

**Natural pH indicators integrated into biopolymers as visual monitoring systems in biological environments**

**Indicadores naturales de pH integrados en biopolímeros como sistemas de monitoreo visual en ambientes biológicos**

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**Abstract:**

Natural pH-sensitive pigments, such as anthocyanins, betalains, carotenoids, and curcuminoids, have distinctive color changes that depend on acidic or alkaline environment. Their potential use as visual monitoring systems, when combined with natural biopolymers, has recently been highlighted as a valuable tool for the food and biomedical sectors. This review aims to compile information on these pigment-polymer mixtures for the development of pH sensors with applications like food preservation monitoring and diagnosis testing design, among others. The properties of these pigments are still under research, particularly carotenoids and curcuminoids, where color-transitions are not as wide. However, challenges such as pigment stability and bioavailability motivate the ongoing development of encapsulation methods and innovative active materials.

**Keywords:** Natural pH indicator, biopolymer, biosensor, food, biomedicine.

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**Resumen:**

Los pigmentos naturales sensibles al pH, tales como antocianinas, betalaínas, carotenoides y curcuminoides, presentan cambios de color característicos dependiendo del ambiente ácido o base en el que se encuentran. Por ello, resalta la importancia que tienen en conjunto a biopolímeros naturales para convertirse en sistemas de monitoreo visual que benefician principalmente al sector alimentario y biomédico. Esta revisión tiene por objetivo recopilar información de estas moléculas extraídas de fuentes naturales en combinación con biopolímeros para el desarrollo de sensores de pH para aplicaciones tanto en la vigilancia alimentaria como en la salud. Aún siguen en estudio las propiedades de estos pigmentos, principalmente en carotenoides y curcuminoides ya que no presentan cambios de color notablemente diferenciales. Aunque retos como la estabilidad y biodisponibilidad

de los pigmentos motivan el desarrollo continuo de métodos de encapsulación y materiales activos innovadores.

**Palabras clave:** Indicador natural de pH, biopolímero, biosensor, alimentos, biomedicina.

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## 1. Introduction

Hydrogen potential or pH, is considered an essential biological factor because it is involved in several cellular, physiological, and environmental processes. Based on this premise, current research focuses on the use of synthetic molecules that change color, as a response to acidic, neutral, or alkaline conditions (pH sensors). Natural pigments such as anthocyanins, betalains, carotenoids, and curcuminoids represent an accessible alternative for monitoring biological conditions as pH sensors through color shifts at different environments. Nonetheless, challenges such as the limited stability and bioavailability of such pigments have spurred the search for new encapsulation and protective methodologies to enhance their application in the development of active biosensors. Its combination with natural biopolymers has recently emerged as an innovative strategy for the development of intelligent pH sensing systems for its applications within the food and biomedical fields because these changes are easily perceived at plain sight. This area of research represents a promising trend towards sustainable and effective technologies for environmental, food and medical monitoring, contributing to the generation of new solutions integrating biological functionality in environmental-friendly mixtures by using agro-industrial waste for the manufacture of new biomaterials.

## 2. Natural pH sensors

At the cellular level, maintaining pH values within a narrow range is essential for many processes to occur, including biochemical reactions, as well as the function of transporters, channels, receptors, structural proteins, and regulatory molecules (Occhipinti & Boron, 2015). This range is closely regulated in the milieu of biological set ups and changes to acidic or basic conditions are frequently part of the indications of abnormal processes. Hence, pH monitoring is essential. Traditionally, to identify whether a substance is acidic or basic, certain compounds are used that are capable of color shifting as a function of their chemical surroundings. Most of these are synthetic organic substances generically denominated as pH sensors.

Natural pigments on the other hand, are the main color determinant of many living organisms, due to their chemical nature, including chromophore groups, they are able to capture energy from natural light, which in turn excites electrons from low to high energy orbitals, and ultimately reflect the unabsorbed energy as a form of radiation that is visible to the human eye (Rodríguez-Mena *et al.*, 2023). It has been described that reflections produced by many

