

Production of pH indicators from starch and anthocyanins for use in smart packaging

ABSTRACT

In this research, natural pigments, anthocyanins, were used to produce bio-based films that can be used in smart packaging, as they serve as pH indicators to monitor the freshness of the packaged products. The aim of the research is to analyse the colorimetric and optical differences in the produced films before and after they are exposed to the environment with different pH values. In order to evaluate the possibility of using the produced films in printing processes, the adhesion of the produced pH indicators to packaging material was determined. This research has shown that it is possible to produce pH indicators from anthocyanins extracted from plant residues as well as from different types of starch. The influence of the anthocyanins immobilised in the starches on the colorimetric properties of the produced films and the adhesion of the films to the packaging material was evaluated. The research results showed that it is possible to produce a pH-responsive indicator based on the ingredients used. Different types of starch showed no significant difference in the visual appearance of the films, but a difference was found when different anthocyanins were used. It was proposed to use potato starch with anthocyanins from red cabbage and maize starch with anthocyanins from red onions to produce pH indicators. These polymer composites showed clear color shifts that are easy to recognise visually.

KEY WORDS

smart packaging, pH-indicators, anthocyanins, biodegradable composite films

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Introduction

Over the last two decades, the concept of packaging has been evolved and the basic functions of traditional food packaging have been updated. A new and innovative approach to packaging has been introduced by adding new and enhanced functions to traditional packaging. Beyond the basic functions of protecting and storing goods, functions have been introduced that can monitor, sense, and communicate important information about the condition of the product throughout its life cycle. For food packaging, a number of additional advanced systems have been introduced to improve food quality, increase product safety, extend the shelf life of the

packaged product and provide more information about the packaged product through communication with the consumer (Gregor-Svetec, 2018; Schaefer & Cheung, 2018; Thirupathi Vasuki, Kadirvel & Pejavarana Narayana, 2023). Whether for food, pharmaceuticals or consumer goods, smart packaging systems provide real-time data on factors such as freshness, temperature and integrity, helping to ensure product quality and safety. Basically, smart packaging comprises two types of advanced systems: active packaging that interacts with the contents to extend shelf life or increase safety, and intelligent packaging that provides information about the condition of the product, such as time-temperature and freshness indicators, sensors and even RFID tags

that enable traceability through supply chains. By bridging the gap between the product and the consumer, smart packaging not only improves the user experience, but also plays a crucial role in reducing waste, complying with regulations and promoting sustainability (Brockgreitens & Abbas, 2016; Halonen et al., 2020).

In this research pH indicators that can be used in smart packaging applications, were produced and analysed. This type of indicator is mainly used in food packaging, as food spoilage is often associated with changes in the natural pH of fresh food, so that the freshness or spoilage of perishable food can be detected by monitoring pH changes in the packaging. By incorporating a pH indicator into the packaging, the fluctuating pH of the packaged food can be detected by a visual colorimetric response. pH indicators are usually made from natural, renewable ingredients that are biodegradable and environmentally friendly (Luo et al., 2021; Păușescu et al., 2022; Yong & Liu, 2020). They usually consist of biopolymers such as chitosan, gelatin, starch, agar and cellulose and their derivatives, which ensure the stability and flexibility of the film (Benalaya et al., 2024; Contessa et al., 2023, Liu et al., 2022).

The pH-sensing elements used are mainly the natural pigments found in fruits and vegetables (anthocyanins, red radish extracts, curcumin and beetroot extract), which can change colour in response to pH fluctuations (Chayavanich et al., 2023; Mahović Poljaček et al., 2024a). In addition, various types of plasticizers are added to ensure the flexibility and processability of the functional films, as well as crosslinking agents and additives such as various antioxidants and antimicrobial agents (Mahović Poljaček et al., 2024b).

Currently, most studies focus on the application of freshness indicators in food packaging, and the applications of smart packaging with freshness indicators in the food sector are limited. Before widespread commercial use can be achieved, several limitations associated with freshness indicators that rely on a broad color spectrum need to be addressed. A more accurate correlation between color response, product type, target metabolites and organoleptic quality and safety is needed. For this reason, further studies that provide a reliable indication of actual spoilage should be conducted to ensure the use of indicators without the risk of false negative results. In this research, potato and maize starch were used to produce biodegradable composite films. Anthocyanins extracted from the peels of red onions and red cabbage leaves were used as a pH-sensing element. The colorimetric and optical properties of the produced films were measured before and after exposure to environments with different pH values. To evaluate the possibility of using the produced films in the printing process, the adhesion of the pH indicators to the packaging material was determined.

