Original Scientific Paper https://doi.org/10.24867/GRID-2026-p77

ph-Responsive Starch Films Incorporating Purple Sweet Potato Anthocyanins and Bacterial Nanocellulose

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ABSTRACT

This study investigated the development and colorimetric properties of pH-sensitive indicators by incorporating anthocyanins extracted from purple sweet potato into a starch matrix. The ability of pH-sensitive indicators to change color with variations in food conditions enables their application in real-time freshness and safety monitoring of food products. The assumption that purple sweet potato anthocyanins act as pH-sensitive indicators is based on well-established anthocyanin chemistry and experimentally observed color transitions under varying pH. In this study, anthocyanins were extracted from different purple sweet potato sources – fresh flesh, fresh peel, and dried peel – to evaluate their behavior. The aim was to determine which part of the purple sweet potato provides the greatest source of anthocyanins. The obtained extracts were incorporated into starch matrices based on maize and wheat starch. Bacterial nanocellulose was added to each matrix to enhance the functional performance of the produced films. The results of the research showed that fresh flesh extract provided the highest anthocyanin content (296.07 mg/100 g), followed by fresh peel (206.65 mg/100 g) and dried peel (115.72 mg/100 g). The colorimetric and optical differences between the produced films were observed for dry films and after their immersion in buffer solutions with pH values ranging from 3.0 to 10.0. All prepared films exhibited distinct pH-dependent color changes throughout the investigated pH range, with transitions from reddish-pink under acidic conditions through purple near neutral pH to green, blue, and teal shades under alkaline conditions. The fresh flesh extract generally produced the most pronounced color response, while the incorporation of bacterial nanocellulose preserved the pH-responsive behavior and modified the optical and colorimetric properties of the films, with more consistent color trajectories observed for some formulations, especially films with fresh peel extract and 30% bacterial nanocellulose. The findings demonstrate the potential for producing starch-based pH-sensitive films by incorporating anthocyanins extracted from purple sweet potato and bacterial nanocellulose. The developed bio-based films show potential for use as natural visual pH indicators in intelligent food-packaging applications.

KEY WORDS
Smart packaging, pH indicators, anthocyanins, purple sweet potato, BNC, starch matrix

1. Introduction

Smart packaging has emerged as an advanced approach in food packaging technology, providing functions that go beyond the primary roles of conventional packaging, such as product protection, containment, and communication. Unlike traditional packaging systems, smart packaging incorporates indicators, sensors, or other func-

tional components that can provide information about the condition, quality, or safety of the packaged product and its surrounding environment. Among the different types of smart packaging, visual indicators that respond to changes in pH have attracted considerable attention because of their potential to provide a simple, non-destructive means of monitoring food quality and freshness (D'Almeida & de Albuquerque, 2024). Such systems can be particularly useful for detecting changes associated with food deterioration, including the formation of volatile basic compounds and shifts in acidity during microbial growth (Ludwicka, Kaczmarek & Białkowska, 2020).

Natural pigments have gained increasing interest as active components for the development of pH-responsive colorimetric indicators, particularly due to the growing demand for sustainable, safer materials for food packaging applications. Among naturally occurring pigments, anthocyanins are especially promising because they are water-soluble compounds belonging to the flavonoid family and exhibit distinct color changes in response to pH. Their colorimetric response is associated with structural transformations between different molecular forms, resulting in a range of visible colors under acidic, neutral, and alkaline conditions (Qin et al., 2021). In addition to their pH sensitivity, anthocyanins are widely available in nature and can be obtained from various plant sources, including red cabbage, purple sweet potato, black carrot, berries, grapes, and other fruits, vegetables, and agricultural by-products (Chen et al., 2020; Faisal et al., 2023; Xiao et al., 2023). The use of such sources also supports the valorization of agro-industrial residues and contributes to the development of more sustainable functional materials.

To develop pH-responsive films with anthocyanins as natural pigments, they can be incorporated into a variety of natural and synthetic matrices, including starch, cellulose and cellulose derivatives, chitosan, gelatin, alginate, pectin, polyvinyl alcohol, and other biodegradable polymer systems (Koshy et al., 2021; Qin et al., 2021). These matrices provide structural support for the pigments while enabling the fabrication of films, coatings, and indicator labels. Among biopolymeric materials, starch is particularly attractive because of its abundance, low cost, biodegradability, renewability, and film-forming ability. Starch-based films can therefore serve as suitable matrices for incorporating natural pH-sensitive pigments, offering the possibility of developing environmentally friendly indicator materials for smart packaging applications. Recent studies have demonstrated the successful development of starch-based anthocyanin films for monitoring the freshness of products such as pork, shrimp, and other perishable foods (Bao et al., 2022; Faisal et al., 2023; Koshy et al., 2021). The performance of these films may depend on the type and structure of the starch matrix, with the amylose content also influencing the functionality and stability of anthocyanin-based pH indicators (Faisal et al., 2023). Nevertheless, the mechanical and barrier properties of starch films may be limited, which has encouraged the incorporation of reinforcing materials and nanostructured components. Bacterial nanocellulose (BNC) is a promising biobased material for improving the functional properties of polymeric films. Due to its nanoscale fibrillar network, high specific surface area, mechanical strength, and ability to form interconnected structures, BNC can contribute to the reinforcement of biopolymer-based matrices. Its combination with starch may therefore provide a suitable platform for developing films with improved structural properties while maintaining the advantages of renewable and biodegradable materials (Ludwicka, Kaczmarek & Białkowska, 2020; Mahović Poljaček et al., 2024). Furthermore, the incorporation of anthocyanins into starch/BNC-based systems may enable the development of multifunctional films that combine structural performance with pH-responsive colorimetric functionality. Recent research has demonstrated the potential of starch-based films containing BNC and natural anthocyanins extracted from red cabbage as pH-responsive materials for smart packaging applications (Lee, Durst & Wrolstad, 2005; Mahović Poljaček et al., 2024).

