Review

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Review

Active and Intelligent Biodegradable Packaging Based on Anthocyanins for Preservation and Monitoring Rich Protein Foods

Bifen Zhu, Yu Zhong, Danfeng Wang and Yun Deng *

Department of Food Science & Technology, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai 200240, China

* Correspondence: y_deng@sjtu.edu.cn

Abstract: Currently, active and intelligent packaging have been developed to solve the spoilage problem for rich protein foods during storage, especially by adding anthocyanin extracts. In such film system, the antioxidant and antibacterial properties were dramatically increased by adding anthocyanins. The physicochemical properties were enhanced through interactions between active groups in anthocyanins and reactive groups in polymer chains. Additionally, active and intelligent film could monitor the spoilage of rich protein foods in response to pH change. Therefore, this film could monitor the sensory acceptance, and extending the shelf life of rich protein foods simultaneously. In this paper, the structure and functional properties of anthocyanins, the composite actions of anthocyanin extracts and biomass materials, and their reinforced properties of active and intelligent film were discussed. Also, the applications of this film in quality maintenance, shelf-life extension and quality monitoring for fresh meat, aquatic product and milk were summarized. The film that achieves high stability and continuous release of anthocyanins on demand will become an underlying trend in packaging applications for rich protein foods.

Keywords: Food packaging; Anthocyanins; Biodegradable materials; Food quality

1. Introduction

Rich protein foods, including fish, shrimp, meat and dairy products, are important sources of protein, fat-soluble vitamins, micronutrients and special flavor. Thus, rich protein foods have a high demand and are crucial for maintaining human health. However, along the supply chain, oxidative reactions and microbial infections lead to deterioration of flavor, texture, and color of rich protein foods. At the same time, ketones, aldehydes, and aromatic amines produced by oxidation of lipids and proteins are potentially toxic. And food contamination caused by pathogenic microorganisms can easily lead to serious foodborne illness [1]. It is difficult for consumers to evaluate these changes in most cases. Meanwhile, misjudgments on food quality and safety can lead to food waste [2,3]. In order to mitigate and monitor these potential risks, appropriate packaging is needed.

Traditional plastic packaging has excellent mechanical, barrier and thermal properties, mitigating the impact of the external environment on food. However, plastics are difficult to be biodegraded and recycled, and mainly treated by incineration and landfill, which are easy to pollute water, soil, air and cause severe environmental problems. As a result, natural materials such as polysaccharides, protein and lipids, which are biodegradable, environmentally friendly and readily available. In addition, they have a good film forming ability [4,5]. However, the functionality of single-component film is restricted. For example, pure starch film has poor water resistance and tensile strength, but good barrier properties [6]. Similarly, gelatin film possesses good physical properties, but lacks antioxidant and antibacterial ability [7]. Studies have shown that the addition of natural active ingredients, in particular anthocyanin extracts, could enhance the functional of biodegradable films. Thereby expanding its application in rich protein foods preservation and spoilage monitoring [8–10].

Anthocyanins, as a natural pigment, is safe, non-toxic and rich in resources, and is commonly used as food colorants, additives in pharmaceuticals and health products, highly favored by consumers [11,12]. Previous studies have shown that anthocyanins have good color responsiveness to pH changes caused by spoilage of rich protein foods. Moreover, the added anthocyanins into biomass materials could improve the UV blocking capacity, reduced oxygen and WVP of film, inhibited the growth of microorganisms in rich protein foods [1,13,14].

In previous reviews, the preparation, function and application of active or intelligent packaging with natural polymers and functional additives including anthocyanins, essential oils and nanocomponents as main components in food preservation have been comprehensively discussed [15–17]. Different from previous reviews, this article was to highlight the active and intelligent biodegradable packaging based on anthocyanins for preservation and monitoring rich protein foods. Firstly, the extraction and functional properties of anthocyanins were briefly introduced. Also, the mixing of anthocyanins with different bio-based materials, the improvement of anthocyanins on film function and the on-demand release of anthocyanins by substrates were discussed. Additionally, the preservation and monitoring of active and intelligent film for meat, aquatic products and milk were summarized. Finally, the future research and commercial prospect of active and intelligent packaging were predicted.

