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Review

Recent advances in the development of smart, active, and bioactive biodegradable biopolymer-based films containing betalains

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ABSTRACT

Betalains are natural nitrogenous water-soluble pigments found in species belonging to the *Caryophyllales* order and in mushrooms. Betalains can be considered multifunctional molecules due to their diverse bioactivities such as antioxidant, antimicrobial, anticancer, and anti-inflammatory. Furthermore, they can detect pH variations in foods and are considered promising colorimetric bioindicators. The bioactivities of betalains have improved their use as active and bioactive agents, and colorimetric indicators in the development of edible and biodegradable films for foods, which are trends in the food packaging market. Thus, this review presents the state-of-art information on the use of betalains as a multifunctional molecule in the development of smart, active, and bioactive edible and biodegradable packaging for foods. Studies have revealed that betalains can be successfully used to develop: smart films to indicate the freshness and spoilage of foods such as shrimp, fish, and chicken; active films with antimicrobial and antioxidant potentials to increase the shelf life of sausage and shrimp; and bioactive films with health benefits.

1. Introduction

Traditional food packaging, based on synthetic and non-biodegradable polymers, cause serious environmental problems. Therefore, developing biodegradable polymer- and renewable source-based packaging has become an important line of research in the food field (Ncube et al., 2020). Edible and biodegradable films are generally developed from biological macromolecules such as proteins or protein isolates (e.g., zein, gelatin, casein, and sunflower), lipids (e.g., carnauba wax and beeswax), and polysaccharides (e.g., pectin, chitosan, starch, and alginate) (Hassan, Chatha, Hussain, Zia, & Akhtar, 2018). These films can carry natural compounds with bioactivities and physicochemical properties that make them usable to develop smart, active, and bioactive films (Oliveira Filho et al., 2021).

Smart films can monitor food quality and environmental conditions inside the package, such as spoilage metabolites, presence of oxygen, and temperature, and also communicate with consumers providing, for example, qualitative information through visual colorimetric changes (Priyadarshi, Ezati, & Rhim, 2021). Active films contain active agents (such as antioxidant and antimicrobial compounds) that desirably interact with the food product, to protect, prolong shelf life, and preserve sensory properties (appearance, aroma, consistency, texture, and flavor) (Umaraw et al., 2020). These components act directly on food processes, being involved in physiological (fruit and vegetable respiration), chemical (fat oxidation), physical, and microbiological changes (Nair et al., 2020; Oliveira Filho et al., 2021; Oliveira Filho, Lemes, Braga, & Egea, 2020).

Bioactive films, in turn, are edible packages that contain substances with bioactivity (such as prebiotics, probiotics, vitamins, and flavonoids), which promote consumers health benefits (Lopez-Rubio, Gavara,

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& Lagaron, 2006). Bioactive films are an innovation in the concept of edible packaging linked to the concept of functional food (Espitia, Batista, Azeredo, & Otoni, 2016).

Pigments are chemical compounds present in plant and animal tissues that are visible to the human eye in varying colors and which provide beneficial effects for health. Pigments are necessary for plant protection and reproduction (Stintzing & Carle, 2004; Tanaka, Sasaki, & Ohmiya, 2008). Natural pigments in plants, such as curcumin, betalains, carotenoids, chlorophylls, and anthocyanins, have been studied as natural agents of plant origin to develop biodegradable smart, active, and bioactive edible films (Bhargava, Sharanagat, Mor, & Kumar, 2020; Vargas, Loaiza, & González, 2021). Betalains, which are subclassified into betaxanthines (yellow-orange) and betacyanins (red-violet), are natural plant pigments widely used as colorants in food products because they present strong and stable colors (pH 3–7) (Tossi, Tosar, Pitta-Álvarez, & Causin, 2021).

Betalain change color under different pH conditions, and can be used as natural pH indicators (Naghdi, Rezaei, & Abdollahi, 2021; Yao et al., 2021). In addition, they have strong antioxidant (Mello et al., 2014), antimicrobial (Yong, Dykes, Lee, & Choo, 2018), anti-inflammatory (Martinez et al., 2015), antilipidemic (Sawicki, Juśkiewicz, & Wiczkowski, 2017) properties, among others (Hussain, Sadiq, & Zia-Ul-Haq, 2018), and can be applied as natural agents in food preservation, as well as in the development of functional foods, which provide beneficial effects for health.

Due to these characteristics, betalains are multifunctional molecules with potential for application in the development of smart, active, and bioactive food films. However, studies on this topic are still limited, primarily focusing on the efficiencies of the pigments in food systems; *in vivo* studies evaluating the potential of betalains in human and animal models are even more difficult to find (Hadipour et al., 2020; Khan, 2016; Rahimi, Abedimanesh, Mesbah-Namin, & Ostadrahimi, 2019; Rahimi et al., 2019). In addition, to the best of our knowledge, there are no studies in the literature with bioactive polymeric films containing betalains that assess the bioavailability and bioaccessibility of the pigments once incorporated to the films. Thus, this review aims to present the state-of-art information on the use of betalains as a multifunctional molecule in the development of edible and biodegradable smart, active, and bioactive food packaging.

2. Betalain: A promising bioactive molecule

Betalains (Fig. 1**A**) are red-violet and yellow-orange water-soluble nitrogenous compounds normally found in roots, fruits, stems, and flowers (Otálora, de Jesús Barbosa, Perilla, Osorio, & Nazareno, 2019; Ramamoorthy et al., 2016). Structurally, betalains are classified into betacyanins (pink/red/violet) composed of betalamic acid (the chromophore) and *cyclo*-3,4-dihydroxyphenylalanine (cycloDopa) (Fig. 1**B**) and betaxanthines (yellow/orange) formed by the condensation of betalamic acid and amino acids or amines (Fig. 1**C**) (Delgado-Vargas, Jiménez, & Paredes-López, 2000; Khan, 2016).

In the plant kingdom, these pigments are restricted to Caryophyllales order (Table 1) and mainly distributed in families such as Amaranthaceae (*Beta vulgaris* L), Amaranthaceae (*Amaranthus* spp.), Cataceae (*Opuntia* xoconostle and *Hylocereus* sp.), Nyctaginaceae (*Bougainvillea*),

Table 1
Average quantity of betalains found in plants belonging to Caryophyllales order.

Plants	Mean of total betalains related by the authors	References
Beta vulgaris L.	~90.20 mg/100 g of fruit weight	Zin and Bánvölgyi (2021)
Amaranthus caudatus L (total betacyanins)	~52.97 mg/g	Roriz et al. (2021)
Amaranthus leaf	~85.02 mg/100 g	Kanatt (2020)
Hylocereus polyrhizus	~0.54 mg/g	Utpott et al. (2020)
Bougainvillea glabra	~2.81 mg/g	Kuhn, de Azevedo, and Noreña (2020)