Overall, the development of pH-responsive starch films incorporating natural anthocyanins and bacterial nanocellulose represents a promising approach for the design of sustainable smart packaging materials. In this study, purple sweet potato anthocyanins were incorporated into starch-based films containing bacterial nanocellulose to develop pH-responsive materials with potential application as colorimetric indicators for food smart packaging. The resulting films were evaluated in terms of their visual response to different pH conditions, with the aim of assessing their potential for use as indicator labels for monitoring changes in food quality and freshness.

2. Materials and methods

In this study, purple sweet potato (Ipomoea batatas L.) has been used as a source of anthocyanins. Purple sweet potato anthocyanins are characterized by relatively high stability compared with some other natural anthocyanin sources and exhibit pronounced color changes under different pH conditions. These properties make them attractive for the development of natural colorimetric indicators for food quality monitoring. To determine the most suitable source of anthocyanins from sweet potato (SWP), pigments were extracted from three different sources: fresh peel, fresh flesh, and dried, ground peel. Ethanol, distilled water, and hydrochloric acid were used as the extraction solvent. A solvent mixture was prepared by combining 189 mL of ethanol, 2 mL of HCl, and 50 mL of water. For each extraction, 25 g of SWP material, either fresh peel, fresh flesh, or dried and ground peel, was added to the solvent. The mixture was stirred at 60

°C for 30 min to facilitate pigment extraction. Subsequently, the mixture was cooled to 4 °C and kept at this temperature for 24 h. The extracts were then vacuum-filtered through filter paper to remove solid residues and collect the anthocyanin-containing solution. All extracts were stored in glass bottles at 4 °C until further analysis.

After extraction, the anthocyanin solutions were incorporated into maize- and wheat-starch-based film-forming solutions at concentrations of 14% (v/v) and 12% (v/v), respectively. The film-forming solutions were prepared by dissolving starch in distilled water. Glacial acetic acid (CAS No. 64-19-7) was first diluted to a 1% (v/v) solution and then added to the film-forming solutions at 3% (v/v) for the maize starch (MS)-based solutions and 5% (v/v) for the wheat starch (WS)-based solutions. Glycerol (99.5% purity, CAS No. 56-81-5) was added at concentrations of 15% (v/v) for MS and 20% (v/v) for WS.

Additionally, a set of samples containing bacterial nanocellulose (BNC) was prepared in this study. Our previously published research demonstrated that the incorporation of BNC into starch-based films positively influenced their colorimetric properties, resulting in more vibrant and stable colorimetric responses (Mahović Poljaček, Tomašegović & Donevski, 2026; Mahović Poljaček et al., 2024). In the present study, BNC was added to the film-forming solutions at concentration of 30% (v/v), based on the volume of the BNC suspension, which contained 0.15% dry matter. Bacterial nanocellulose (BNC) was produced from the cellulose-rich biofilm generated during the acetic fermentation of apple juice, following the method described by Lavrič, Medvešček and Skočaj (2020).

The mixtures containing predetermined amounts of dissolved starch, glycerol and acetic acid, with and without BNC, were heated on a temperature-controlled hotplate (Tehtnica, Rotamix 550 MMH, Slovenia) and continuously stirred using a DLS Digital Overhead Stirrer (Velp Scientifica Srl, Italy). After approximately 30 min of mixing, equal volumes of the film-forming solutions were poured into Petri dishes to obtain films of uniform thickness. The poured solutions were then dried and stored in a ventilated environmental chamber at 25 °C and 50% relative humidity (RH) for 10 days.

The produced films were labelled according to the starch matrix, anthocyanin source, and BNC content. “MS” and “WS” denote maize starch and wheat starch films, respectively, while “SWP FP”, “SWP FF”, and “SWP DP” refer to extracts obtained from fresh purple sweet potato peel, fresh flesh and dried peel, respectively. Films without BNC were designated as “0BNC”, while films containing 30% (v/v) of BNC suspension were designated as “30BNC”.