2. Extraction, structure and function of anthocyanins

2.1. Extraction of anthocyanins

Solvent extraction usually used to extract anthocyanins, with ethanol, methanol, acetone, water or mixtures of the aforementioned solvents as the medium. In addition, anthocyanins may degrade during extraction, and the stability of anthocyanins can be improved by adding formic acid or hydrochloric acid [15]. However, alcoholic extracts have poor heat stability and short half-life. In recent years, the use of natural deep eutectic solvent (NADESs) to extract anthocyanins has become a hotspot. NADESs is homogeneous liquid consisting of hydrogen-bond acceptor (HBA) and hydrogen-bond donor (HBD) [18]. NADESs has non-toxicity, biodegradability, and reusability. Additionally, the hydroxyl or carboxyl groups could form hydrogen bonds with extracts, bring higher extraction yields, enhance stability, and increase antimicrobial and antioxidant ability of anthocyanins. Bi et al. experimented with the extraction of mulberry anthocyanins using 6 different NADESs, and found all of them exhibited higher extraction yields compared to acidified ethanol, with the best results obtained using ChCl/lactic acid [19]. Jovanović et al. developed a NADES-based extraction process for elderberry anthocyanins. It showed that anthocyanins extracted using ChCl/xylitol NADES exhibited the highest antioxidant and antimicrobial activities, and had better stability [20]. Moreover, the acidity of the solution can affect the extraction efficiency of anthocyanins. Acid NADESs can improve the extraction efficiency of anthocyanins, by preventing the degradation of non-acylated anthocyanins [21].

2.2. Chemical structure and color indication mechanism of anthocyanins

The flowers, leaves, stems and fruits show different colors due to different types and contents of pigments, which are mainly affected by flavonoids, carotene and beet pigments. Anthocyanins is the most important color developing substances in flavonoids, with C_6-C_3-C_6 as the carbon skeleton, and 3,5,7-trihydroxy-2-phenylbenzopyran as the basic structural unit (Figure 1a) [15,22,23]. Due to the methylation and hydroxylation of different carbon positions in anthocyanins, more than 700 anthocyanins in 27 classes have been discovered [24]. There are 6 common anthocyanins (Figure 1b), including pelargonidin, cyanidin, delphinidin, peonidin, petunia and malvacin [15]. Meanwhile, anthocyanins exhibit highly reactivity to pH values. In H^+ or OH^- environment, the distribution of π electrons in the pigment molecules changes, resulting in changes in the structure of anthocyanins. This results in changes in the absorption and reflection of light by anthocyanins, leading to the display of different colors [25,26] (Figure 2). In addition, the molecular structure of anthocyanins also affects antioxidant and antimicrobial activity [27,28]. This will be analyzed in detail in the following sections.