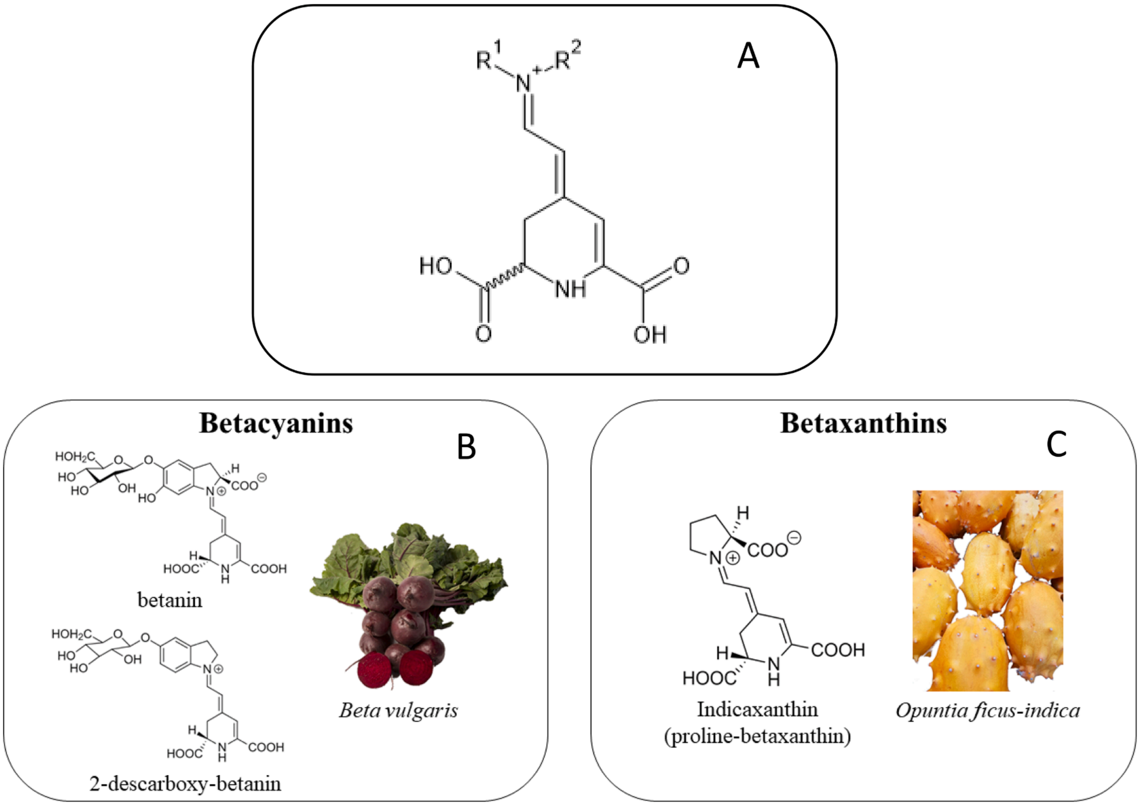

Fig. 1. Molecular structure of betalains (A), structure of betacyanins (Beta vulgaris) (B) and betaxanthis (Opuntia ficus-indica) (C).

Phytolaccaceae (*Pytolacca amareciana*), and Portulacaceae (*Portulaca grandiflora*) (Martins, Roriz, Morales, Barros, & Ferreira, 2017; Tesoriere, Allegra, Butera, & Livrea, 2004; Zin, Anucha, & Bánvölgyi, 2020). In addition, betalains can also be produced in *Amanita muscaria*, which generates a red pigmented mushroom in the cap skin through the combination of orange, yellow, and purple pigments (Gill & Steglich, 1987). Betalain concentration varies according to the species and is the highest in sugar beet (944.57 mg/L) (Wruss et al., 2015).

Contrary to the biosynthetic pathway of anthocyanins and carotenoids, betalain biosynthetic pathway in plants is partially understood (Gandía-Herrero & García-Carmona, 2013). The biosynthetic pathway of betalains includes the hydroxylation of L-tyrosine, an aromatic amino acid that participates in protein synthesis from shikimate pathway, to L-3,4-dihydroxyphenylalanine (L-DOPA) by tyrosinase in the presence of molecular oxygen or tyrosine hydroxylase (Gandía-Herrero, García-Carmona, & Escribano, 2004; Yamamoto, Kobayashi, Yoshitama, Teramoto, & Komamine, 2001). L-DOPA is then oxidized by tyrosinase to form dopaquinone (Gandía-Herrero & García-Carmona, 2013).

CycloDOPA and betalamic acid are important intermediates for betacyanin and betaxanthin synthesis, which can be obtained using multiple techniques. CycloDOPA can be formed by spontaneous dopaquinone cycling (Gandía-Herrero & García-Carmona, 2013) or converting L-DOPA catalyzed by an *CYP76AD1*-encoded enzyme belonging to the cytochrome P450 family present in *Beta vulgaris* root (Hatlestad et al., 2012). On the contrary, betalamic acid is obtained by L-DOPA 4,5-dioxygenase (DODA), which converts L-DOPA to 4,5-dry-DOPA, an intermediate compound that is rearranged to form betalamic acid (Timoneda et al., 2019). Betalamic acid performs spontaneous condensation reactions with amino acids or amines, producing betaxanthines, or with cycloDOPA, producing betacyanins (Gandía-Herrero & García-Carmona, 2013).

Regarding betalain extracts, different extraction methods have been studied, which will directly influence the properties of this molecule (Table 2). The main solvents described for betalain extracts production are distilled water (Apriliyanti, Wahyono, Fatoni, Poerwanto, & Suryaningsih, 2018), methanol/water combination (Cejudo-Bastante, Cejudo-Bastante, Cran, Heredia, & Bigger, 2020), different ethanol concentrations (Aparicio-Fernández, Vega-Ahuatzin, Ochoa-Velasco, Cid-Pérez, Hernández-Carranza, & Ávila-Sosa, 2018; Yao, Hu, Qin, & Liu, 2020), and citric acid solution (Kanatt, 2020).

Betalains have a long history of usage as food colorings, such as pokeberry juice, which was used in the 19th century to enhance the color of red wine and later banned because of its toxicity due to the presence of saponins and lectins (Petit-Paly, Andreu, Chénieux, & Rideau, 1994). Presently, raw, canned, or even cooked red beet extract is the only commercially used source of betalains as a food coloring (Mereddy, Chan, Fanning, Nirmal, & Sultanbawa, 2017; Rodriguez-Amaya, 2019); it is applied in the production of different foods such as strawberry-flavored ice cream, jam, ice sherbets, cupcake, beetroot jelly, biscuits fortified with red beetroot, multigrain snacks fortified with beetroot powder, probiotic beetroot drink, noodles fortified with beetroot power, cheese cracker fortified with beetroot, betalain-rich capsule, and beetroot wine (Nirmal, Mereddy, & Maqsood, 2021).

However, betalain application in the food industry is limited because its color can degrade when the foods are exposed to high temperatures and to light. Simultaneously, this color degradation could indicate excessive thermal processing, as the color changes according to process changes (Herbach, Stintzing, & Carle, 2006). Moreover, betalains are advantageous than other red colored pigments such as anthocyanins, due to their higher solubility, significantly higher tinting strength, and stability at pH 3–7, making them the most suitable for application in foods with low acid content or neutral pH (Stintzing & Carle, 2004). This lack of stability of the molecule at different conditions demonstrates the potential of betalain extracts as natural indicators of the food quality in the storage process, highlighting their application strategy in smart films.

Table 2
Extraction methods for obtaining betalain extracts to be applied to different polymer-based films.

Sources	Extraction Method	References
Prickly Pear (*Opuntia ficus-indica* L.)	Extraction from the dried prickly pear husks in citrate–phosphate buffer (pH 6.5), which was filtered, centrifuged, and diluted in 90% ethanol (ratio 1:10).	Aparicio-Fernández et al. (2018)
Dragon fruit (*Hylecereus polyhizus*)	Extraction from fresh red dragon fruit husks in water heated followed by filtration.	Apriliyanti et al. (2018)
Red beetroots (*Beta vulgaris*. L. sp)	Extraction from lyophilized red beets in methanol and water (plus 50 mM sodium ascorbate) to complete discoloration, which have been centrifuged and the extract were combined.	Cejudo-Bastante et al. (2020)
Red beetroots (*Beta vulgaris*. L)	Extraction from red beets in water followed by filtration and centrifugation.	Zamudio-Flores et al. (2015b)
Red beetroots (*Beta vulgaris*. L)	Extraction from red beets in water followed by filtration, centrifugation, and lyophilization.	Zamudio-Flores et al. (2015a)
Amaranth (*Amaranthus tricolor* L.)	Extraction from amaranth in ethanol (60% v/v, 4 °C/24 h) followed by filtration, centrifugation, separation in AB-8 macroporous adsorption resin, and vacuum concentration.	Hu et al. (2020)
Amaranth (*Amaranthus tricolor* L.)	Extraction from dried amaranth leaves in water containing citric acid (0.1 M) (1:10, w:v) followed by centrifugation and filtration.	Kanatt (2020)
Red pitaya (*Hylocereus polyrhizus*)	Extraction from fresh red pitaya bark in 30% ethanol solution (4 °C) followed by filtration, centrifugation, separation with column of macroporous resin D101 using 30% ethanol, and concentration.	Qin et al. (2020)
Cactus pears (*Opuntia ficus-indica*)	Extraction from pears cactus in 60% ethanol solution (4 °C) followed by centrifugation, concentration, separation with column loaded with resin AB-8 using 60% ethanol solution, and dehydration.	Yao et al. (2020)
Red pitaya, red beetroot, red amaranth, prickly pear, and globe amaranth	Extraction from red pitaya pulp, red beetroot, red amaranth, prickly pear, and globe amaranth in 60% ethanol solution (4 °C) followed by filtration, eparation with column loaded with resin AB-8 using 60% ethanol solution, and lyophilization.	Yao et al. (2021)

In addition to their application as a natural dye, betalains also have potent antimicrobial and antioxidant activities (Cai, Sun, & Corke, 2003; Veličanski, Cvetković, Markov, Vulić, & Đilas, 2011), which may corroborate their application in active and bioactive films.