To determine the concentration of anthocyanin pigments extracted from the different SWP sources, the total monomeric anthocyanin content was determined using the pH differential method described by Lee, Durst and Wrolstad (2005). A Shimadzu UV-1900i UV–Vis spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan) was used for the measurements. The method is based on the difference in anthocyanin absorbance at pH 1.0 and pH 4.5, measured at a wavelength of 520 nm. This difference in absorbance results from structural transformations of anthocyanins between the two pH conditions. The absorbance spectra of the samples were recorded over the wavelength range of 250–750 nm. The anthocyanin pigment concentration, expressed as cyanidin-3-glucoside equivalents, was calculated using Equation (1), according to Lee, Durst and Wrolstad (2005).

$$\text{Anthocyanin pigment (cyanidin-3-glucoside equivalents,mg/L)} = (A \times MW \times DF \times 1000)/(\varepsilon \times l) \quad (1)$$

where $A = (A_{520\ nm} - A_{700\ nm})_{at\ pH\ 1.0} - (A_{520\ nm} - A_{700\ nm})_{at\ pH\ 4.5}$; MW = 449.2 g moL^{-1} for cyanidin-3-glucoside; DF = dilution factor, l = path-length (cm), ε = 26.900 molar extinction coefficient, in L/mol cm for cyanidin-3-glucoside and 10^3 = factor for conversion from g to mg.

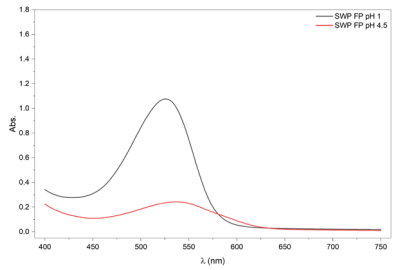
The colorimetric properties of the pH indicators were measured for the dried films and after immersion for 15 min in buffer solutions in buffer solutions ranging from pH 3.0 to pH 10.0. A Techkon SpectroDens spectrophotometer (TECHKON GmbH, Königstein, Germany) was used for the measurements. The spectral reflectance of the films was measured relative to a calibrated white reference, and the CIE L*a*b* color coordinates were calculated from the reflectance data. The results are expressed as lightness (L*) and chromatic coordinates (a* and b*) in the CIE L*a*b* color space (Fairchild, 2013). All measurements were performed using a D50 illuminant and a 2° standard observer with an M1 filter. Each sample was measured at ten different points, and the results are reported as mean values. To evaluate the pH-dependent color changes of the indicator films, the mean total color difference (ΔE^*_{ab}) was also calculated.

3. Results and discussion

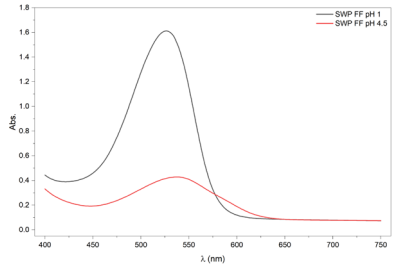
3.1. Determination of the total monomeric anthocyanin pigment content

The UV–Vis absorption spectra of anthocyanin extracts obtained from different SWP sources at pH 1.0 and 4.5 are shown in Figure 1. Figure 1.a presents the absorption spectra of the anthocyanin extract obtained from fresh peel, Figure 1.b shows the spectra of the extract obtained from fresh flesh, and Figure 1.c presents the spectra of

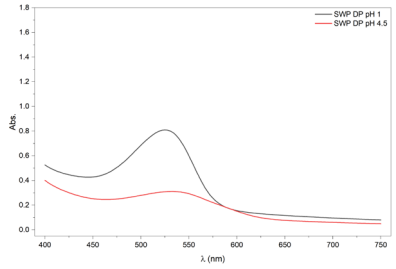
the extract obtained from dried peel. Table 1. presents the absorbance values of the extracts at 520 and 700 nm. According to the calculations based on Equation (1), the anthocyanin concentrations in the extracts from fresh peel, fresh flesh, and dried peel, expressed as mg of cyanidin-3-glucoside equivalents per 100 g, were 206.65, 296.07, and 115.72 mg/100 g, respectively.



(a)



(b)



(c)

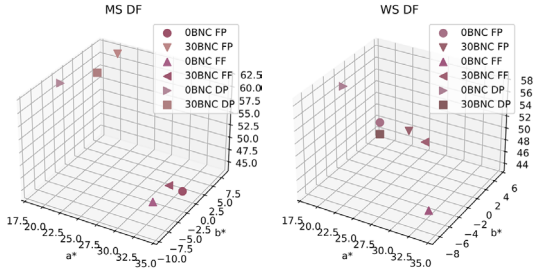
» **Figure 1:** *UV-Vis absorption spectra of SWP anthocyanin extracts, a) fresh peel, b) fresh flesh and c) dried peel*

Table 1
Absorbance values of extracts at 520 and 700 nm at pH values of 1.0 and 4.5

Absorbance	SWP fresh peel		SWP fresh flesh		SWP dried peel	
	pH 1.0	pH 4.5	pH 1.0	pH 4.5	pH 1.0	pH 4.5
A_{520}	1.061	0.228	1.585	0.402	0.802	0.305
A_{700}	0.022	0.014	0.078	0.077	0.096	0.061