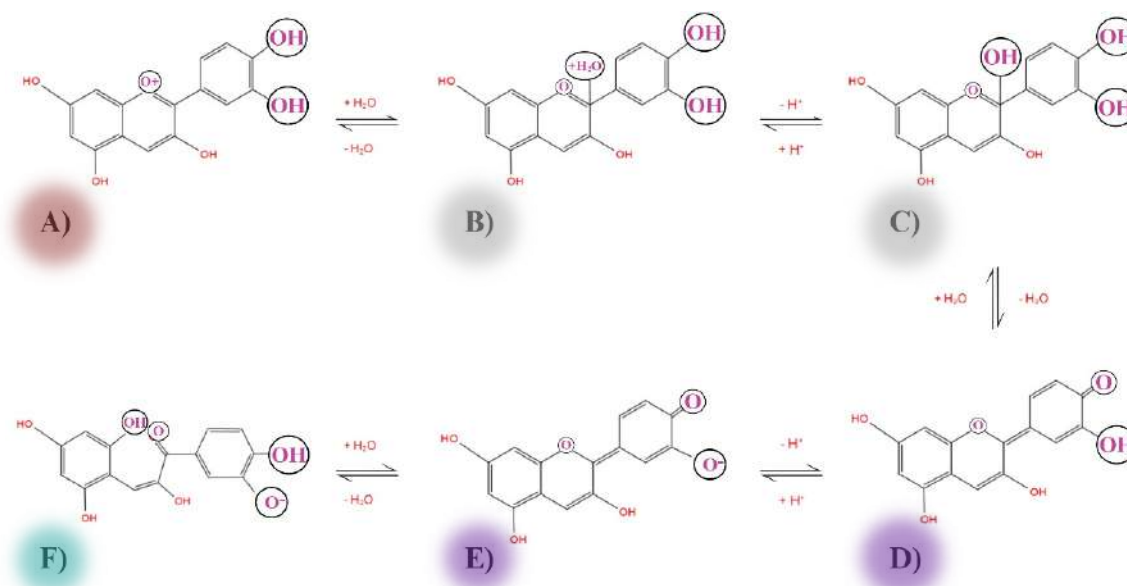
of these pigments is pH-dependent, this is why vegetables or plants harvested at different seasons or from diverse soil types are chromatically divergent from each other. This behavior constitutes the base of natural pH sensors, and the chemical characteristics of some could be found in the following sections.

## 2.1 Relevant natural compounds

### 2.1.1 Flavonoid Derivatives: Anthocyanins

Anthocyanins are a group of naturally occurring, water-soluble pigments belonging to the flavonoid family. These pigments are found in a wide variety of natural sources, the main one being red cabbage. They can also be found in fruits such as blueberries, blackberries, raspberries, strawberries, cherries, plums, pomegranates, and red grapes, as well as vegetables like beets and red onions, and others such as purple corn and black tea (Jang *et al.*, 2023).

Structurally, anthocyanins are composed of the glycosylated portion of anthocyanidins and adopt different molecular forms depending on the pH (**Scheme 1**). Briefly, in acidic environments, protonation stabilizes the red flavylium cation, giving the solution a red appearance. As pH increases, this cation is converted to the colorless pseudobase known as carbinol, reducing the intensity of the red color. At neutral pH, the equilibrium between protonated and deprotonated forms produces a purple tincture. Under alkaline conditions, deprotonation generates the quinoidal base, which shifts the solution's color to blue-green accents. In highly alkaline environments, anthocyanins degrade into chalcones, resulting in a yellow coloration (Nadi *et al.*, 2025) (**Scheme 5**).



**Scheme 1.** Representation of the structural changes of the anthocyanins molecule and its color transitions according to pH. A) In acidic environments (pH <4), protonation stabilizes the red flavylium cation. B, C) As the pH increases (pH 4-5), the flavylium cation is converted into the

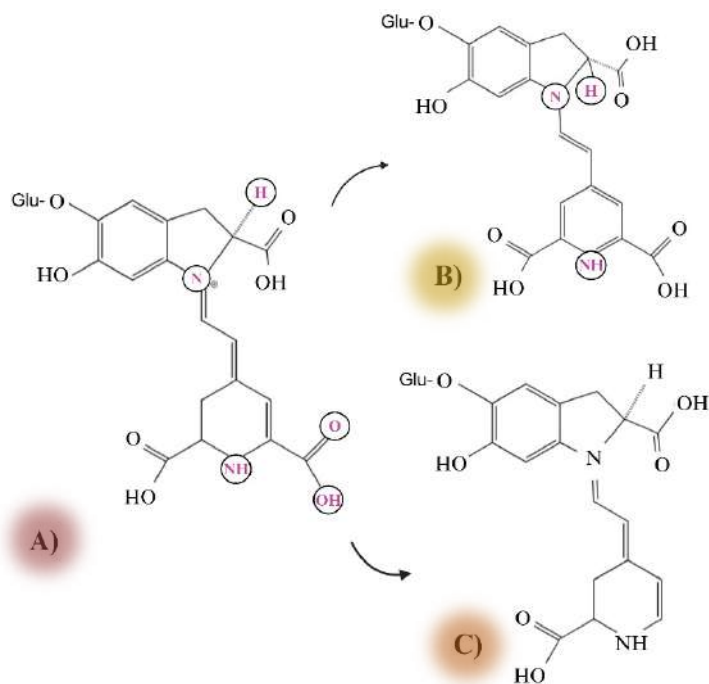
colorless carbinol pseudobase. D) At pH 6-7, the equilibrium between the protonated and deprotonated forms produces a purple color. E) At pH 7-8, deprotonation generates the quinoidal base, shifted to blue, changing the color of the solution to bluish green. F) In highly alkaline environments, anthocyanins degrade into chalcones, resulting in a yellow coloration.

