https://doi.org/10.24867/GRID-2024-p48 Original scientific paper

IMMOBILIZATION OF ANTHOCYANINS BY BACTERIAL NANOCELLULOSE IN PRODUCTION OF INDICATORS FOR PH-CONTROLLED SMART PACKAGING

Sanja Mahović Poljaček [1], Tamara Tomašegović [1], Sonja Jamnicki Hanzer [1], Maja Strižić Jakovljević [1], Ivana Murković Steinberg [2], Iva Žuvić [2], Gregor Lavrič [3], Urška Kavčič [3], Igor Karlovits [4]

[1] University of Zagreb, Faculty of Graphic Arts, Zagreb, Croatia

[2] University of Zagreb, Faculty of Chemical Engineering and Technology, Zagreb, Croatia

[3] Pulp and Paper Institute, Ljubljana, Slovenia

[4] Danfoss Trata d.o.o., Šentvid, Slovenia

Abstract: *The main objective of this research was the production and characterization of bio-based indicators for pH-controlled smart packaging. Since pH-sensitive indicators can change color depending on the freshness or spoilage of the food, they can be used to monitor the freshness level and safety of food products. Potato and maize starch without and with the addition of bacterial nanocellulose (BNC) were used as a polymer matrix for the immobilization of pH-sensitive indicators. The natural pigment anthocyanin, extracted from the peels of red onions, was used as a pH-sensing element. In addition, an ultrasound treatment of the extracted anthocyanins was performed to determine the influence of the treatment on the color response of the produced films. The colorimetric and optical differences between the different starch films with ultrasound-treated and untreated anthocyanins were observed. To observe the color response of the prepared indicators when exposed to different pH environment, their optical and colorimetric changes were monitored before and after exposure to altered pH values. The results obtained show the potential for the production of pH-sensitive films immobilized by BNC. In addition, the results will provide the information about the influence of ultrasound treatment during anthocyanin extraction on the optical and colorimetric changes of the pH-sensitive starch-based films.*

Key words: pH indicators, BNC, anthocyanins, UAE, colorimetric properties

1. INTRODUCTION

pH-controlled smart packaging is an innovative approach to maintaining and monitoring the quality of the packaged product through the use of conventional and advanced technologies. This type of packaging can provide real-time information about the quality of the packaged product and the environmental conditions inside the package, helping to ensure freshness and safety, detect spoilage or verify the effectiveness of the packaging. pH-controlled smart elements used in the food packaging industry include various types of indicators and sensors that can be used to monitor the freshness, stability and condition of perishable products (Mustafa & Andreescu, 2020; Rodrigues et al., 2021). In meat or dairy products, for example, spoilage often leads to a drop in pH levels, which can be detected by the color-changing element that provides a clear visual indication to the consumer. In addition, smart packaging systems can provide various safety warnings to ensure that the product remains within a safe pH range throughout its shelf life (Figure 1).

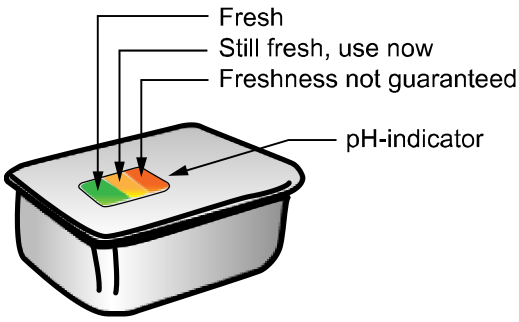

Figure 1: Example of the pH indicator in the packaging container

pH indicators used in smart packaging to control food safety can indicate spoilage, contamination or degradation of packaged food, allowing timely intervention and better information to consumers. They are mainly composed of natural compounds that are applied to the packaging in the form of labels, films or coatings and change color depending on the pH variations in the packaging. In particular, different types of biodegradable and bio-based materials with natural sensing elements are being researched. Among the various materials, chitosan, starch and alginate composites and their by-products are most commonly used (Zhao et al., 2022). By utilizing the natural properties of starch and combining these properties with advanced technologies, the materials produced offer environmentally friendly ways to monitor and improve the freshness and safety of packaged products. By incorporating pH-sensing elements, produced materials can visually indicate changes in the acidity of the packaged product and alert consumers to possible spoilage.

One of the most interesting natural sensing elements that has recently been widely used in smart packaging is anthocyanins. Anthocyanins are a class of plant phenolic pigments and food ingredients that have various health and other benefits due to their antioxidant and antimicrobial properties (Eng Khoo et al., 2017; Samota at al., 2022; Zhou et al., 2023). In smart packaging, anthocyanins are used as pH-sensing elements responsible for the red, orange, pink and blue colors when exposed to different pH environments. They are very interesting for these applications due to their availability, as they can be extracted from various natural resources such as vegetables (red cabbage, red onions, purple carrots, etc.), fruits (grapes, blueberries, raspberries, etc.) and flowers (butterfly pea, red rose, etc.) (Chayavanich, et al., 2023; Kurek, et al., 2019; Oladzadabbasabadi et al., 2022; Zhao, et al., 2022). Their main advantage in the production of pH indicators is the ability to change color in response to different pH values. On the other hand, by-products and waste from some food crops are cost-effective sources of anthocyanins, which further contribute to sustainable development. pH sensing elements, i.e. anthocyanins, can be embedded in a polymer composite or applied to the packaging material to detect a pH change in the packaging environment and inform the consumer about the potential spoilage of food. There are many methods for extracting anthocyanin pigments from various sources and they are being intensively researched. These include solvent extraction method (SEM), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical carbon dioxide extraction (SCDE) and various combined methods (Chesnokova et al. 2021; Tan et al., 2022). Since anthocyanins are highly unstable, all methods aim to preserve the stability and shelf life of anthocyanins, which are directly related to the beneficial properties of these compounds.