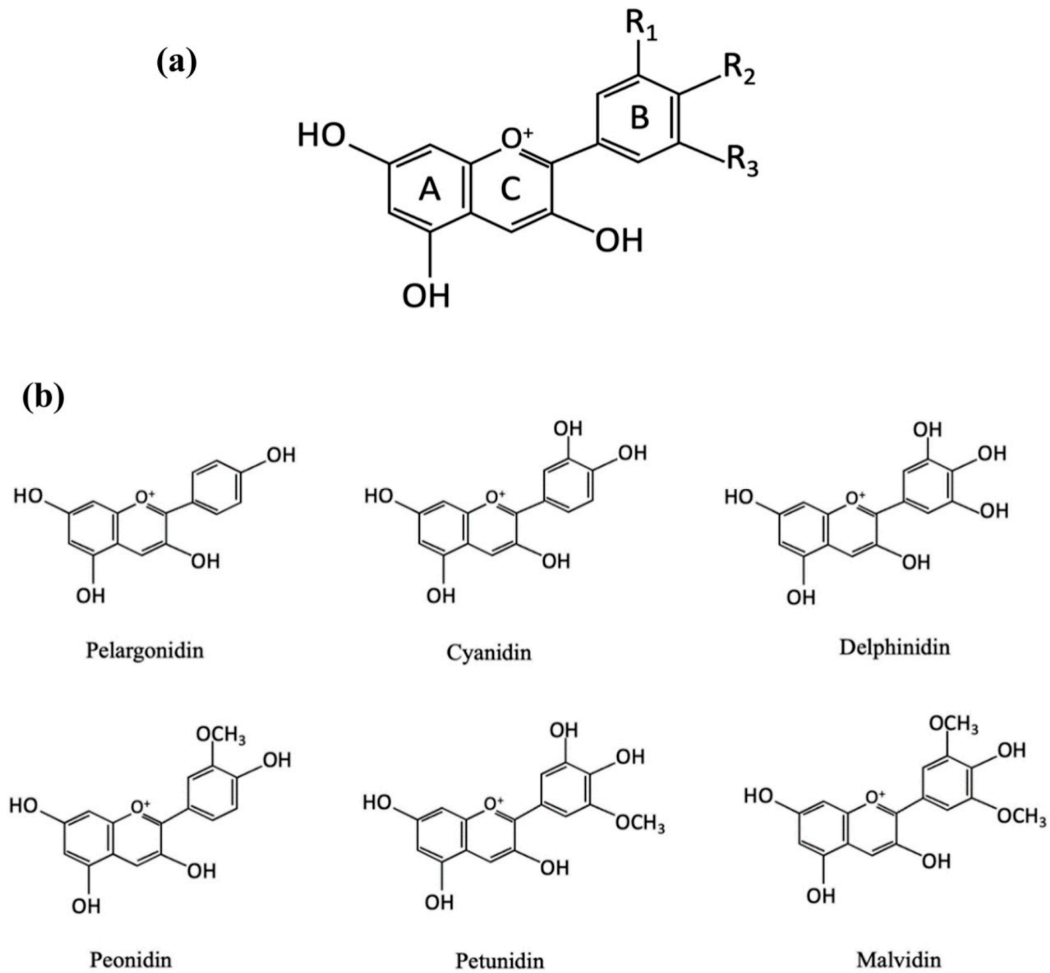

Figure 1. The basic structure of a typical anthocyanin (a), and the chemical structures of different types of anthocyanidins (b) [15].

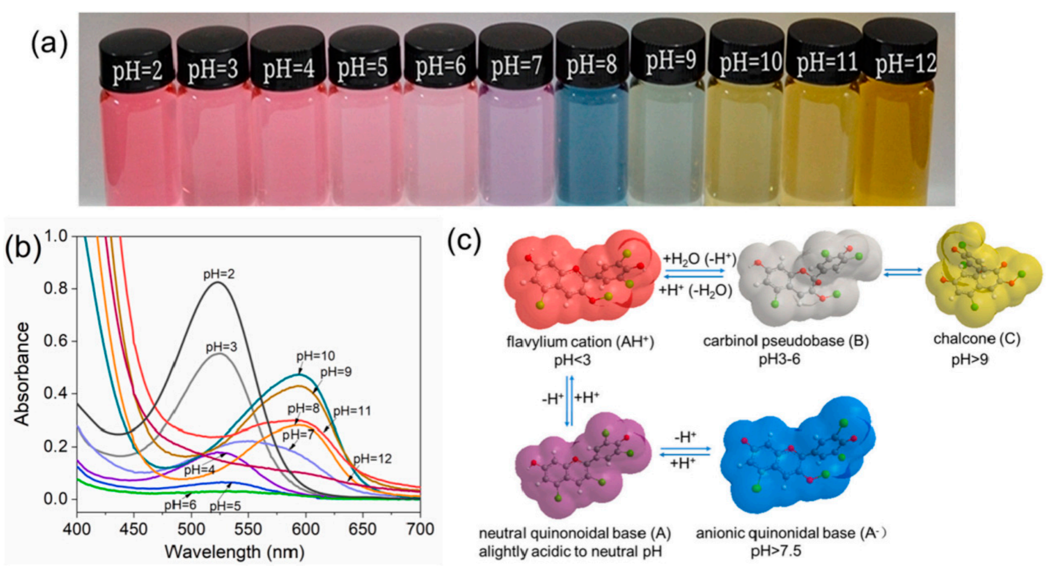

Figure 2. Color changes (a) and absorption spectra (b) of *Perilla frutescens* (L.) Britt. anthocyanins solutions at pH 2-12, corresponding structural transformation (c) [26].

2.3. Antioxidant mechanism of anthocyanins

As summarized in Table 1, anthocyanins from jambolao skins [29], black rice bran [30], shikonin [31], blueberry [32] and so on had scavenging effects on ABTS and DPPH. This is attributed to the conjugated structure, hydroxyl functional groups, o-diphenol structure and polar substituents present in the molecular structure of anthocyanins, which endow it with antioxidant activity and inhibitors of oxidative reactions [33,34]. Moreover, it has been demonstrated that the anthocyanin glycosides antioxidant was higher than glycosides, monoglycosides was higher than polysaccharides, and anthocyanosides was higher than acylated anthocyanosides [35]. Typically, anthocyanins have multiple aromatic rings that form a conjugated structure. This conjugated structure gives anthocyanins good electron transfer properties, allowing them to capture and neutralize free radicals. In addition, the o-diphenol structure of anthocyanins enhances their ability to capture free radicals. The o-diphenol structure at the 3' and 4' positions on ring B of anthocyanins forms a more stable o-quinone structure or conjugated semiquinone by two single-electron transfer reactions with RO·. Moreover, the hydroxyl functional group in anthocyanins can provide hydrogen atoms or electrons, thus stabilizing free radicals and reducing the occurrence of oxidation reactions. For example, the 5'-hydroxyl group on ring A of anthocyanins can be easily oxidized, releasing H^+. Which had a strong scavenging effect on RO·. Then, the RO· combined with 3', 5', and 7' hydroxyl groups to form pseudo-semiquinone structures and through keto-enol tautomerization to improve stability [12]. Unlike other polyphenols, anthocyanins lack an electron in the C ring, forming a secondary oxonium ion, which easily attracts free radical attack.

