. Significant differences were further examined using Duncan's multiple range test (DMRT) at the 5% level of significance. The analysis was performed using MiniTab.

3. Results and Discussion

3.1 Color Stability of the Freshness Indicator Labels at Room Temperature (25^{0}C)

The observed changes in the color intensity of untreated indicator labels indicated a continuous increase in ΔE*ab values, ultimately reaching 13.33 after 10 days of storage (Figure 1). This consistent increase suggests that untreated anthocyanin indicators are prone to degradation, leading to significant perceptible color changes that reflect the potential loss of functionality (Abedi-Firoozjah et al., 2022). This result aligns with the literature, indicating that anthocyanin stability can be severely compromised under various storage conditions due to factors such as temperature, light, and pH, which can catalyze degradation (Luo et al., 2022).

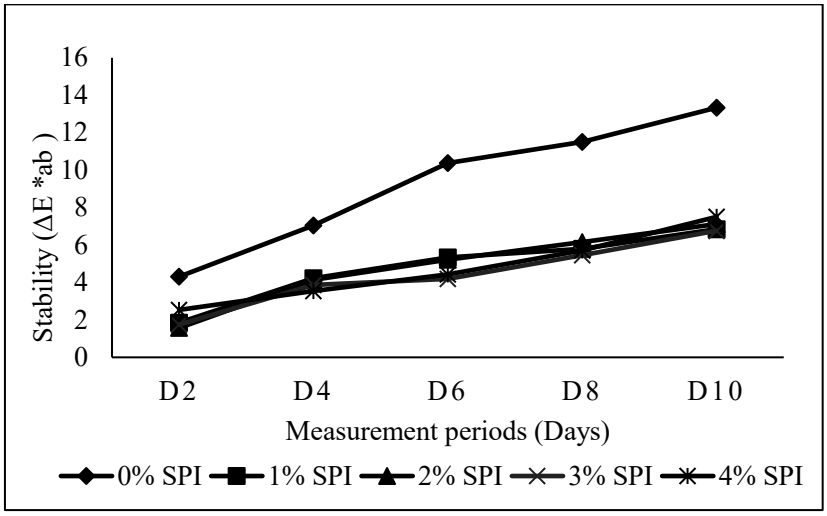

Figure 1. Color stability graph of freshness indicator labels when stored at room temperature (25°C)

Conversely, the anthocyanin-based indicators treated with SPI demonstrated a significantly lower overall color change, with the highest recorded ΔE*ab value of 7.50 at a 4% SPI concentration. This finding suggests that incorporating SPI into the indicator system may confer a stabilizing effect, buffering against the rapid degradation typically observed in untreated indicators (Ścibisz & Ziarno,

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2023; Zhao et al., 2023). Notably, the ΔE*ab values of the SPI-treated labels did not differ significantly among concentrations, indicating a plateau in the stabilizing effect of SPI (including lower and higher concentrations of SPI) within the tested range, indicating a plateau effect in color stability that may be optimal for practical applications (Raharjo et al., 2019; Santoso et al., 2023).

3.2 Color Stability of the Freshness Indicator Labels at a Cold Temperature (4°C)

Figure 2 shows that on the second day of testing, treatment with 0% SPI concentration showed the greatest color change, and its ΔE*ab value of 3.23 was higher than all other SPI concentration treatments, namely 1.55 (1% SPI), 1.69 (2% SPI), 1.83 (3% SPI), and 1.43 (4% SPI).

