Red Cabbage Anthocyanins in Smart Food Packaging, Sensors

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Anthocyanins, as one of the water-soluble phenolic compounds, are able to generate a wide range of colors (for example, blue, purple, orange, and red) that are widely isolated from flowers, cereals, fruits, and vegetables. In addition, based on the pH values of the solution, anthocyanins can be found in different colors and chemical forms that can monitor food quality parameters, and eventually, keep track of food products over the shelf life period. The reversible color attributes of anthocyanins-rich solutions are associated with the source, composition, and configuration of anthocyanins.

intelligent packaging | active packaging | natural pigment | pH-responsive indicator

1. Halochromic (pH-Sensitive) Properties of Red Cabbage Anthocyanins

Generally, changes in the safety and quality of food products can occur during production, storage, distribution, shipment, and consumption. Consumers commonly detect and evaluate the freshness and quality of packaged foods using the shelf life date printed on the package. However, the shelf life date alone cannot be enough to evaluate the freshness and quality of some food products such as fresh fruits and vegetables [1]. Color, as a natural indicator of food quality, is regarded as one of the key factors to identify and monitor the physicochemical alterations of food products [2][3]. In this regard, the use of intelligent color sensors and labels is an innovative and smart system for detecting, tracking, protecting, and assuring production of safe and high-quality products. The utilization of natural colorants (mainly plant-based ones) from various sources has been suggested as proper alternatives to unsafe synthetic (chemical) dyes. Recently, the interaction (sensitivity) of natural colorants (especially anthocyanins) toward environmental variations, triggered a huge interest in their application as suitable tools to produce pH, gas, or temperature-responsive smart packaging systems [4][5].

2. Effects of Red Cabbage Anthocyanins on Properties of Smart Bio-Based Films

2.1. Physical Properties

2.1.1. Thickness

The thickness of packaging films is an important factor due to its impact on mechanical, light transmittance, and gas barrier properties of the fabricated composites. This factor is highly influenced by the film composition, dispersibility, and flow properties. Several ones confirmed that the low contents of anthocyanins cause no significant difference in film thickness because they can uniformly distribute in the film matrix [6][7][8][9][10]. Chen et al. [7] indicated that the film thickness did not significantly change by incorporation of various contents of red cabbage anthocyanins (RCAs), probably due to the low RCAs concentration, and proper compatibility of RCAs within the chitosan/oxidized chitin nanocrystals (CS/OCN) composite matrix. Similarly, Park et al. [11] reported no significant difference in the thickness of edible chitosan-based films, which could be due to the low amount and low dry matter content of the extract in film formulations.

However, loading high amounts of anthocyanins could disrupt the integrity of the film matrix and influence the film thickness. In this regard, Prietto et al. [12] noted that the thickness of pH-sensitive corn starch films increased by the addition of anthocyanins content. In agreement with these results, the addition of RCE caused a linear increase in the thickness of colorimetric films based on polyvinyl alcohol/sodium carboxymethyl cellulose (PVA/CMC·Na) [13] and cellulose acetate [14]. In contrast, do Nascimento Alves et al. [15] stated that various RCE-loaded biodegradable films including polyvinyl chloride, gelatin, and green banana starch fabricated slender film layers compared with the control films without RCE. In accordance with these, it can be inferred that the incorporation of RCAs has different effects on film thickness in smart packaging films, probably depending on the composite matrix and the RCA characteristics (composition and amounts).

2.1.2. Moisture Absorbency and Swelling Index

Moisture absorbency and swelling index (SI) are important factors for developing pH-sensitive smart packaging systems due to their influences on color response efficiency within the film matrix [16]. This is more critical in water-sensitive hydrophilic composite films since the moisture content and water activity could significantly alter their structure and functionality [17]. The higher SI value causes a rapid color release which is not a desirable reaction in colorimetric composite films [13][18].

2.1.3. Water Solubility

Water solubility reflects the water sensitivity of films. Smart packaging films with remarkable water resistance are preferred to preserve food items with intermediate or high moisture content. Intelligent films with low water resistance can dissolve quickly which causes a significant loss and release in colorimetric agents [19]. Wu et al. [10] reported that the water solubility of konjac glucomannan films with oxidized chitin nanocrystals (KGM/O-ChNCs) was significantly increased as a result of RCAs addition. This effect is correlated to the extremely hydrophilic nature of anthocyanins. Similarly, Prietto et al. [12] reported that the addition of RCAs in pH-sensitive corn starch films increased their water solubility due to the increased hydrophilic spots within the biopolymer matrix. They also reported that the acylation and glycosylation of anthocyanins can reduce and increase the water solubility of the films, respectively. Likewise, Kuswandi et al. [18] found that the addition of RCAs into the bacterial cellulose (BC) membrane significantly increased solubility in water. Thus, the interaction of RCAs with the film matrix often enhanced the water solubility of films due to their hydrophilic nature.