The antimicrobial activity of betalains may play an important role in food preservation as well as the control of pathogenic bacteria present in the intestine. In this context, different sources of betalain extracts with different antimicrobial potentials have been described, such as *Beta vulgaris* L. pomace extract, which demonstrated antimicrobial activity against *Salmonella typhymurium* (ATCC 14028), *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 11632), *Bacillus cereus* (ATCC 10876), and wild isolates such as *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Enterobacter cloacae*, *Bacillus* spp., *Enterococccus faecalis*, *Staphylococcus epidermidis*, *Staphylococcus cohni* spp. *Cohni,* and *Listeria monocytogenes* (Veličanski et al., 2011); beet root (*Beta vulgaris*) extract showed potential activity against *Escherichia coli*, *Salmonella enteritidis*, and *Staphylococcus aureus* (Zia, Sunita, & Sneha, 2021); *Opuntia ficus-*

indica L. inermis extracted oil showed antibacterial potential against *Enterobacter cloacae* and *Candida parapsilosis* as well as antifungal potential against *Penicillium*, *Aspergillus,* and *Fusarium* (Khémiri, Essghaier Hédi, Sadfi Zouaoui, Ben Gdara, & Bitri, 2019); and betacyanin isomers from *Gomphrena globosa* L. flowers demonstrated antibacterial activity against *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 35218, *Escherichia coli* ATCC 25922, *Enterobacter cloaceae*, and *Pseudomonas aeruginosa* ATCC 27853 (Spórna-Kucab, Bernaś, Grzegorczyk, Malm, Skalicka-Woźniak, & Wybraniec, 2018).

The antioxidant activity of betalains, in addition to being dependent on the extract source, can also be altered by the type of molecule that constitutes the extract. For example, between the extracts from plants of the Amaranthaceae family (EC_{50} ranging from 3.4 to 8.4 μM using the DPPH method) (Cai et al., 2003), the EC_{50} of betacyanins of the Gomphrenin type was 3.7 μM, and of betaxantins was 4.2 μM, which shows an antioxidant activity 3–4 times higher than that of ascorbic acid (13.9 μM) and twice higher than that of catechin (7.2 μM) and rutin (6.1 μM).

The antioxidant activity, in addition to being important for the application of these extracts in active films, can also be important in bioactive films (which will act as a vehicle for this effect in the human body). Thus, it is important that this activity should be demonstrated along the gastrointestinal tract. Montiel-Sánchez, García-Cayuela, Gómez-Maqueo, García, and Cano (2021) studied the antioxidant activity of cactus fruit extracts *(Myrtillocactus geometrizans)* through gastrointestinal *in vitro* system and demonstrated losses of this activity of 48% in the oral phase, 28% in the gastric phase, and 75% in the intestinal phase, resulting in 82% instability of this extract in the human body.

Interestingly, even with these instability values within the human body, betalain extracts have demonstrated important health-promoting effects such as anticancer, hepatoprotective, and antidiabetic activities (Polturak & Aharoni, 2018). To prevent cancer development, betalains can act scavenging free radicals and activating the nuclear erythroid factor 2-related factor 2 (Nrf2) (Esatbeyoglu et al., 2014).

As for its hypolipidemic and hepatoprotective activities, Tesoriere et al. (2004) reported that volunteers ($n = 8$) showed resistance to copper-induced oxidation after a single ingestion of 500 g cactus pear fruit pulp, which provides 28 and 16 mg indicaxanthin and betanin, respectively; they suggested that betalains may be involved in the protection of low-density lipoprotein (LDL) against ex vivo induced oxidative changes. Effects on decreasing total serum cholesterol and triacylglycerols levels in rats fed with dislipidaemic diets were also observed by Wroblewska, Juskiewicz, and Wiczkowski (2011), corroborating the results described above. In addition, these authors also demonstrated the antidiabetic activity of betalains extracts by reporting serum glucose level reduction after ingesting beetroot crisps for four weeks.

The stability of betalains is affected by several factors such as light, oxygen, $pH < 3$ or >7, temperature, and water activity (Castro-Enríquez et al., 2020). With the exception of water activity that is an important factor due to hydrolytic reactions, the other physical-chemical factors have been shown to act in synergy, favoring the further degradation of betalains (Reshmi, Aravindhan, & Devi, 2012).

The structure of betalains also plays an important role in the stability of these molecules. For example, betacyanins show higher stability at acidic pH and are less prone to oxidation than betaxanthines, but betaxanthines show greater stability at pH 7 and hydrolytic enzymes. The higher stability of betacyanins in relation to betaxanthins may be due to the fact that some of them have a glycosylated structure, which has a high oxidation–reduction potential (Herbach, Stintzing, & Carle, 2006; Castro-Enríquez et al., 2020).

Due to the easy degradation of betalains, studies have required ways to stabilize them to enhance their applications in food. The incorporation of betalains in biopolymer films represents a promising method for the stabilization of these molecules. In this sense, the incorporation of betalain-rich extracts in biopolymer matrices for the development of smart, active, or bioactive films represents a strategy to explore the full potential of these multifunctional molecules and enhance their applications.

3. Smart films containing betalains

Consumers demand safe foods with a long shelf life, leading researchers and the food industry to explore new technologies for food packaging (Biji, Ravishankar, Mohan, & Gopal, 2015). Smart films are a kind of packaging that communicates with consumers, providing timely information about the freshness and/or spoilage of food. These films monitor the quality of the packaged products along with the conditions inside the package, such as temperature, pH, oxygen, and microbial metabolites (Oliveira Filho et al., 2021).

Several smart packages have been developed in the recent years, including pH indicator films, which provide qualitative information through visual color changes. Although most smart films are produced from non-renewable sources such as synthetic petroleum products, biodegradable alternatives have been receiving a lot of attention (Apriliyanti et al., 2018; Hu, Yao, Qin, Yong, & Liu, 2020; Jamróz, Kulawik, Guzik, & Duda, 2019; Kanatt, 2020; Naghdi et al., 2021; Qin, Liu, Zhang, & Liu, 2020; Yao et al., 2020; Yao et al., 2021). Biopolymers such as polysaccharides and proteins from different sources have been explored to prepare biodegradable smart films due to their abundance, non-toxicity, and biocompatibility as a polymer matrix (Alizadeh-Sani, Mohammadian, Rhim, & Jafari, 2020).

Traditionally, compounds such as bromocresol purple, cresol red, bromocresol green, and bromophenol blue are used as colorimetric indicators in smart packaging (Poyatos-Racionero, Ros-Lis, Vivancos, & Martínez-Máñez, 2018). However, the use of these indicator compounds to monitor food is limited due to the potential toxicity of some of them (which can make the food unsafe for consumption by posing a risk to health), as well as due to the inedible nature of these compounds (which can change the sensory characteristics of the packed food) (Alizadeh-Sani et al., 2020; Liu et al., 2019). Thus, the search for preferably edible, nontoxic, and pH-sensitive bioindicators extracted from natural sources have been the aim of studies for the development of smart food packaging (Moradi, Tajik, Almasi, Forough, & Ezati, 2019).

Natural plant pigments, such as curcumin, anthocyanins, chlorophyll, and betalains, can change color in different pH ranges due to structural instabilities; thus, they can be used as natural and nontoxic pH indicators with high potential of application in smart packaging (Priyadarshi et al., 2021). Betalains generally show structural stability at pH 3–7; however, they show structural and color variations from pink/red/violet (Fig. 2) to yellow/orange (Fig. 2) under alkaline conditions ($pH > 7$) (Qin et al., 2020).