Materials and methods

Materials and film preparation

The polymer composite for the preparation of a film-forming solution consisted of potato starch (extra pure, CAS: 9005-25-8) (Carl Roth, Germany), maize starch (extra pure, CAS: 9005-25-8) (Carl Roth, Germany), distilled water, glycerol (purity 99.5%, CAS: 56-81-5, Gram-Mol d.o.o., Croatia) and glacial acetic acid (1% v/v, CAS: 64-19-7, VWR BDH Prolabo, USA) in different concentrations. Red cabbage (*Brassica oleracea* L.) and red onions (*Allium cepa* L.) used for the extraction of anthocyanins were obtained from the local market. Commercially available pH buffers (Gram-Mol d.o.o., Croatia) (pH2-pH4 [$C_6H_8O_7$ (1-hydrate), NaOH, HCl, H_2O], pH5-pH7 [$C_6H_8O_7$ (1-hydrate), NaOH, H_2O], pH8 [H_3BO_3 , NaOH, HCl, H_2O], pH9-pH10 [H_3BO_3 , NaOH, $CaCl_2$, H_2O] were used for the detection of color changes.

Anthocyanins were extracted from the outer leaves of red cabbage and the peels of red onions using the modified method described in the previously published study (Tan et al., 2022). The samples were milled and mechanically mixed in a 96% (v/v) ethanol solution (Pharmachem, Slovenia). Extraction was performed by mixing 200 g of sliced samples in 300 ml of ethanol. The resulting mixture was placed in a water bath at 60 °C for 90 minutes. The samples were then cooled and filtered using Whatman® Quantitative Filter Paper, ashless, grade 40 (Cytiva, USA). The anthocyanin extracts were stored in the refrigerator before use.

The film-forming solutions containing potato and maize starch were prepared by mixing starch with other components in different concentrations. The first set of samples was based on potato starch (PS) and the second on maize starch (MS). Each starch sample was dissolved in distilled water to obtain 2% (w/v) film-forming solutions and heated on a temperature-controlled hotplate (Tehtnica, Rotamix 550 MMH, Domel, Slovenia) under stirring (DLS Digital Overhead Stirrer, Velp Scientifica Srl, Italy). The pH-sensing elements, anthocyanins, extracted from red cabbage leaves (RCA) and peels of red onions (ROA) were added to each film-forming solution. Acetic acid and glycerol was slowly added to the film forming solution by gradually increasing the temperature to ensure solubilization and complete gelatinization of the ingredients. The PS film-forming solution was heated to 60 °C and the MS solution to 80 °C, as they are different types of starch and different processes occur during the production of the film samples (Pounds et al., 2021). The preparation of each starch-based film took about 30 minutes, after which the film-forming solution was poured into a Petri dish. The films were dried and stored in a ventilated climate chamber at 25 °C and 50% relative humidity (RH) for seven days.

A total of six starch-based films were produced: two samples based on PS and MS starch without anthocyanins and four samples based on PS and MS starch with anthocyanins (RCA and ROA). The samples produced and their ingredients are summarized in Table 1. The amount of ingredients refers to the total amount of film-forming solutions required for the production of starch-based films.

Table 1

The composition of film-forming solutions

Produced films	Acetic acid (%)	Glycerol (%)	ROA (%)	RCA (%)	Designation of samples
PS starch	15	3	/	/	PS
	15	3	/	14	PS_RCA
	15	3	12	/	PS_ROA
MS starch	20	5	/	/	MS
	20	5	/	14	MS_RCA
	20	5	12	/	MS_ROA

Methods

The CIE $L^*a^*b^*$ color values were measured on dry film samples before and after they were immersed in different buffer solutions. The lightness value (L^*) of a color describes its relative lightness, the a^* chromatic coordinate defines the position of the measured color between green and red and the coordinate b^* defines the position of the color between yellow and blue in the CIE color space (Fairchild, 2013). The Techkon Spectro-Dens spectro-densitometer (TECHKON GmbH, Germany) was used for the measurements. The measurement conditions were set to illuminant D50/2°, M1 filter according to ISO 13655:2017 (International Organization for Standardization, 2017). 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. After immersing the anthocyanin-containing samples in different pH buffers for twenty minutes, the colorimetric parameters were also measured.