3.2. Properties of produced starch-based films

Figure 2 presents the CIE L*a*b* values of the dry films (DF) prepared from different starch types and compositions. Figure 2a presents the results for samples without and with BNC based on maize starch (MS), while Figure 2b presents the corresponding results for samples based on wheat starch (WS). The films exhibited colors ranging between the red and yellow regions of the CIE L*a*b* color space. In Figure 2a, the results are distributed into two distinct regions. The 0BNC DP, 30BNC DP and 30BNC FP samples form a high-lightness and low-redness cluster. In contrast, the 0BNC FP, 0BNC FF and 30BNC FF formulations are grouped at lower lightness and significantly higher redness values. The introduction of BNC (30BNC vs. 0BNC) resulted in changes in redness, with the most pronounced differences observed for the 30BNC FP and 30BNC DP samples. Figure 2b shows that the samples based on wheat starch exhibited greater dispersion in terms of both lightness and the yellow–blue coordinate compared with the maize starch-based films. The 0BNC DP sample remained an outlier, characterized by high lightness and low redness. In contrast, 0BNC FF exhibited the lowest lightness and b* value, indicating a shift toward the blue region. The remaining samples (30BNC DP, 0BNC FP, 30BNC FP, and 30BNC FF) formed a central cluster with intermediate lightness and higher b* values in comparison to other samples. This grouping suggested a similar balance between the chromatic contribution of the anthocyanin extracts and the optical properties of the film matrix. In the BNC-containing films, the formation of the nanocellulose network likely enhanced light scattering and reduced the relative dominance of the anthocyanin coloration, resulting in a shift toward intermediate lightness and higher b* values. This effect was particularly evident for the DP extract, which had the lowest anthocyanin concentration (Figure 1.c). The similar position of the 0% BNC FP samples indicated that a comparable optical response can also be obtained without BNC when the contribution of the FP anthocyanins is balanced by the optical characteristics of the starch matrix.



(a) (b)

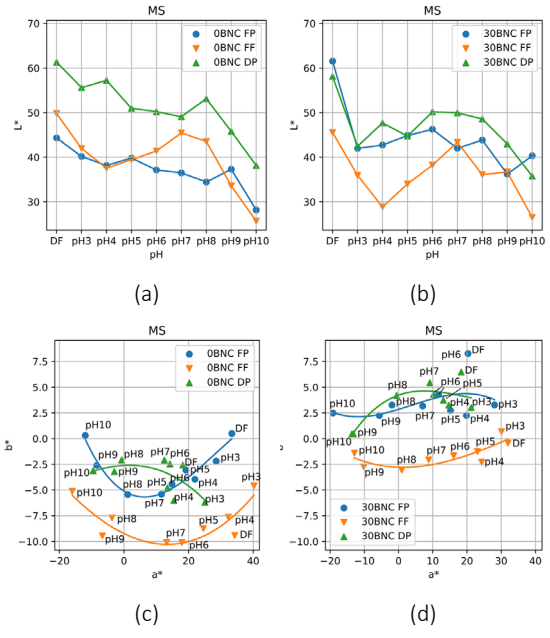
» **Figure 2:** *CIE L*a*b* values of starch-based films: (a) maize starch and (b) wheat starch*

The comparable CIE L*a*b* values obtained for the 0% BNC films containing SWP DP extract, irrespective of the starch type, suggest that the color of these films was primarily determined by the relatively low pigment contribution of the dried-peel extract and the optical characteristics of the starch matrix, while the differences between maize and wheat starch had a comparatively minor effect on the overall color. Similarly, the 0% BNC films containing SWP FF extract exhibited comparable CIE L*a*b* values for both starch types. In this case, the relatively high anthocyanin content of the fresh-flesh extract likely contributed strongly to the overall film color, reducing the relative influence of differences in the starch matrix. The similar color coordinates may therefore indicate that, in the absence of BNC, both starch matrices provided comparable optical environments for the incorporated anthocyanins.

3.3. Colorimetric properties of maize-based films

Absolute CIE L*a*b* values measured for the maize starch-based films after immersion in buffer solutions (pH 3.0–10.0) are presented in Figure 3, where DF on the horizontal axis denotes dry film. Figures 3a and 3b show the lightness (L*) values for films without and with BNC, respectively. The 0BNC samples showed distinct differences in lightness, with the DP (dried peel) sample exhibiting the highest values, while FF (fresh flesh) and FP (fresh peel) showed lower and more variable responses across the pH range. Among the 30BNC films, DP and FF also showed considerable changes in lightness, whereas FP remained relatively stable from pH 3.0 to 9.0. Overall, the 0BNC films exhibited higher maximum lightness values than the 30BNC films. The DP samples consistently showed the highest lightness within both groups, while highly alkaline conditions (pH 10.0) caused a sharp decrease in lightness for almost all samples, except for 30BNC FP, which remained relatively high.

The higher lightness (L*) observed for the 0BNC films compared with the 30BNC films may be related to differences in the optical properties and interactions between the starch matrix and the BNC network. The consistently higher lightness observed for the DP samples in both groups may be associated with the drying of the SWP peel, which may have altered the pigment composition and reduced the concentration of extractable anthocyanins. In contrast, the higher anthocyanin concentrations measured in the FF and FP extracts may be associated with their lower baseline lightness. These observations are consistent with the calculated anthocyanin concentrations presented in Section 4.1, where the lowest anthocyanin concentration was measured in the SWP DP extract and the highest in the SWP FF extract. The pronounced variations observed for SWP FF may be related to the pH-dependent structural changes of anthocyanin compounds (Sohany et al., 2021). Under highly alkaline conditions (pH 9–10), the lower lightness observed for the 0BNC films may be associated with changes in the starch matrix and degradation of anthocyanin compounds. The presence of BNC may have contributed to maintaining the structural integrity of the film matrix and may have limited pigment degradation under alkaline conditions (Abdelkader, Hamed & Gomaa, 2024).



