Naturally occurring pigments for intelligent food packaging applications: Recent applications and future perspectives

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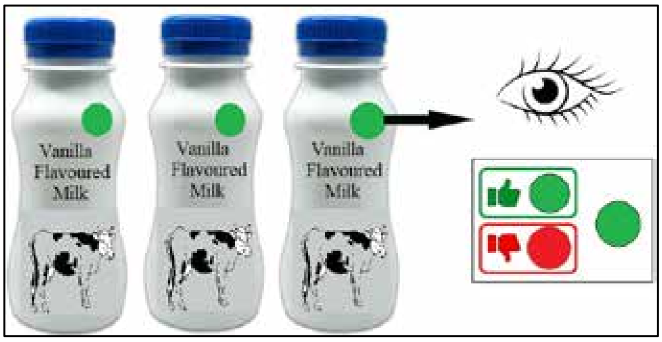
Fresh food products deteriorate rapidly due to physical damage caused by handling, transport, and storage, as well as inherent causes such as chemical reactions, enzyme activity, and microbial deterioration. Packaging is amongst the most important techniques for preserving food quality during transport, storage, and end use. It helps to ensure the food quality and safety for both sellers and buyers, resulting in increased consumer safety and less food wastage. Packaging, on the other hand, may help to keep food fresh by acting as a storefront for information about the food that is inside. This helps to cut down on food waste and deterioration. In this regard, active and intelligent packages are being designed, and they make up a significant contribution to the food packaging sector.

Smart packaging includes active and intelligent packaging solutions that aim to provide consumers with more accurate information on packaged food conditions while also providing a protective function for the food product by incorporating active compounds. The use of compounds that may release directly or indirectly to the food in order to retain quality or prevent deterioration is known as active packaging. Active compounds such as antioxidants, oxygen scavengers, moisture absorbers or antibacterial are added to the polymer matrix used in this method of package.

Intelligent food packaging refers to the techniques that oversee the state of packed food or its surrounding environment by monitoring changes and communicating the conditions which helps to make decision about the packaged food product. In order to achieve this, intelligent packaging contains indicators, data carriers, or sensors. Mainly the indicators are based on time-temperature detection, freshness detection, gas production detection, and convey information through a visual colour variation that the customer may clearly identify. Data carriers are a type of information carrier used in the food supply chain. Barcodes and Radio Frequency Identification chips (RFID) are the most prevalent data carriers. In sensors, physical or chemical changes in food are detected by a transducer and converted to an electrical signal.



The most common types of indicators used in intelligent food packaging are based on a visual colour change, which is caused due to chemical or microbial alterations in food which interacts with the indicator, indicating alterations in food, which is often caused by pH variation. Indicators function by changing the colour of pigments that have been incorporated to the indicator, which are responsive to pH alterations. Methyl red, bromocresol purple, bromophenol blue, and chlorophenol red are synthetic dyes that have been shown to response the alterations pH effectively. Although they have been proved to be effective, their toxic, carcinogenic, and mutagenic properties make them unsuitable for use in food. Natural pigments, rather than synthetic dyes, are in line with the current consumer trend toward more sustainable and natural healthier alternatives. Natural pigments derived from plant based materials are renewable, nontoxic, and have low impact on the environment.

Many studies on the development, characterization, and use of bio-based indicators in intelligent food packaging have emerged in recent years, and some of them are reviewed and summarized here. Attention has been paid to bio based indicators like anthocyanin, curcumin, and betalains as they have been widely used in recent applications due to their clear and effective colour responses to pH alterations in intelligent food packaging applications.

Anthocyanin as an indicator for intelligent food packaging

Anthocyanins are naturally occurring water-soluble pigments found in a variety of plants and fruits. These are categorized as flavonoids, and research has shown that they have antioxidant, anti-inflammatory, and anticarcinogenic properties. Anthocyanins are accountable for the red, purple, and blue colours seen in a wide range of plants and fruits, and they are very reactive to pH changes (Figure 1) [1].

