Comparison of Carriers in Anthocyanin Smart Packaging from Water Hyacinth Flower for Soy Milk Freshness

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ABSTRACT

Soy milk is highly susceptible to spoilage, making the detection of freshness essential. This study investigates the use of anthocyanin extract from water hyacinth flowers as a natural pH indicator in smart packaging. Water hyacinth, often considered a waste product, was selected due to its rarely studied anthocyanin content and eco-friendly benefits. The effectiveness of two biopolymer carriers—starch and cellulose—was evaluated in terms of their ability to support anthocyanin-based indicators. The parameters tested included organoleptic properties, moisture absorption, FTIR characterization, and real-time pH responsiveness in soy milk over 48 hours. Results showed that both starch and cellulose carriers could indicate pH changes via visible colour shifts, particularly at pH 9 and 40°C, where spoilage progresses faster. Starch demonstrated greater chromatic contrast than cellulose. FTIR analysis revealed more intense absorption bands in starch (1416 cm^{-1} for C=C and 1646 cm^{-1} for C=O), indicating better interaction with anthocyanins. RGB intensity analysis confirmed the ability of both carriers to detect spoilage, but Euclidean Distance (ED) measurements highlighted the superior sensitivity of starch. At pH 9, the ED value for starch reached 85.67, compared to only 33.50 for cellulose. These findings demonstrate that starch is a more effective carrier material than cellulose for anthocyanin-based smart packaging. It offers better sensitivity, colour contrast, and stability in detecting soy milk freshness, especially under higher temperature conditions. This innovation not only promotes food safety but also provides an eco-friendly use for invasive plant species, such as water hyacinth.

1. Introduction

Soy milk is a plant-based beverage derived from soybeans, rich in high-quality protein, unsaturated fats, and phytochemicals, including isoflavones and phytosterols. This makes soy milk a suitable alternative to animal-based milk [1]. Soy milk is highly beneficial for health, as it contains dietary fiber and is cholesterol-free. Additionally, since it does not contain lactose, soy milk is safe for consumption by individuals with lactose intolerance [2], [3], [4]. Soy milk contains lower levels of saturated fat compared to cow's milk and can be fortified to provide essential nutrients such as calcium and vitamin D [5]. Due to its high nutritional content and the absence of lactose, soy milk has a relatively short shelf life, lasting only about one day at room temperature, or even less [6]. Pathogenic microorganisms such as bacteria and fungi can contaminate soy milk, resulting in noticeable changes in taste, texture, color, and odor [7], [8]. During food production, poor hygiene practices can lead to microbial contamination, accelerating spoilage and further posing risks to consumer health [9], [10]. When dealing with this, consumers are advised to assess the freshness of

packaged food by observing any color changes that may be caused by potential microbial contamination or chemical reactions [11].

Smart packaging incorporates sensors that monitor the condition of food products and the internal environment of the packaging. For products like milk, for example, expiration dates alone are regarded as insufficient; thus, such indicators are used to define food condition and enable consumers to assess its quality and freshness directly [12], [13]. According to previous research [14], to ensure safety and facilitate quality control, various chemical sensors have been developed, including colorimetric indicator labels that are directly integrated into smart packaging. Through shifts in pH and temperature within the packaging environment, smart packaging provides early information to both consumers and food producers regarding food spoilage [15].

Among the various technologies embedded in smart packaging, colorimetric indicators are especially appealing due to their simplicity and visual immediacy. One of the key sensory characteristics influencing the quality and acceptance of a food product is color [16]. While pigments and dyes are commonly used as pH sensors, due to their toxic properties, synthetic dyes are increasingly being replaced by natural dyes, which are considered safer [17], [18]. Among natural pigments such as curcumin, betalain, and carotenoids, anthocyanins have been widely used in smart food packaging and colorimetric pH indicator devices, as they have a non-toxic nature and a distinctive ability to change color in response to pH variations [19], [20], [21]. According to previous research [22], Water hyacinth can be extracted using various solvents and tested for its phytochemical content (anthocyanins), which can be detected in acidic or aqueous extracts.