### 2.1.2 Heterocyclic nitrogen derivatives: Betalains

Betalains are water-soluble natural pigments composed of betacyanins and betaxanthins. In addition to having various functional properties, they are one of the most found natural pigments in fruits, bracts, stems and flowers of edible plants (Pratiwi et al., 2025). These molecules are present in the vacuoles of the Caryophyllales order and in fungi of the genera *Amanita*, *Hygrocybe* and *Hygrophorus* (Sadowska-Bartosz & Bartosz, 2021). Also, they are frequently isolated from beetroot (*Beta vulgaris*), prickly pear (*Opuntia spp.*), djulis (*Chenopodium formosanum*), amaranth (*Amaranthaceae*), pitayas (*Stenocereus ssp.*) and pitayas (*Hylocereus undatus*) (Ortega & López, 2024). The structure of betacyanins includes an iminium adduct of cyclodioxypheylalanine (cyclo-DOPA) and betalamic acid, while betaxanthins are condensation products of betalamic acid with  $\alpha$ -amino acids or amines (Sadowska-Bartosz & Bartosz, 2021). Betalamic acid is the main chromogenic structural unit and the key biosynthetic precursor of betalains, with a chromophore in a protonated 1,7-diazaheptametine system, which has variations in its ascyll groups and sugar fractions in betacyanins and conjugation with amines and amino acids in betaxanthins (Ortega & López, 2024), which were originally classified according to their color as red-purple betacyanins or yellow-orange betaxanthins (Esteves *et al.*, 2022). They exhibit an invariant spectrum at pH values ranging from 3 to 7; in acidic environments (pH<3), there is a hypsochromic shift of approximately 2 nm and a hypochromic effect in the absorption band at 535–540 nm. Furthermore, betaxanthins fluoresce with maximum excitation range of 463–535 nm and have an emission peak detected at 508–608 nm. Betalains are optimally stable in the pH range of 5–6 and can be stored at 4°C for 20 days and frozen at -30°C for 275 days at pH 7. They are heat-sensitive and degrade at temperatures above 50°C (Sadowska-Bartosz & Bartosz, 2021). Likewise, there are chemical reactions that affect color stability such as oxidation, which darkens the product and causes color loss; dehydrogenation, which raises the temperature of betacyanin, forming betanin, which changes to a yellow color; and decarboxylation, which preserves the color by isomerizing and decarboxylating C15. However, at C17, decarboxylation generates a hypsochromic change, resulting in an orange-red color (Ortega & López, 2024) (**Scheme 2**).

Several techniques have been implemented to extract betalains, each with its own advantages and limitations. Solid-liquid extraction is economical and suitable for heat-sensitive compounds, although slow and with low yields. Microwave-assisted extraction improves efficiency and reduces solvent use but requires expensive equipment and can cause thermal degradation. Ultrasound-assisted extraction is also effective and suitable for thermolabile compounds, although it requires precise calibration. Finally, supercritical fluid extraction

produces solvent-free, high-purity extracts, but involves high costs and a complex process (Calva-Estrada *et al.*, 2022).



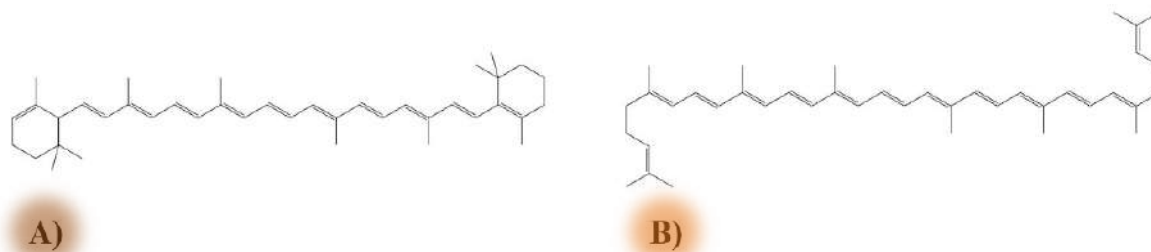
**Scheme 2.** Representation of the structural changes of the isobetanin molecule. A) Isobetanin (red), B) After dehydrogenation, Neobetatin (yellow), C) After decarboxylation, 17-Decarboxy-isobetanin (red orange)

### 2.1.3 Isoprenoid Derivatives: Carotenoids

Carotenoids, also called tetraterpenoids, are natural lipophilic bioactive pigments found in some foods of natural origin (Y. Kumar *et al.*, 2024; Ndwandwe *et al.*, 2024a). They are hydrophobic substances, yet lipophilic and readily soluble in lipids and other organic solvents such as acetone, alcohol, and chloroform. They are classified into two groups: 1) Carotenes, composed of linear hydrocarbons capable of cyclizing at one or both ends, such as lycopene,  $\beta$ -carotene,  $\alpha$ -carotene, and 2) Xanthophylls, characterized by their oxygenated functional group, some examples are lutein, violaxanthin, neoxanthin, and zeaxanthin. Most carotenoids are tetraterpenoids (C<sub>40</sub>), meaning they are chains of 40 carbon atoms, composed of eight isoprenoid units connected in a linear and symmetrical pattern, with a reversed order in the center of the molecule. Their basic cyclic structure can be modified by hydrogenation, dehydrogenation, cyclization, and oxidation. Chromophores in carotenoids reflect red, orange, and yellow light (Beltrán *et al.*, 2012).