Images of the films immersed in different buffer solutions were taken to observe the visual change in the starch-based films produced.

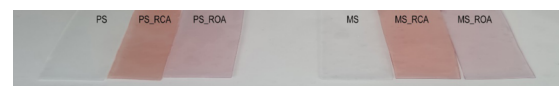
The surface properties of the dry films were investigated by calculating the surface free energy (SFE) of the samples. The SFE of the solid surface is usually measured indirectly using the results of contact angle measurements (θ) with sample liquids of known surface tension. In this study, demineralized water, glycerol and diiodomethane were used. The total surface tension, dispersive surface tension and polar surface tension,

expressed in mJ/m^2 , were 72.8, 21.8 and 51.0 for water, 64.0, 34.0 and 30.0 for glycerol and 50.8, 50.8 and 0 for diiodomethane, respectively. The contact angles were calculated according to Young's equation and the SFE according to the Owens-Wendt-Rabel and Kaelble method (Israelachvili, 2011; Owens & Wendt, 1969; Żenkiewicz, 2007). The measurements were carried out using a DataPhysics OCA 30 goniometer (DataPhysics Instruments GmbH, Germany). To determine the adhesion properties of the produced starch-based films and the polypropylene substrate, which is most commonly used for food packaging, the adhesion parameters were calculated, i.e., the spreading coefficient (S_{12}), work of adhesion (W_{12}) and the interfacial tension (γ_{12}) (Israelachvili, 2011).

Results and discussion

Optical properties of produced films

Figure 1 shows the samples of the starch-based films produced. The images were taken after the drying process, which took about seven days in a ventilated climate chamber. The films were separated from the Petri dishes for image capturing, cut into strips and applied to a transparent substrate. The transparent films (on the left side of the film sets, labeled MS and PS) are films that were produced without the addition of anthocyanins. In the middle are film samples that were produced by adding anthocyanins from red cabbage and have a pale pink color (MS_RCA and PS_RCA). The samples on the right were produced by adding anthocyanins from red onion peels and have a pale purple color (MS_ROA and PS_ROA).



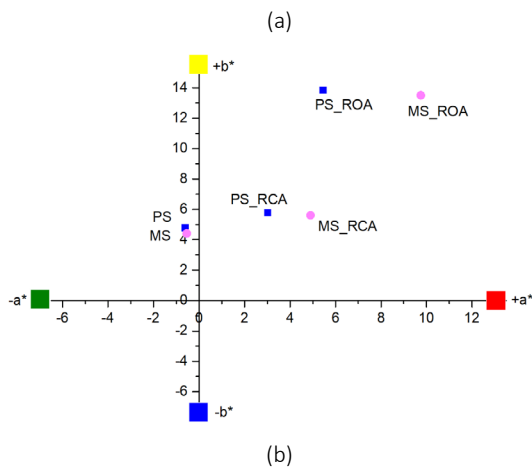
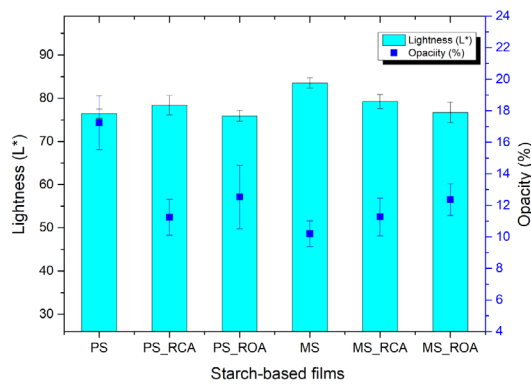
» **Figure 1:** Potato (PS) and maize (MS) starch-based films without and with anthocyanins

Figure 2 shows the optical properties of the dry films produced. The results of absolute lightness (L^*) and opacity are shown in Figure 2a. When looking at the lightness results, it can be seen that the values for all samples are relatively uniform and are between 75 and 80 lightness units. The lightness values are slightly higher for the film samples made from maize starch.

When looking at the opacity values, it can be seen that the film made from potato starch has the highest opacity (approx. 17.25 %) and the film made from maize starch has the lowest value (approx. 10.2 %). The addition of anthocyanins reduces the opacity values in the films made from potato starch and slightly increases them in the samples made from maize starch. The different types of anthocyanins show no significant difference in the optical properties of the films.