The color of betanins directly depends on their stability as a function of pH. They are generally stable between pH 3–7, with the optimum pH being between 4 and 5. In this pH range, betanins are bright bluish-red in color and turn blue-violet as the pH increases. Once the pH reaches alkaline levels, betanin degrades by hydrolysis, resulting in a yellowish-brown color (Delgado-Vargas, Jiménez, & Paredes-López, 2000).

Betalain-rich extracts obtained from different sources, mainly pitaya fruit peels (*Hylocereus polyrhizus)*, paperflower (*Bougainvillea glabra*), fresh red pitaya fruits (*H. polyrhizus*), beetroot (*Beta vulgaris*), amaranth leaf and flour (*Amaranthus tricolor* L.), and cactus pear extract (*Opuntia ficus-indica*), have been used as natural indicators for the development of smart films (Fig. 3). These smart films can be based on different biopolymers (such as glucomannan–polyvinyl alcohol, quaternary ammonium chitosan/fish gelatin, potato starch, Furcellaran, starch/polyvinyl alcohol, polyvinyl alcohol, gelatin, and tara gum/polyvinyl alcohol) and monitor the quality of fresh products (mainly shrimp, chicken, and fish) (Apriliyanti et al., 2018; Hu et al., 2020; Jamróz et al., 2019; Kanatt, 2020; Naghdi et al., 2021; Qin et al., 2020; Yao et al., 2020; Yao et al.,

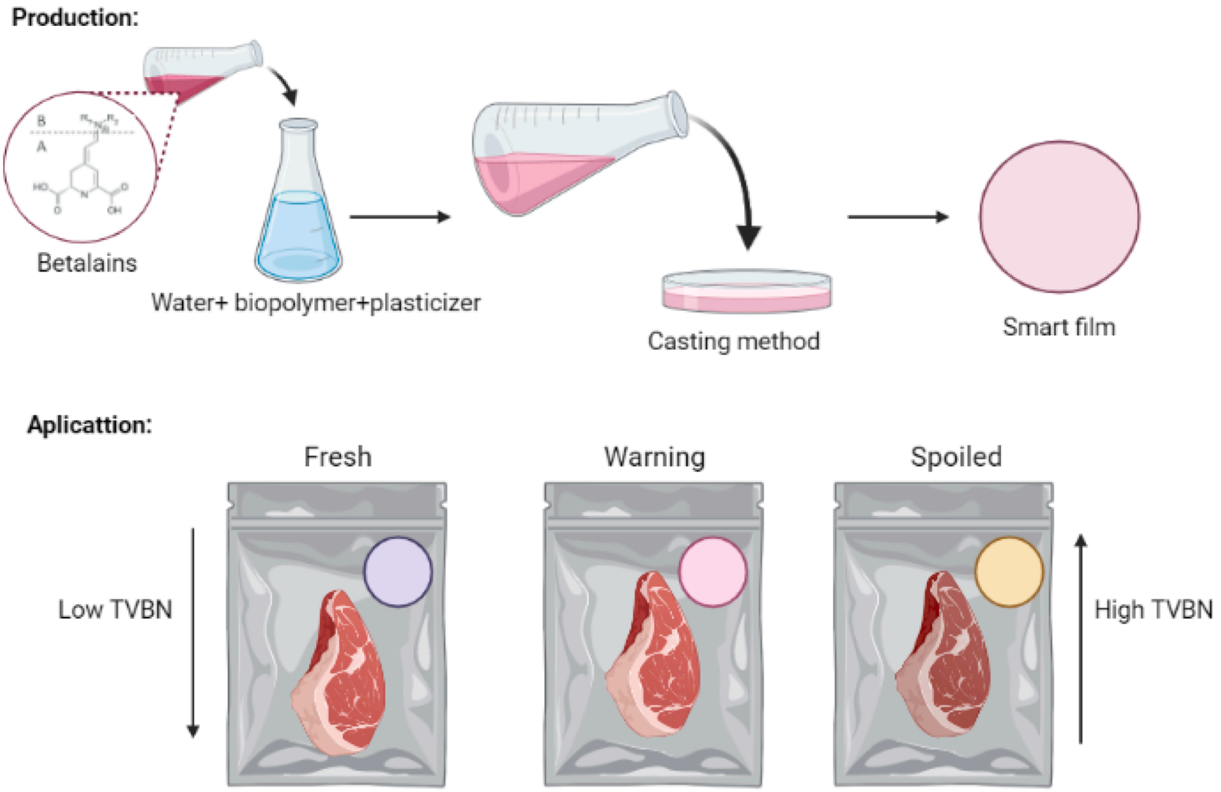

Fig. 2. Overview of the production and application of smart films containing betalain-rich extracts in food quality monitoring.

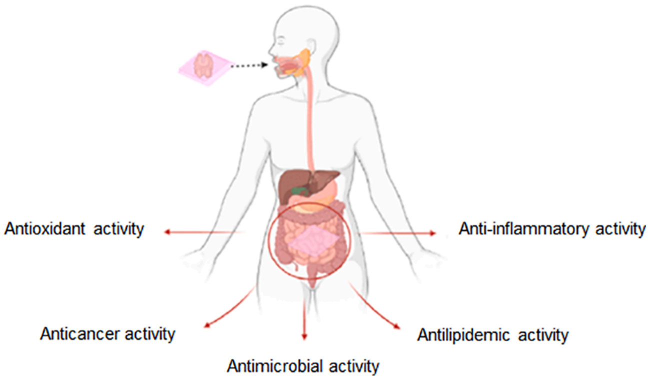

Fig. 3. Possible beneficial effects of ingesting betalain-incorporated bioactive films.

2021). Smart films with pH control function can be formulated by combining biopolymers with betalain-rich extracts and produced by casting technique. After drying, the smart films can be applied directly in contact with the food or coupled to the primary packaging material to interact with compounds released during deterioration, mainly total volatile basic nitrogen (TVBN), in the headspace of the packaging. The interaction of the smart film with the food or with its headspace at a particular pH changes the color of the film, visually indicating whether the product is fresh or spoiled (Fig. 3).

Studies have revealed the promising nature of smart films formulated with betalain-rich extracts to monitor the quality of fresh food products (Table 3). Protein-rich foods such as meat and seafood are susceptible to microbial and chemical spoilage; their deterioration process is accompanied by the release of different volatile nitrogen compounds such as ammonia and biogenic amines, that can lead to sensory rejection and off-flavor formation in these products (Comi, 2017). These compounds are closely linked to changes in the pH of foods and their surrounding environment, which can be detected and shown to the consumer using indicator films containing pH-sensitive compounds (Rodrigues, Souza, Coelhoso, & Fernando, 2021).

Shrimp spoilage increases the production of alkaline compounds such as ammonia, dimethylamine, and trimethylamine. Smart films based on biopolymers added with betalain-rich extracts detect the presence of these compounds and indicate the quality of shrimp during storage. Qin et al. (2020) have developed starch/polyvinyl alcohol films incorporated with betalain-rich pitaya bark extracts to indicate the freshness of shrimp; they showed perceptible changes in color from pink to yellow in response to volatile nitrogen compounds (TVBN 19.38 mg/100 g) accumulated during the shrimp deterioration process. Hu et al. (2020) have also monitored the freshness of shrimp stored at 20 °C for 48 h using films based on the combination of quaternary ammonium chitosan/fish gelatin added with betalain-rich extracts from amaranth; a perceptible change in color from pink to yellow was observed when the TVBN accumulation reached the maximum limit established by the Chinese standard for seafood (20 mg/100 g). Films produced using 5 and 10 % amaranth extracts showed high sensitivity to detect the deterioration, with color change over 24 h, the time for the product to reach the maximum acceptable limit of TVBN accumulation.

Yao et al. (2021) developed smart films formulated with starch, polyvinyl alcohol, and betalain-rich red pitaya pulp, prickly pear fruit, red beet, globo amaranth flower, and red amaranth leaf extracts to monitor shrimp quality. The film color gradually changed from purple/red to yellow with the increase in the TVBN accumulation, which reached 21.88 mg/100 g in 24 h. The study revealed that the red pitaya extract was the most suitable to monitor the freshness of shrimp, with the highest color variation ($\Delta E > 5$).