» **Figure 3:** *CIE L*a*b* values of maize-based films: a), c) without BNC and b),d) with BNC*

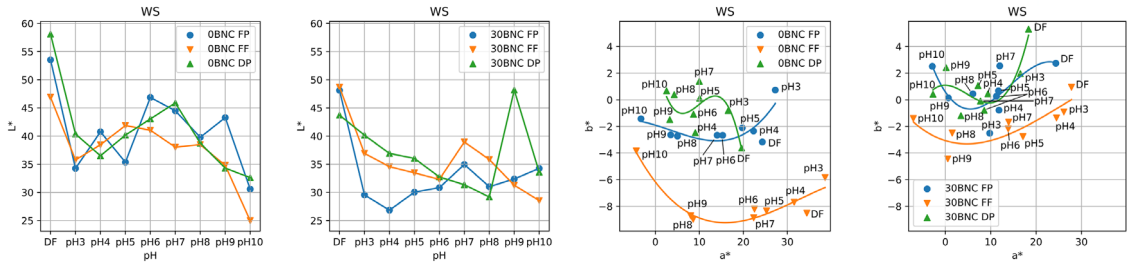
Figures 3c and 3d show chromaticity diagrams illustrating the color changes across pH values from 3 to 10 along the a* (green-red) and b* (blue-yellow) axes for the maize starch-based indicator films. The DF label denotes dry films. Across all samples, increasing pH shifted the a* values from positive (red) toward negative (green) values, reflecting pH-induced changes in the structure and equilibrium of anthocyanin chromophores. Across both formulations, the extracts exhibited a clear pH-responsive chromatic shift: acidic conditions (pH 3.0-5.0) were associated with red to purple hues and higher positive a* values, neutral conditions (pH 6.0-8.0) represented an intermediate transition region, while alkaline conditions (pH 9.0-10.0) resulted in a pronounced shift toward green hues and negative a* values. These color changes are consistent with the known pH-dependent structural transformations of anthocyanins, including changes in the relative proportions of flavylium, quinoidal, and other structural forms, followed by degradation under strongly alkaline conditions (Mahović Poljaček, Tomašegović & Donevski, 2026). Among the pigment sources, FF (fresh flesh) exhibited the most pronounced red coloration at pH 3.0, whereas DP (dried peel) showed an arched trajectory toward higher b* values around pH 6.0-7.0. The incorporation of 30% BNC

resulted in shifts in the color trajectories, particularly along the b* axis, which may be related to changes in the optical properties and light-scattering behavior of the nanocellulose-containing matrix. Similar effects of BNC incorporation on the colorimetric response of starch-based anthocyanin films have been reported, with changes in the a* and b* coordinates depending on BNC concentration and pH (Ghadiri Alamdari et al., 2022). Furthermore, the 30BNC films showed more consistent color trajectories compared with the 0BNC samples, with 30BNC FP (fresh peel) exhibiting a relatively smooth transition along the a* axis while maintaining positive b* values. This behavior indicates a relatively consistent color response of the 30BNC FP formulation over the investigated pH range and highlights its potential for application as a pH-responsive indicator film.

3.4. Colorimetric properties of wheat-based films

Absolute CIE L*a*b* values measured for the wheat starch-based films after immersion in buffer solutions (pH 3.0–10.0) are presented in Figure 4. The CIE L* (lightness) results revealed clear differences between the control (0BNC) and nanocellulose-reinforced (30BNC) wheat starch formulations across the tested pH range. For the 0BNC samples, lightness values remained relatively high, ranging primarily between 35 and 47, with the highest values observed under near-neutral conditions at pH 6.0-7.0. However, all 0BNC samples exhibited a pronounced decrease in lightness under strongly alkaline conditions, reaching their minimum values at pH 10.0, particularly for 0BNC FF. In contrast, the addition of 30% BNC generally decreased the baseline lightness across most of the investigated pH range. The relatively high lightness measured for the 30BNC DP sample at pH 9 represents an exception to the trend observed for the other samples. This deviation may have resulted from variability occurring after immersion in buffer solutions and swelling. Despite the low baseline, BNC incorporation altered the pH-dependent lightness response. The 30BNC FP sample showed a gradual increase in lightness from pH 4.0 to pH 10.0, without the pronounced decrease observed for the corresponding 0BNC sample. Overall, the analysis of the lightness results indicated that incorporating 30% BNC into the wheat starch matrix generally resulted in lower lightness compared with the 0BNC samples. This difference may be related to changes in the optical properties and light-scattering behavior associated with the denser BNC-containing network. Similar effects of the film matrix on the colorimetric response of anthocyanin-based starch films have been reported, indicating that interactions between anthocyanins and the polymer matrix can influence the observed CIE L*a*b* values and pH-dependent color changes (Choi et al., 2017; Koshy et al., 2021). For the 0BNC samples, the relatively high lightness observed at pH 6.0–7.0, followed by a pronounced decrease at pH 10.0, may be associated with pH-induced changes in the starch matrix and structural transformations and degradation of anthocyanin compounds under strongly alkaline conditions, which may alter the optical properties of the films. Purple sweet potato anthocyanins are known to undergo pH-dependent structural transformations, resulting in substantial changes in color and colorimetric parameters, with degradation becoming more pronounced under strongly alkaline conditions (Ke et al., 2024; Koshy et al., 2021; Sohany et al., 2021). In contrast, the presence of BNC may have contributed to maintaining the structural integrity of the film matrix under alkaline conditions, resulting in a more stable lightness response, particularly for the 30BNC FP formulation. This interpretation is consistent with previous studies showing that the polymer matrix can influence the stability and pH-responsive behavior of anthocyanin-containing films.