Because of the nontoxic and biodegradability properties, different types of biopolymers, such as chitosan, cellulose, starch, polylactic acid (PLA) etc., have been widely used in food packaging. The incorporation of bio based indicators for biopolymer food packaging materials provides a sustainable alternative to synthetic dyes and synthetic polymer-based intelligent food packaging materials. For example, Pereira Jr, de Arruda [2] prepared intelligent food packaging film by incorporating anthocyanins extracted from red cabbage into chitosan/PVA films. The film was designed to detect the pH alterations of packed food, when it was exposed to incorrect storage temperatures. When the anthocyanin incorporated chitosan/PVA films were exposed to solutions of varying pH (from pH 1.0 to pH 12.0), a noticeable colour change was observed, varying from bright red to bright green. When milk is heated to a certain temperature, which is higher than the temperature suitable for cooling (7 °C), it starts to breakdown, changing the pH of the milk and decreasing the milk's stability. The films indicated a distinct colour variation after getting in contact with the milk, starting with a dark grey colour at pH 6.7 (fresh milk) and progressively becoming clearer as the pH declined from 6.7 to 5.0. The films showed a dark pink colour at pH 4.6 (due to the spoilage of milk), clearly demonstrating milk deterioration.

Due to its high abundance, film-forming property, and nontoxicity, starch seems to be one of the most appealing biopolymers. Vedove, Maniglia [3] prepared an intelligent food packaging film using cassava starch and anthocyanin to detect changes in pH of meat that was stored at 6 °C. Cassava starch sheets with anthocyanin was prepared using co-rotating twin-screw extruder connected with sheet-forming mold. Results showed that 20 mg anthocyanin/100 g film produced the best pH change indicator performance with most visually

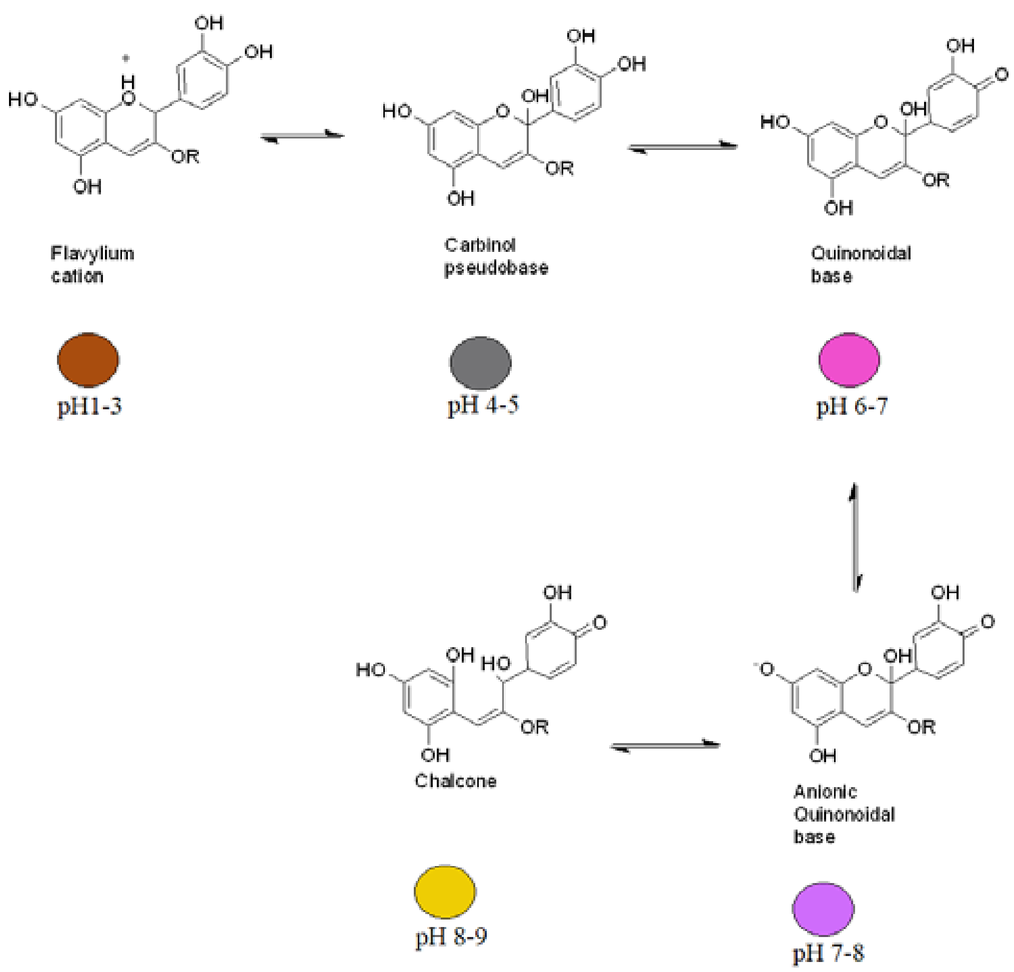


Figure 1. Anthocyanin structural changes and colour variations at various pH levels.

notable colour change. The high meat pH is caused due to evolving volatile nitrogenous compounds such as ammonia as a result of the act of microorganisms on meat proteins. When exposed to the sheet, this gas diffuses into it and subsequently hydrolyses the indicator anthocyanin. The change in anthocyanin colour with the increase of pH from 5 to 9 is due to the anthocyanin structure changing from a flavylium cationic form to a pseudo-base carbinol form and finally to its anionic form, which reduces the contribution to red colour.