The use of anthocyanin extract from water hyacinth flowers is an appealing option because it is not only naturally safe but also practical. Water hyacinths, which often grow abundantly in polluted water bodies, can also serve as natural indicators of water quality [23], [24]. One of the active compounds found in water hyacinth flowers is anthocyanin, which remains relatively underexplored in smart packaging applications. According to a study conducted by Priyanka and Jayakumari [25]Water hyacinth flowers have been used as a natural dye for fabrics. These flowers, which exhibit a purple hue, contain anthocyanins with a single delphinine glycoside, making them suitable for dye extraction. The ionic structure of anthocyanins in solution varies depending on the pH level [26]. Anthocyanins produce a red color in acidic conditions (low pH) and shift to purple or blue in alkaline conditions (high pH) [27]. In consideration of environmental pollution and the risks posed by synthetic polymers, various materials such as polysaccharides are being used as matrices for anthocyanin-rich packaging films. Therefore, this study focused on biodegradable packaging made from natural materials, specifically tapioca starch and cellulose, which are suitable for pH indicators due to their colorless and flexible properties that do not interfere with the color or chemical structure of anthocyanins. These materials are widely used due to their compatibility with water-soluble substrates and ease of processing [21], [28], [29]. Cellulose acts as a stable matrix with moisture resistance and can improve material flexibility, while starch increases polymer rigidity, forms good films, but has lower gas barrier properties [30].

Previous studies have explored the development of smart packaging for detecting the freshness of sliced watermelon, as seen in the previous research [7], which utilized purple sweet potato extract and tapioca starch-based carrier in smart packaging. Similarly, previous researchers [29], investigated the use of red cabbage as a natural indicator and cellulose-based carrier. However, no research has yet compared different carrier types for water hyacinth flower anthocyanin extract as an indicator label for smart packaging. Therefore, this study aims to determine the most effective carrier type for anthocyanin-based indicator labels in detecting the freshness of soy milk through various analytical tests.

2. Research Methodology

2.1. Materials

The materials used in this research include Eichhornia crassipes (water hyacinth) flowers, soy milk, NaOH (Merck), 37% HCl (SAP), tapioca starch, Whatman No. 1 filter paper, glycerol (local), and distilled water (technical grade), UV-Vis Spectrophotometer, Fourier-Transform Infrared Spectroscopy (FTIR), micrometer screw gauge, analytical balance, 140 mL transparent glass bottles, thread, needle, smartphone, glassware set, hotplate stirrer, magnetic stirrer, universal indicator,

26×16 cm glass plates, oven, baking tray, cutter, ruler, thermometer, tweezers, dark bottles, black box, baking tray, scissors, and adhesive tape.

2.2. Procedures

1) Preparation of Water Hyacinth Flower Anthocyanins

The extraction of anthocyanins from water hyacinth flowers was conducted using the infusion method. A total of 25 g of water hyacinth flowers was added to 250 mL of distilled water and heated to 70°C. The resulting extract was then cooled to room temperature and filtered using Whatman No. 1 filter paper. The filtered extract was stored in a dark bottle to protect it from light exposure and kept in a refrigerator.

The extracted anthocyanin pigment was adjusted to different pH levels (3, 5, and 9) using NaOH for alkaline conditions and HCl for acidic conditions. The pH solution was confirmed using a universal pH indicator. As a blank control, anthocyanin extract at pH 7 was prepared and used for comparison in the subsequent tests. The absorbance was then measured at wavelengths ranging from 400 nm to 800 nm, with a maximum absorbance (λmax) observed between 465 nm and 560 nm using a UV-Vis Spectrophotometer. Distilled water was used as the baseline for UV-Vis's measurements. All procedures were carried out in duplicate (duple).