2.1.4. Oxygen and Water Vapor Permeability

Oxygen permeability (OP) and water vapor permeability (WVP) are two determining factors to track the permeability attributes of food packaging systems as important criteria in food quality and safety [7]. They are of great importance in extending the shelf life of packaged food by maintaining a suitable equilibrium of moisture and oxygen contents and controlling physical or chemical deterioration. Prevention or reduction in moisture and oxygen transfer between the food and the surrounding environment is a primary function of food packaging and low WVP and OP are generally required for food packaging [8].

The film permeability depends on many factors, such as the integrity and mobility of the polymeric chain, the ratio between crystalline and amorphous zones, the hydrophilic/hydrophobic ratio and so on [20]. The presence of RCA in the biopolymeric film matrix could affect these factors and modify the WVP and/or OP properties.

2.2. Mechanical Attributes

The integrity and sustainability of food products could be guaranteed by composite films possessing suitable mechanical strength. Tensile strength (TS), and elongation at break (EAB) are two important mechanical criteria to monitor the strength and flexibility of packaging films, respectively. TS is defined as the maximum tolerance of composite films against the applied stress while being pulled or stretched before breaking occurs. Moreover, EAB is defined as the maximum capability of composite films to maintain the alterations in the length and shape of the films deprived of any crack formation [21][22].

Generally, the mechanical properties (TS and EAB) of RCAs-rich films can be affected by various factors. In this regard, Liang et al. [21] reported that the TS of the RCA-blended ASKG/CMC·Na composite films decreased by loading RCA, while their EAB showed an increasing pattern. It might be due to the formation of some interactions between RCA and composite matrix, and plasticizing effects of RCA, both varying the state of hydrogen bonds within the polymer chains, resulting in enhanced molecular mobility and hence a damaged integrity network [21]. Similarly, Park et al. [11] also indicated that the addition of RCA decreased the TS value and remarkably increased the EAB value of edible chitosan films. Pourjavaher et al. [16] reported that the mechanical properties of anthocyanins-rich BC nanofibers can be influenced by anthocyanins concentration. They observed a concentration-dependent decrease in the TS and an increase in EAB, which was in agreement with Chen et al. [7] on chitosan/oxidized–chitin nanocrystals (CS/OCN) composite films and Freitas et al. [14] on cellulose acetate-based films. On the other hand, the phenolic compounds of RCAs in the BC nanofibers matrix probably act as the plasticizer agent and reduce the interactions among the BC membrane macromolecules. Kuswandi et al. [18] observed similar effects of the RCA phenolic compounds on the mechanical properties of BC membrane.

2.3. Thermal Characteristics

Mapping the thermal degradation profile of composite films is introduced as an efficient tool to understand their thermal stability. For this purpose, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) are usually employed to monitor the thermal stability of composite films. Some have shown that the incorporation of

RCAs decreases the thermal stability of the films because the RCAs weaken the intermolecular interactions among film components, which facilitates its decomposition at lower temperatures. For instance, Silva-Pereira et al. [23] found that the addition of RCE into chitosan/starch film caused lower thermal stability compared with the control film. Similar findings were reported by Prietto et al. [12] in the starch films incorporated with RCE.

In contrast, some studies have informed that the thermal stability of composite films could be improved by loading RCAs-rich extracts due to the generation of robust intermolecular connections between RCAs and polymer chains [7]. Freitas et al. [9] reported that the thermal properties of hydroxypropyl methylcellulose (HPMC) packaging films were influenced by loading RCA, as well as the pH variations of the film-forming solution. Based on the results, the incorporation of RCA increased the film's thermal stability and consequently exhibited higher maximum values of thermal degradation temperatures, which can be related to the formation of hydrogen bonds between anthocyanins and polymer chains, leading to a decrease in the accessibility of hydroxyl groups in HPMC that interact with water molecules. Chu et al. [24] also observed the maximum decomposition temperature of anthocyanins-rich composite cationic guar gum–hydroxyethyl cellulose films was significantly higher than other reference films. In line with these findings, Eskandarabadi et al. [25] reported an increase in the thermal stability of biodegradable ethylene-vinyl acetate nanocomposite films added with anthocyanin. However, Liang et al. [21] indicated that the incorporation of RCA didn't change the thermal stability of the ASKG packaging films. As a result, the thermal properties of anthocyanins-loaded packaging films may be also affected by various factors such as polymer type, the interaction among film components, as well as the source and content of the incorporated anthocyanins.