Though previous studies were focused on pH-induced anthocyanin composite film colour changes, Zhang, Zou [4] highlighted that the colour is directly affected by the stability of the composite film, thus it is important to analyze the film's stability before using it in intelligent food packaging applications. Their results depicted that these indicator films stored at high temperature (25 °C) showed a higher colour change compared to the films stored at lower temperatures (4° C). This was due to the pyrylium ring opening of anthocyanins at high temperatures, producing a chalcone structure and yellow coloration. Among the films prepared, the starch/PVA hydrogel films showed highest colour stability compared to the starch/chitosan hydrogel and PVA/chitosan hydrogel films. The starch/PVA/anthocyanin (extracted from roselle calyx) hydrogel film was used to monitor the pork freshness and it showed a purple colour at first and brown colour after 48 hours and lastly a yellow colour at 72 hours. Meat proteins are particularly susceptible to bacterial and mildew growth. During spoiling, many volatile nitrogenous chemicals, such as ammonia and amines, are formed. The mechanism is that ammonia is diffused into the film, then hydrolysed to release hydroxyl ions, resulting in increased pH levels of the film. This leads to a change in the anthocyanin structure to chalcone, causing the colour of the composite hydrogel films to change.

Curcumin as an indicator for intelligent food packaging

Curcumin is a hydrophobic polyphenol derived from turmeric that has antioxidant, antibacterial, and anti-inflammatory properties and a bright yellow colour. Curcumin has a yellow colour between pH 1 and 7, and a red–orange colour at pH 8 [1].

Besides anthocyanin, curcumin has also been employed as a bio-based indicator in intelligent packaging applications. Curcumin's colour changes are less diverse than anthocyanin's, but its sensitivity to alkalinity is highly noticeable. Musso, Salgado [5] prepared smart edible films using gelatine and curcumin. A casting method was used to prepare the film with water and an ethanol-water mixture (1:1 v/v) as solvents. Their findings revealed that films at pH of 6 become orange-red when exposed to basic gases, fluids, and semi-solids, and films at pH 11 become yellow when exposed to acidic medium. Further, they mentioned that the film reactions were rapid and distinct with liquids and gases than those with semisolid substrates, particularly for films formed from aqueous dispersions. The decreased diffusive processes are most likely the reasons for slower turning kinetics in semisolid media.

According to some studies, the relative humidity of the environment influences the colour variation of curcumin-incorporated food packaging films. However, this effect also varies with the type of the film, whether it is hydrophobic or hydrophilic. For example, Zhai, Wang [6] used a melting extrusion process to prepare a hydrophobic film from low density polyethylene (LDPE) and curcumin. With exposure to NH_3 at various relative humidity (RH) levels, the film's colour changed from light yellow to brown. According to the results, the film's colour change was more intense at low RH values compared to high relative humidity values. At higher RH, more NH_3 dissolved in water and form $NH_3{\cdot}H_2O$, and then ionize into ammonium and hydroxyl ions. The hydrophobic feature of the film prevented hydroxyl ions in water drops from diffusing into the film and causing curcumin colour change. The impact of RH on the curcumin colour change for hydrophobic LDPE films was quite different compared to the curcumin incorporated hydrophilic films reported in previous studies. Films changed from light yellow-to-light brown with the storage of beef and silver carp at 4 °C due

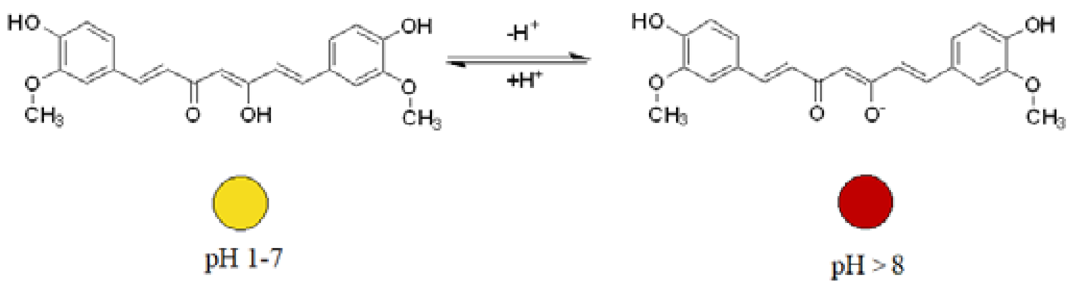


Figure 2. Curcumin structural changes and colour variations at various pH levels.

to the increased levels of total volatile basic nitrogen compounds.