2) Preparation of Indicator Labels

The starch-based indicator label was prepared by dissolving 4.5 g of tapioca starch and 4.5 g of glycerol in 30 mL of distilled water. The solution was then heated to 75°C while stirring at 50 rpm using a magnetic stirrer until gelatinization occurred. The gelatinized starch was poured onto a 26 cm × 16 cm glass plate and dried at 100°C for 3 hours. Once dried, the starch film was immersed in 25 mL anthocyanin extract at different pH levels and air-dried for 3 hours to ensure proper dye absorption and integration with the indicator film. The indicator film was then cut into 2 cm × 2 cm squares. For the cellulose-based indicator label, Whatman No. 1 filter paper was cut into 2 cm × 2 cm pieces. The paper pieces were then immersed in the 25 mL water hyacinth flower extract for 20 minutes, followed by air-drying at room temperature.

3) Smart Packaging Testing

The indicator label was evaluated through organoleptic testing, moisture uptake analysis, and FTIR characterization. To assess the label's ability to detect soy milk freshness, 30 mL of soy milk was placed in a sealed transparent glass bottle. The indicator label was suspended from the bottle cap, maintaining a distance of approximately 5 cm from the surface of the soy milk without direct contact. The bottles were stored under three different temperature conditions: 4°C (refrigerator), 23°C (room temperature), and 40°C (oven) for 48 hours to monitor color changes due to milk spoilage. The color changes of the indicator label were captured using a smartphone camera and analyzed in a black box setup to obtain RGB values. The observed color changes were scanned and recorded using the Euclidean Distance (ED) equation, where the RGB values were converted into ED values.

3. Results and Discussion

3.1. Anthocyanin Extraction from Water Hyacinth Flowers

The extraction of anthocyanins from water hyacinth flowers was performed using the infusion method. The light purple color of the flowers indicates the presence of anthocyanin compounds. The total anthocyanin content obtained was 3.77 mg cyanidin-3-glucoside/100 g of water hyacinth flower. Anthocyanins are polar compounds, meaning they dissolve easily in water-based solvents like distilled water (aquades) and tartaric acid [31]. Therefore, the infusion method was employed to extract anthocyanins from water hyacinth flowers, as described by Khasanah et al. [32]Heating plant materials (simplisia) in water at 90°C for 15 minutes allows the extraction of active compounds that are water-soluble or polar.

However, anthocyanins are highly unstable pigments. Several factors influence their stability, including temperature, chemical structure, light exposure, water activity, enzymatic activity, metal ions, pressure, and other chemical components [31]. Polyphenol oxidase and peroxidase are plant enzymes that can advance anthocyanin degradation after the extraction [33]. Temperature plays a crucial role in the degradation of anthocyanins, both during storage and processing. To slow down anthocyanin degradation, enzymes should be inactivated by storing the extract at low temperatures.

At elevated temperatures, anthocyanins can break down into their chalcone form, which is colorless, leading to fading and decomposition [34]. Generally, anthocyanins are less stable in neutral or alkaline solutions, and exposure to light accelerates their degradation, resulting in a fading of their color over time. Therefore, storing anthocyanin extracts in low-light and low-temperature conditions is recommended to maintain their stability [35].

3.2. Stability Test of Water Hyacinth Anthocyanin Extract at Different pH Levels

The pH variation significantly influences the color stability of natural anthocyanin extracts [18], [36]. As shown in Fig. 1., the extract undergoes noticeable color changes when treated with HCl and NaOH. This occurs due to the ionic nature of anthocyanin molecules [37]. At pH 3, the extract appears magenta, shifting to purple at pH 5, and turning green at pH 9. These color changes result from structural modifications in anthocyanins under different pH conditions. At higher pH levels, anthocyanins transform into their anhydrobase form, leading to an expansion of delocalized bonds, which intensifies the color change [38].

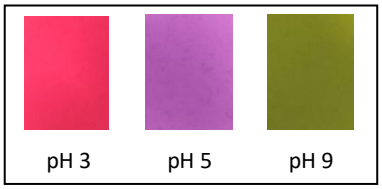

Fig. 1. Color variations of water hyacinth anthocyanin extract

The UV-Vis spectrum of the anthocyanin extract at different pH levels is shown in Fig. 2. at pH 3, the maximum absorption peak occurs at 550 nm, while at pH 5, the peak shifts to 400 nm. At pH 9, the highest absorption is observed at 450 nm. However, at pH 9, the change in absorption wavelength suggests anthocyanin degradation, leading to reduced stability. Fig. 2. illustrates structural changes in anthocyanins, where their form transitions from quinoidal to chalcone [39]. According to previous research [36], anthocyanins remain stable at low pH. In acidic conditions, cyanidin molecules are protonated, forming positively charged ions (H^+) or cations. However, as pH increases, deprotonation occurs, and at high pH levels, the molecule adopts a negative charge (anion). These results indicate that the anthocyanin extract is most stable and effective at pH 3.