2.4. Structural Properties

The structural properties of biopolymer films containing RCAs were commonly determined by FTIR analysis, which investigates the inter- and intra-molecular interactions between the composite film components.

The characteristic FTIR bands of RCAs were introduced in [9][13][17][26][27]. Freitas et al. [9] reported a broad strong absorption band at the wavenumber of 3360 cm^{-1}, demonstrating the presence of O–H stretching vibration as a result of the formation of hydrogen bonds in RCE [16]. The peaks at about 2980 cm^{-1} and 1645–1735 cm^{-1} are assigned to C–H stretching vibration present in aromatic rings, and the stretching vibration of C=O flavonoids, respectively, confirming the presence of aromatic compounds in RCE. A peak at the range of 1620–1640 cm^{-1} is probably generated due to the formation of stretching vibrations of aromatic rings of C=C bands. The band at the range of 1410–1415 cm^{-1} belongs to the C–O groups which displays the angular deformation of the phenols [13]. However, Freitas et al. [9] found these groups in tiny absorption bands in the range of 1300–1380 cm^{-1}. The peaks at about 1090 cm^{-1} and 1050 cm^{-1} are related to C–O–C stretching vibration and C–O–C–O–C alkyl aryl ether (or anhydroglucose ring of O–C) stretching vibration, respectively. The peaks exist in the cyanidin-3,5-O-diglucoside structure, known as the main cyaniding in red cabbage. The bands from 990 cm^{-1} to 1000 cm^{-1} are also assigned to C=O stretching vibrations.

3. Applications in Food Packaging and Sensors

3.1. Intelligent Characteristics in Natural Biopolymeric Films

Natural biopolymers, especially polysaccharide and protein-based ones, have been widely used to produce the halochromic active and intelligent packaging films due to their biocompatibility/biodegradability, nontoxicity, stability, easy availability, and good film-forming ability. Kuswandi et al. [18] reported good performance of an edible pH sensor based on the immobilized RCA into BC membrane as it was employed to track the pH alterations of some beverages. Additionally, the pH sensor can be used for observing the milk freshness, as it can easily differentiate the deteriorated milk from fresh milk by using color detection sensors that display the pinkish-gray to the bluish-gray color range. In this regard, do Nascimento Alves et al. [15] fabricated biodegradable films using green banana starch, gelatin, and alginate incorporated with RCA for monitoring the quality of sheep meat freshness. They observed changes in color parameters due to the increase in the pH of the meat. The pH of sheep meat was increased due to the volatile alkaline compounds formed in the samples during the storage period and thereby all films indicated changes in color parameters.

Chitosan is the most common natural biopolymer that has been used in the fabrication of smart films with RCA. Silva-Pereira et al. [23] monitored fish deterioration using chitosan/corn starch blended film with RCA. They observed color change in the film depending on the different conditions of the fish sample during storage at room temperature. Based on the result, no color change was observed in the indicator film after 12 h. After 16 h of storage, the color began to shift to blue, which showed initial spoilage and pH increase. After 72 h of storage, the color completely turned to yellow, indicating complete fish spoilage [23]. Bento et al. [28] developed and evaluated a pH-sensitive packaging film using chitosan, gelatin, PVA, and RCE to monitor ricotta cheese spoilage. Despite the high sensitivity of film to pH changes, the results demonstrated that the initial light brown color of the indicator film was not significantly changed after seven days of refrigerated storage, which may be correlated with keeping pH at 4.48 as well as viable numbers of mesophilic microorganisms compared with initial time. Recently, antimicrobial activity and indicator properties of edible chitosan-based films prepared with RCE (as spoilage indicator) and clove bud oil (CBO; as antimicrobial agents) were investigated by Park et al. [11]. They reported that the pH of fish peptone agar including *Pseudomonas fluorescens* enhanced from near 6 to 9, and the initial purple color of the films changed to deep blue during the growth of fish-spoiling bacteria. They claimed that the edible films containing CBO and red cabbage have a high potential for use in fish preservation. Vo et al. [29] tested the freshness of pork belly using an intelligent film prepared with chitosan/PVA/RCA for 24 h at room temperature. They observed that the initial sea green color of the film changed to pink color at 12 h, representing an acidic condition near pH 5–6 on the pork slices surface. After 24 h, the film wrapping the meat turned pale pink, displaying it in the slightly alkaline range. The color change appeared due to the meat spoilage during the growth of microorganisms and the deterioration of samples caused by biochemical reactions.