2.4. Antimicrobial mechanism of anthocyanins

Table 1 summarizes the inhibitory effects of different sources of anthocyanins against common foodborne pathogens such as *S. Enteritidis*, *P. fluorescens*, *E. coli* and *S. aureus* [36–39]. The molecular structure of anthocyanins contains several hydroxyl functional groups, such as the hydroxyl groups in phenyl rings and aromatic alcohols. These hydroxyl groups react with lipid peroxides on microbial cell membranes, leading to membrane damage and lysis. In addition, the hydroxyl functional groups can undergo ionization and exhibit acid-base properties. Under acidic conditions, anthocyanins molecules, in the form of positively charged ions, bind to negatively charged components (proteins and cell wall polysaccharides) of the bacterial surface. Changes the membrane potential of bacteria and disrupts the cycle of bacterial. Additionally, anthocyanins can interact with the electron transport chains in microbial cells, interfering with enzyme systems and ATP synthesis, thereby affecting microbial metabolism and proliferation. This is attributed to the existence of multiple conjugated aromatic rings in the anthocyanins structure, which gives them electron-transferring ability. In summary, anthocyanin-microbe interactions occur through specific structural interactions between anthocyanins and microorganisms. Thus, structural differences result in different responses of microorganisms to the same anthocyanin. For example, anthocyanins may exhibit excellent inhibitory and bactericidal properties against one type of microorganism, while having no effect on another. Research demonstrated that red onion skin extract had an inhibitory effect on *S. aureus* DSM 20,231, but had no inhibitory effect on *E. coli* DSM 30083 and *Salmonella* DSM 13,772 [38]. This was part contrary to the founds of Sagar et al. they discovered that onion extracts have antimicrobial activity against Gram- bacteria [40]. This may be related to the onion species.

3. Active and intelligent films containing anthocyanins

Anthocyanins is susceptible to light, heat, humidity and oxygen. To promote the application of anthocyanins into food package, it is important to compound anthocyanins into suitable matrix. Biomass materials are more sustainably sourced and less environmentally polluting than non-renewable resources. Additionally, biomass materials can effectively disperse anthocyanins and bind them through hydrogen bonding or electrostatic interaction [5]. Many researches have indicated that the incorporation of anthocyanins causes the film color changes in response to conditions, and improves the film's mechanical properties, UV blocking ability, antibacterial and antioxidant capacity

[5,10,41–44]. After a preliminary literature search, the substrates obtained directly from natural products polysaccharides (cellulose, chitosan and starch) and protein (gelatin) that have been used to develop active-freshness indicator packaging were summarized (Table 1).

3.1. Cellulose-based film

Cellulose is the most abundant renewable resource in the world and has a linear and high molecular weight structure. The linear arrangement of cellulose gives it a comparatively ordered crystalline structure, providing cellulose film with good mechanical strength and stability. However, cellulose is highly crystalline in its natural state and only soluble in a few organic solvents, which makes it less susceptible to film formation [16,45]. Cellulose molecules are abundance of hydroxyl group, and could form strong intermolecular and intramolecular hydrogen bonds [46]. Compared to natural cellulose, the cellulose derivatives such as cellulose acetate [26], methylcellulose [29], hydroxypropyl methylcellulose [47] possess good film-forming, high modulus, and strong barrier properties, making them favored for active-freshness indicator packaging. However, the hydroxyl group of cellulose is replaced by methoxy group, resulting in high solubility of methylcellulose. It was found that jambolao extracts (50%) added could enhance the water resistance of the methylcellulose film, which was attribute to the interaction of jambolao extracts with methylcellulose via intermolecular hydrogen bonds. In addition, the extracts could improve the mechanical property of the methylcellulose film, while decrease the water vapor permeability [29]. Moreover, anthocyanins as pH color index, endows cellulose derivatives film to indicate food freshness [47,48]. You et al. fabricated carboxymethyl cellulose/konjac glucomannan composite film incorporated blackcurrant anthocyanins (BCA). Composite film was red at pH 2-3, light pink at pH 4-8 and yellow-green at pH 9-13, respectively. In addition, composite film inhibited both *E. coli* and *S. aureus* [9].