Smart films developed with betalain-rich extracts also monitor the deterioration of other protein-rich products such as fish and chicken. Kanatt (2020) developed smart films based on polyvinyl alcohol/gelatin and betalain-rich amaranth leaf extract to monitor the freshness of fish and chicken. The color of the films changed from red to yellow in both

Table 3
Primary effects of betalain incorporation on the properties of active and smart biopolymeric films (2012–2021).

Matrix	Betalain source	Food	Type of film	Effects/Results	Reference
Hydroxypropyl methylcellulose (HPMC)	Beetroot juice and purple carrot extract	–	Active	Positively affected: color change (increased by ~3,400% in ΔE).Negatively affected: thickness (increased by ~12%).	Akhtar et al. (2012)
Hydroxypropyl methylcellulose (HPMC)	Beetroot juice and purple carrot extract (NRC – natural red color)	–	Active	Positively affected: elongation at break (increased by ~102%) and oxygen permeability (decreased by about 4 10^{-18} m^3m^{-1} s^{-1} Pa^{-1}).Negatively affected: tensile strength (decreased by ~38%), Young's modulus (decreased by ~56%), and WVP (increased by ~63%).	Akhtar, Jacquot, Jamshidian, Imran, Arab-Tehrany, and Desobry (2013)
Banana starch	Fresh beets	Sausages	Active	Positively affected: sausage color (stabilized about 12% more in coated sausages with betalains), pH (decreased 3x more in control sausages during storage) and TBARS prevention (malonaldehyde content about 7x higher in control sausages on day 20).	Zamudio-Flores et al. (2015)
Oat and banana starch films	Fresh beets	–	Active	Positively affected: elongation at break (around 5x higher with betalains). Negatively affected: WVP (increased by ~92%) and solubility (increased by ~20% at 25 °C).	Zamudio-Flores et al. (2015b)
Starches of different amylaceous sources	Beet flour	–	Active and smart	Positively affected: color change and flexibility (increased by 6% in strain at break).Negatively affected: contact angle (decrease by 8°) .Not-significantly affected: solubility (increase by 1%) .	Gutiérrez, Guzmán et al. (2016)
Native and modified plantain flour	Beet flour	–	Active and smart	Positively affected: transparency and color difference (ΔE increased by ~8 units). Negatively affected: moisture (~8% higher with beet flour) and L* (decrease by about 6 units).	Gutiérrez, Suniaga et al. (2016)
Residues of gelatin capsules	Beetroot	–	Active	Positively affected: water solubility (decrease by ~5%) and WVP (decreased by ~22%).Negatively affected: elongation at break (decreased by ~165%). Not-significantly affected: thermal stability.	Iahnke et al. (2016)
Chitosan-starch	Beetroot and pitaya	–	Active	Positively affected: UV–vis light barrier properties and antimicrobial activity (increased by ~7% in the diameter of inhibition zone against fungi).	Lozano-Navarro et al. (2017)
Carboxymethyl cellulose (CMC)	Red Prickly Pear (*Opuntia ficus-indica* L.) peel powder and extract	–	Active	Positively affected: tensile strength (increased by 7 MPa), total phenolic content (around 1,130 × higher), and antioxidant activity (138 mg GAE/100 g)Negatively affected: elongation at break (decreased by 5.2 mm) and penetration force (decreased by ~10 N).	Aparicio-Fernández et al. (2018)
Glucomannan-Polyvinyl alcohol	Peel of dragon fruits	Freshwater fish *Osphronemus gouramy* (O. gouramy).	Smart	Positively affected: when in contact with the fish, the color of the films changed from purple to yellow after 8 days.	Apriliyanti et al. (2018)
Chitosan-starch	Beetroot and pitaya	–	Active	Positively affected: antioxidant activity (imparted to the films) and thermal stability (weight loss around 10% lower at 135 °C). Negatively affected: permeability (increased by ~8% in apparent density).	Lozano-Navarro et al. (2018)
Furcellaran (FUR)	Beetroot (BTR), elderberry (EB), blueberry (BB), green tea (GT) and yerba mate (YM) extracts	Fresh Atlantic mackerel	Smart	Positively affected: color change during fish spoilage and TVB-N of fish (variation lower than 3 mg/100 g in 14 days).	Jamróz et al. (2019)
Gelatin/chitosan	Betanin nanoliposomes	Fresh beef	Active	Positively affected: improved crystallinity, antioxidant activity (increase by ~77%), and pH and color changes.	Amjadi et al. (2020)
Quaternary ammonium chitosan (QC)/fish gelatin (FG) blend films	Amaranth (*Amaranthus tricolor* L.) extract	Shrimp	Active and smart	Positively affected: UV–vis light barrier, elongation at break (increased by almost 20%), antimicrobial activity, and WVP (decreased by ~10%).Negatively affected: thickness (increased by ~15%).	Hu et al. (2020)

(continued on next page)

Table 3 (*continued*)

Matrix	Betalain source	Food	Type of film	Effects/Results	Reference
Polyvinyl alcohol (PVA) and gelatin	*Amaranthus* leaf extract	Chicken and fish	Active and smart	Positively affected: WVP (decreased by ~30%), UV–vis light barrier, tensile strength (increased by 3.5 MPa), antioxidant (increased by ~40%) and antimicrobial activities.	Kanatt (2020)
Tara gum/polyvinyl alcohol (PVA)	Fresh red pitaya (*H. polyrhizus*)	Shrimp	Smart	Positively affected: WVP (decreased by ~24%), UV–vis light barrier, color change, mechanical (increased by ~7% in EAB), antioxidant and antimicrobial properties.	Qin et al. (2020)
Quaternary ammonium chitosan (QAC)/ polyvinil alcohol (PVA) blend	Cactus pears extract (*Opuntiaficus-indica*)	Shrimp	Active and smart	Positively affected: UV–vis light barrier, WVP (decreased by ~6%), elongation at break (increased by ~14%), antioxidant (increased by ~30% in scavenging activity), antimicrobial, and sensitivity to ammonia properties.	Yao et al. (2020)
Potato starch	Paper flower	Fish	Smart	Positively affected: color change, contact angle (increased by ~40°), and WVP (decreased by ~67%).Negatively affected: tensile strength (decreased by ~6 MPa).	Naghdi et al. (2021)
Starch/polyvinyl alcohol (PVA)	Red pitaya fresh extract (RPFE), prickly pear fruit extract (PPFE), red beetroot extract (RBRE), globe amaranth flower extract (GAFE) and red amaranth leaf extract (RALE).	Shrimp	Active and smart	Positively affected: tensile strength (increased by >1 MPa with all the extracts), WVP (decreased with all the extracts), and color change.	Yao et al. (2021)

products correlating with the increase in pH, TVBN accumulation, and bacterial growth pattern of the products. However, such color change was more evident in fish than in chicken.

Films based on glucomannan/polyvinyl alcohol and fresh red pitaya peel extract were effective in monitoring fish fillet deterioration. The films color visibly changed from pink to yellow after storage for 8 d at 4 °C. This change coincided with an increase in TVBN accumulation from 2.4 mg/100 g to 39.74 mg/100 g, which was detected when the accumulation was >20 mg/100 g (Apriliyanti et al., 2018).

Smart starch-based films incorporated with betacyanin extract from paperflower were developed by Naghdi et al. (2021) to detect the deterioration of whole Caspian Sea sprat; a visual color change from pink to yellow was observed in parallel with the increase in TVBN accumulation from 7.01 mg/l00 g to ~30 mg/l00 g, and with the increase in microbiological counts from 2.41 log CFU/g to 7.59 log CFU/g. The color change started on the eighth day of storage, reaching an almost completely yellow film on the twelfth day, when the fish was considered microbiologically and chemically deteriorated.

The efficiency of indicators is highly dependent on their chemical composition (Mei et al., 2020) and chemical interactions with the polymer matrix (Naghdi et al., 2021). Jamróz et al. (2019) reported that furcellaran films developed with beetroot, elderberry, blueberry, green tea, or yerba mate extracts were not suitable as pH indicators to monitor deterioration of Atlantic mackerel fillets stored at 2 °C, as the color change in smart films was insufficient.