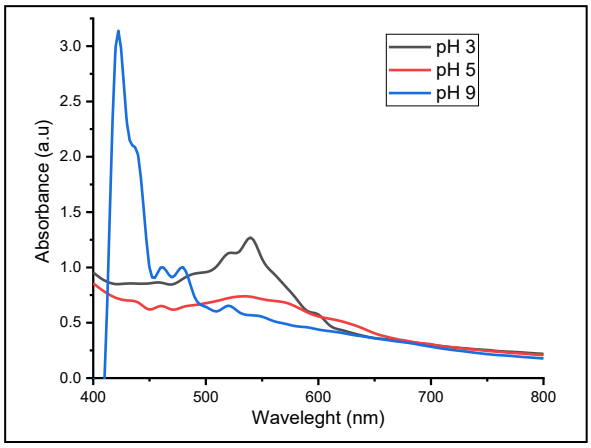

Fig. 2. UV-Vis peak shift with pH, showing stability at pH 3

The structure of anthocyanins is strongly influenced by pH, where they are more stable in acidic conditions than in alkaline ones, as shown in Fig. 3. At pH 3, anthocyanins exhibit greater stability, displaying a magenta color. In this state, they exist in the flavylium cation and carbinol forms. At pH 5, anthocyanins primarily adopt quinoidal and chalcone forms [40]. When pH increases beyond 4, colorless carbinol base and chalcone equilibrium occur [38]. Conversely, at pH 9, the dominant quinoidal form results in a green hue, indicating a more degraded anthocyanin structure [41]. This occurs as anthocyanins are unstable in high-pH conditions, where the pyrilium ring opens and forms a chalcone structure due to easy hydration and oxidation at the C_2 position [42].

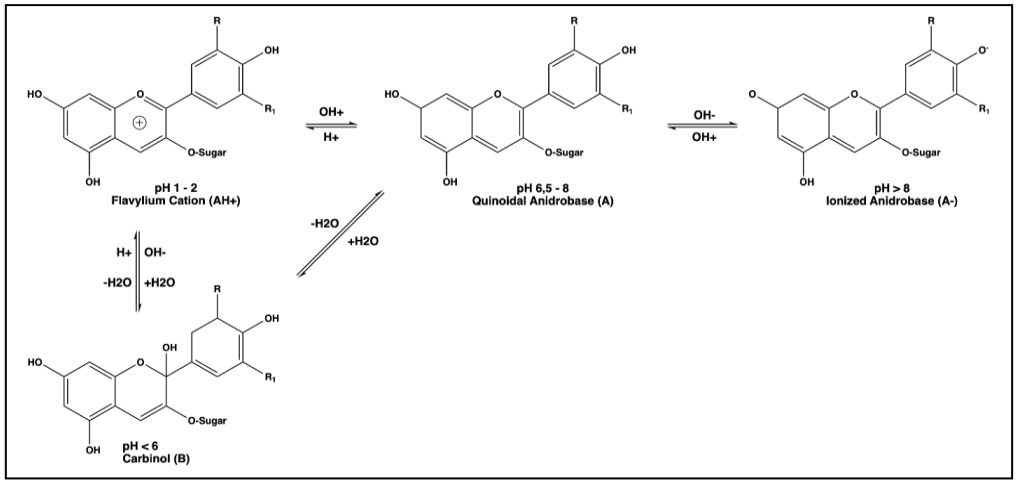

Fig. 3. Structural transformation of anthocyanins at pH 3, 5, and 9 [40]