3.2. Chitosan-based film

Chitosan, derived from deacetylated chitin, has obvious inhibitory effect on bacteria and fungi [4,13,49,50]. The positively charged -NH^{3+} group in chitosan molecules can adsorb onto negatively charged bacteria, disrupting the integrity of cell wall, increasing membrane permeability and causing leakage of cellular contents. Inside the cell, chitosan can adsorb and bind to proteins and nucleic acids, influencing the normal physiological functions of microorganisms, inhibiting their growth and reproduction. Moreover, a large number of amino and hydroxyl groups exist on the molecular chain of chitosan, and can selectively bind metal ions (such as Mg^{2+} and Ca^{2+}) on the outer membrane of bacteria, inhibiting the production of bacterial toxins [10]. Furthermore, chitosan exhibits excellent film forming properties and is made into highly transparent, non-toxic, and edible film. However, the mechanical properties, water resistance and gas barrier of pure chitosan film is deficient [51]. Incorporation of anthocyanins into chitosan film improves its mechanical properties and air barrier capacity. This is mainly due to the interaction of the hydroxyl groups of anthocyanins with the hydroxyl and amino groups of chitosan. Yong et al. developed an active/intelligent film by added purple cabbage anthocyanins (PCA) and purple sweet potato anthocyanins (PSA) into chitosan/polyvinyl alcohol (CP), κ-carrageenan/polyvinyl alcohol (KP) and locust bean gum/polyvinyl alcohol (LP) matrices. The results indicated that the incorporation of PSA and PCA improved film homogeneity, light barrier, antioxidant, pH and ammonia sensitivity through electrostatic interactions and hydrogen bonding between anthocyanins and the matrix. Due to the pH-sensitivity of PSA and PCA, films showed obvious color changes (Figure 3a) under pH value from 3-12. In addition, the color changes of PSA and PCA films in the presence of ammonia were significant (Figure 3b) [8]. Similarly, Li et al. found the pure chitosan film showed some antioxidant activity, and the mulberry anthocyanins added film significantly improved the DPPH free base scavenging ability [52].

Moreover, chitosan could control the release of anthocyanins, achieving the goal of extending shelf life of rich protein foods and immediate indication the edibility endpoints [36,52]. Wang et al. fabricated a chitosan/esterified chitin film loaded with eggplant peel (EE) derived anthocyanins. They found that the release rate and cumulative release of EE in different food simulation systems were different. CS has a well solubility in acetic acid, its structure is more stretched, thus allowing rapid release of EE in 3% acetic acid. Meanwhile, the release rate of EE decreased sequentially in 50% ethanol, 10% ethanol and distilled water, which may be due to the better solubility of EE in alcoholic solutions. In addition, attributed to the electrostatic interactions and hydrogen bonds between CS and EE, the release of total anthocyanins in all films is incomplete, which favors a sustained release effect [53].

3.3. Starch-based film

Starch has become the most promising material for the production of biodegradable polymers with low cost and good film forming [54–56]. The formulation of starch film is critical and determines the barrier and mechanical properties. Adding anthocyanin extracts to formulations could prevents film cracking and brittleness, increasing flexibility and ductility, making starch film with rich color variation via pH changes. Meanwhile, starch could encapsulate anthocyanins effectively through electrostatic interactions, hydrogen bonding and hydration, and enhances the stability of anthocyanins, preventing it from precipitating or dissolving [57,58]. Additionally, the branching structure of starch molecules has more voids and adsorption sites, which would enhance the encapsulation of anthocyanins. The release of anthocyanins from film can be controlled by adjusting the shape and size of the starch molecule. Besides, the crystalline form of the starch can affect the release behavior of anthocyanins. Compared to crystalline regions, amorphous regions are looser and favor anthocyanins diffusion [59,60]. Zhang et al. compared the release of shikonin from starch and agar-based films in 50% ethanol and water. The results showed that the release of shikonin was very rapid in the first 30 minutes, and due to its alcohol solubility, the release of shikonin in 50% ethanol was faster. In addition, the release of shikonin from agar-based film was faster due to the higher swelling rate of agar than that of the starch [31]. Furthermore, molecular interactions between starch and anthocyanins affect its conformation, which explains the color change of anthocyanins may not match the anthocyanins film even at the same pH value [6,61–63].

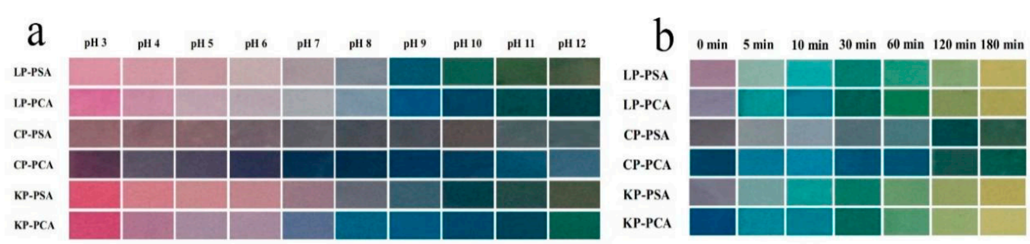

Figure 3. (a) Color changes of different polysaccharide/PVA films with PSA or PCA after being immersed in buffer solutions (pH 3-12) for 1 min. (b) Color changes of different polysaccharide/PVA films with PSA or PCA after being exposed to ammonia (1 mol/L) at 20 °C for 5-180 min [8].