Successful smart films should be developed using sensitive pH indicators and matrices with high sensitivity for TVBN detection; they also must be used in specific storage temperature conditions. Furthermore, the sensitivity of the films should also be investigated in relation to other spoilage metabolites, such as biogenic amines (like histamine, caverine, and putrescine), and not just in relation to ammonia. The temperatures used in the studies should also simulate the actual conditions that the products are exposed to during storage. In general, films based on biopolymers and betalains have shown promising results for applications as smart packaging materials for food.

4. Active films containing betalains

Active films based on synthetic polymers are a consumer demand for the development of sustainable and eco-friendly products aiming food preservation (Han et al., 2018; Oliveira Filho et al., 2021; Yong & Liu, 2021). There are some reports of active films containing betalains from red beetroot powder using starch and synthetic polymers commonly used in the food industry, such as polydimethylsiloxane, for the production of a bio-elastomer film (Tran, Athanassiou, Basit, and Bayer, 2017), as well as of ethylene vinyl alcohol copolymer with betalains from red beetroot powder or extract (Cejudo-Bastante et al., 2020). Table 3 lists active films produced with natural polymers containing betalains.

Active films produced with bio-based polymers containing betalains have been developed as alternatives to mitigate the impacts of food packaging on environmental pollution as well as to preserve the safety, nutritional, and sensory qualities of the food. Furthermore, food byproducts such as minimal beet root processing residues and gelatin capsule residues can be used to develop these films, contributing to increase their sustainability (Iahnke, Costa, de Oliveira Rios, & Flores, 2016; Tran et al., 2017).

Betalains have higher antioxidant activity than vitamin C, catechin, and some anthocyanins, which is explained by their electron donation ability and the presence of several phenolic hydroxyl groups in their structures (Cai et al., 2003; Gengatharan, Dykes, & Choo, 2015; Qin et al., 2020; Yao et al., 2020). Once betalains or betalains-rich extracts are incorporated into the film, they can impart or contribute to the antioxidant activity of the films, providing an interesting property to the developed materials that prevent food oxidation, avoid rancidity and nutritional loss during storage (Cejudo-Bastante et al., 2020; Hu et al., 2020; Iahnke et al., 2016; Kanatt, 2020; Naghdi et al., 2021; Qin et al., 2020; Yao et al., 2020).

The antioxidant activity of betalains is proportional to the hydroxyl or imino groups, besides being affected by glycosylation and hydroxyl group positions; moreover, gomphrenins and acylated gomphrenins have high antioxidant activities in this class of pigments (Cai et al., 2003; Yao et al., 2021). Yao et al. (2021) produced starch/polyvinyl alcohol films incorporated with betalain-rich extracts from five different plant species. Higher antioxidant activity (EC_{50} 0.47 mg/mL) was obtained in films developed with globe amaranth (*G. globosa* L.) extract, rich in gompherinin III and isogompherinin III, than in films produced with red amaranth (*A. tricolor L.*), prickly pear (*O. ficus-indica L.*), red pitaya (*H. polyrhizus*), and red beetroot (*B. vulgaris L.*) extracts, with EC_{50} values of 1.67, 4.45, 5.01, and 6.10 mg/mL, respectively.

The antioxidant potential of betalains is also dependent on the type of molecule that is present in higher concentration in the extract incorporated into the smart film, as well as on the concentration of this extract (Cejudo-Bastante et al., 2020; Hu et al., 2020; Iahnke et al., 2016; Kanatt, 2020; Naghdi et al., 2021; Qin et al., 2020; Yao et al., 2020). Controversially, Lozano-Navarro et al. (2018) reported no relevant differences in the antioxidant activity (0.20994–0.21008 mg/mg gallic acid equivalent, GAE) of chitosan/starch films produced with 0.5–5% beetroot (*Beta vulgaris*) or pitaya (*Hylocereus undatus*) extracts.

Betalains, which are hydrophilic, can be incorporated into films as aqueous or powder extracts (Aparicio-Fernández et al., 2018; Cejudo-Bastante et al., 2020). Aparicio-Fernández et al. (2018) demonstrated that carboxymethyl cellulose (CMC) films incorporated with red prickly pear (*Opuntia ficus-indica* L. cv. San Martín) aqueous extracts presented higher antioxidant capacity (0.92 mg GAE/g), reducing power (8.35 mg AAE/g), and total phenolic compounds (4.24 mg GAE/g) than those incorporated with the powder extract (0.76 mg GAE/g, 6.34 mg AAE/g, and 3.55 mg GAE/g, respectively), even with low contents of betacyanins and betaxantins. After incorporating betalains in aqueous or powder extract forms, these authors obtained a high antioxidant potential in films incorporated with aqueous extracts; such result was attributed to a reduced activity of the powders, since the higher interaction between betalains in that form and proteins/polysaccharides of the polymeric matrix may reduce their availability. Cejudo-Bastante et al. (2020) reported no differences in the antioxidant potential for samples with 0.1, 0.5, and 1% red beetroot (*Beta vulgaris* L., sp. Var. Pablo) powder or extract; however, increasing differences were noted with the increase in betalain concentration, highlighting the higher activities for the films incorporated with the extract.

The antioxidant activity of the films can contribute to the preservation of food inside packaging and during storage, as these foods are exposed to light and heat. Akhtar et al. (2012) demonstrated <20% reduction in the antioxidant potential of edible hydroxypropyl methylcellulose films containing pure natural red color compound (beetroot red E162 and purple carrot extract E163) over 20 days under light exposure, which can contribute to reduce the changes that can occur in foods due to this exposure.

Zamudio-Flores, Ochoa-Reyes, Ornelas-Paz, Aparicio-Saguilán et al. (2015) demonstrated that banana oxidized starch films containing betalains from beet juice applied on sausage surface slightly reduced the color changes with storage; such fact was attributed to the antioxidant activity of betalains present in the film, since they can inhibit the process of darkening in foods, associated to lipid oxidation and malondialdehyde formation. However, Iahnke et al. (2016) reported low peroxide values of sunflower oil protected with gelatin-based active films containing beetroot powder, which may have interacted with the gelatin used in film formation and contributed to the antioxidant activity in the lipid system.

Antimicrobial property is another property of the active films, which is developed not only by a mechanical barrier function of the film, but also due to the antimicrobial activity of the film components, that can extend the lag phase of the microorganisms or induce bacteria death (Kanatt, 2020). When a polymer possesses antimicrobial activity, such as chitosan or its derivatives, the incorporation of betalain extract can enhance this property in their films. The action of these active films against the microorganisms is explained by the effects of the interaction with betalains on their cellular membrane, causing destabilization and affecting their permeability and function, eventually leading to cell death (Gengatharan et al., 2015; Hu et al., 2020; Kanatt, 2020; Qin et al., 2020). Kanatt (2020) highlighted the reduced action of betalain films against gram-negative bacteria (such as *Escherichia coli* and *Pseudomonas fluorescence*), which can be associated with the presence of lipopolysaccharide in the membrane, reducing the transfer of active compounds across the membrane.

The incorporation of betalains into films with no intrinsic antimicrobial activity resulted in the growth inhibition of gram-positive and gram-negative bacteria (Kanatt, 2020), while an increase in the concentration of these compounds raised the size of the inhibition zones for all films (Hu et al., 2020; Lozano-Navarro et al., 2017; Qin et al., 2020; Yao et al., 2020). However, the increase in *Escherichia coli* inhibition in chitosan films according to the increase in betalains concentration was not significant, indicating that this activity must be also strain-dependent (Hu et al., 2020; Yao et al., 2020).

The antimicrobial activity of active films containing betalains was investigated for several bacteria, such as the common foodborne pathogens *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella typhimurium*. The activity against *Bacillus cereus* and *Pseudomonas fluorescence* and the fungi commonly found during food decomposition such as *Penicillium notatum*, *Aspergillus niger*, and *Aspergillus fumigatus* was also investigated (Table 3). Unfortunately, the antimicrobial activity of active films containing betalains (Table 3) was exclusively assessed by the agar diffusion method, an approach that simulates film application, but does not classify their effect as bacteriostatic or bactericidal (Hu et al., 2020; Yao et al., 2020).