3.3. Results of Indicator Label Production

The variation in carrier or matrix materials was conducted using starch and cellulose. Based on previous research [43], these two carriers were selected because they serve as effective carrier matrices for sappanwood extract, making them potentially suitable for anthocyanins extracted from water hyacinths. The interaction between anthocyanins and cellulose derivatives results in a softer cellulose matrix, which eventually reduces tensile strength and increases flexibility. On the other hand, interaction with starch tends to increase polymer rigidity and reduce gas permeability [30]. The indicator labels were categorized into two types: standard labels (before immobilization) and immobilized labels. The resulting pH variations produced magenta at pH 3, light purple at pH 5, and light green at pH 9, reflecting the acid-base response of the indicator labels shown in Fig. 4.

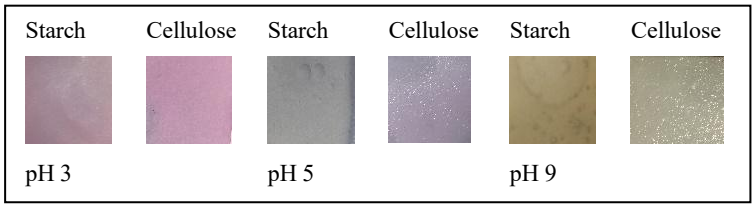

Fig. 4. Color changes of indicator labels according to pH and carrier type

3.4. Indicator Label Specification Testing

The results of organoleptic testing for each type of indicator label carrier, presented both before and after immobilization with water hyacinth anthocyanins, are shown in Table 1. However, differences in color variations were observed. Regarding thickness testing, the starch-based carrier was found to be thicker, which makes it less resistant to moisture and potentially shortens its shelf life and reliability, as shown in Fig. 5. This is likely due to uneven starch distribution during the film-forming process. In contrast, the cellulose-based carrier has a more uniform thickness and lower moisture uptake. The uneven thickness of different sections within the same carrier sheet contributed to variations in weight loss after the drying process. Additionally, the drying process after contact with anthocyanins also influenced the moisture content of the labels. Uneven drying resulted in differences in moisture levels in various areas of the same carrier sheet [43].

Table 1. Results of the Indicator Label Organoleptic Test

Group	Organoleptic test (shape and color)	
	Starch	***Cellulose***
Indicator label without anthocyanin	Square, slightly clear white	Square, white
Indicator label pH 3	Square, magenta	Square, magenta
Indicator label pH 5	Square, light purple	Square, light purple
Indicator label pH 9	Square, light green	Square, greenish purple