Carotenoids are found in fruits and vegetables like mango, papaya, peaches, spinach, broccoli, carrots, red bell peppers, and tomatoes (Beltrán *et al.*, 2012; W. Zhou *et al.*, 2020). Color expression comes from their conjugated double bonds, and pH-sensitivity has been previously described (Y. Kumar *et al.*, 2024). The carotenoid chain has 3 to 15 conjugated

bonds, and each molecule's maximum absorption wavelength is determined by its chain length. This conformation is responsible for their absorption in the visible light region (400-500 nm), resulting in yellow, orange, and red colors. The number of carbon-carbon double bonds is proportional to the color intensity and determines the maximum absorption in the visible light spectrum, therefore differing for each type of carotenoid molecule (**Scheme 3**). Coloring behavior is mostly stable under low acidity and neutral conditions (pH=2-7). On the other hand, its stability is reduced under extremely alkaline and acidic conditions due to oxidation and isomerization processes, thus compromising the stability of the carotenoids and their color (**Scheme 5**) (de Oliveira Filho *et al.*, 2022).

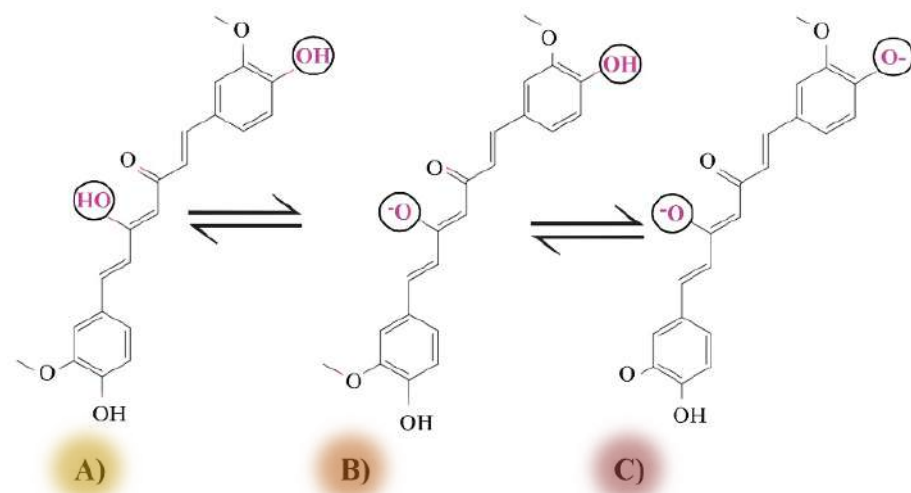


**Scheme 3.** Structural representation of two carotenoids. A) B-carotene B) Lycopene.

#### 2.1.4 Heterocyclic Derivatives of Turmeric: Curcuminoids

Polyphenols are widely distributed in the plant kingdom and possess biological functions such as antibacterial, antioxidant, anticancer, and anti-inflammatory properties (Yadav *et al.*, 2020). One of the polyphenolic compounds, curcumin, is a bioactive natural compound isolated from *Curcuma longa L.*, belonging to the Zingiberaceae family (Araiza-Calahorra *et al.*, 2018). Turmeric, contains between 3% and 5% curcuminoid derivatives such as curcumin (70%), demethoxycurcumin (20%), and bisdemethoxycurcumin (10%), rendering an orange-yellow color (Sabet *et al.*, 2021).

The color change of curcumin is due to an acid-base equilibrium. In acidic and neutral solutions (pH < 7.4), it remains in its neutral (protonated) molecular form, where its yellow color is most visible. Under alkaline conditions (pH > 8.6), the curcumin molecule is deprotonated, causing a rearrangement of its electrons and therefore a conformational change in its structure, resulting in the formation of its anionic species (**Scheme 4**). This structural alteration modifies the conjugated bond system, which changes its visible light absorption spectrum and causes a color transition to reddish-orange or dark red (**Scheme 5**) (Aliabbasi *et al.*, 2021).



**Scheme 4.** Representation of the structural changes of the curcumin molecule and its color transitions during pH sensing. A) Curcumin molecule in acidic or neutral pH ( $\text{pH} < 7.4$ ), where it remains in its protonated molecular form and exhibits a yellow color. B) Curcumin molecule under alkaline conditions ( $\text{pH} 8.6\text{-}10$ ), where one hydroxyl group is deprotonated and exhibits a reddish-orange color. C) Curcumin molecule under more alkaline conditions ( $\text{pH} > 10$ ), where another hydroxyl group is deprotonated and exhibits a dark red color.