Various natural and synthetic polymers can be used to immobilize anthocyanins in a solid material and achieve an improved colorimetric response (Moradi et al., 2019). In this study, bacterial nanocellulose (BNC) was added to the film-forming solution in the production of pH indicators. It has been published that BNC can enhance the absorption of liquids and gases that cause pH change in the production of pH-sensitive films, thus facilitating the color change of the pH sensing element embedded in the BNC network. In addition, BNC has been shown to be useful in protecting anthocyanins from degradation processes and to have a positive effect on their stability, ensuring the excellent barrier properties (Beluhan et al., 2022; Mahović Poljaček et al., 2024; Perdani & Gunawan, 2021; Pourjavaher et al., 2017).

There are a number of publications on the production of various pH indicators for use in smart packaging systems, but the production of pH indicators based on starch matrices with the addition of BNC and anthocyanins from red onions has not yet been reported to our knowledge. The aim of the research was to evaluate the colorimetric and optical differences in the prepared potato and maize starch-based films with UAE treated and untreated anthocyanins and BNC. The prepared films were measured before and after exposure to the environment with different pH values.

2. MATERIALS

In this study, polymeric composites were prepared by dissolving two types of starch in distilled water. A potato starch (extra pure, CAS: 9005-25-8) (Carl Roth, Germany) and maize starch (extra pure, CAS: 9005-25-8) were used. In addition to the starch, glycerol (purity 99.5 %, CAS: 56-81-5) and glacial acetic acid (CAS: 64-19-7) (1 % v/v) were used in varying concentrations to produce a film-forming solutions.

Fresh red onion (Allium cepa L.) for extraction of anthocyanins was supplied from the local market. The anthocyanins were extracted from the peel of red onions (ROA). The peel of red onions was used because the flavonoid compounds, which are mainly responsible for the purple/red color of the onion, are highly concentrated in the outer peel of the onion (Samota et al., 2022). Two methods were used for the extraction. The first was the SEM method, which was performed according to the modified method described in the publication (Tan et al., 2022), and the second was the combination of SEM and UAE methods. In the extraction, the red onion peels were ground and mechanically mixed within a 96 % (v/v) ethanol solution (Pharmachem, Ljubljana, Slovenia). Extraction was performed at 60°C for 90 minutes, after which the samples were cooled and filtered with Whatman® Quantitative Filter Paper, ashless, grade 40 (Cytiva, USA). Ultrasonic (pre)treatment was performed with an ultrasonic probe (Sonics Vibracell VCX 750) at a power of 350 W for 10 minutes. After one minute of probe operation, a one-minute break was taken. This procedure in combination with a cold water-bath prevented excessive heating of the suspension. The anthocyanin extracts were stored in the refrigerator before use.

BNC was produced from the cellulose-rich biofilm formed after acetic fermentation of apple juice according to the method described in (Lavrič et al., 2020).

Various buffers were used to test the color-changing effect of the films produced: commercially available pH buffers pH 2 - pH 4 [$C_6H_8O_7$ (1-hydrate), NaOH, HCl, H_2O], pH5-pH6 [$C_6H_8O_7$ (1-hydrate) (Gram-Mol d.o.o., Croatia) and additionally prepared buffers: pH 7 - pH 8.05 [Na_2HPO_4, $C_6H_8O_7$] and pH 9.17 - pH 10.5 [Na_2CO_3 - $NaHCO_3$].

3. METHODS

Film-forming solutions were prepared by mixing starch in distilled water on a temperature-controlled hotplate (Tehtnica, Rotamix 550 MMH, Domel, Slovenia) with stirring (DLS Digital Overhead Stirrer, Velp Scientifica Srl, Italy). The films were prepared at different temperatures; the film-forming potato starch solution was heated to 60°C and the maize-starch solution to 80°C. Anthocyanins, glycerol and acetic acid were slowly added to the diluted starch solution at different concentrations. Glycerol was used because it acts as a plasticizer and the incorporation of glycerol into the film-forming solutions increases the flexibility of the films produced and positively influences other film properties (Pounds, et al., 2021). Acetic acid was added to the film-forming solutions to ensure that the branched amylopectin molecules in the starch break into straight-chain amylose molecules. Our previous studies have shown that different amounts of the components are required to obtain compact and stable films. For this reason, a series of tests were carried out to form the films that have optimal properties for the measurements (Mahović Poljaček et al., 2024).

A total of eight samples were prepared, four samples based on potato starch and four samples based on maize-starch. The films were prepared by adding untreated anthocyanins (ROA) in one set of starch-based films and by adding anthocyanins with ultrasound-assisted extraction (ROA-UAE) in another. In addition, the samples were prepared without BNC and with BNC addition. The BNC used was added to the film-forming solutions at a concentration of 50 % (v/v) (dry matter of BNC in a suspension was 0.15 %). The preparation of each starch-based film took approximately 30 minutes. Twenty-five grams of each film-forming solution was poured into a glass Petri dish to obtain a constant film thickness. The films were dried and stored in a ventilated environmental chamber at 25°C and 50 % relative humidity (RH) for ten days. Table 1 contains the films and their ingredients. The amount of ingredients refers to the total amount of film-forming solutions needed to produce the films.