Moreover, the antimicrobial activity of pure betalains extracts (i.e., not incorporated to any polymeric matrix) has also been evaluated for different pathogens (different from the common strains mentioned above) by Gengatharan et al. (2015) and Melgar et al. (2017). Melgar et al. (2017) reported that the minimal inhibitory concentrations and minimal bactericidal concentrations of the betalains extracts they investigated were comparable or even lower than those of commercial antibiotics (like streptomycin and ampicillin) and antifungal drugs (like ketoconazole and bifonazole) for some strains; this result also demonstrates the antimicrobial potential of this pigment, not only for foodborne pathogens, another factor that encourages its incorporation into active packaging films.

In food systems, the incorporation of betanin (free or nanoliposomes) into zinc oxide nanoparticles that were used in gelatin/chitosan nanofiber films improved the stability of the betanin molecules during preparation processes, which increased the antioxidant and antimicrobial activities and preserved beef inoculated with *Escherichia coli* and *Staphylococcus aureus* (Amjadi, Nazari, Alizadeh, & Hamishehkar, 2020). The authors reported a synergistic effect of betanin with chitosan nanofiber, which may be related to the ability of chitosan to donate hydrogen ions to betanin, reactivating its antioxidant capacity, or a synergistic effect with zinc oxide nanoparticles due to the contributions of the phenolic groups of betanin on the permeability through bacterial cellular membrane.

Kanatt (2020) developed smart and active polyvinyl alcohol/gelatin films with 5% (v/v) *Amaranthus* leaf extract —that is rich in betacyanins, betaxanthins, betalamic acid, chlorophylls, and carotenoids— to preserve chilled meat and fish. The total bacterial count (TBC), as well as *Staphylococcus aureus* and fecal coliform counts was reduced in the presence of the active film. The acceptable limit of TBC in both chicken and fish was exceeded on the third day of storage (2–4 °C) using a film with no extract (control), while these products were preserved up to day 10 when the active/smart film was applied. However, the acceptable limit of *Staphylococcus aureus* and fecal coliform in both fish and chicken was exceeded between days 3–7 using control films, which were also visibly deteriorated in this duration. In contrast, the application of smart films effectively controlled the growth of these microorganisms within acceptable limits even after refrigerated storage for 12 days. In addition, oxidative rancidity and volatile amine formation were well-controlled with the use of the smart and active films, indicating that not only the antimicrobial activity, but also the antioxidant activity measured *in vitro*, can directly preserve the sensory and nutritional aspects of food.

The incorporation of betalain-rich plant extracts into polymeric eco-friendly films has proved to be a promising sustainable option for producing active films for food packaging, as these compounds provide or enhance the antioxidant and antimicrobial activities of these packages, contributing to preserve food properties. In addition to being naturally found, these compounds (that are commonly incorporated in the form of powder or aqueous extracts) can even be encapsulated to enhance these activities.

5. Bioactive films containing betalains

Bioactive films contain at least one component that is intended to reach the human body and is beneficial to human health. These compounds can be vitamins, probiotics, prebiotics, or others and can be obtained directly from food or isolated and ingested in this way (Arroyo et al., 2019; Ortega & Campos, 2019).

Bioactive compounds could be ingested through frequent consumption of fruits, vegetables, grains, oils, and nuts in a balanced diet (Teodoro, 2019). However, with the change in the eating habits of the majority of the population, the consumption of these foods has decreased; therefore, providing new sources or vehicles that can carry and/or replace these bioactive compounds has become a priority for the food industry (Jia, Luo, Li, Zheng, Xiao, & Luo, 2019). Accordingly, bioactive films can be an effective alternative as vehicles to provide bioactive compounds important for human health and that cannot be supplied by usual diets (Pavli, Tassou, Nychas, & Chorianopoulos, 2018).

Bioactive compounds provide several benefits to human health, which can range from preventing cardiovascular disease risk factors (such as atherosclerosis, obesity, and diabetes mellitus) to preventing chronic diseases (such as cancer, diabetes, and neurodegenerative diseases) (Ortega & Campos, 2019; Raiola, Errico, Petruk, Monti, Barone, & Rigano, 2018; Sharifi-Rad et al., 2020). Currently, these diseases are prominent causes of death, and malnutrition is one of the main factors associated with them (Stratton et al., 2018). In addition, many bioactive phytochemicals delay cell aging through reduced oxidative agents, besides having anti-inflammatory properties and regulating the expression of enzymes involved in epigenetic changes (Si & Liu, 2014). Betalains, in particular, are a class of bioactive compounds that stand out due to their important pharmacological activities, such as anticancer (HogenEsch & Nikitin, 2012), anti-inflammatory (Martinez et al., 2015), antioxidant (Čanadanović-Brunet et al., 2011), antilipidemic (Clemente & Desai, 2011), and antimicrobial (Gengatharan et al., 2015) (Fig. 3).

The antioxidant activity of betalains, already discussed in the previous section, is the main target of studies that assess their bioactivity when incorporated into films and coatings. This antioxidant activity may be the reason of betalains promoting the biological effect associated with cancer control (Hussain et al., 2018). The scavenging of free radicals can decrease oxidative stress and reactive oxygen species formation contributing to the prevention of anti-inflammatory processes, which are important factors in tumor development (Das, Williams, Das, & Kukreja, 2013; Rodriguez, Vidallon, Mendoza, & Reyes, 2016).

In preventing cardiovascular disease, betalains inhibit lipid peroxidation and LDL oxidation (Clifford, Howatson, West, & Stevenson, 2015). The scavenging of radicals by betalains is directly related to their chemical structure as it occurs through electron transfer between molecules and can be enhanced when the compound has a high amount of hydroxyl and amino groups, as well as catechol (Esatbeyoglu et al., 2015). In addition, betalains may also prevent DNA damage, regulate gene activity, and induce detoxifying enzymes (Hussain et al., 2018).

There are some challenges in the application of betalains to bioactive films such as the stability of these molecules. To try to solve this problem, Kumar, Chauhan, and Giridhar (2020) proposed the nanoliposomal encapsulation of betalains using a lipid capsule that preserves hydrophobic and hydrophilic molecules with an envelope. They observed that the antioxidant activity of the encapsulated betalains was entirely preserved, which makes this a promising alternative for the application of stable betalains to films.

Another problem related to the administration of betalains as bioactive compounds is their apparent low oral bioavailability (Milton-Laskibar, Martínez, & Portillo, 2021). Tesoriere et al. (2004) elucidated the reasons behind this limited bioavailability and identified that they can be degraded in the gastrointestinal tract or distributed throughout the body after absorption. Studies have reported that the availability of betanin decreases by up to 54% when the pigment is subjected to a simulated digestion by gastric fluid and is barely absorbed in the small intestine, being significantly absorbed in the large intestine (Khan, 2016; Silva et al., 2019).

On the contrary, *in vitro* tests have identified that the indicaxanthin pigment almost does not undergo degradation by the gastrointestinal tract and metabolic changes, is not absorbed by the paracellular junctions of intestinal epithelial cells through non-polarized transport, and is unaffected by membrane transporter inhibitors (Tesoriere, Fazzari, Angileri, Gentile, & Livrea, 2008). After being absorbed from the gastrointestinal tract, betalains are distributed into the bloodstream and reach the liver. Studies have shown that some betalains are not metabolized by hepatocytes and their renal excretion is low (Frank et al., 2005; Tesoriere et al., 2004). Therefore, the metabolic pathways through which betalains could undergo structural changes, forming bioactive secondary metabolites, may have not yet been fully elucidated (Khan, 2016; Milton-Laskibar et al., 2021).

Betalains also seem to play an important role interacting with the gastrointestinal microbiota and probiotics, by stimulating the production of short-chain fatty acids (SCFA), responsible for protecting against inflammation and maintaining intestinal wall integrity (Oliveira, Nascimento, Sampaio, & Souza, 2021; Silva, Bernardi, & Frozza, 2020). However, studies focusing on betalains and their interaction with GTI microorganisms generally use crude plant extracts, which compromises the understanding of the real mechanism behind their possible prebiotic activity (Ghasempour et al., 2020).