3.4. Gelatin-based film

Gelatin is arranged by proline, hydroxyproline and glycine repeating units [16]. The gelatin molecular contains a number of hydroxyl groups, which enables it to form colloidal particles in aqueous solution, and the cross-linking effect could form a stable, flexible and barrier property gelatin film [7,30,64]. The formation of intermolecular hydrogen bonds between anthocyanins and gelatin significantly increased the tensile strength, WVP and UV-visible barrier and antioxidant capacity of gelatin film [65]. Moreover, the addition of naturally anthocyanins would enhance pH-color response of the gelatin film, which could be used to maintain food quality and monitor food freshness [23,65,66]. For instance, a novel pH-sensitive and active indicator incorporated with *Coleus scutellarioides* anthocyanin extracts (CSAE) was fabricated for fish conservation by Hematian et al. Compared with gelatin film, film containing CSAE had good EAB and UV light barrier capacity, but lower TS. The CSAE film in acidic pH was purple, and green in alkaline pH [66].

3.5. Film forming methods

For the preparation of active and intelligent biodegradable packaging, casting method is the most commonly used, which is simple and convenient. However, starch and gelatin that need to be heated to higher temperatures to form film, and anthocyanins is easily denatured at high temperatures, which affect the color indication, antibacterial and antioxidant effects of anthocyanins [31,62]. Electrostatic spinning can continuously prepare sub-micron or nano-sized ultrafine film without high temperature and pressure. Moreover, the film formed by electrospinning has unique pore structure, large specific surface area and easily modified surface, which provide significant advantages in stimulus sensing and release control of anthocyanins [7,26]. In addition, three-dimensional (3D) printing can quickly and accurately print composite labels with specified shapes, avoiding shape and size errors and each film can be loaded with the same amounts of anthocyanins [52]. Li et al. found that 3D printing made anthocyanins located in the right place of the indicator film, which could reduce the over-oxidation of anthocyanins and did not affect its antimicrobial, antioxidant and color response [52].

Table 1. Preparations of active and intelligent film containing anthocyanins.

Substrates	Extracts	Methods	Effects of anthocyanins	Reference
carrageenan	jaboticaba peels	casting	improves the opacity property, UV-vis light barrier, against *E. coli*, scavenging DPPH	[14]
methylcellulose	jambolao skins	casting	scavenging ABTS and DPPH, increase mechanical and barrier properties	[29]
gelatin, oxidized chitin nanocrystals	black rice bran	casting	UV–vis light barrier, scavenging ABTS, DPPH and FRAP	[30]
potato starch	onion	casting	against *Staphylococcus aureus* DSM 20,231, *Salmonella bongori* DSM 13,772 and *Escherichia coli* DSM 30083, scavenging DPPH	[38]
potato starch	purple corn cob	casting	improve light barrier, against *Escherichia coli*	[62]
quercetin-loaded chitosan, agar, sodium alginate	purple sweet potato	casting	UV blocking, water vapor barrier	[67]
starch, agar	shikonin	casting	UV-light barrier, mechanical strength, scavenging DPPH and ABTS, against *Listeria monocytogenes*	[31]
chitosan	black rice bran	casting	UV–vis light barrier, sensitive and rapid response to pH/NH_3, scavenging DPPH, reduce spoilage bacteria	[36]
alginate, carboxymethyl chitosan	purple cauliflower	casting	improved mechanical strength, reduced the swelling degree, improved the sensitivity of the colorimetric response	[10]
hydroxypropyl methylcellulose	epigallocatechin-3-gallate	casting	enhanced mechanical strength, superior water vapor barrier, UV protection, detect bacterial growth, kill bacteria on-demand	[47]

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zein	blueberry	casting	scavenging DPPH and ABTS, against *Escherichia coli* and *Staphylococcus aureus*	[32]
chitosan, cassava starch	mulberry anthocyanin	casting	reduced oxygen and water vapor transmittance, scavenging DPPH, against *E. coli* and *S. aureus*	[52]
gelatin, carrageenan	shikonin	casting	UV blocking, against *E. coli* and *L. monocytogenes*	[64]
cellulose nanofiber	*Brassica oleracea*	casting	UV blocking, improved the physicochemical, scavenging DPPH and ABTS	[48]
gelatin	Alizarin	casting	rapid response to pH/NH_3, light barrier, hydrophobicity, scavenging ABTS, against *E. coli* and *S. aureus*	[23]
cellulose acetate	*Perilla frutescens*	electrospinning	scavenging DPPH, enhanced hydrophobicity, against *E. coli* and *S. aureus*,	[26]
locust bean gum, polyvinyl alcohol, chitosan, κ-carrageenan	purple sweetpotato, purple cabbage	casting	improved light barrier, scavenging DPPH, ammonia sensitivity	[8]
potato starch	blueberry	casting	improved mechanical, ammonia responsive	[63]
gelatin, zein	blueberry	electrospinning	Fe^{2+} enhances the color response of anthocyanins	[7]
gelatin	Coleus *scutellarioides*	casting	increased film flexibility, decreased tensile strength and UV-vis light transmittance	[66]
gelatin	haskap berries	casting	increased water vapor, UV-vis light barrier and tensile strength, scavenging DPPH	[65]