Table 1: Ingredients for film-forming solutions

Produced films	Acetic acid (%)	Glycerol (%)	ROA (%)	ROA-UAE (%)	BNC (%)	Designation of samples
PS starch	15	3	12	/	/	PS_0BNC_ROA
	15	3	/	12	/	PS_0BNC_ROA-UAE
	15	3	12	/	50	PS_50BNC_ROA
	15	3	/	12	50	PS_50BNC_ROA-UAE
MS starch	20	5	12	/	/	MS_0BNC_ROA
	20	5	/	12	/	MS_0BNC_ROA-UAE
	20	5	12	/	50	MS_50BNC_ROA
	20	5	/	12	50	MS_50BNC_ROA-UAE

To observe the influence of UAE on the pigment content in the extracts the total monomeric anthocyanin pigment content was calculated using the pH differential method (Lee et al., 2005). A Shimadzu UV-1280 UV-Vis spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan) was used. The method utilises the different absorbance of anthocyanins at pH 1.0 and pH 4.5 at a wavelength of about 520 nm. The change in absorbance is caused by the structural transformation of anthocyanins between the two pH values. The absorbance spectra of the samples were recorded in the range of 250 to 750 nm, and the anthocyanin pigment concentration, expressed as cyanidin-3-glucoside equivalents, was calculated using Equation (1) (Lee et al., 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.
Density of the produced films was measured according to the Equation (2) (Lavrič et al., 2021):

$$Density = m/(d \times S)\ (g/cm^3) \quad (2)$$

where m is the mass of the tested sample, d is the thickness of the film in cm, and S is the area of the sample. Films were cut into pieces of 3×3 cm^2 in size. The thickness was measured with DGTB001 thickness gauge (Enrico Toniolo, Milan, Italy) and weighed on an analytical scale.
The colorimetric and optical properties of the produced films were measured. Samples were measured before immersion in the different pH buffers and after they were exposed to different buffer solutions for 20 minutes. The results are shown as a lightness value (L*) and chromatic coordinates (CIE a*/b*) from CIE L*a*b* color space (Fairchild, 2013). The spectro-densitometer Techkon SpectroDens (TECHKON GmbH, Germany) was used for the measurements. The measurement conditions were set to illuminant D50/2°, M1 filter according to ISO 13655. Calibration was carried out on the integrated absolute white standard and the relative CIE L*a*b* values (with paper as white point) were calculated and presented in the results.

4. RESULTS AND DISCUSSION

4.1 Determination of the total monomeric anthocyanin pigment content

The UV-Vis absorption spectra of red onion extracts at pH values 1.0 and 4.5 are shown in Figure 2. Figure 2a is the absorption spectra of untreated extract (ROP) and Figure 2b extract after ultrasound treatment (ROP-UAE). Table 2 gives the absorbance values of both samples at 520 and 700 nm respectively. According to calculation (Equation 1), the anthocyanin concentration for untreated sample (ROA), expressed as mg of cyanidin-3-glucosides per 100 g of dry peels of red onion, has a value of 13.97, and a value of 14.85 was found for the UAE treated sample (ROA-UAE).

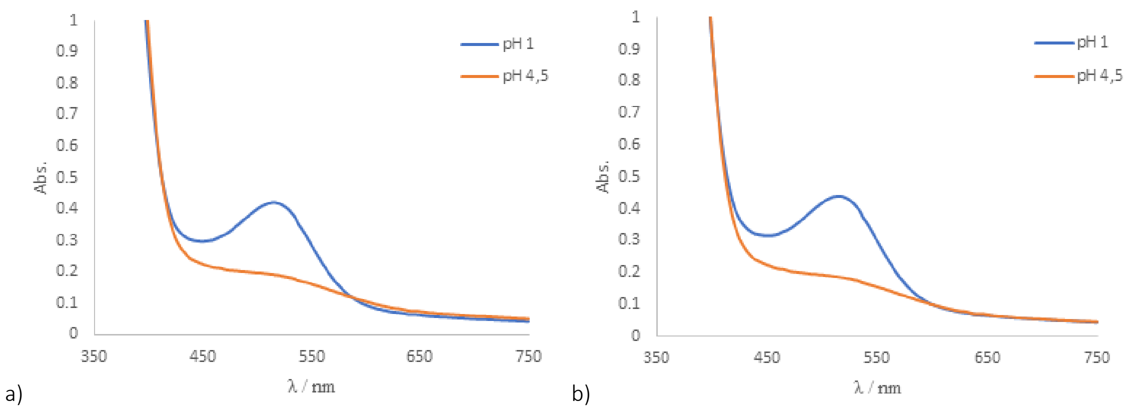

Figure 2: UV-Vis absorption spectra of anthocyanin extracts, a) untreated ROA and b) ultrasound treated ROA (ROA-UAE)

Table 2: Absorbance values of red onion samples at 520 and 700 nm at pH values of 1.0 and 4.5

Absorbance	ROA sample		ROA-UAE sample	
	pH 1.0	pH 4.5	pH 1.0	pH 4.5
A_{520}	0.418	0.187	0.438	0.183
A_{700}	0.051	0.059	0.054	0.053

4.2 Properties of starch-based films

Figure 3 shows the optical properties of the samples measured on dried starch-based films. Figure 3a shows the results of the calculated film density (according to Equation 2) and the results of the measured lightness (L*). It can be seen that the type of starch used has little influence on the density of the films produced. In general, the density value fluctuates between 1.05 and 1.25. Films based on potato starch have slightly higher density values compared to films based on maize starch. The addition of BNC primarily leads to a reduction in density for samples based on potato starch and to a slight increase when BNC was added to maize starch. In addition, it can be seen that samples containing BNC and ROA-UAE anthocyanins have higher density values compared to the samples without BNC. When comparing the lightness results, it can be seen that the samples without BNC addition (PS_OBNC_ROA and MS_OBNC_ROA) measured the lower brightness. The addition of anthocyanins extracted by ultrasonic treatment led to an increase in lightness values for all samples. Figure 3b shows the results of the relative CIE a*/b* color coordinates measured on the films. The films produced have an appearance between red and yellow color coordinates. These results were expected as the peel of the red onion contains flavonoids, which are responsible for the purple/red color of the onion (Samota et al., 2022). Slight differences can be seen between the films produced on the same starch base. Films with different composition based on potato starch show a lower color distribution than samples based on maize starch. Samples without BNC (MS_0BNC_ROA and MS_0BNC_ROA-UAE) show a color shift in the reddish direction and samples with BNC (MS_50BNC_ROA and MS_50BNC_ROA-UAE) show a color shift in the yellowish direction.