Since the starch-based films produced are relatively translucent, the color change of the samples varied depending on the composition and could sometimes be observed and visually compared better when placed on a white substrate.

For this reason, the samples were measured while placed on a white paper that served as a substrate. The relative CIE a^*/b^* color values (with paper as the white point) measured on the PS and MS film samples are shown in Figure 2b. It can be seen that the PS and MS films without the addition of anthocyanins have similar CIE a^*/b^* color coordinates with no significant differences. The addition of anthocyanins to the films shifted the color. Samples containing anthocyanins from red cabbage have lower CIE a^*/b^* color values (MS_RCA and PS_RCA) compared to samples prepared by adding anthocyanins from the peels of red onions to the films (MS_ROA and PS_ROA).



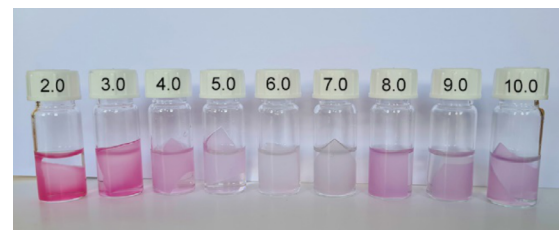
» **Figure 2:** (a) CIE lightness (L^*) values and the opacity and (b) CIE a^*/b^* color coordinates of the starch-based films

It can be said that these results were expected and that the addition of anthocyanins has a significant effect on the color shifts of the starch-based films produced. Since the intention of this research is to produce an indicator that shows a visually detectable color shift by varying the pH value, these results show the potential of the anthocyanins used for the production of a pH indicator.

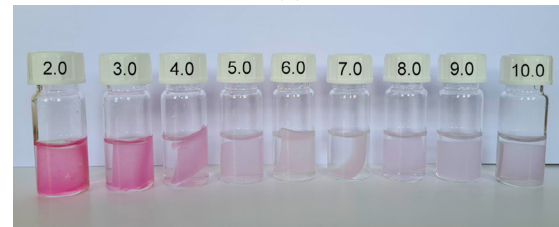
Visual analysis of produced films in varied-pH environment

To observe the color changes in the films when exposed to an environment with different pH values, the anthocyanin-containing samples were immersed in buffer solutions with a pH value of 2 to 10. The images of the samples immersed in buffer solutions for 20 minutes are shown in Figure 3.

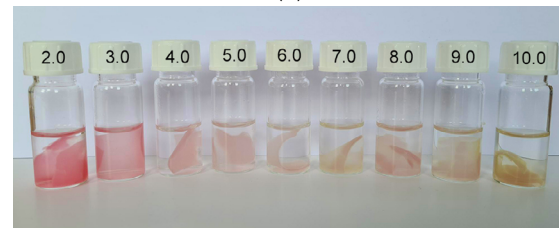
Figures 3a and 3b show PS and MS films containing anthocyanins from red cabbage and Figures 3c and 3d show films containing anthocyanins from red onion peels. It can be seen that the samples show considerable color differences at different pH values, which is primarily due to the fact that the anthocyanins are extracted from different plants. The color of the starch-based films with red cabbage anthocyanins changed from pink (pH2) to colorless (pH7) and light pink at pH10. The color of the starch-based films with anthocyanins from the peel of red onions changed from reddish-pink (pH2) to light red (pH7) and pinkish-yellowish at pH10.



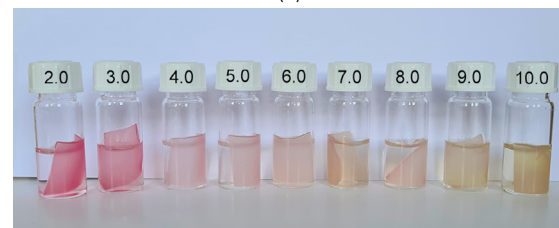
(a)



(b)



(c)



(d)

» **Figure 3:** Images of the films immersed in different buffer solutions: (a) PS_RCA, (b) MS_RCA, (c) PS_ROA and (d) MS_ROA

These visual color changes observed on starch-based films proved that the films produced can provide information on whether the pH of the food or the pH of the environment is varying. Different fresh meat must have a pH in the range of 5.5 to 6.2 (Sujiwo, Kim & Jang, 2018). The initial spoilage of meat or fish occurs at a pH of around 6, and then the pH continues to drop in small intervals to around 4 or 5. When the pH drops to around 2, yeasts and other microorganisms can continue to grow on the food and make it unusable and spoiled (Pounds et al., 2021). On the other hand, increased pH is associated with the production of nitrogenous basic compounds, mainly amino groups, which also cause the microbial spoilage of packaged foods (Triki et al., 2018).