Results of the chromatic coordinates (Figures 4c-d) of wheat starch-based films demonstrated distinct pH-responsive trajectories from pH 3 to pH 10, varying significantly by extract source. Fresh flesh (FF) formulations consistently maintained lower b* values compared to fresh peel (FP) and dried peel (DP) extracts, which exhibited lower redness (a*) and higher yellowness (b*) under acidic conditions. The addition of bacterial nanocellulose (30BNC) narrowed the chromatic range of the pH-sensitive films while maintaining the overall direction of the pH-dependent colur transition. The shift toward the yellow direction (positive b* values) may be associated with changes in the optical properties of the film matrix caused by the incorporation of the BNC network. This observation is consistent with the results obtained for



» **Figure 4:** *CIE L*a*b* values of wheat-based films: a), c) without BNC and b), d) with BNC*

the maize-starch-based films, although the effect was slightly more pronounced for the wheat-starch films, suggesting that interactions between BNC and the starch matrix may contribute to the observed color response.

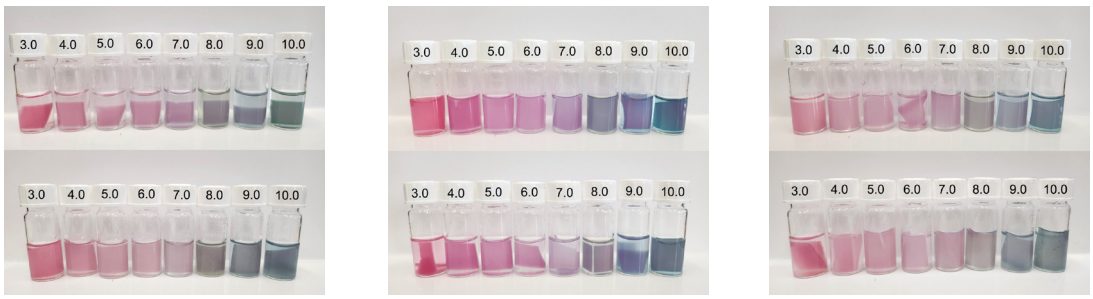
3.5. Visual analysis of starch-based films in varied-pH environment

Visual color changes of the pH-responsive MS-based and WS-based films after 15 min of immersion in buffer solutions (pH 3-10) are presented in Figures 5 and 6. Figures 5a-c show the immersed maize starch films containing SWP anthocyanin extracts from fresh peel, fresh flesh, and dried peel, respectively. The upper images show films without BNC, whereas the lower images show films containing 30% BNC. The MS-based indicator films exhibited a distinct pH-dependent color transition, progressing from pink/red under acidic conditions (pH 3.0-6.0) through purple at near-neutral conditions (pH 7.0-8.0) to green-blue under alkaline conditions (pH 9.0-10.0). These color changes are consistent with the pH-dependent structural transformations of anthocyanins, in which the flavylium cation predominates under acidic conditions, while quinoidal and other deprotonated forms become increasingly important as pH increases, followed by degradation under strongly alkaline conditions (Ke et al., 2024; Koshy et al., 2021). Similar pH-dependent color transitions have also been reported for starch-based films containing BNC and anthocyanins (Mahović Poljaček, Tomašegović & Donevski, 2026). Among the pigment sources, the FF (fresh flesh) extract produced the most visually pronounced color response, followed by FP (fresh peel), whereas DP (dried peel) exhibited darker and more muted tones. The latter may be related to changes in pigment composition or stability during the drying process. The incorporation of 30% BNC slightly reduced the apparent lightness of the films, which may be associated with the optical properties and light-scattering behavior of the nanocellulose network, while the overall pH-dependent color transitions remained clearly distinguishable (Mahović Poljaček, Tomašegović & Donevski, 2026)

The results presented in Figure 6 demonstrate that wheat starch-based film formulations containing SWP fresh peel (FP), fresh flesh (FF), and dried peel (DP) extracts exhibited clear and distinct pH-dependent color transitions across the tested pH range of 3.0-10.0.