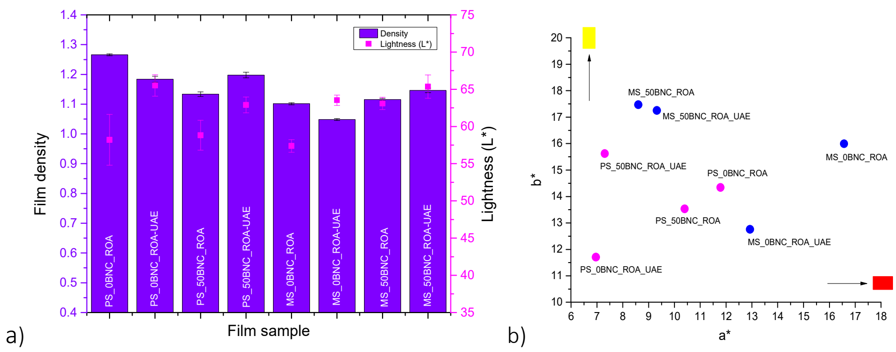

Figure 3a) Film density and CIE lightness (L) values*
and b) CIE a/b* coordinates of the produced starch-based films*

4.3 Visual analysis of starch-based films immersed in different pH-buffers

Figure 4 shows the colour changes of the produced potato-based films after immersion in different buffer solutions (pH 2.0 - pH 10.5). Figures 4a and b show the samples without the addition of BNC (PS_0BNC_ROA and PS_0BNC_ROA-UAE) and Figures 4c and d show the samples with the addition of BNC (PS_50BNC_ROA and PS_50BNC_ROA-UAE).

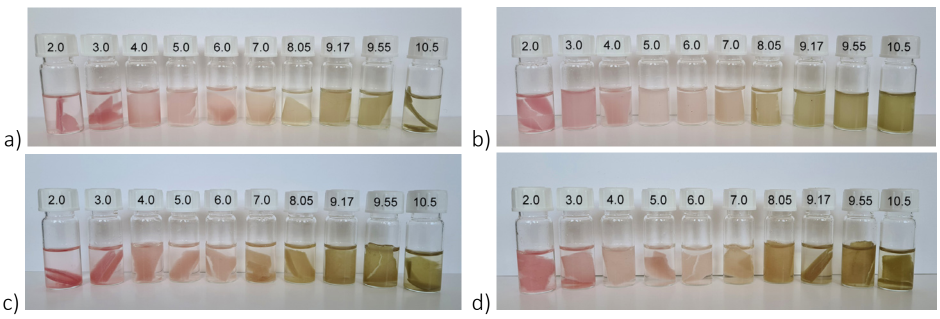

Figure 4: Potato starch-based films immersed in different buffers, (a) PS_0BNC_ROA, (b) PS_0BNC_ROA-UAE, (c) PS_50BNC_ROA and (d) PS_50BNC_ROA-UAE

All samples show a gradual transition from pink to light pink to light green and brownish. There is a slight difference in colour appearance for the samples containing anthocyanins extracted with ultrasound assistance (Figures 4b and d); it appears that the colours are slightly enhanced across the range of buffers. On the other hand, the addition of BNC (Figures 4c and d) has a visible effect on the colour change. The films in the different buffers show a more pronounced colour transition when BNC is added than the samples without BNC.

Figure 5 shows the colour changes of the produced maize-based films after immersion in different buffer solutions (pH 2.0 – pH 10.5). Figures 5a and b show the samples without the addition of BNC (MS_0BNC_ROA and MS_0BNC_ROA-UAE) and Figures 5c and d show the samples with the addition of BNC (MS_50BNC_ROA and MS_50BNC_ROA-UAE). As can be seen in the images, the colour transition is quite uniform in all samples. The transitions from pink and light pink to green-brownish are clearly visible. Considering the different composition of the films, there are no significant differences in the colour response in the samples. However, it can be seen that the samples treated with ultrasound have a slightly more pronounced yellowish tone in all samples. The colour transitions are least pronounced in sample MS_50BNC_ROA-UAE.

When looking at the images of film samples exposed to different pH buffers, as shown in Figures 4 and 5, overall more pronounced visual and colour responses were observed in films containing maize starch. In addition, corn starch-based films immersed in buffer solutions are visibly stable, while potato starch-based films begin to dissolve in the solutions. This appearance can significantly affect the stability of the pH indicators produced, so further research on this topic is required.

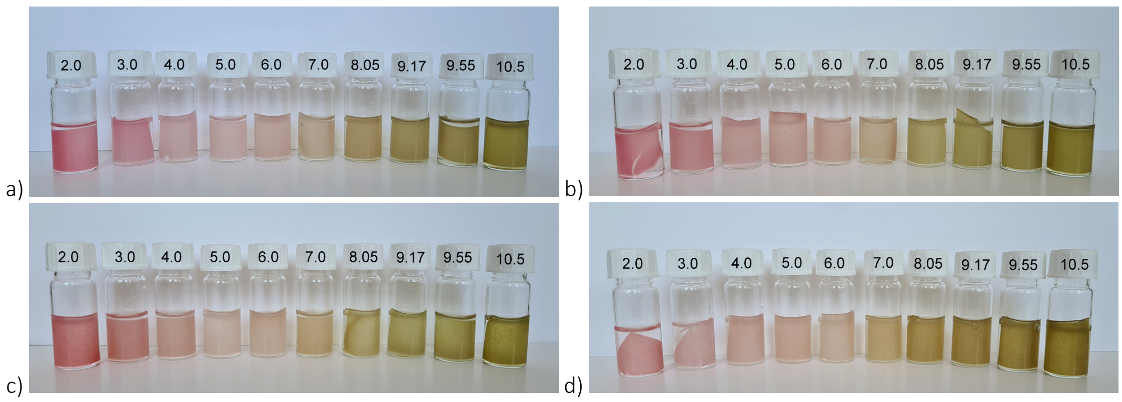

Figure 5: Maize starch-based films immersed in different buffers: (a) MS_0BNC_ROA, (b) MS_0BNC_ROA-UAE, (c) MS_50BNC_ROA and (d) MS_50BNC_ROA-UAE