Colorimetric properties of produced films in varied-pH environment

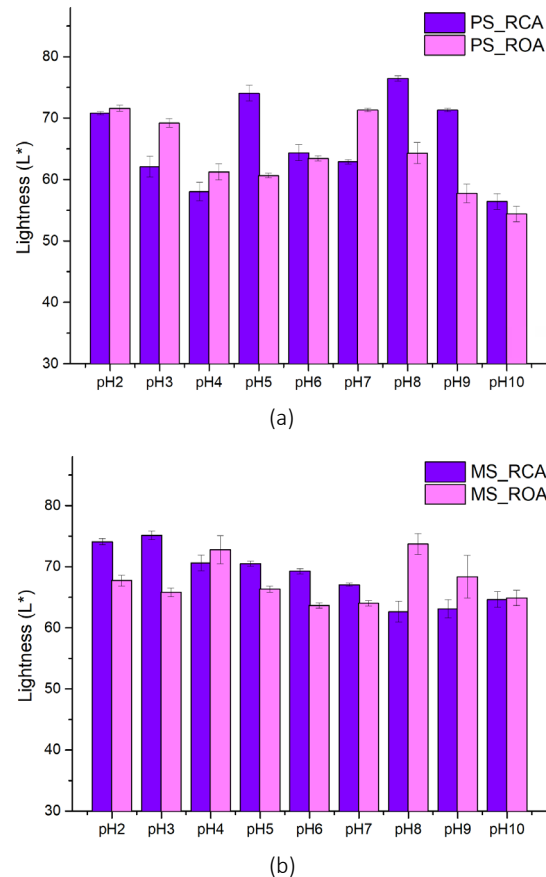
After immersion, the films were dried and additional measurements of the colorimetric properties were carried out. The results of the lightness are shown in Figure 4. Figure 4a shows the PS-starch-based films with RCA and ROA anthocyanins and Figure 4b shows the MS-starch-based films with RCA and ROA anthocyanins. It can be seen that the lightness values for the films produced with the same starch type are similar and that the addition of different anthocyanins has no significant effect on the lightness. There are some exceptions with PS-based films where it appears that the RCA causes the higher lightness values, but these results may be the consequence of the irregular measurement conditions due to the uneven surfaces and thickness of the films. Overall, the lightness results show that higher values are obtained for MS films than for PS films, which may be due to the higher initial value of MS films (compared to PS films) produced without anthocyanins, as shown in Figure 2a.

Figures 5 and 6 show the changes in the CIE color coordinates a^* and b^* of the dried PS- and MS-based films after immersion in different buffer solutions from pH2 to pH10; a polynomial trendline was added to the plots. The results are presented according to the type of anthocyanins used in the film production. Figure 5 shows the relative CIE a^*/b^* values of the PS_RCA and MS_RCA films and Figure 6 shows the relative CIE a^*/b^* values of the PS_ROA and MS_ROA films. The circled ellipses show the ranges of the chromatic CIE a^*/b^* coordinates for films immersed in pH5 and pH6 solutions corresponding to the pH values of fresh meat and seafood (depending on type of product). Deviations from these pH values may indicate possible spoilage of the packaged food due to irregular storage, improper handling, expiration date and other causes.

The results shown in Figure 5 show that the changes in the CIE a^* and b^* values of PS_RCA and MS_RCA films (films with red cabbage anthocyanins) lie in the range

between the red and blue color coordinates. It can be said that PS films show a slightly wider range of color shifts than MS films after immersion in different buffer solutions and that the PS_RCA films show a more pronounced shift to the reddish hue ($+a^*$ values).

These results may indicate that the color changes that can occur due to the variation in pH are visually more pronounced in the PS-based films compared to MS films.