4. Application in rich protein foods

4.1. Fresh meat

Fresh meat is prone to lipid peroxidation, microbial contamination during processing, transportation, storage and consumption. It would result in color, odor and pH changes that impact the acceptable nature of the meat product [68]. For these reasons, fresh meat has a very short shelf life (3-5 days) at 4 °C [69]. Active and intelligent film containing anthocyanins could delay meat spoilage and indicate the freshness of meat [70]. For example, Hao et al. prepared a CS-OEO-BRBA film use chitosan embedded with black rice bran anthocyanin (BRBA) and oregano essential oil (OEO). CS-OEO-BRBA film could improve the quality indexes of sensory and color of pork, slowed down the rise of pH and TVB-N value at 4°C (Figure 4). The inclusion of BRBA and OEO reduced the abundance of spoilage bacteria associated with resistance, pathogenicity, and biofilm formation in pork and delayed the emergence of odor volatiles. Moreover, the CS-OEO-BRBA film turned to bottle green on day 12 (red color at the beginning), indicating that the pork has lost its commercial value [36]. Wang et al. developed an active and intelligent film to monitor the freshness of pork. According to the Chinese Standard GB 2707–2016, the limit of TVB-N for pork should be < 15 mg/100 g. The TVB-N value (15.16 ± 1.15 mg/100 g) of the pork slightly exceeded the standard limit on the first day, which indicated that the pork was not fit for consumption, but the change of appearance pork was

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difficult to be observed with naked eye. However, the color of the film had changed from blue to navy blue. On the second day, the color of the film changed to green and the TVB-N value of the pork had increased to 24.32 ± 1.02 mg/100 g, which indicated the pork had been severely spoiled [53].

Moreover, the antioxidant and antibacterial of anthocyanins can also extend the meat shelf life. The addition of *Amaranthus* leaf extract (ALE) significantly delayed the growth of total bacterial count (TBC) and *Staphylococcus aureus* during chicken preservation kept at chilled. *Staphylococcus aureus* and TBC increased to 3.88 log cfu/g and 6.53 log cfu/g on day 3 in the control group, while the ALE film packaged group increased to 2.91 log cfu/g and 6.00 log cfu/g after 12 days. At the same time, the film changed from red to yellow when the chicken goes from fresh to rotten [71]. Liu et al. showed that add butterfly bean anthocyanins could extend the freshness of beef stored at 4 ℃ for 2 days. When the film color changed from purple, blue to blue-green, it indicated that the beef has changed from fresh to sub-fresh and corrupt [72].

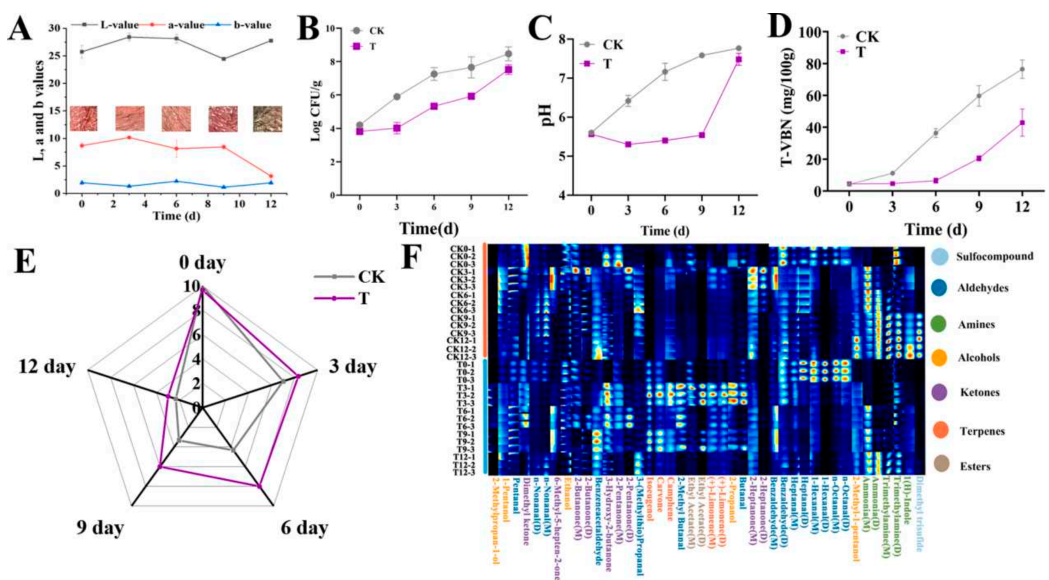

Figure 4. The color value (A), total viable counts (B), pH value (C), TVB-N value (D), changes in sensory index (E), fingerprint of volatile compounds (F) of pork samples during storage at 4 ℃ [36].