The visual colour changes observed on potato and maize starch-based films with anthocyanins extracted from red onion peels proved that the films produced can provide information on whether the pH of the food or the pH of the environment varies. According to literature (Sujiwo, 2018), fresh meat must have a pH value in the range of 5.5 to 6.2. If stored improperly, fresh meat begins to spoil and has a pH below 5.3 or rises. It can be seen that all films produced in the pH range between 5 and 6 have a pale pink to pink colour. Lowering the pH leads to a colour shift towards a more pronounced pink and increasing the pH leads to a colour shift towards a greenish brown. These results indicate a positive response in the colour change during the production of pH indicators based on potato and maize starch.

4.4 Colorimetric properties of starch-based films exposed to different pH-buffers

Relative CIE L*a*b* values measured on the films placed on the white paper surface after immersion in buffer solutions (pH 2.0 - pH 10.5) were calculated and presented in Figures 6 to 9 Lightness (L*) was presented separately since previous research (Mahović Poljaček et al., 2024) showed that the addition of BNC affected the films' swelling behaviour, as well as other properties of the films that could directly influence L* values. Furthermore, observing the changes in CIE a* and b* values on a separate diagram can help with the analysis of the shifts in hue range and anthocyanin stability (Mahović Poljaček et al., 2024).

Lightness of the films produced without the UAE of anthocyanins was lower than lightness of the films with UAE-anthocyanins in the pH range between 3 and 10.5 (Figure 6a). However, the addition of BNC, which is not completely optically transparent, disrupted this property of the films produced using UAE during anthocyanin extraction (Figure 6b). Higher L* values of 50_BNC_ROA-UAE films are observable only in the acidic and neutral pH range and are not consistently higher than L* values of 50_BNC_ROA film samples. The reduction in the lightness of the films containing 50% BNC compared to the films without BNC may be attributed to the optical and physical properties of BNC (Almeida et al., 2023), as well to the interactions of BNC and ROA.

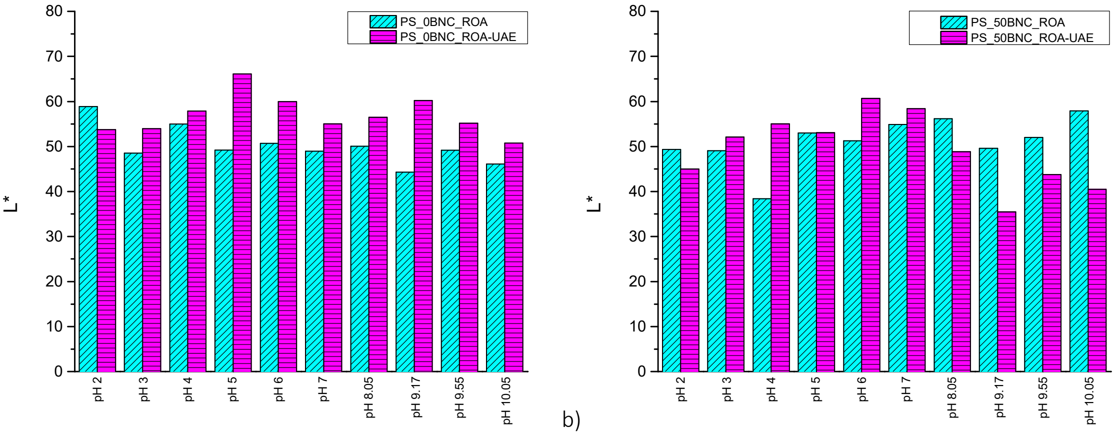

Figure 6: Lightness (L) of potato starch-based films produced without and with UAE,*
a) PS_0BNC and b) PS_50BNC

Figure 7a presents the changes in CIE a* and b* values of PS-based films while Figure 7b presents the changes in MS-based films resulting from the changed pH of the environment.