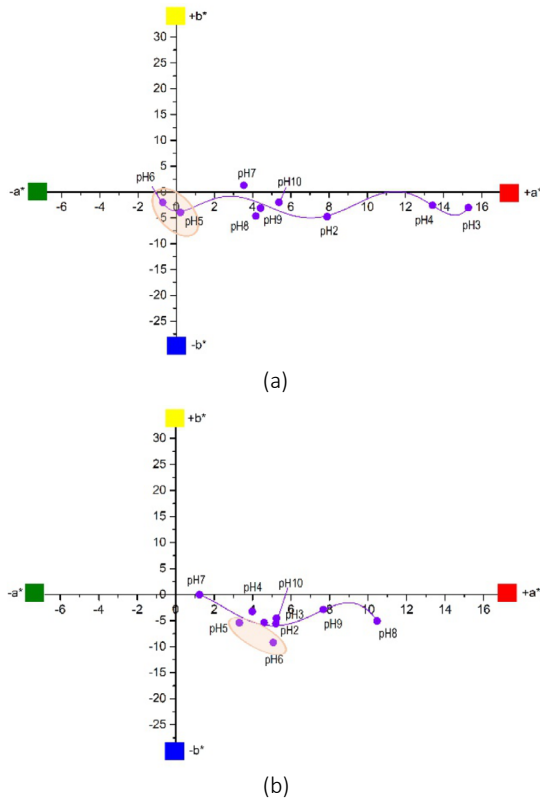


» **Figure 4:** Relative CIE L^* values of the films immersed in different buffer solutions: (a) PS_RCA and PS_ROA, (b) MS_RCA and MS_ROA

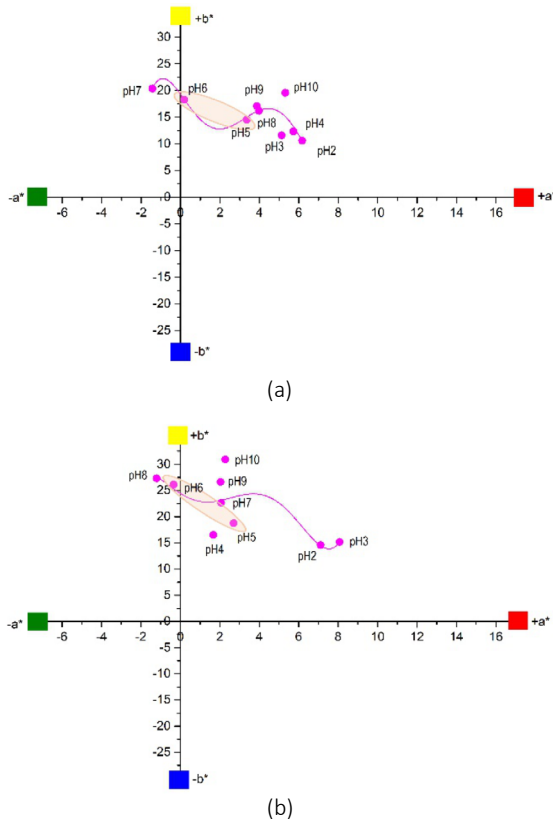
The results of the PS and MS films with anthocyanins from the peels of red onions are shown in Figure 6 and show the color shifts in the areas between the yellow and red coordinates. Compared to the MS_ROA films, the PS_ROA films show a slightly stronger color shift towards the yellowish ($+b^*$ values) and reddish hue ($+a^*$ values).

From the results shown in Figures 5 and 6, it can be concluded that the produced films show visible color shifts when exposed to a different pH environment.

It can be suggested that to prepare a pH indicator from PS and MS starch, PS starch with red cabbage anthocyanins and MS starch with red onion anthocyanins should be used. These polymeric composites show expressed color shifts that are easy to detect visually.



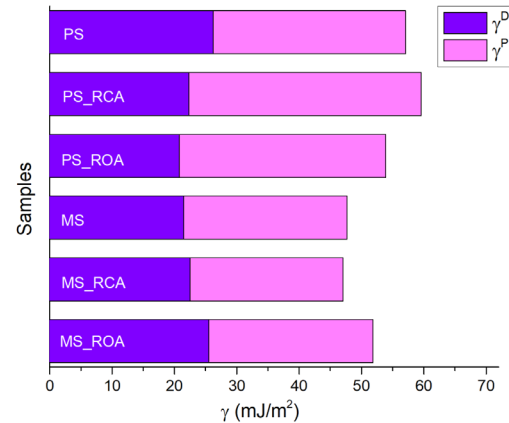
» **Figure 5:** Relative CIE a^*/b^* values of the films immersed in different buffer solutions: (a) PS_RCA and (b) MS_RCA