4.2. Aquatic products

Aquatic products are popular among consumers, but they are susceptible to microbial infestation and enzymatic reactions during storage after inactivation, triggering biochemical reactions that lead to spoilage. It's not only causes the loss of the eating quality and nutritional value of aquatic products, but also brings food safety problems [67]. Bacterial-induced spoilage of aquatic products produces basic compounds resulting from the degradation of proteins, such as trimethylamine and dimethylamine, and the consequent pH increase is considered to be an important indicator of deterioration in quality [43]. Changes in pH value can alter the molecular structure or conformation of the anthocyanins within the active and intelligent film, inducing a change in the film color to determine the quality of the aquatic products [66,68]. In conclusion, the color of the film is highly correlated with the sensory quality, lipid peroxide, colony count, volatile salt nitrogen, and pH value of the food.

Ezati et al. observed that the controlled release of shikonin from complex films exhibited strong antioxidant (ABTS and DPPH). In addition, the film supplemented with shikonin had obvious inhibitory effect on *Listeria monocytogenes*. After 12 h, the growth rate of *Lactobacillus monocytogenes* was 3-fold lower in the shikonin-added films compared to the pure film. Particularly, the films showed quick color changes when exposed to ammonia vapor and different pH values. The films show a characteristic color change from reddish-pink to bluish-purple when used for shrimp packaging, indicating the onset of shrimp spoilage [31]. Wu et al. also found that after fresh shrimp was stored at 25 °C for 24 hours, the *Clitoria ternatea* extracts added film changed from blue to blue-green, indicating that the shrimp changed from fresh to rotten [73]. Moreover, Kanatt developed an *Amaranthus* leaf extract added film, which could effectively reduce the total bacterial count of fish stored at 4 °C, inhibit the growth of *Staphylococcus aureus* and oxidative rancidity. Increased the shelf life of fish from 3 days to 12 days. When the film color changed from red to yellow, it means the edible end of the fish [71].

4.3. Milk

Milk, as a nutritious and comprehensive food, contains high quality proteins, oligosaccharides, fats and vitamins. It is also highly susceptible to the growth of microorganisms, carbohydrate fermentation, fatty acid failure and protein denaturation, and thus spoiling nutrition. A lot of milk is wasted due to spoilage during distribution and consumption. Milk stored in supermarkets or at home has to be checked for freshness before consumption. Currently, the commonly used methods to monitor milk freshness are nuclear magnetic resonance, near-infrared spectroscopy, and mid-infrared. Which need expensive equipment and tedious operations [7]. In this regard, active and intelligent packaging has emerged to provide convenience, real-time monitoring food safety and quality and reduction food waste. Carrageenan/gelatin-based film containing shikonin exhibited terrific against *L. monocytogenes* and *E. coli*. The film showed pH-dependent color variation, red at pH 2-7, purple at pH 9, blue at pH 10-12. After three months, the film still exhibited good color stability. The film changed from purple to reddish-pink when it immersing in fresh, under decaying, and spoiled milk for 10 min. At the same time, the RCS index, pH and the degree of spoilage of milk corresponded to each other [64]. Also, Gao et al. prepared indicator film for monitoring milk freshness by incorporating gelatin, blueberry anthocyanins, and Fe^{2+} into a corn protein matrix using the electrostatic spinning method. The change of film color was perceptible visually, from purple-black (fresh milk), royal purple (spoiled milk) to purple-red (spoiled milk). At the same time, the color parameters of the film (L^*, a^*, R, G and B) were highly correlated with the pH of the milk during storage [7]. Moreover, the freshness monitor could reflect by digitized color information via intelligent phone [67].

5. Summary and future prospects

Compositing anthocyanins can improve the physicochemical properties of the biological matrices, endow the films with antimicrobial and antioxidant capabilities, and have a high potential for developing active and intelligent packaging in rich protein foods. Meanwhile, rich protein foods contain an abundance of fat, the rancidity of lipids would produce undesirable flavors and promote food spoilage. However, fewer studies have been conducted on the inhibition of lipid oxidation and specific spoilage bacteria in rich protein foods. Much of the research on slow release of anthocyanins was stuck in simulated conditions, but the situation inside the actual pouch is much more complex and more worth to research. Therefore, future research should focus on the above issues. The simplified preparation and industrialized production of the active and intelligent film will provide protection for the rapid development of fresh food in electricity suppliers.

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Conflict of 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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