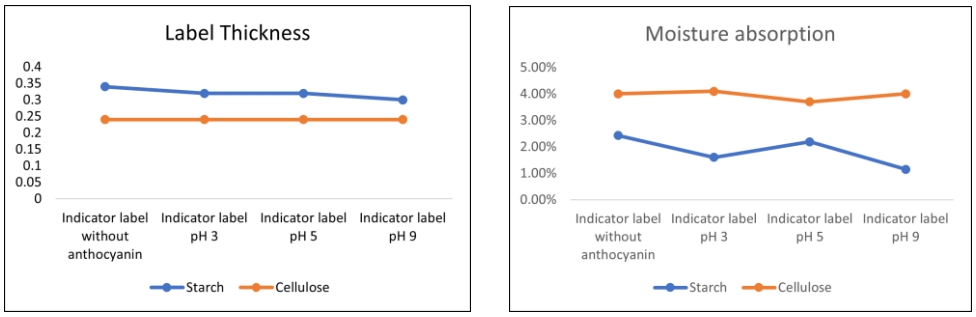

Fig. 5. Thickness and moisture variations affect label stability and reliability

The FTIR functional group analysis revealed significant differences between starch-based and cellulose-based carriers, as shown in Fig. 6. The anthocyanins present in water hyacinth are responsible for the blue and red pigmentation in the plant. Cyanidin is the primary aromatic structure of anthocyanins. For starch-based labels, the O-H functional group was detected at wavenumbers 3262.06 cm^{-1}, 3258.85 cm^{-1}, 3265.63 cm^{-1}, and 3264.04 cm^{-1}, while for cellulose-based labels, the O-H functional group was observed at lower wavenumbers, specifically at 3339.88 cm^{-1}, 3336.56 cm^{-1}, 3337.58 cm^{-1}, and 3337.91 cm^{-1}. the O-H shift hydrogen bonding between anthocyanins and the matrix, because anthocyanin easily form bonds with hydroxyl groups of the matrix. Furthermore, the C=C aromatic structure in the starch-based label was detected at 1416 cm^{-1}, followed by C-H bonds at 760 cm^{-1} and 855 cm^{-1}. Meanwhile, in cellulose-based indicator labels, the C-H bonds were detected at 660 cm^{-1} and 997 cm^{-1}.

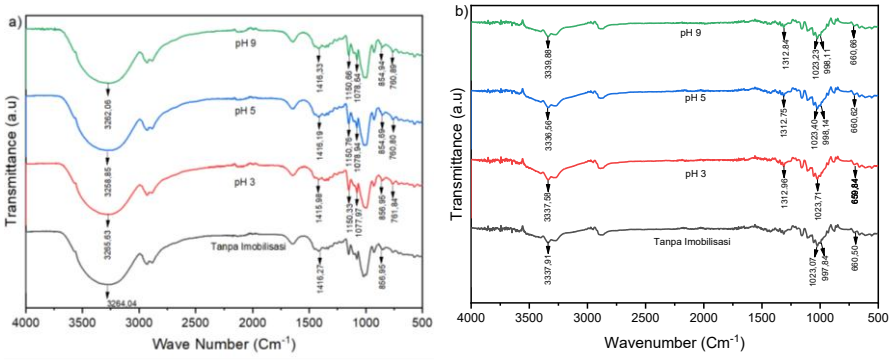

Fig. 6. Starch-based Carrier, (b) Cellulose-based Carrier: showing differences in O-H intensity and the presence of C=O in starch, indicating stronger anthocyanin interaction with the starch matrix

For the starch-based carrier, the absorption bands at 1646.87 cm^{-1}, 1647.54 cm^{-1}, 1646.91 cm^{-1}, and 1647.49 cm^{-1} indicate the presence of a C=O functional group, which likely relates to flavonoid structure in anthocyanin. In contrast, a functional group was not detected in the cellulose-based carrier. The C-O functional group within the anthocyanin glycoside structure was detected at 1150 cm^{-1} and 1078 cm^{-1} for the starch-based label. In contrast, for the cellulose-based label, the observed range was 1023 cm^{-1} – 1312 cm^{-1}. The presence and more vigorous intensity of C=O and C-O bands in starch-based materials suggest stronger interactions, possibly weak chemical or stronger hydrogen bonding. In contrast, in cellulose-based materials, the interactions are mainly physical entrapment with conventional hydrogen bonding. Based on the FTIR analysis, the absorption bands in the starch-based carrier were more intense compared to those in the cellulose-based carrier.

3.5. Smart Packaging Carrier Testing

The indicator label test using water hyacinth flower anthocyanin extract with starch and cellulose carriers is presented in Fig. 7. The indicator label test showed that at pH 9 under 25°C and 40°C, the highest ED increase of 85.6688 occurred at 40°C. For the starch-based carrier, the indicator label at pH 5 exhibited poor performance in detecting soy milk freshness due to indistinguishable color changes, despite an increase in ED values. At pH 3 and temperature 40°C, the color change was less pronounced, with an ED difference of only 3.5909. According to previous research [44], this minor difference does not significantly impact detection, as it is not perceptible to the naked eye. Conversely, for the cellulose-based carrier, at pH 3 and 4°C, with an ED increase of 23.102,

indicating the onset of soy milk spoilage. Although color changes were also observed at 25°C and 40°C, they were not as significant, despite the rising ED values. At pH 5, color changes were observed at all tested temperatures, accompanied by substantial increases in ED and a noticeable transition. For pH 9, the highest ED increase of 33.4957 was observed at 40°C.