Betalains are compounds with multiple benefits to human health; thus, these molecules can be used for the development of bioactive films. Their antioxidant potential, the main feature behind their bioactivity, has been extensively elucidated and can be preserved when incorporated into films, making them a promising target in the development of bioactive edible films. However, their molecular characteristics need to be kept intact and stable to guarantee the mechanisms through which the betalains act.

6. Effect of betalains incorporation on film properties

The inclusion of betalains in polymeric smart, active, and/or bioactive films can bring significant changes to the intrinsic physicochemical characteristics of the polymers. Water-related properties (such as solubility, swelling, and water vapor barrier), optical properties such as light barrier and film color changes, as well as structural, mechanical, and thermal properties are among the most important properties to be evaluated, aiming at the application of films as food packaging or coatings (Table 2). Furthermore, water-related properties such as the solubility of materials can directly interfere with their use as bioactive films, since betalains release will be affected by the ease of their detachment from the polymeric matrix and their solubility in the aqueous medium of application (Oliveira Filho et al., 2021).

Factors such as betalain source and the way in which these pigments will be added to the polymeric matrix interfere with the final properties of the films, since the interactions between them and the polymers may be more or less evident. Aparicio-Fernández et al. (2018) have evaluated the effect of the concentration of red prickly pear (*Opuntia ficus-indica* L.) peel powder and aqueous extract, both betalain-rich, on the physical and antioxidant characteristics of CMC films; according to them, the peel powder changed more significantly the mechanical properties of the films than the aqueous extract, probably due to the higher abundance of fibers in the peel powder than that in the extract. Furthermore, the characteristics of the polymer itself can affect the final properties of the material, according to its interaction with betalains. Gutiérrez, Guzmán, Jaramillo, and Famá (2016) reported the development of native and phosphated plantain flour films with the inclusion of beet flour; betalains from beet interacted better with the phosphated flour than with the native flour, limiting its immediate response to pH changes.

The most common water barrier properties of polymeric films that can be affected by betalains inclusion are wettability (related to the degree of hydrophilicity of the matrix), moisture content, swelling capacity (related to the water diffusion among the polymeric chains), and water solubility, which is also related to the hydrophilicity of the film and the interactions between its components. Different effects of betalains incorporation on the moisture content of polymeric films have been reported: Aparicio-Fernández et al. (2018) demonstrated an increase in the moisture content of CMC films incorporated with red prickly pear peel powder and extract, which they attributed to the hydrophilic property of the peel and to the extract's numerous –OH groups, while Qin et al. (2020) reported that adding betalains from red pitaya decreased the moisture content of starch and polyvinyl alcohol (PVA) films, which they related to the lower interaction between the film components with the inclusion of pigments and their consequent lower water sorption ability.

Kanatt (2020) did not report significant differences in the moisture content of PVA–gelatin films incorporated with *Amaranthus* leaf extract; in turn, the solubility of the films, as well as their swelling capacity, was significantly reduced. Decreased solubility can be a positive effect when it is desirable to maintain the integrity of the film for a long period of time when in contact with water; however, high solubilities may be desired for bioactive edible films proposed as vehicles for the release of bioactive compounds: Gutiérrez, Guzmán et al. (2016) reported an increase in the solubility of native and modified plantain flour films with betalain inclusion, which they related to the sugars present in beet flour. They also pointed out that the sugars (of a polar character) present decrease the contact angle of the films, consequently increasing their wettability.

One of the most important water-related properties to be evaluated with the inclusion of betalains in active films is water vapor permeability (WVP); it is directly related to the ability to act as a barrier to the passage of water, thus controlling the gain or loss of moisture of the food that will be coated or covered by the developed material. This property is dependent on the free volume in the polymeric matrix available for the diffusion of water molecules, which can be altered by the number of interactions between the film components and betalains. In most cases, the inclusion of these pigments positively affected the water barrier property of the films, decreasing WVP (Hu et al., 2020; Kanatt, 2020; Naghdi et al., 2021; Yao et al., 2020; Yao et al., 2021); only Zamudio-Flores et al. (2015a) reported a negative effect of increasing WVP of oat and banana starch films incorporated with betalains, which they attributed to the increase of the solubility of the films.

Water-related properties should be extensively studied in materials with potential application such as food films and coatings —particularly for smart and active—, with their ability to act as a barrier to the passage of light. The color, transparency, and opacity of polymeric films can be significantly affected by the inclusion of betalains, to a greater or lesser degree depending on the origin, concentration, and structural properties of both polymers and pigments. One of the functions of food packaging is to prevent or retard oxidation induced by exposure to light, slowing down the decomposition of more sensitive foods; in general, adding betalains positively affects the UV and visible light barrier properties of polymeric films (Akhtar et al., 2012; Hu et al., 2020; Kanatt, 2020; Lozano-Navarro et al., 2017; Qin et al., 2020; Yao et al., 2020), which may be related to the presence of unsaturated bonds (such as $C{=}O$, $C{=}N$, and $C{=}C$) that absorb UV–vis radiation in the structure of these pigments, thus decreasing the transmittance of the films (Hu et al., 2020).

The inclusion of pigments can also significantly affect the mechanical properties of polymeric films, such as tensile strength, elongation at break, and toughness; bond formation between betalains and polymers can form a more compact and tougher polymeric network, which minimizes the damage transmitted to the foods covered by them, during their handling in situations of transport, storage, and commercialization. Most studies report an increase in tensile strength with betalain inclusion, combined with a decrease, often not significant, in the elongation at break, which is related to film flexibility (Aparicio-Fernández et al., 2018; Gutiérrez, Guzmán et al., 2016; Hu et al., 2020; Kanatt, 2020; Naghdi et al., 2021; Qin et al., 2020; Yao et al., 2020; Zamudio-Flores et al., 2015a). Usually, the thermal properties of polymers can also be affected with betalains incorporation: Gutiérrez, Suniaga, Monsalve, and García (2016) observed an increase in the glass transition temperature (T_g) of native and modified plantain flour films with beet flour incorporation, which they attributed to a plasticizer effect of the sugar molecules present in the flour.

Therefore, a complete physicochemical characterization of polymeric films incorporated with betalains is necessary, since several factors can change the characteristics of interest of these materials, such as pigment origin, concentration, and the mechanism of addition to the film, as well as the origin, concentration, and type of polymers used. The same tendencies will not always be observed with betalains incorporation, which is why evaluating the water and light barrier properties, as well as mechanical and thermal properties, is necessary. Once the material and all its properties have been fully elucidated, it will then be possible to assess its effects on the proposed applications.

7. Final considerations and outlook

Betalains are natural plant pigments widely recognized for their beneficial effects on human health, and have been used as a natural coloring agent in foods. In this review, we discuss the potential of betalains for application as an active, smart, and bioactive agent in the development of edible and biodegradable food packaging.

Betalains have the ability to change their color depending on changes in the chemical characteristics of food, being able to provide information on food quality in a non-destructive way and in real time. The application of betalains as colorimetric bioindicators in smart food packaging proved to be a promising strategy, as the films were able to indicate the freshness and spoilage of foods such as shrimp, fish, and chicken. The combination of betalains with other pH indicator pigments can increase the sensitivity of the bioindicator, making it more accurate in monitoring food quality, expanding its range of color variation. Future studies should evaluate the possibility of developing applications that will help consumers to interpret the color changes of smart films to avoid dubious interpretations by consumers.

Active films containing betalains can protect food against spoilage or pathogenic microorganisms during its shelf life, in addition to promoting an antioxidant effect against lipid oxidation caused by free radicals. Active films with betalains showed antimicrobial and antioxidant potentials, and were able to increase the shelf life of sausage and shrimp. The properties of betalains as an active agent can be enhanced by the combination of antimicrobial nanoparticles and essential oils, enhancing their applications in food preservation.

While smart and active packaging containing betalains seem to have been extensively studied till date, studies on bioactive packaging are still lacking, mainly due to the lack of information on the mechanism of their action in the human body. Although this mechanism is not yet well understood, films containing betalains may represent an alternative mechanism of protection and controlled release for the biological activity of these molecules.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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