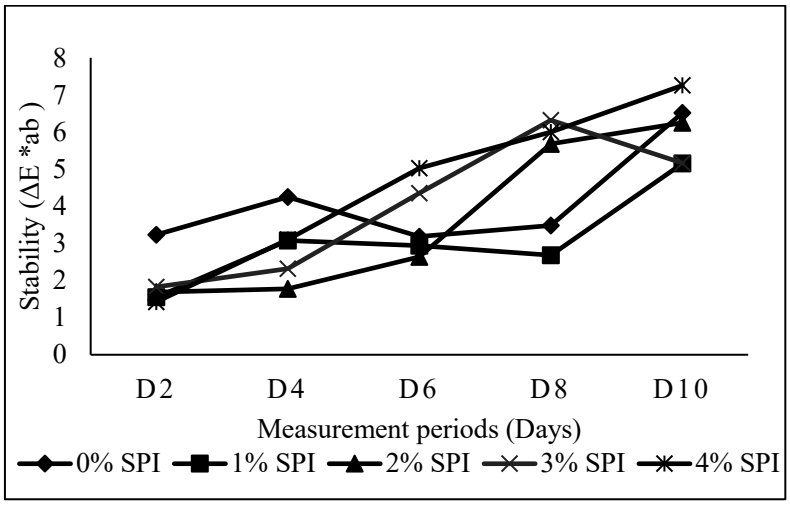

Figure 2. Color stability graph of freshness indicator labels during cold storage (4°C)

This outcome suggests that the untreated labels quickly undergo color changes, likely reflecting pH alterations or degradation of anthocyanin stability due to environmental factors or intrinsic instability (Kishore et al., 2024). In contrast, SPI-treated labels demonstrated better color retention, which could be attributed to SPI's film-forming ability, which provides a protective barrier for anthocyanins against environmental factors that contribute to color degradation (Jang et al., 2025; Leandro et al., 2024).

The findings indicate a threshold relationship regarding the influence of SPI concentration on color stability. While SPI addition generally enhances stability, the variations in ΔE*ab among the different SPI concentrations were not statistically significant, indicating that higher concentrations do not correlate with enhanced stability beyond a certain point (Özünlü & Ergezer, 2022). This observation supports earlier research suggesting that specific biopolymer matrices improve the overall colorimetric response and stability of anthocyanins incorporated into films (Lin et al., 2019; Tan et al., 2024b; Zhao et al., 2023).

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3.3 Comparison of Color Stability at Room Temperature (25°C) and at a Cold Temperature (4°C)

The test results revealed that the color change (ΔE*ab) was greater at room temperature than at cold temperatures. The color change at cold temperatures was also relatively stable, despite fluctuations. The trend in Figure 1 shows that the labels stored at room temperature exhibited a consistent increase in ΔE*ab values, indicating that the color of the freshness indicator became increasingly visible. At lower temperatures, although there was a color change, it was less pronounced than that at room temperature. This finding indicates that lower temperatures are more effective in maintaining color stability.

As reported by Enaru et al. (2021), the color of the freshness indicator label at room temperature is unstable because the anthocyanins contained in the label are flavonoid pigments that are sensitive to high temperatures, pH, and light. At low temperatures, the rate of chemical reactions can decrease due to reduced molecular kinetic energy, thereby slowing the chemical and enzymatic degradation processes that lead to color changes or structural damage to anthocyanins.

This finding aligns with Cheng et al. (2022), who reported that freshness indicator labels showed better color stability when stored at low temperatures than at room temperature. Sun et al. (2022) also showed that freshness indicator labels remained stable at 4°C until day 16, whereas those stored at 25°C remained stable only until day 3 of testing.

Although the color of the freshness indicator label remained stable at low temperatures, the labels in both conditions underwent color changes, as indicated by an increase in the ΔE*ab value. This change occurred because the freshness indicator labels in both conditions were exposed to light during storage. According to Enaru et al. (2021), light exposure can accelerate anthocyanin degradation and stimulate oxidation reactions that can damage the structure. Therefore, although low temperatures can slow the rate of label color change, color degradation still occurs because of light exposure.

3.4 Biodegradability of the Freshness Indicator Labels

The results of the DMRT test (Table 1) indicated a significant difference between the freshness indicator labels and SPI concentrations.

Table 1. Results of the biodegradability test for freshness indicator labels.