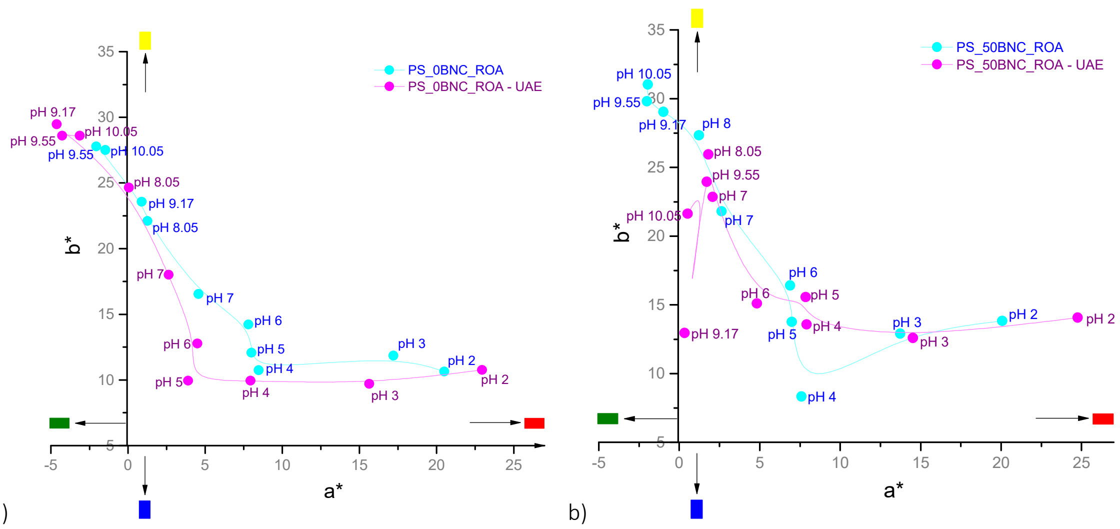

Figure 7: CIE a/b* values for potato starch-based films produced without and with UAE,*
a) PS_0BNC, b) PS_50BNC

By observing Figure 7a, it can be seen that UAE caused the expansion of the colour shift range in both a* and b* axis when film samples without BNC were exposed to buffer solutions. This expansion can be linked to the increased concentration of anthocyanins in the film and certainly contributes to the vividness of the film colours after their exposure to varied-pH environments. On the other hand, UAE expanded the colour shift range only in highly acidic environment (pH up to 3) for the film with BNC and UAE (Figure 7b). At alkaline pH levels, colour changes of the films among the neighbouring pH steps remained modest comparing to the colour changes of the films produced without UAE of anthocyanins. It is noteworthy that the L* values of the PS-based films with BNC produced using UAE of anthocyanins significantly decreased in alkaline medium, as well (Figure 6b). This could point to the interaction between BNC and anthocyanins at their higher concentrations, and in alkaline medium. Specifically, when comparing respective a*/b* plots in Figures 7a and b, it is visible that the addition of BNC resulted in more expressed shift to yellow hues for the film without UAE of anthocyanins. As a contrast, the film with

BNC and UAE of anthocyanins did not show such expressed shift to yellow hues at alkaline pH compared to the film without BNC. This occurrence could be both the advantage and disadvantage, depending on the intended range of pH values during the utilization of the films. More expressed colour change with increased pH is certainly easier to notice, but at the same time points to the anthocyanin degradation (Ohno et al., 2021).

L* values of the MS-based films are presented in Figure 8. Contrary to the behaviour of PS-based films, the increased lightness of the MS-based films containing 50% BNC compared to the films without BNC is visible when comparing Figures 8a and b, especially when UAE is included in the film production. The results obtained are consistent with previous research showing that BNC could stabilize and preserve anthocyanins in the pH-sensing films. This is because BNC can form a network that physically protects anthocyanins from degradative factors. Additionally, previous research has demonstrated that incorporating BNC into pH-sensing films resulted in more vibrant and stable colorimetric responses under various conditions, and that maize starch is superior matrix to potato starch for the encapsulation of anthocyanins due to the better and stronger interactions with BNC and anthocyanins (Cao et al., 2023; Mahović Poljaček et al., 2024).

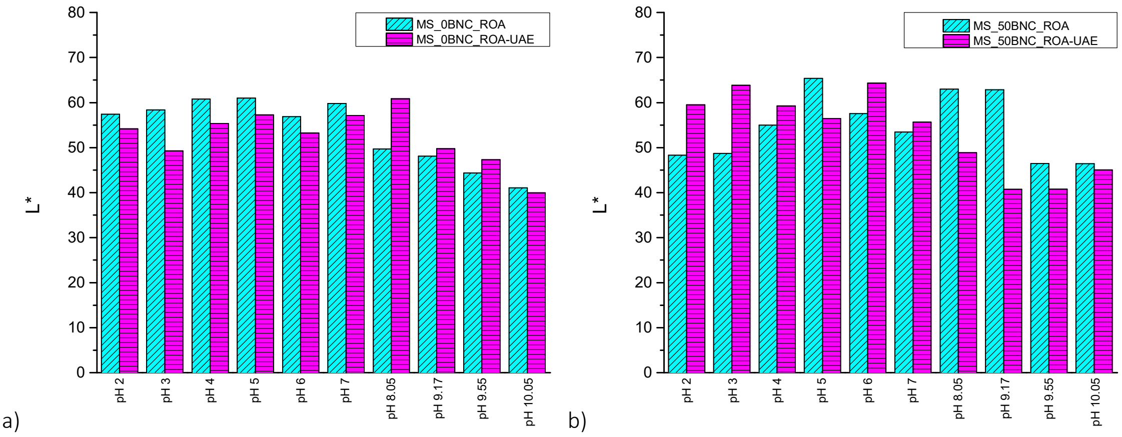

Figure 8: Lightness (L) of maize starch-based films,*
a) MS_0BNC and b) MS_50BNC

By observing Figure 9a, it can be seen that UAE caused the expansion of the colour shift range when MS-based film samples without BNC were exposed to buffer solutions, similar to PS-based films. However, this expansion is observable only on a* axis. When BNC was added to the film composition, UAE did not result with the colour shift expansion (Figure 9b). This could be related to the increased stability of the ROA in the starch matrix, due to their interaction with BNC.