Overall, the total ED intensity of both starch and cellulose carriers increased, from before detection to after detecting soy milk freshness. These findings align with previous research [45], who reported that indicator labels fade and brighten when detecting spoiled milk. Based on the results, soy milk freshness can be evaluated following the Indonesian National Standard (SNI) 3141.1:2011, by measuring pH, conducting an organoleptic test, and observing color changes in indicator labels using different carriers (starch and cellulose) combined with water hyacinth anthocyanin extract. The results confirmed that spoiled soy milk could be directly detected using this indicator label, with pH 9 showing the most noticeable color change and the highest ED values for both starch and cellulose carriers.

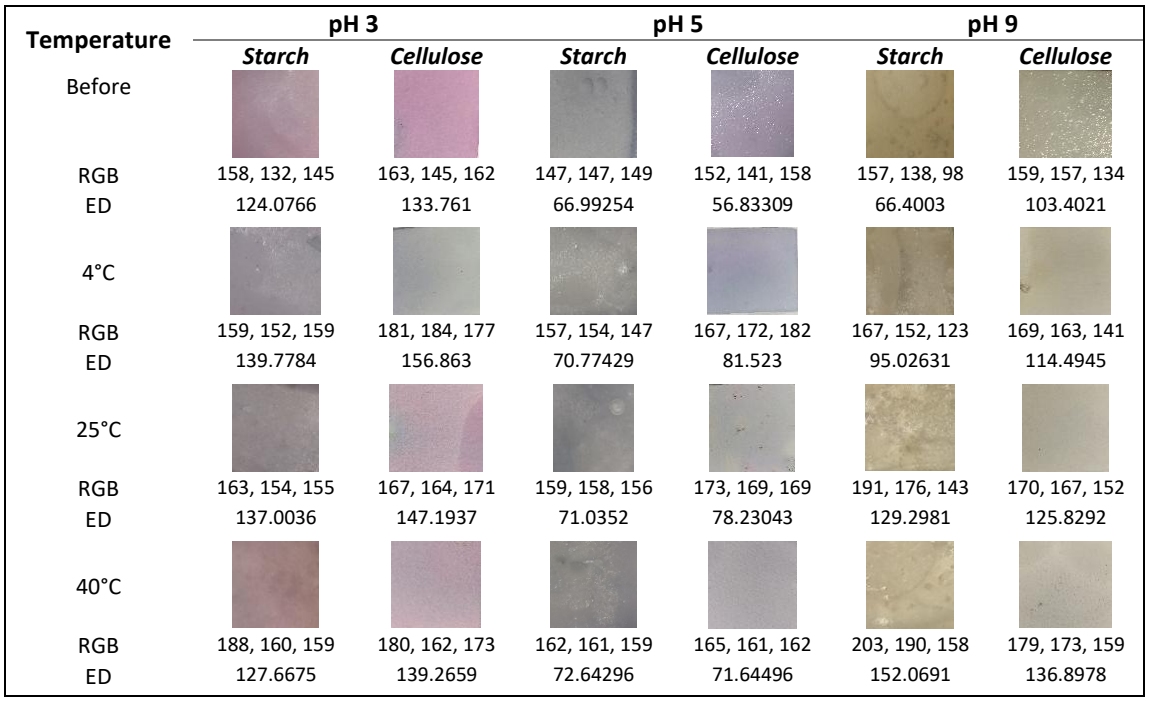

Fig. 7. Color changes and ED values indicate spoilage detection, most distinct at pH 9

4. Conclusion

The use of starch and cellulose as natural carriers for water hyacinth anthocyanin indicator labels has demonstrated effectiveness in detecting the freshness of soy milk, with the best pH-responsive color change observed at pH 9 at 40°C, and the highest ED for starch (85.6688) compared to cellulose (33.4957). Additionally, FTIR absorption bands of starch, particularly O-H and C=O, showed approximately 25% greater intensity than those of cellulose. Therefore, starch is the preferred carrier for water hyacinth anthocyanin application in smart packaging indicator labels. However, the practical difference is moderate. Future research should focus on scaling up production, improving film durability, and evaluating performance under real-world storage and distribution conditions to support commercial application.

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