No.	Treatment	Biodegradability of freshness indicator labels (%)				
		H1	H2	H3	H4	H5
1	0% SPI	19.71 ± 1.55^{a}	38.60 ± 3.29^{a}	59.18 ± 6.06^{a}	81.70 ± 6.39^{a}	97.70 ± 3.98^{b}
2	1% SPI	22.42 ± 3.16^{ab}	54.37 ± 1.53^{b}	67.95 ± 4.31^{b}	97.33 ± 2.67^{b}	100 ± 0.00^{b}
3	2% SPI	27.23 ± 5.08^{bc}	54.67 ± 4.68^{b}	68.47 ± 4.17^{b}	94.81 ± 5.57^{b}	100 ± 0.00^{b}
4	3% SPI	29.49 ± 2.24^{c}	56.21 ± 7.04^{b}	72.07 ± 4.04^{b}	93.61 ± 6,43^{b}	100 ± 0.00^{b}
5	4% SPI	29.93 ± 4.04^{c}	55.42 ± 2.45^{b}	75.72 ± 2.47^{b}	96.36 ± 3.81^{b}	100 ± 0.00^{b}

Note: Different letters in the same column indicate a significant difference ($P < 0.05$) according to the Duncan's test.

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The biodegradability test results showed that the treatment with 0% SPI had the lowest biodegradability value among the SPI concentrations (Table 1). In contrast, the samples containing SPI exhibited higher biodegradability. Figure 3 shows that the higher the SPI concentration, the higher the biodegradability value. This trend may be attributed to the increased availability of protein substrates for microbial degradation as the SPI concentration increases. Additionally, higher SPI levels can stimulate microorganisms to produce more proteolytic enzymes because they have more substrates to break down. Consequently, higher SPI concentrations may enhance enzymatic activity, thereby accelerating biodegradation (Zink et al. 2016).

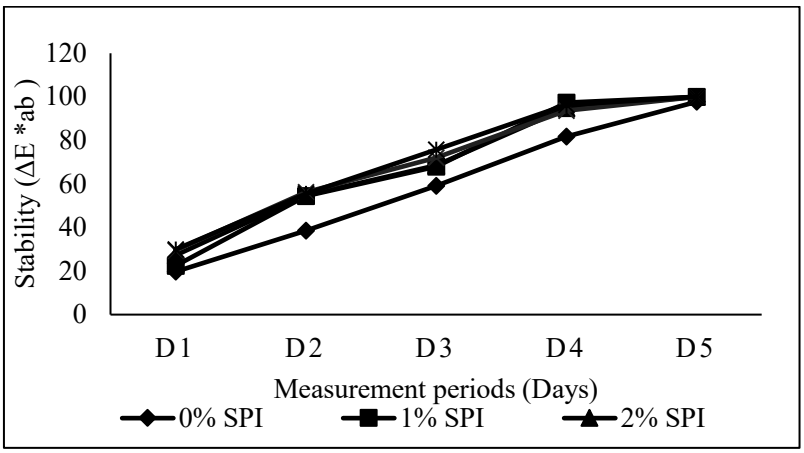

Figure 3. Graph of biodegradability measurements of freshness indicator labels.

The label degradation process begins when the label absorbs water from the soil, causing the protein structure on the label to swell and open, making it more accessible to microorganisms (Álvarez-Castillo et al., 2021). These soil microorganisms produce proteolytic enzymes that cleave peptide bonds in SPI proteins, yielding smaller polypeptides or amino acids. These products are then used as sources of carbon and nitrogen, which further supports microbial growth and accelerates the degradation of the label until most of the material is broken down and disappears (Silva et al., 2023).

3.5 Analysis of the water absorption capacity of the freshness indicator labels

Figure 4 shows that the water absorption capacity of the freshness indicator label decreased from 0% SPI (62.31%) to 1% SPI (52.51%) and then to 2% SPI (48.78%) and increased for 3% SPI (52.28%) and 4% SPI (51.52%). The lowest water absorption capacity was observed in the 2% SPI treatment and the highest in the 0% SPI treatment. This finding aligns with Zhang et al. (2021), who reported that increasing the SPI concentration in film-forming materials decreases the water absorption capacity because the hydrophobic nature of the protein can reduce its water-absorbing capacity. The decrease in water absorption capacity is consistent with the addition of protein, which strengthens the film structure and reduces water penetration into the film.

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The increase in water absorption at 3% and 4% SPI concentrations may be due to other factors affecting film structure. Gao et al. (2020) reported that higher protein concentrations can form looser aggregates, which can increase the water absorption capacity.