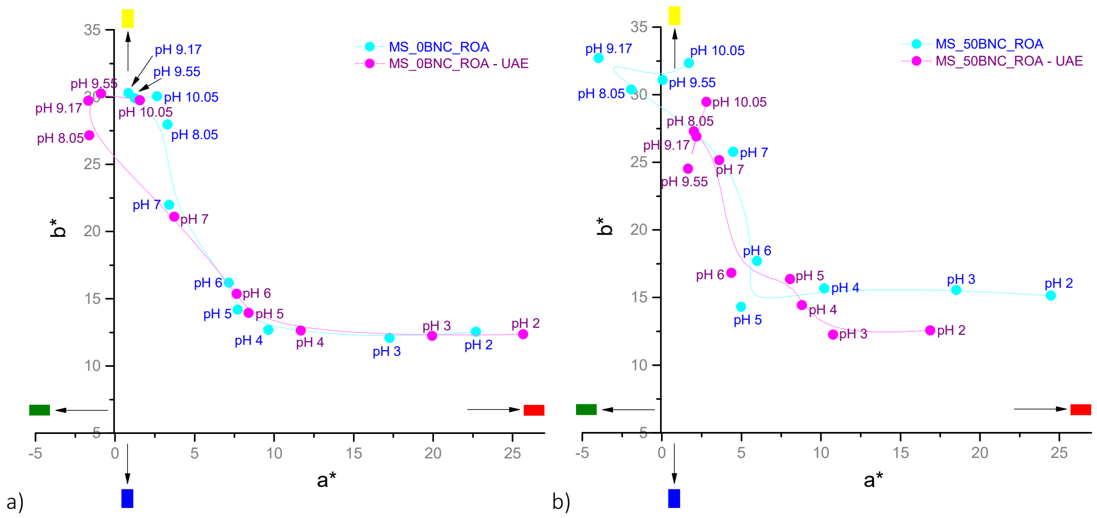

Figure 9: CIE a/b* values for maize starch-based films,*
a) MS_0BNC, b) MS_50BNC

When comparing Figures 9a and b, it can be thereby concluded that the addition of BNC expanded the colour shift range only for the films produced without using UAE of the anthocyanins. However, increased L* values of the MS-based films with BNC in acidic and neutral media comparing to the films without BNC contributed to the differences in the colour appearance of the films.
These research findings suggest that adding BNC to starch-based films, especially in the case of MS-based films, offered advantage for applications in indicator systems and smart packaging due to the stabilization of the anthocyanins in the starch matrix. However, depending on the application of such films, the expansion of the colour shift range should be considered. Moreover, the colorimetric measurements, particularly the analysis of CIE lightness (L*) in a separate diagram and the use of CIE a*/b* plots, can help interpret the interactions of different anthocyanins with starch base and additives such as BNC.

5. CONCLUSIONS

In this research, pH indicators with polymeric bio-based matrices, anthocyanins from red onions and bacterial nanocellulose were investigated to observe their potential for use in smart packaging applications. Potato and maize starch were used as matrix for the preparation of films, bacterial nanocellulose was added to immobilize anthocyanins, acetic acid and glycerol were added in film-forming solutions to obtain films with optimal properties for measurements. Ultrasound-assisted treatment of the extracted anthocyanins was performed to determine the influence of the treatment on the colour response of the produced films. To observe the colour response of the prepared starch-based films when exposed to different pH values, their optical and colorimetric changes were monitored before and after exposure to altered pH values.
The research results have shown that it is possible to produce bio-based films that can be used in pH-controlled smart packaging. The pH-sensitive films produced showed a visually detectable colour change when the pH of the environment changed, proving their potential and the use of anthocyanins to produce a pH indicator.
When determining the total monomeric anthocyanin pigment content, ultrasound-assisted extraction was found to cause a slight increase in the anthocyanin content of the extracts. Since pH-sensitive indicators can change colour depending on the pH of the environment, it was found that the addition of bacterial nanocellulose to starch-based films, especially in the case of maize-based films, offers advantages for applications in indicator systems and smart packaging.
The results show that the produced films could be used in smart packaging or as an improved packaging material. Further research on mechanical properties, water barrier properties, antioxidant properties and other interactions with different packaging materials should be conducted to further investigate the effectiveness of the presented pH indicators.

6. REFERENCES

Almeida, T., Karamysheva, A., Valente, B.F.A. & Silva, J.M. (2023) Biobased ternary films of thermoplastic starch, bacterial nanocellulose and gallic acid for active food packaging. *Food Hydrocolloids*. 144, 108934. Available from: doi:10.1016/J.FOODHYD.2023.108934

Beluhan, S., Herceg, F., Pavunc, A.L. & Djaković, S. (2022) Preparation and Structural Properties of Bacterial Nanocellulose Obtained from Beetroot Peel Medium. *Energies*, 15, 9374. Available from: doi:10.3390/EN15249374

Cao, S., Wang, S., Wang, Q. & Lin, G. (2023) Sodium alginate/chitosan-based intelligent bilayer film with antimicrobial activity for pork preservation and freshness monitoring. *Food Control*. 148, 109615. Available from: doi:10.1016/J.FOODCONT.2023.109615

Chayavanich, K., Kaneshige, R., Thiraphibundet, P., Furuike, T., Tamura, H. & Imyim, A. (2023) pH-responsive nanofibrous membrane fabricated from gelatin and red radish anthocyanins for meat spoilage monitoring, *Dyes and Pigments*. 216, 111331. Available from: 10.1016/j.dyepig.2023.111331

Chesnokova, N.Y., Chebukin, P.A., Levochkina, L.V., Prikhod'Ko, Y.V. & Kuznetsova, A.A. (2021) Influence of ultrasound and extraction conditions on the intensity of extraction of anthocyanin pigment from berry raw materials. *IOP Conf. Ser.: Earth Environ. Sci*. 848. 012211. Available from: doi: 10.1088/1755-1315/848/1/012211

Hock Eng Khoo, Azlan, A., Tang, S.T. & See Meng Lim (2017) Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food Nutr Res*., 61(1), 1361779. Available from: doi: 10.1080/16546628.2017.1361779