### 3. Biopolymers

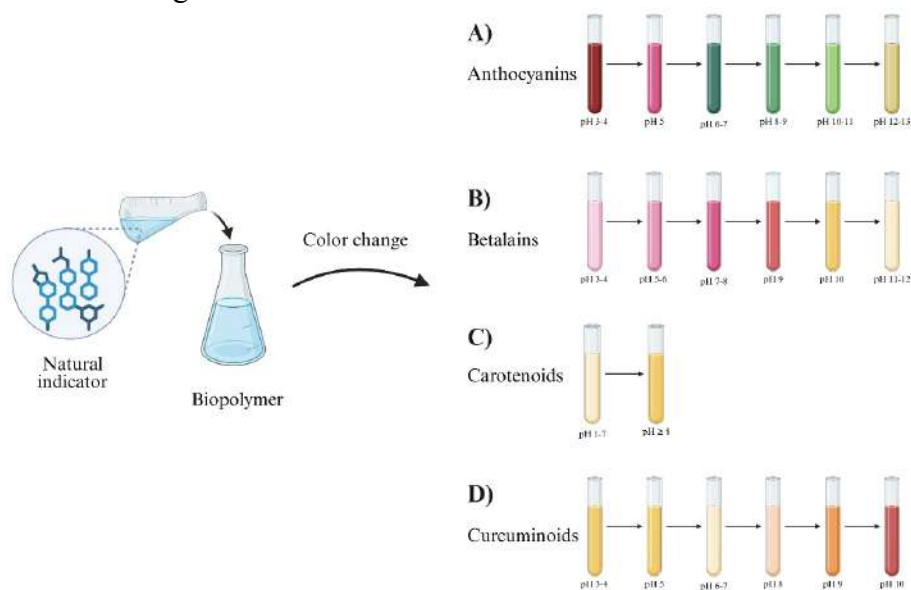
Biopolymers are natural macromolecules formed by monomers such as carbohydrates, proteins or produced in animals, plants, bacteria, and algae. These materials are notable having good film-forming properties, for being biodegradable, environmentally friendly, non-toxic, accessible and inexpensive, as they are often obtained from agricultural waste. Because of these characteristics, biopolymers represent a sustainable and viable option for developing smart packaging sensitive to natural pH (Ndwandwe *et al.*, 2024b). Among them, the most used for this application are those based on carbohydrates (such as starch, agar, and chitosan) and proteins (such as gelatin and zein).

#### 3.1. pH sensing biopolymers

Various biodegradable polymers have been developed from different natural resources, such as polysaccharides, proteins, and lipids, to produce smart packaging films with incorporated natural color indicators, due to their biodegradability, biocompatibility, non-toxicity, and abundance (Kumar *et al.*, 2020).

The use of biopolymers is often limited due to a lack of suitable physical and functional properties, as well as unsatisfactory properties for direct use in active packaging applications (Roy *et al.*, 2022). Methods have been proposed to develop active films that play an active

role in the preservation and monitoring of biological systems through the application of various natural active agents from diverse sources.



**Scheme 5.** Representation of the color changes of natural sensors at different pH. A) Anthocyanins (Abedi-Firoozjah *et al.*, 2022), B) Betalains (Khan & Liu, 2022), C) Carotenoids (de Oliveira Filho *et al.*, 2024a), D) Curcuminoids (Roy *et al.*, 2022).

### 3.2. Biosensing based on anthocyanins

Anthocyanins have gained relevance over the years due to their diverse biological and colorimetric properties, which have been successfully applied in various fields within the food and biomedical sectors.

The human body rapidly metabolizes and excretes anthocyanins, reducing their beneficial activity. Beyond their low bioavailability, anthocyanins also have low stability and are highly sensitive to external factors, making them susceptible to decomposition (Rosales-Murillo *et al.*, 2024). For this reason, current research focuses on developing strategies to improve the stabilization and bioavailability of anthocyanins.

Among the strategies aimed at stabilizing anthocyanins, encapsulation has become the most effective and researched technique. It is based on developing a protective coating for anthocyanins to provide advanced properties and performance. Combining anthocyanins with biopolymers such as chitosan, cellulose, starch, zein, gelatin, pectin, agarose, and xanthan gum has become an alternative for addressing the challenge of encapsulating these natural pigments, as well as enabling the development of smart biosensors and active materials. (Rosales-Murillo *et al.*, 2024)

### 3.2.1 Applications

The growing interest in understanding the applications and advantages of adding anthocyanins to natural biopolymers has led researchers to explore these benefits primarily in two sectors: the food industry and the healthcare sector. Regarding the food industry, one of the central objectives is to study the beneficial effects of this combination, which can respond to external stimuli. In this context, anthocyanins act as natural indicators for monitoring the quality of food products in response to interactions with temperature, pH, and microbial activity.

As described in the preceding paragraphs, anthocyanins are found in a wide variety of natural products, one example being the hibiscus flower (*Hibiscus sabdariffa*). In a study published by Khezerlou *et al.*, anthocyanins from *H. sabdariffa* (HSA) were incorporated into colorimetric chitosan labels for packaging to monitor fish spoilage and detect changes in quality and pH of fresh food during storage (**Table 1**). In this study, fish slices were placed in plastic containers, and an HSA label was affixed to the inside of each container's lid. These containers were stored at 25 °C for three days. pH values, TVB-N (total volatile basic nitrogen) levels, and label color were recorded every 12 h at 25 °C. The results showed a visible change in the color of the labels, from a light brown (fresh) before storage to a grayish color indicating poor condition after storage. This phenomenon is attributed to the increase in TVB-N and pH due to the breakdown of the fish's protein tissue by enzymes and microorganisms, leading to ammonia production. In fact, the pH of the samples increased from 6.8 to 8.4, and the TVB-N concentration increased from 6.7 to 53.3 mg/100 g after 72 h of storage. These results suggest that the fish samples would be inedible after three days of storage (Khezerlou *et al.*, 2023).

Another sector studied for the application of biopolymers with natural pH indicators is the medical sector. Because anthocyanins exhibit structural changes in response to varying concentrations of hydrogen ions, and consequently change color, they play an important role as biocompatible pH sensors.