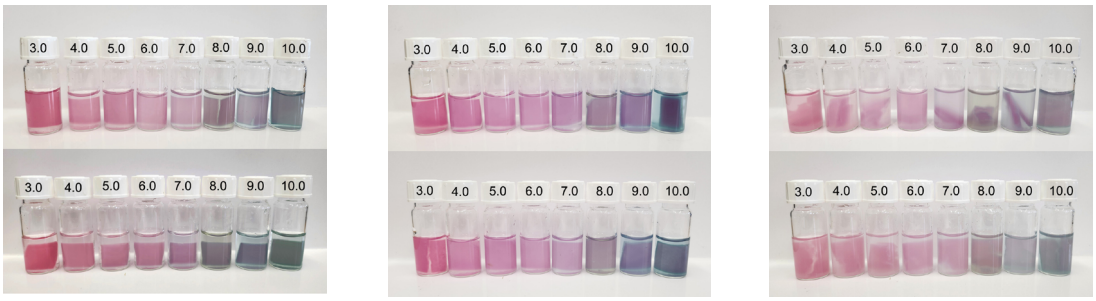
Under acidic conditions (pH 3.0-6.0), the films maintained reddish-pink hues characteristic of anthocyanin-based indicators. These hues gradually changed to light purple near neutral pH (pH 7.0), followed by transitions to dark purple, blue, and deep teal/grey under increasingly alkaline conditions (pH 8.0-10.0). These changes are consistent with the well-known pH-dependent structural transformations of anthocyanins, including the conversion of flavylium cations into quinonoidal and other deprotonated forms at higher pH values (Castañeda-Ovando et al., 2009). The more intense coloration observed for the films containing the SWP FF extract can be attributed, at least in part, to its higher anthocyanin content compared with the FP and DP extracts. The higher concentration of anthocyanins provides a greater amount of pH-sensitive chromophores, resulting in more pronounced and visually distinguishable color changes. In contrast, although fresh peel is also rich in anthocyanins, peel extracts may contain other co-extracted compounds, including phenolic compounds and other constituents, which can influence the resulting color through copigmentation and light-scattering effects (Castañeda-Ovando et al., 2009). The weaker coloration and less pronounced color transitions observed for the DP extract may be related to its lower anthocyanin content, which may have resulted from the drying process. Anthocyanins are susceptible to degradation during drying, particularly under elevated temperatures and in the presence of oxygen, which can lead to the formation of less intensely colored or colorless degradation products (Patras et al., 2010). Consequently, the lower anthocyanin content of the DP extract resulted in lower initial color intensity and less pronounced visual changes across the investigated pH range.

The results presented in Figure 6 demonstrate that wheat starch-based film formulations containing SWP fresh peel (FP), fresh flesh (FF), and dried peel (DP) extracts exhibited clear and distinct pH-dependent color transitions across the tested pH range of 3.0-10.0.



(a) (b) (c)

» **Figure 5:** *Visual color changes of maize starch-based films at different pH values (3.0–10.0): a) fresh peel extract, b) fresh flesh extract, and c) dried peel extract; upper images: 0BNC, lower images: 30BNC*



(a) (b) (c)

» **Figure 6:** *Visual color changes of wheat starch-based films at different pH values (3.0–10.0): a) fresh peel extract, b) fresh flesh extract, and c) dried peel extract; upper images: 0BNC, lower images: 3OBNC*

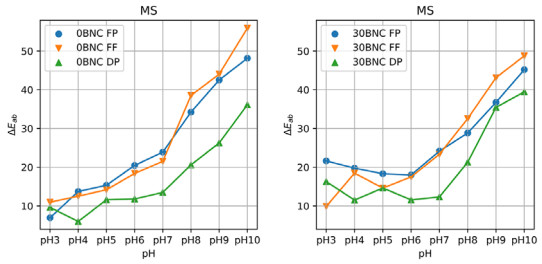
Under acidic conditions (pH 3.0-6.0), the films maintained reddish-pink hues characteristic of anthocyanin-based indicators. These hues gradually changed to light purple near neutral pH (pH 7.0), followed by transitions to dark purple, blue, and deep teal/grey under increasingly alkaline conditions (pH 8.0-10.0). These changes are consistent with the well-known pH-dependent structural transformations of anthocyanins, including the conversion of flavylium cations into quinonoidal and other deprotonated forms at higher pH values (Castañeda-Ovando et al., 2009). The more intense coloration observed for the films containing the SWP FF extract can be attributed, at least in part, to its higher anthocyanin content compared with the FP and DP extracts. The higher concentration of anthocyanins provides a greater amount of pH-sensitive chromophores, resulting in more pronounced and visually distinguishable color changes. In contrast, although fresh peel is also rich in anthocyanins, peel extracts may contain other co-extracted compounds, including phenolic compounds and other constituents, which can influence the resulting color through copigmentation and light-scattering effects (Castañeda-Ovando et al., 2009). The weaker coloration and less pronounced color transitions observed for the DP extract may be related to its lower anthocyanin content, which may have resulted from the drying process. Anthocyanins are susceptible to degradation during drying, particularly under elevated temperatures and in the presence of oxygen, which can lead to the formation of less intensely colored or colorless degradation products (Patras et al., 2010). Consequently, the lower anthocyanin content of the DP extract resulted in lower initial color intensity and less pronounced visual changes across the investigated pH range.