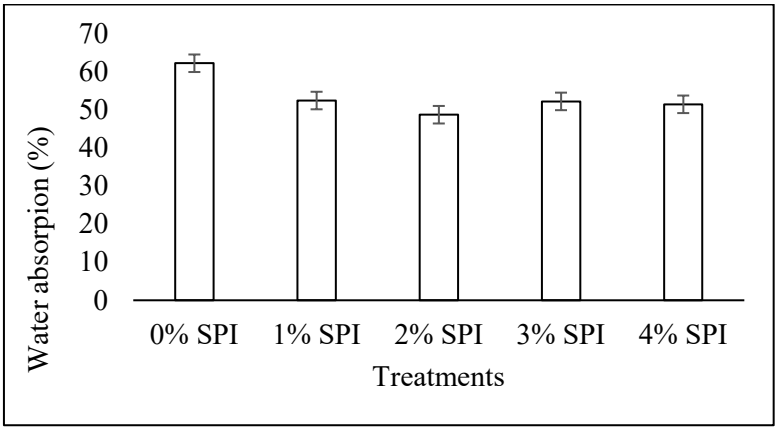

Figure 4. Water absorption graph of freshness indicator labels.

3.6 Analysis of the reversibility of the freshness indicator labels in acidic conditions

The color change of the freshness indicator labels was assessed for reversibility and stability when treated with several concentrations of SPI and tested under acidic (Table 2) and alkaline (Table 3) conditions. Table 4 shows the ΔE*ab values for color comparison between the initial and final films at pH 7 under acidic and alkaline conditions. Under acidic conditions, the ΔE*ab values for the 0, 1, 2, 3, and 4% SPI treatments were 10.76, 7.89, 2% SPI was 7.28, 3% SPI was 8.03, and 4% SPI was 8.65. The one-way ANOVA and DMRT post-hoc tests also showed a significant difference in color change between the 0% treatment and the 1%, 2%, 3%, and 4% treatments under acidic conditions.

Table 2. Color change of the freshness indicator labels under acidic conditions.

No.	Treatments	Start	pH 7	pH 4	pH 1	pH 7
1	0% SPI					
2	1% SPI					
3	2% SPI					
4	3% SPI					
5	4% SPI					

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Table 3. Color changes in the freshness indicator labels under alkaline conditions.

No.	Treatments	Start	pH 7	pH 9	pH 11	pH 7
1	0% SPI					
2	1% SPI					
3	2% SPI					
4	3% SPI					
5	4% SPI					

Statistical analysis revealed that only the 0% SPI treatment showed a significant difference compared to the other treatments under alkaline conditions. This result indicates that the color change between SPI treatments under alkaline conditions was relatively similar. When comparing the two conditions, the ΔE*ab value under acidic conditions was smaller (i.e., more stable) than that under alkaline conditions, indicating that the indicator label was more stable in response to pH changes under acidic conditions.

Table 4 shows that the indicator label with 0% SPI treatment under both acidic and alkaline conditions had the highest ΔE*ab value. In addition, it can be observed that the anthocyanin color on the labels with 0% SPI concentration faded under both acidic and alkaline conditions. In contrast, the SPI labels at 1%, 2%, 3%, and 4% retained their original anthocyanin color. This finding is consistent with Wang et al. (2024), who reported that SPI can stabilize label color by reducing the solubility of anthocyanins and preventing them from detaching from the label.

Table 4. Analysis of the reversibility of the freshness indicator label color at pH 7 under acidic and alkaline conditions

No	Treatments	Reversibility (ΔE*ab)	
		Acidic condition	Alkaline condition
1	0% SPI	10.76 ± 0.76^{b}	17.9 ± 0.89^{b}
2	1% SPI	7.89 ± 1.05^{a}	13.38 ± 0.29^{a}
3	2% SPI	7.28 ± 0.41^{a}	14.47 ± 0.76^{a}
4	3% SPI	8.03 ± 0.82^{a}	13.61 ± 0.81^{a}
5	4% SPI	8.65 ± 0.71^{a}	13.05 ± 0.88^{a}

Note: Different letters in the same column indicate significant differences ($P < 0.05$) according to Duncan's test.