3.2. Intelligent Characteristics in Nano-Biocomposite Films

In addition, some were considered the nanocomposite matrix as the residence of RCA. Halochromic CS/OCN composite films and RCA were applied for monitoring the hairtail and shrimp freshness by Chen et al. [7]. The films exhibited visible color differences when the pH varied from 3 to 13. In addition, the film color was developed by increasing the anthocyanins level (0–1.2%, *w*/*v*), so that the color of the film with 1.2% RCA was distinctly different compared with the control film (without RCA) during 48 h of hairtail and shrimp storage at 25°C. They concluded that the color changes of smart labels were consistent with three different stages of freshness, including fresh step (reddish purple), medium step (brown), and spoiled step (yellow), which could be recognized by naked eyes. Eskandarabadi et al. [25] designed the intelligent ethylene-vinyl acetate nanocomposite film with different additives such as rosemary extract, anthocyanin extract, and ZnO/Fe-MMT nanoparticles to detect meat deterioration. They investigated the color change behavior of anthocyanin at different pH levels. The meat deterioration was detected due to the released ammonia, causing the color change of films from red (acidic color) to yellow (basic color). Wu et al. [10] prepared and tested a smart system using the incorporation of oxidized chitin nanocrystals and RCA into konjac glucomannan films (KCR). Based on the obtained results, with an increase in pH value, the KCR film's color turned from pink–red to green. The intensity of this color change was significantly dependent on the incorporation amount of anthocyanin.

3.3. Extraction Factors Affecting the Intelligent Characteristics of Bio-Based Films

Anthocyanins extracted from different sources can indicate various colors and stability modes because of the intrinsic characteristics in their chemical configurations. However, on the effect of temperature, light, and time on the stability of pH-sensitive indicators are currently limited. Meanwhile, the functional properties (antioxidant/antimicrobial activity and pH sensitivity) of anthocyanins-rich films mainly depend on the stability and release of anthocyanins from the composite film matrix, which is attributed to their concentration, microstructure of films, and intermolecular interactions of anthocyanins with the film matrix [30][31]. Prietto et al. [12] prepared pH-sensitive packaging films based on corn starch comprising RCAs and black bean anthocyanins (BBAs). They observed that BBAs are more sensitive against different pHs and exhibit color changes very rapidly so that the appearance changed within about 5 s. In contrast, the pH-sensitive films with RCAs presented higher stability and greater color variation compared with BBAs. They concluded that RCAs are suitable candidates to fabricate intelligent packaging films. Hamzah et al. (2021), the color change of sago starch-based film (with different concentrations) indicated that the RCA was released from the film into the water during 32 h with different colors on release, which was observed as an increasing trend in color intensity. It was also claimed that the color change was observed among pHs 4, 9, and 13. The release of color from the films shows the stability of anthocyanin which plays a key role in the efficiency of smart packaging films. Thus, if the anthocyanins are not appropriately sustainable/compatible with the film structure, the packaging system will face some challenges to be regarded as an intelligent packaging system [27].

The extraction procedure of RCA is also an important factor in pH sensing. Musso et al. [32] prepared gelatin-based smart films using RCA and claimed that the anthocyanin-rich film was pink at pH < 4 but turned yellow at pH > 11. They reported that the alcoholic extract had a purple coloration while the aqueous one had a pink hue. In addition, the alcoholic-extracted anthocyanins present better functionalities and higher effectiveness as a result of higher contents and varieties of anthocyanins as compared with aqueous-extracted anthocyanins. Thus, the gelatin/anthocyanin film showed pH sensitivity and strong antioxidant activity that were associated with the extraction conditions of anthocyanins.