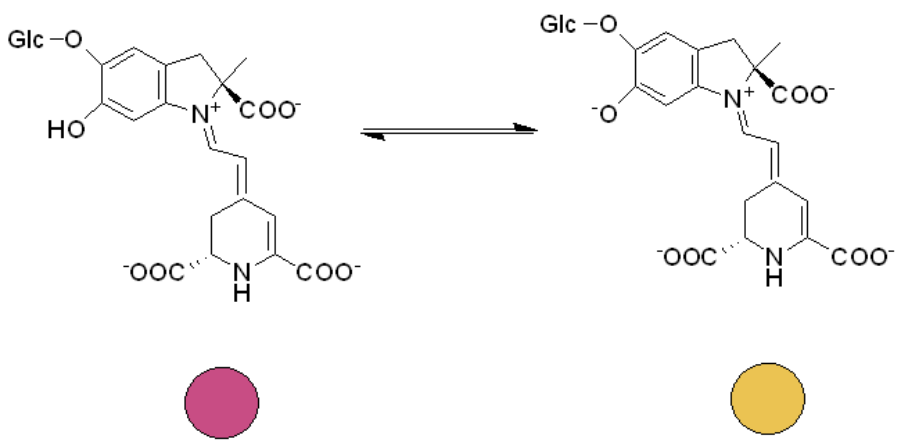
Curcumin has been shown to lose its functionalities as a consequence of exposure to high temperatures, light, and oxidation during the production and storage processes. Because curcumin is highly unstable, it is possible that intelligent packaging won't accurately reflect the quality of the food it contains. Liu, Dang [7] incorporated pickering emulsion for curcumin-loaded corn starch/ PVA films for use in intelligent packaging to increase the stability of curcumin. The films' antioxidant activity was determined, indicating that pickering emulsion could minimize curcumin degradation throughout the production and storage phase. During the monitoring of the fish freshness, the colour of the film changed (bright yellow to reddish-brown) considerably when the fish was close to deteriorating after 48 hours. In contrast, the curcumin and pickering emulsion incorporated films showed a more noticeable colour change compared to the films loaded with curcumin only, and the colour change became more intense as the quantity of curcumin increased.

Betalains as an indicator for intelligent food packaging

Betalains are naturally occurring nitrogen-containing pigments present in the vacuoles of certain plant tissue. They have antioxidant properties and serve as radical scavengers, inhibiting lipid peroxidation and heme breakdown at low concentrations. Betalamic acid is a common biosynthetic precursor, and it seems to be stable at pH ranges between 3 and 8 [1].

Betalains can be extracted from plant species belonging to the families of Caryophyllales. Yao, Hu [8] extracted betalains from cactus pears (order- Caryophyllales) to develop quaternary ammonium chitosan (QAC), polyvinyl alcohol (PVA) and betalains film to use as freshness indicators of shrimp. Cactus pears extract (CPE) is rich in betalains, which have antioxidant and antibacterial properties. QAC and PVA were dissolved in distilled water with various concentrations of CPE and 10% glycerol to form the films. At pH 3–8, CPE solution had a purple/red colour and a noticeable absorption band at 535 nm. Exposure to ammonia with varying exposure times was used to assess the pH sensitivity of films containing different contents of CPE. The film containing 3 wt% CPE exhibited notable colour variations from purple to yellow as the exposure duration was increased. This was confirmed by placing the films in the headspace of a container containing fresh shrimp and monitoring their colour change with the deterioration of fresh shrimp. The colour of the film added with 5 wt% and 3 wt% CPE changed from purple (0–12 h) to orange (24 h) and yellow (36 h), showing that these two films could be used to detect shrimp freshness.

Hu, Yao [9] extracted betalains from vegetable amaranth (order-Caryophyllales) to prepare quaternary ammonium chitosan/fish gelatin and betalains film for multifunctional food packaging applications. pH sensitivity studies showed that the amaranth extract changed its colour from purplish red to yellow with the increase of pH from 8 to 12. This was due to the structural conversion of betalains to betaxanthins in alkali environment. Because volatile ammonia may penetrate into the film and produce $NH_3.H_2O$, which subsequently converts to NH_4^+/OH^- and creates an alkaline pH, films changed colour from pink to yellow when exposed to ammonia. Furthermore, during the monitoring of the freshness of shrimp, films changed their colour to yellow at 24 h, proving the film's suitability to monitor shrimp freshness.