Furthermore, comparison of the upper rows (0BNC) and lower rows (30BNC) indicates that the incorporation of bacterial nanocellulose (BNC) preserved the pH-responsive color-changing behavior of the films while maintaining visually distinguishable color differences. These results demonstrate the potential of the developed starch/BNC films containing SWP anthocyanins as visual pH indicators for intelligent food-packaging applications.

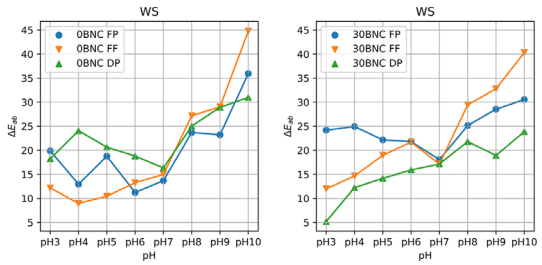
3.6. Color differences of starch-based films in varied-pH environment

Figures 7 and 8 show the total color difference (ΔE^*_{ab}) of the maize and wheat starch-based pH indicator films calculated after immersion in buffer solutions at different pH values (pH 3.0-10.0). Each point represents the ΔE^*_{ab} difference between the means of ten CIE L*a*b* measurements taken on dry film and immersed samples. A ΔE^*_{ab} value of approximately 1 corresponds to a just noticeable color difference, while values of approximately 3 and 5 indicate moderate and highly noticeable color differences, respectively. Figure 7a shows the colorimetric differences for the maize starch without BNC. Colorimetric differences between the immersed films and the corresponding dry films increase with pH. For all three anthocyanin sources (FP, FF and DP) the slope is noticeably steeper from pH 7.0, for the alkaline pH levels. The DP sourced films show smaller colorimetric differences compared to FP and FF which are more similar in that regard. In other cases, displayed in Figure 7b and Figure 8a and 8b, the acidity of solutions appears to have no or very little effect on colorimetric difference. While the colorimetric differences of films immersed in acidic solutions from the dry films are quite large, they are similar for different acidic pH levels. Furthermore, it can be noted that curves become steep for the alkaline pH levels. At alkaline pH levels, the addition of BNC appears to reduce the colorimetric differences in MS films to some extent, although the observed differences remain very noticeable. In all cases, at alkaline pH levels, the largest colorimetric differences are visible on FF films. The MS FP films, Figure 7a and 7b, show colorimetric differences close to those of FF films. For WS films, Figure 8a and 8b, the differences between FF and FP are somewhat larger, but FP still exhibits very large colorimetric differences. It can also be noted that colorimetric differences of FP films do not increase monotonically along the alkaline

pH range for the WS films without BNC, but they do increase monotonically for the WS films with BNC.



» **Figure 7:** *Total color difference (CIE ΔE*ab) of maize starch-based pH indicator films at different pH values: a) without BNC and b) with BNC*



» **Figure 8:** *Total color difference (CIE ΔE*ab) of wheat starch-based pH indicator films at different pH values: a) without BNC and b) with BNC*

4. Conclusions

In this research, purple sweet potato anthocyanins sourced from fresh peel (FP), fresh flesh (FF), and dry peel (DP) were successfully incorporated into maize- and wheat-starch-based films to obtain pH-responsive colorimetric indicators. Among the investigated anthocyanin sources, the fresh flesh extract exhibited the highest total monomeric anthocyanin content (296.07 mg/100 g), followed by the fresh peel (206.65 mg/100 g), while the dried peel extract showed the lowest content (115.72 mg/100 g). Bacterial nanocellulose (BNC) incorporation influenced the colorimetric properties of the produced dry films (before their immersion in buffer solutions), with the effect depending on starch type and anthocyanin source. In BNC-free films, maize and wheat starch exhibited comparable color responses, indicating a relatively minor influence of starch type on the overall film color. All produced starch-based films exhibited distinct pH-dependent color changes over the investigated pH range from 3.0 to 10.0 after immersion in buffer solutions. The films changed from reddish-pink hue under acidic conditions, through purple shades near neutral pH, to green, blue, and teal tones under alkaline conditions, confirming their potential as visual pH indicators. The films with FF generally produced the most intense and visually pronounced color response, consistent with the higher anthocyanin content. For maize-starch-based films, the incorporation of 30% BNC altered the color trajectories, particularly along the b* axis, while providing a more consistent color response across the investigated pH range, and specifically highlighting the potential of the 30BNC FP formulation as a pH-responsive indicator film. Furthermore, the incorporation of 30% BNC narrowed the chromatic range of the wheat-starch-based films while preserving the overall pH-dependent color transition, but with a general shift towards positive b* values- suggesting that BNC-starch interactions influence the optical and colorimetric response of the films. Overall, the results of this research demonstrated that purple sweet potato anthocyanins combined with starch and bacterial nanocellulose can provide visually distinguishable pH-responsive films. The produced materials therefore show promising potential for use as natural colorimetric indicators in intelligent food packaging applications, particularly for visual monitoring of changes associated with food quality and spoilage.

5. Funding

This research was supported by the NextGenerationEU project NPOO MAHOVIĆ POLJAČEK 2025/2029 2025106.

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