Skin wounds are one of the main conditions where pH variations can be found as depending on the stage of wound development; their treatment presents a significant challenge, employing various techniques including the use of drugs, dressings, and hydrogels. The latter is of particular interest due to its novelty and limited exploration. In a study published by Herrera *et al.* Membranes were fabricated from a combination of different biopolymers, such as chitosan-polyvinyl alcohol (PVA), fibroin-chitosan-PVA, and fibroin-collagen-PVA, with the addition of anthocyanins from red cabbage (*Brassica oleracea*) to evaluate their potential as pH indicators (**Table 1**). Once the membranes were manufactured, they were cut to 1 x 1 cm dimensions and placed in media with different pH values ranging from 3 to 9. After monitoring each prepared membrane in the different pH media, it was confirmed that the anthocyanin indicator effect was observed in all cases. A notable color change was observed in all cases, caused by the pH values to which the membranes were subjected, resulting in colors ranging from red to yellow as the pH increased (Herrera *et al.*, 2022). Another example

is GelDerm (Mariani et al. 2017), a 3D-printed alginate-based hydrogel dressing integrated with a natural pH sensor from red cabbage extract (anthocyanins) and gentamicin-loaded fibers, this design enables simultaneous wound monitoring and localized antibiotic delivery. Such a dressing sensed pH within the wound via an array of cabbage juice beads which color transitions were visible to the naked eye or by smartphone imaging, using this map for infection and healing dynamics measurement. *In vitro* and *ex vivo* models with *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed that it can also detect infection-associated pH shifts while providing bactericidal release of gentamicin in a sustained manner, all in a cytocompatible hydrogel that additionally maintains a moist wound environment. These studies contribute to the creation of updated scientific evidence and pave the way for new monitoring methods in the evolution and treatment of skin wounds.

### 3.3. Biosensing based on Betalains

Biopolymers are optimal materials for developing pH indicators as biosensors due to their biodegradable characteristics and their interaction with natural pigments, exhibiting biocompatibility to chemical changes. Among the natural pigments, betalains stand out for their sensitivity and rapid visual response to pH variations, making them ideal for colorimetric biosensors. The most studied biopolymers used with betalains are starch, agar, carrageenan, chitosan, and cellulose derivatives, as well as proteins such as zein and gelatin (de Oliveira Filho *et al.*, 2022b).

Betalain components are ammonium derivatives of betalamic acid. They exhibit remarkable structural stability in a moderate pH range between pH 3 and 7, but their colorimetric spectrum changes considerably upon exposure to extreme values (Scheme 5) from red violet under acidic conditions to yellow or brown under alkaline conditions (Pratiwi et al., 2025).

#### 3.3.1 Applications

In the food industry, preserving food quality until it reaches the consumer is of utmost importance. Smart packaging is an innovative and sustainable strategy. The incorporation of sensors capable of detecting variations in temperature, microbial activity, or pH (Abedi-Firoozjah et al., 2023) into biopolymers allows for visual monitoring through colorimetry and provides consumers with information about the food's characteristics (Zheng et al., 2022). The incorporation of betalains into polymeric matrices such as chitosan (Yao et al., 2020), PVA (Kanatt, 2020), starch (Qin et al., 2020), and fish gelatin (Hu et al., 2020) not only detects the freshness and quality of perishable products but also contributes to improving the material's functional properties, such as antioxidant and antimicrobial activity, thus extending the product's shelf life and protecting consumer health from the formation of toxic compounds such as acetic acid, n-butyrate, and biogenic amines generated from food decomposition (Rodrigues *et al.*, 2021).

Several studies have demonstrated the effectiveness of these systems in foods with a high risk of spoilage, such as seafood, meats, fruits, and vegetables, as shown in Table 1. These

studies incorporate different pigment sources derived from betalains, such as prickly pear extract (*Opuntia ficus-indica*) (Yao *et al.*, 2020), paper flower (*Bougainvillea glabra*) (Naghdi *et al.*, 2021), and beetroot (*Beta vulgaris*) (Khazada *et al.*, 2025). Similarly, PVA and starch films were processed with betalains from red pitaya peel (Qin *et al.*, 2020). The observable color change, linked to changes in pH, serves as a reliable indicator of the food's condition, providing the end user with information about its state of spoilage.

### 3.4. Biosensing based in Carotenoids

The adaptation of pH-sensitive films has enabled the monitoring of food spoilage and quality, due to their interaction with degradation molecules resulting from each metabolic process. This is because the spoilage of foods like fish and meat initiates protein metabolism, generating the production of nitrogenous compounds, which consequently raises the pH. Conversely, dairy products, fruits, and vegetables release organic acids, which lowers the pH inside the packaging (Y. Kumar *et al.*, 2024). Carotenoids have been reported to exhibit yellow, orange, red, and purple colors due to their tetraterpene pigments. The most used natural carotenoid pigments in food packaging as freshness indicators are beta-carotene, beta-cryptoxanthin, alpha-carotene, lutein, beta-apo-8-carotenal, astaxanthin, zeaxanthin, and canthaxanthin. The stability of carotenoids is pH-dependent and is expressed through color changes. Incorporating natural carotenoid pigments into biopolymer-based films as pH-dependent indicators has not presented a risk of poisoning compared to synthetic indicators. These films provide consumers with real-time visual information about label color changes related to food condition, thus improving food safety and quality. These biopolymer films incorporate active plant compounds in the form of pigments, such as carotene from carrots and lycopene from tomatoes (Roy *et al.*, 2023).