$3 < pH < 8$ $\quad$ $pH \geq 8$

Figure 3. Betalains structural changes and colour variations at various pH levels.

In another study, Qin, Liu [10] extracted betalains from red pitaya (order-Caryophyllales) peel to incorporate into starch/PVA film in intelligent food packaging applications. At pH 3–7, red pitaya peel extract appeared in red colour, indicating that betalains remained stable at this pH. At high pH values, red pitaya peel extract solution appeared in orange colour (at pH 8–9) or yellow colour (at pH 10–12). During the ammonia test, the pink colour of the films faded after 10 min exposure to ammonia. After 20 minutes of exposure to ammonia, the films became yellow and the yellowness level steadily increased. The pink colour of the films remained after 12 hours of monitoring the freshness of shrimp. The film changed to pale yellow at 24 h and completely changed to yellow at 48 h, showing that proteins in shrimp had degraded into organic amines.

The different types of naturally occurring pigments used in intelligent packaging applications are summarized in Table 1.

Table 1. Summary of the different types of naturally occurring pigments used in intelligent packaging applications.

Matrix	Indicator(pigment)/source	Colour change	Food type	
Chitosan/PVA	Anthocyanin/Red cabbage	Dark grey colour at pH 6.7 (fresh milk). Dark pink colour at pH 4.6 (due to the spoilage of milk).	Milk	[2]
Cassava starch	Anthocyanin/Purchased	Decreased the reddish contribution with increase of the pH from 5 to 9	Fish Meat	[3]
Starch/PVA	Anthocyanin/Roselle calyx	Varied the colour during the storage of pork (purple colour at first, brown colour after 48 hours and yellow colour at 72 hours)	Pork	[4]
Gelatine	Curcumin/Purchased	At pH 6 become orange-red, At pH 11 become yellow	Food head space, Semisolid food, Solid food	[5]
LDPE	Curcumin/Purchased	Colour changed from light yellow-to-light brown due to the increased levels of total volatile basic nitrogen compounds	Beef and silver carp	[6]
Corn starch/ PVA	Curcumin/Purchased	Varied the colour during the storage of fish. Bright yellow changed to reddish-brown after 48 hours	Fish	[7]
QAC/PVA	Betalains/Cactus pears	Varied the colour during the storage of shrimp. Purple (0–12 h) to orange (24 h) and yellow (36 h)	Shrimp	[8]
Chitosan/fish gelatin	Betalains/Vegetable amaranth	Varied the colour during the storage of shrimp. From pink to yellow at 24 h	Shrimp	[9]
Starch/PVA	Betalains/Red pitaya peel	Varied the colour during the storage of shrimp. The pink colour remained after 12 h, changed to pale yellow at 24 h and completely changed to yellow at 48 h	Shripm	[10]

Concluding remarks and future perspectives

Intelligent food packaging offers a novel approach to communicate the quality of food which it is being packed and identify the spoiled food not just for suppliers, but most specially for customers. Furthermore, it can be used to develop biobased food packaging solutions to address environmental problems created with petroleum-based materials.

Anthocyanins, curcumin, and betalains are pigments that are extracted from different parts of plants and that have been the most commonly investigated options for developing bio-based sensors. The above pigments are both antibacterials and antioxidants, and when integrated with packaging, they may offer functionality to the polymer matrix, increasing the shelf life of the foods being packaged. As a result, these naturally extracted indicators have the possibility of being used in smart packaging solutions in the food industry.

In addition to their potential for use as pH indicators, there are some issues related to their stability when exposed to certain temperatures, O_2, and light. Therefore, investigations are required to increase the indicators' effectiveness and stability in the polymer matrix. Incorporation of the pigments within solid particles or liquid vesicles and the use of UV-light blocking agents such as ZnO nanoparticles have shown encouraging results in terms of pigment stabilizers. In addition, a combination of pigments can be used to improve the stability and effectiveness of indicators. For example, the mixing of water-soluble and alcohol-soluble pigments allows one to detect the condition of water-soluble and oil-soluble foods. Moreover, these pigments have certain limitations such as less contact with solid or semi solid food stuff and some diffusivity problems with food packaging materials. As a consequence, further research is required to fully comprehend how these natural pigments interact with food.

Additionally, if intelligent packaging systems could monitor leakages in packing or respond to pathogens, toxins, allergens, or how long a temperature breach has persisted throughout cold storage food supply chains, the food supply sector would be delighted. This would contribute to minimizing food wastage and improving the sustainability of the food supply chain.

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