3.4. Intelligent Characteristics in Electrospun Fibers

In recent years, electrospun nanofibers prepared through the electrospinning technique have been utilized in active and intelligent food packaging systems equipped with halochromic indicators. Maftoonazad and Ramaswamy [17] produced an electrospun nanofiber mat based upon PVA, and RCE as a pH-biosensor to monitor the pH-dependent quality attributes of rutab (a kind of soft date fruit). Based on the obtained results, relying on the temperature fluctuations over the storage, the pH variations could be correlated to the physiological activity of the fruit itself, and metabolites generated during the microbial growth. At 25 °C, the pH of rutab strongly decreased during 96 h of storage (complete fruit spoilage), causing the mat color to alter slightly after 72 h and was completely changed to purple after 96 h. However, at 5 °C, the pH value gradually dropped, and the color completely changed to purple after 12–20 days. As a result, the pH biosensor can be used as a real-time pH indicator to monitor the progression of spoilage of packaged rutab. Safitri et al. [33] also incorporated the RCA into the poly (ethylene glycol) diacrylate-based hydrogel containing lignocellulose nanofiber (PEGDA/LCNF) hydrogel as a colorimetric pH indicator film, which successfully indicated multicolor response at specific pH buffers. Pourjavaher et al. [16] prepared a pH indicator based on BC nanofibers by adding RCA. The BC-diluted anthocyanin label exhibited the highest response to the tested range of pH (pH 2–10). However, labels containing concentrated anthocyanins indicated to be the least sensitive to the pH variations. Prietto et al. [34] developed halochromic electrospun fibers based on zein (30% *w*/*v*) and RCAs (3% *w*/*v*, 4% *w*/*v*, and 5% *w*/*v*), which showed a color alteration in fibers from red to green by enhancing pH from 1 to 10. The results also displayed that an increase in the concentration of RCAs from 3 to 5% led to an increase in the intensity of color variation at different pH values.

3.5. pH-Sensing Applications in Health Monitoring

In addition to food packaging, the RCA-rich electrospun biopolymers could act as a colorimetric pH sensor in the health monitor. Pakolpakçıl et al. [35] also showed that the pH-responsive electrospun nanofibers based on the PVA (12% *w*/*w*), sodium alginate (1% *w*/*w*), and red cabbage extract (2–3%) display color differences from red to pink, blue, and finally to green by increasing pH from 4 to 10, which potentially can be used for monitoring wound healing. In another, Devarayan and Kim fabricated an eco-friendly and reversible pH sensor by immobilizing RCAs on electrospun cellulose acetate fiber mats as a potential substitute for diagnosing the alcoholic individuals and monitoring the evolution of certain illnesses. The effectiveness of pH-responsive nanofibers was evaluated after exposure to different temperatures and pH values during storage for 30 days. No significant differences were found in color responses of composites after 1 day of storage at −50 °C compared with the fresh samples. While the color response pattern was different after treatment at 100 °C. After one month, the responsiveness of the pH sensor did not change. Thus, it was confirmed that the pH sensor was constant at different temperatures and storage times, with the capability of sensing pH in the range of 1–14. Additionally, the sensors were reversible when dipped in different buffer solutions [36].

3.6. Active Characteristics in Bio-Based Films

In addition to the pH-responsive color-changing properties of RCAs for monitoring the freshness or spoilage of food products in the intelligent packaging, they could provide new opportunities for the development of active packaging for extending the shelf life of the packaged products. Some are described the acceptable antioxidant and antimicrobial activities of RCAs-rich films [8][10][11][25][32]. For instance, Chu et al. [24] indicated that the diameter of the inhibition zone of RC3 film (CGG-HEC10 with 3% RCA) against *E. coli* and *S. aureus* was higher than that of the CGG–HEC10 composite film (without RC). Thus, RC3 film represented good antibacterial activity because of the presence of red cabbage pigments. Similar results were obtained by Wu et al. [10], who observed an RCA concentration-dependent antimicrobial activity in KCR films against *E. coli* and *S. aureus*. They also observed that the DPPH radical scavenging ability increased with increasing RCA concentration in KCR films due to the strong antioxidant activity of anthocyanins. Furthermore, Eskandarabadi et al. [25] detected the highest antioxidant activity against DPPH in ethylene-vinyl acetate films with RCA stabilized on montmorillonite compared with the other active materials such as ZnO nanoparticles and rosemary extract. In addition, RCA films had higher antimicrobial activities against *E. coli* and *S. aureus* than the ZnO film because of the higher penetration rate of anthocyanins [25].

The extraction procedure of RCAs from red cabbage also affects the active capabilities of packaging. Musso et al. reported the significantly increased antioxidant properties (by $ABTS^{\cdot+}$ and FRAP assays) of the gelatin films incorporating the aqueous (GAw) and alcoholic (GAe) RCE. In addition, the antioxidant activity of GAe was remarkably higher than GAw. This difference could be related to the higher content and varieties of anthocyanins in alcoholic red cabbage extract. However, in this work, the addition of RCE (extracted with different solvents) showed no significant antimicrobial activity against different bacterial strains [32]. This could probably be attributed to the low concentration of anthocyanins in both extracts and also specific interactions between antimicrobial agents of anthocyanins and gelatin in the film matrix.

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