#### 3.4.1 Applications

Starch-based smart films with green tea and basil extracts, rich in carotenoids, show in films with basil extract in an acidic solution (pH 3), a color change from greenish yellow to a very light yellow. Immersion in an alkaline solution (pH 12) resulted in a color change from dull greenish-yellow to a slight darkening of the film, demonstrating its potential as pH-sensitive colorimetric indicator films for monitoring food quality (**Table 1**). However, it has been noted that these color changes may not be solely due to the presence of carotenoids and could also be attributed to the chlorophyll in the Brazilian extract, which did not undergo purification before being added.

Recent research on stimuli-responsive or hydrogel dressings point to natural colorants (e.g., anthocyanins, purple potato extract, curcumin) as attractive pH-sensitive components for smart wound materials; however, carotenoids in these contexts are more often discussed for bioactivity and environmental sensitivity rather than pH-reporting chromophores. For instance,  $\beta$ -Carotene-laden gelatin/polyglyceryl stearate/graphene oxide (GPGO) hydrogels have been developed as burn wound dressings, where  $\beta$ -carotene, encapsulated for sustained

antioxidant and antibacterial activity, proved good hemocompatibility, cytocompatibility, and promotion of *in vitro* and *in vivo* wound closure (Darban et al. 2024).

Other reviews underscores carotenoids (e.g.,  $\beta$ -carotene, lycopene) as bioactive molecules that can be incorporated into dressings due to their antioxidant, anti-inflammatory, and antibacterial properties, but pH sensing is typically achieved with other chromophores rather than these molecules (Serpico et al. 2023; Viaña-Mendieta, 2022).

Currently, carotenoids are still unexplored as bioindicators in the development of smart films compared to other natural pigments. All of this is due to the stability of carotenoids in an acidic and neutral state (pH 2-7), visually limiting pH variations and, consequently, color changes. Combining them with other natural pigments has been suggested, based on the hypothesis of increasing their sensitivity and expanding their applications. Some combinations already studied, such as anthocyanins with betalains and with curcumin, have proven to be better than their individual use (de Oliveira Filho et al., 2024b). As can be seen in Scheme 1, color transition in carotenoids is more subtle than other extracts probably withdrawing attention from its potential use in medical and food preservation applications.

### 3.5. Biosensing based on Curcuminoids

Curcumin has been added to biopolymer films such as starch, gelatin, chitosan, pectin, and carrageenan to enhance their functional properties. The development of curcumin-based composite films for food packaging has been significant because it provides additional enhanced properties such as increased mechanical strength, UV protection, antioxidant properties, and antimicrobial activity (Aliabbasi et al., 2021).

When incorporated into biopolymer films, curcumin exhibits a pH-dependent color change. (Aliabbasi et al., 2021). The color-changing properties of curcumin allow us to monitor food quality in real time, as its main structure changes depending on the pH (Priyadarshi et al., 2021). Various methods are used to manufacture films with added curcumin, such as solution molding, compression molding, and extrusion. Blending and solution casting are the simplest ways to produce films at a laboratory scale (Roy *et al.*, 2022).

#### 3.5.1 Applications

Curcumin is a colorant known since antiquity, with properties of great interest for the development of smart packaging for use in the food industry. It prevents food spoilage and contamination and provides biological benefits, such as antioxidant, antibacterial, antiviral, antitumor, and anti-inflammatory effects. In the field of food safety, progress has been made in manufacturing films that change color according to the pH to which they are exposed. These films were enhanced with curcumin and sulfur nanoparticles (SNPs). Subsequent analyses revealed that both ingredients were correctly distributed within the pectin film, resulting in a uniform film. The addition of curcumin and SNPs resulted in a noticeable color change, improved UV light blocking capacity, and increased heat resistance. The film also inhibited the growth of *E. coli* and *L. monocytogenes* and exhibited strong antioxidant

activity. When applied to shrimp packaging, the film reacted to pH changes and ammonia vapor, displaying a distinctive color change at pH 6 and 1, from yellow to orange, as the shrimp quality varied. (**Table 1**) (Ezati & Rhim, 2020).

In another study, a two-layer indicator film was created. The first layer consisted of carrageenan with curcumin and/or anthocyanin, while the second layer was an emulsion made with konjac glucomannan and camellia oil. Subsequent analyses showed that all components bonded effectively, forming two well-defined layers. The emulsified layer improved its resistance to heat, humidity, and UV radiation, as well as the mechanical properties of the indicator layer. In a four-day chicken meat spoilage test at room temperature (approximately 25°C), both the curcumin-containing film and the curcumin/anthocyanin-containing film showed a distinct color change in both the fresh and spoiled states. (**Table 1**) (X. Zhou et al., 2021).