Kurek, M., Hlupić, L., Ščetar, M., Bosiljkov, T. & Galić, K. (2019) Comparison of Two pH Responsive Color Changing Bio-Based Films Containing Wasted Fruit Pomace as a Source of Colorants. *J. Food Sci.* 84, 2490–2498. Available from: doi: 10.1111/1750-3841.14716

Lavrič, G., Medvešček, D. & Skočaj, M. (2020) Papermaking properties of bacterial nanocellulose produced from mother of vinegar, a waste product after classical vinegar production. *Tappi J*. 19, 197–203. Available from: doi:10.32964/TJ19.4.197

Lavrič, G., Oberlintner, A., Filipova, I., Novak, U., Likozar, B. & Vrabič-Brodnjak, U. (2021) Functional Nanocellulose, Alginate and Chitosan Nanocomposites Designed as Active Film Packaging Materials. *Polymers*. 13, 2523. Available from: doi:.3390/polym13152523

Lee, J., Durst, R.W., Wrolstad, R.E., Barnes, K.W., Eisele, T, Giusti, M., Haché, J., Hofsommer, H., Koswig, S., & Krueger, D.A. (2005) Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study. *J. AOAC Int*. 88, 1269–1278. Available from: doi: 10.1093/JAOAC/88.5.1269

Mahović Poljaček, S., Tomašegović, T., Strižić Jakovljević, M., Jamnicki Hanzer, S., et al. (2024) Starch-Based Functional Films Enhanced with Bacterial Nanocellulose for Smart Packaging: Physicochemical Properties, pH Sensitivity and Colorimetric Response. *Polymers* 2024. 2216 (16), 2259. Available from: doi:10.3390/POLYM16162259

Moradi, M., Tajik, H., Almasi, H., Forough, M. & Ezati, P. (2019) A novel pH-sensing indicator based on bacterial cellulose nanofibers and black carrot anthocyanins for monitoring fish freshness, Carbohydrate *Polymers*. 222, 115030. Available from: doi: 10.1016/j.carbpol.2019.115030

Mustafa, F. & Andreescu, S. (2020) Nanotechnology-based approaches for food sensing and packaging applications. *RSC Adv.* 10, 19309–19336. Available from: doi: 10.1039/D0RA01084G

Ohno, S., Yokota, M., Yamada, H. & Tatsuzawa, F. (2021) Identification of Chalcones and their Contribution to Yellow Coloration in Dahlia (Dahlia variabilis) Ray Florets. *The Horticulture Journal*. 90 (4), 450–459. Available from: doi: 10.2503/HORTJ.UTD-305

Oladzadabbasabadi, N., Nafchi, A.M., Ghasemlou, M., Ariffin, F., Singh, Z. & Al-Hassan, A.A. (2022) Natural anthocyanins: Sources, extraction, characterization, and suitability for smart packaging, *Food Packaging and Shelf Life.* 33, 100872. Available from: doi: 10.1016/j.fpsl.2022.100872

Perdani, C.G. & Gunawan, S. (2021) A short review: Nanocellulose for smart biodegradable packaging in the food industry. *IOP Conf. Ser. Earth Environ. Sci.* 924, 12032. doi:10.1088/1755-1315/924/1/012032.

Pounds, K., Jairam, S., Bao, H., Meng, S., Zhang, L., Godinez, S., Savin, D.A., Pelletier, W., Correll, M.J. & Tong, Z. (2021) Glycerol-Based Dendrimer Nanocomposite Film as a Tunable pH-Sensor for Food Packaging. *ACS Appl. Mater. Interfaces.* 13, 23268–23281, Available from: doi: 10.1021/ACSAMI.1C05145/SUPPL_FILE/AM1C05145_SI_001.PDF

Pourjavaher, S., Almasi, H., Meshkini, S., Pirsa, S. & Parandi, E. (2017) Development of a colorimetric pH indicator based on bacterial cellulose nanofibers and red cabbage (Brassica oleraceae) extract. *Carbohydrate Polymers*, 156, 193-201. Available from: doi: 10.1016/j.carbpol.2016.09.027

Rodrigues, C., Souza, V.G.L., Coelhoso, I. & Fernando, A.L. (2021) Bio-Based Sensors for Smart Food Packaging—Current Applications and Future Trends. *Sensors.* 21, 2148. Available from: doi: 10.3390/S21062148

Samota, M.K., Sharma, M., Kaur, K., Sarita, Yadav, D.K., Pandey, A.K., Tak. Y., Rawat, M., Thakur, J. & Rani, H. (2022) Onion anthocyanins: Extraction, stability, bioavailability, dietary effect, and health implications. *Front. Nutr.* 9, 917617. Available from: doi: 10.3389/fnut.2022.917617

Sujiwo, J., Kim, D. & Jang, A. (2018) Relation among quality traits of chicken breast meat during cold storage: correlations between freshness traits and torrymeter values. *Poultry Science*. 97(8), 2887-2894. Available from: doi: 10.3382/ps/pey138

Tan, J., Han, Y., Han, B., Qi, X., Cai, X., Ge, S. & Xue, H. (2022) Extraction and purification of anthocyanins: A review. *J. Agric. Food Res*. 8, 100306. Available from: doi: 10.1016/j.jafr.2022.100306

Zhao, L., Liu, Y., Zhao, L. & Wang, Y. (2022) Anthocyanin-based pH-sensitive smart packaging films for monitoring food freshness. *Journal of Agriculture and Food Research.* 9, 100340, Available from: doi: 10.1016/j.jafr.2022.100340

Zhou, X., Xie, W., Jing, H., Fu, J., Li, M., Bian, J., Xu, J., Cai, Y., He, H. & Zhou, D. (2023) Analysis of anthocyanins and total flavonoids content in functional rice and its recombination inbred lines. *Front. Plant Sci.* 14, 1113618. Available from: doi: 10.3389/fpls.2023.1113618