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Under acidic conditions, anthocyanins tend to be in the flavylium cation form, which imparts a bright red color to the label. Under these conditions, the hydroxyl group on the flavonoid ring of anthocyanins is protonated, increasing their stability at a low pH (Dangles & Fenger, 2018). Under alkaline conditions, anthocyanins change into their anionic form by losing protons from the hydroxyl groups on their flavonoid rings. At high pH, anthocyanins degrade more rapidly through hydrolysis, resulting in the loss of structure and color. This degradation can produce colorless or pale compounds, thereby causing the label color to turn blue-green (Wahyuningsih et al., 2017).

Extreme pH changes, such as pH 1 and pH 11, affect the stability of the indicator labels. Therefore, the longer a label is soaked in a buffer solution (under acidic or alkaline conditions), the more likely it is that labels containing more than 1% SPI will become damaged or torn. Under extreme pH conditions, such as pH 1, a label can undergo denaturation due to the protonation of amino acid groups, leading to protein conformational changes and aggregate formation. Similarly, at pH 11, a label can undergo denaturation, thereby increasing its solubility (Tan et al., 2024a).

The higher ΔE*ab value under acidic conditions compared to that under alkaline conditions may be attributed to the higher stability of the flavylium cation form at low pH, which can maintain the red color of anthocyanins. In contrast, at high pH or in an alkaline environment, anthocyanins undergo deprotonation to colorless forms, such as carbinol or chalcone, resulting in a color change and reduced color stability (Khoo et al., 2017).

3.7 Sensitivity analysis of the freshness indicator labels to pH changes

The test results showed that the indicator label in all treatments changed from red to green as the pH increased from 2 to 11. At pH 2, the indicator label showed red, which then turned pink at pH 3 and 4. When soaked in buffer solutions at pH 5–6, the label color changed from pink to brownish and then to greenish-brown at pH 7–8. When soaked at pH 9 to 10, the label showed an increasingly intense green color. However, at pH 11, the label color faded to pale green. The test results are consistent with those of Choi et al. (2017), who reported that a positive a* value indicates a tendency toward red, whereas a negative a* value indicates a shift toward green. Overall, for each treatment, the film color changed from red to brown at pH 2 – 6, then to brown-green at pH 7 – 8, and finally to bright green at pH 9 –10. The b* parameter values showed a similar pattern, where the film color changed from yellow (values above zero) to blue (values below zero).

Changes in color are caused by pH-induced changes in the molecular structure of anthocyanins (Ke et al., 2024). Under very acidic conditions (pH < 2), anthocyanins are in the form of flavylium cations, which gives a red color (Khoo et al., 2017). As the pH increases from 2 to 4, protons begin to detach, producing a blue quinonoidal form. At pH 5 - 6, the color intensity begins to decrease due to the hydration of flavylium cations, forming a colorless carbinol pseudobase and open chalcone (Enaru et al., 2021). Within the pH range of 4–6, the flavylium, quinonoidal, carbinol, and chalcone forms are

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in equilibrium. Further pH increases (pH 8 – 9) lead to the formation of anhydrobase structures, which also cause color changes in the solution and the indicator film (Kossyvaki et al., 2022). At pH 11, the label appears pale because it has changed to the colorless chalcone form, which is more water-soluble.

Table 5. Sensitivity of freshness indicator labels to pH changes.

pH	0% SPI	1% SPI	2% SPI	3% SPI	4% SPI
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					

4. Conclusion

The addition of SPI improved the stability and performance of anthocyanin-based freshness indicator labels fabricated from purple sweet potato waste. A 1% SPI concentration was the optimal formulation, providing good color stability during storage (particularly at low temperatures), high pH sensitivity, adequate physical strength, and rapid biodegradability. The color change of the indicator reliably reflected freshness-related pH changes in the package environment, linking label performance directly to fruit quality during storage. These findings demonstrate that the 1% SPI-based indicator is a promising and sustainable tool for real-time monitoring of fruit freshness.

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6. AI Writing Statement

The author did not use any generative AI tools in writing this article. The introduction, analysis, data interpretation, and conclusion are the result of the author's own work.

7. References

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