Most research studies report halochromism in curcumin, which is yellow at acidic pH but turns orange-red when the pH becomes alkaline. This distinctive color change is positively correlated with TVB-N produced during the spoilage of protein-rich foods (Roy *et al.*, 2022). In addition, sodium cellulose sulfate/chitosan composite films loaded with curcumin offer colorimetric response across pH 3–10 and, though developed for pork freshness monitoring, present formulation and pH sensitivities relevant for translation toward medical devices (Tang *et al.*, 2022).

For applications on healthcare diagnostics, curcumin-containing fibrous composites were synthesized to enable visible color changes from yellow to red-brown within the physiological pH range of 6.0–9.0, specifically targeting wound pH assessment, although data on reversibility and dye leaching were not always reported (Pan *et al.*, 2019). Wearable sensor approaches based on curcumin embedded in electrospun polycaprolactone (PCL) fibers have also been described, allowing for visual or smartphone-based pH readouts with intended integration into wound dressings, though precise pH response range and leaching performance were often unspecified (Pusta et al., 2022).

Cellulose-based biopolymers represent another promising class, in which cellulose nanocrystal (CNC)-reinforced films containing esterified curcuminoids provide broad, reversible color transitions over pH 2–10 and strong resistance to bleaching, particularly in acidic media. While initially designed for smart food packaging, these CNC–curcuminoid systems show direct adaptability to biosensing applications for sweat or wound fluids due to their favorable stability and biocompatibility (Singh, 2024).

Currently, the applications of curcumin as a natural indicator require further research to obtain results applicable across all biological fields, with the potential for future integration into the healthcare sector and the full utilization of its properties.

**Table 1.** Recent applications of biopolymers mixed with natural pH sensors.

Pigment category	Biopolymer source	Pigment source	Pigment concentration	Additives	Application	Color transition	Ref.
Anthocyanins	Chitosan	hibiscus flower ( <i>Hibiscus sabdariffa</i> )	3% w/w	Acetic acid and glycerol	Colorimetric labels for packaging and monitoring fish deterioration	Red, pink, light green to yellow	(Khezerlou et al., 2023)
	Fibroin, chitosan, Collagen	red cabbage ( <i>Brassica oleracea</i> )	5550 mg/ml	PVA	Skin wound care treatment and pH sensing	Red, blue, green, yellow	(Herrera et al., 2022)
Betalains	Ammonium chitosan	prickly pear ( <i>Opuntia ficus-indica</i> )	2% y 3% w/w	PVA	Monitoring shrimp freshness	Purple to orange	(Yao et al., 2020)
	Potato starch	bougainvillea ( <i>Bougainvillea glabra</i> )	5, 10 and 15 mg/g of polymer	None	Monitoring fish freshness	Pink to yellow	(Naghdi et al., 2021)
	Potato and Sago starch	beetroots ( <i>Beta vulgaris</i> )	4 ml/1.5g dissolved starch	glycerol	Monitoring chicken freshness	Red, purple to yellow	(Khanzada et al., 2025)
Carotenoids	Cassava starch	green tea and basil	3g/100 ml	None	pH-sensitive colorimetric indicator films for monitoring food quality	Green-yellow to light yellow	(de Oliveira Filho et al., 2024b)
	Polylactic acid	bixin	1g/Kg	None	Peroxide reduction in sunflower oil to avoid food spoilage	Light yellow to orange	(Stoll et al., 2023)
Curcuminoids	Pectin	<i>Curcuma Longa</i>	1% w/w	sulfur nanoparticles	Monitoring shrimp freshness	Yellow to orange	(Ezati & Rhim, 2020)
	Glucomannan/ Carragenin	<i>Curcuma Longa</i>	0,16 g and 0,08 g	Camellia oil	Monitoring chicken freshness	Yellow to orange	(X. Zhou et al., 2021)
	Sodium cellulose sulfate/chitosan	<i>Curcuma longa</i>	6% w/w	None	Monitoring pork freshness		(Tang et al., 2022)
	Electrospun polycaprolactone (PCL) fibers	<i>Curcuma longa</i>	5%, 10%; 20%; with respect to the weight of PCL	None	Wound healing status	Yellow to orange	(Pan et al., 2019)

## 4. Conclusions

pH is a fundamental parameter in biological systems, where naturally occurring pigments function as natural indicators of its variation. Molecules such as anthocyanins, betalains, carotenoids, and curcuminoids show great potential for developing materials that help solve problems in various fields, including food and biomedicine. When these molecules are integrated into natural polymers such as chitosan, cellulose, or starch, these natural indicators allow for the creation of colorimetric sensors capable of monitoring food freshness or wound healing in real time, in an environmentally friendly and safe manner.

Currently, challenges related to production and industrial integration remain, and new strategies are being researched to improve their stability and sensitivity. We can say that these new biopolymers associated with natural pH indicators could be applied as promising alternatives to reduce the use of single-use plastics and contribute to the monitoring of biological systems with the naked eye.

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## Nomenclature

- **Ciclo-DOPA:** cyclodioxyphenylalanine
- **CMC:** carboximethylcellulose
- **HSA H:** sabdariffa
- **PVA:** polyvinyl alcohol
- **TVB-N:** nitrógeno básico volátil total
- **UV:** ultraviolet
- **UV-vis:** ultraviolet to visible spectra

## Declarations

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