» **Figure 6:** Relative CIE a^*/b^* values of the films immersed in different buffer solutions: (a) PS_ROA and (b) MS_ROA

Surface and adhesion properties of the films

Figure 7 presents the results of the calculated surface free energy (γ) and its dispersive (γ^D) and polar (γ^P) components for PS- and MS-based films. The evaluation of the SFE results of the starch-based films is particularly important for packaging as it can predict the interactions with the specific substrate that comes into contact with the pH-sensitive films. In addition, the SFE of the produced films can be helpful in discussing other interactions that may occur within the packaging surface layer. From the calculated results, it appears that PS films have slightly higher total SFE values compared to MS films and that all film-samples contain higher values of polar (γ^P) than dispersive (γ^D) components. These changes are generally enhanced after the addition of anthocyanins, which is probably due to the polarity of the anthocyanins.



» **Figure 7:** Surface free energy components of PS- and MS-based films, with and without anthocyanins

In order to observe the interaction between polypropylene, the most commonly used substrate for packaging fresh meat, and the films produced, the adhesion parameters were calculated. The SFE of the polypropylene was calculated using the Owens-Wendt-Rabel and Kaelble method by applying probe liquids to the polypropylene surface and using the measured contact angle data. It was calculated that the total SFE of polypropylene is 52.24 mJ/cm², with the dispersive component corresponding to 31.74 mJ/cm² and the polar component corresponding to 19.51 mJ/cm². The results of the adhesion parameters, the interfacial tension (γ_{12}), thermodynamic work of adhesion (W_{12}) and the spreading coefficient (S_{12}) are shown in Table 2. For optimal adhesion, the value of the interfacial tension should be positive or equal to zero, the value of the work of adhesion should be as high as possible and the spreading coefficient should be close to zero.

From the results, the interfacial properties between polypropylene and the starch-based films without and with anthocyanins are similar and show good adhesion

between the films and the substrate. It can be said that starch-based MS films have a slightly poorer adhesion than PS films, as they have a slightly lower work of adhesion value and a slightly lower spreading coefficient value. Although the adhesion results are quite positive, further analyses should be carried out that include additional observations of the interactions between the pH films produced and the substrates used, such as the stability on the surface and sensing properties, the influence of moisture, different storage temperatures, etc.

Table 2

Adhesion parameters between the starch-based films and polypropylene

Sample	γ_{12} (mJ/m ²)	W_{12} (mJ/m ²)	S_{12} (mJ/m ²)
PS-film	2.55	106.75	2.27
PS_RCA	4.92	107.15	2.67
PS_ROA	3.93	102.19	-2.29
MS-film	2.48	97.45	-7.03
MS_RCA	2.08	97.21	-7.27
MS_ROA	1.83	102.26	-2.22

Conclusion

In this research, starch-based films with natural pigments anthocyanins, were used to produce a bio-based pH-responsive indicator. The research results showed that it is possible to produce a pH-responsive indicator based on potato and maize starch with anthocyanins extracted from the peels of red onions and red cabbage leaves.

When measuring the optical properties of the films produced, it was found that the lightness values were slightly higher for the film samples produced from potato starch than for the films produced from maize starch. Immersion of the anthocyanin-containing starch-based films in different buffer solutions (from pH2 to pH10) showed that the prepared films can be used as pH indicators as they changed their color in different pH environments. Different types of starch showed no significant difference in the visual appearance of the films, but the difference was found when different anthocyanins were used. It was proposed to use PS starch with anthocyanins from red cabbage and MS starch with anthocyanins from red onion to produce a pH indicator from PS and MS starch. These polymer composites show expressed color shifts that are easy to detect visually.

The surface free energy of the starch-based films was measured to observe the surface properties of the films and to determine the adhesion of the films to the packaging material. It was found that potato-based films had slightly higher overall surface free energy values compared to maize-based

films and that all samples contained higher values of polar than dispersive components. These results are particularly important for packaging as they can predict the interactions with the substrate that comes into contact with the pH-sensitive films.

The results presented in this study demonstrate the possibility of using starch-based films with anthocyanins as pH indicators in smart packaging. Further research in this area is planned to observe the stability of the produced indicators at different storage temperatures, to further investigate their sensing properties, the influence of moisture, the measurement of mechanical properties and water barrier properties and to define the other interactions with different packaging materials.

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