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Preparation and characterization of PH-responsive polyvinyl alcohol/chitosan/anthocyanin films

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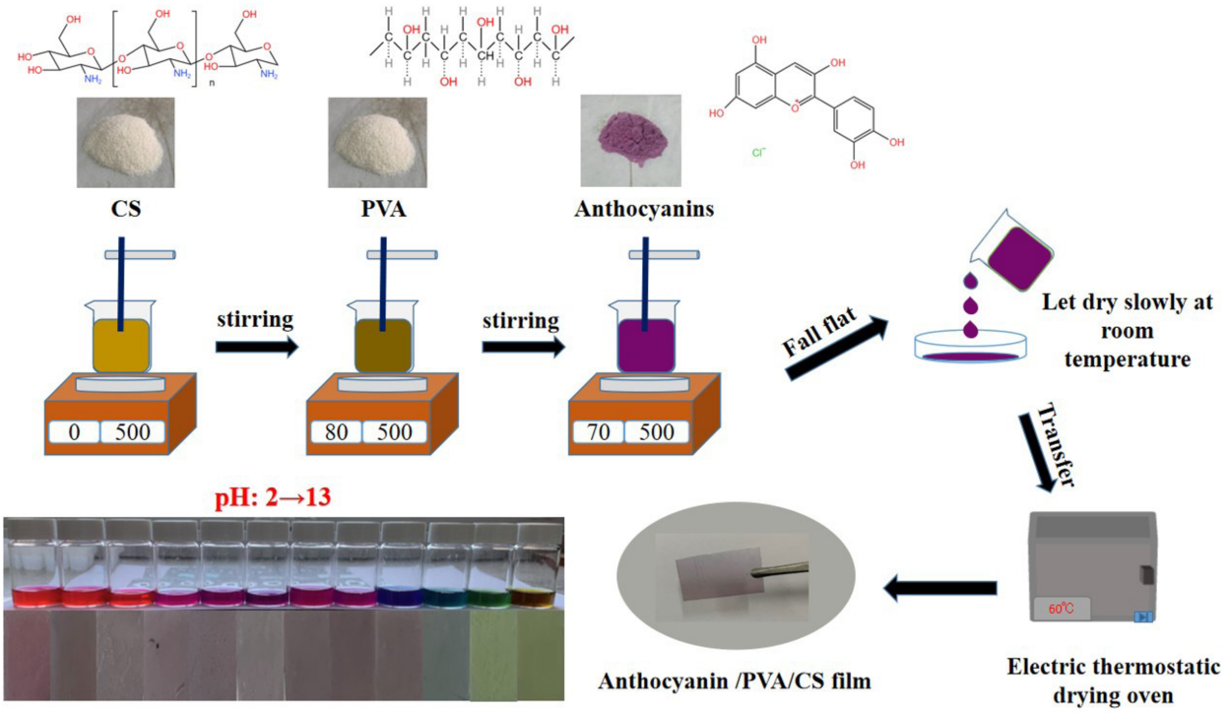
Abstract

Anthocyanin/polyvinyl alcohol/chitosan composite membrane was prepared by solution casting method using chitosan (CS) and polyvinyl alcohol (PVA) as matrix and natural pigment anthocyanin as filler. The structures of the composite films were characterized by Uv-visible reflectance spectrum (UV-VIS), Fourier infrared spectroscopy (FT-IR) and the scanning electron microscopy (SEM). The effects of adding anthocyanins on the physical properties, water resistance, degradation, antibacterial properties and pH responsiveness of the films were investigated. The experimental results showed that the prepared anthocyanin/polyvinyl alcohol/chitosan composite indicator films show color changes from pink to purple to blue and then to yellow-green when the pH value ranges from 2 to 14. The total color difference ΔE value is greater than 12. The antibacterial properties and other properties of the composite film can enhance by adding anthocyanin. The tensile strength of anthocyanin/polyvinyl alcohol/chitosan film is more than 70 MPa, which is larger than that of polyvinyl film and polyvinyl alcohol/chitosanfilm. The film has excellent physical, antibacterial properties and obvious pH response performance, making it ideal for food packaging.

Keywords: chitosan; polyvinyl alcohol; anthocyanins; composite film; characterization.

Practical Application: In this paper, the main application of membrane and food preservation, food detection. For example, it is expected to be applied to detect the freshness of meat. Through the color change of the film, the pH change of meat can be inferred, so as to detect the freshness of meat.

Graphical Abstract



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1 Introduction

Among natural biopolymers, CS is one of the most promising candidates for food packaging materials, because it is edible, non-toxic, biodegradable, biocompatible and other characteristics (Yuan et al., 2021). As a natural degradable polysaccharide, it was non-toxic and harmless, with a broad spectrum of antibacterial, anti-corrosion, promoting wound healing and other functions. Widely used in food, medicine, cosmetics, agricultural environmental protection and other fields (Liang & Qiu, 2020). However, due to the poor mechanical properties of chitosan, its application in packaging field is limited. In order to solve this problem, Chitosan can be compounded with materials with excellent flexibility. PVA is a polar biodegradable synthetic polymer, because of its high tensile strength, it is widely used in polymer blends for its flexibility and excellent film forming properties (Kochkina & Lukin, 2020). PVA is a hydrolytic product of polyvinyl acetate, it has the characteristics of good film forming, good mechanical properties and chemical resistance (Zhong et al., 2019). It is widely used in medical, film material industry. So that it has a certain mechanical strength and can be applied to food packaging (Shetty & Rao, 2022). If the film is used for food preservation, it needs good antibacterial and antioxidant properties, and the film has a certain pH response to food. Therefore, Anthocyanin, a natural pigment, was chosen as the film filler.

Anthocyanins are polyphenol compounds mainly composed of flavanol monomers such as catechins or their derivatives connected by C-C bonds (Chen et al., 2019). Anthocyanins are natural pigments, they have many health benefits, such as antioxidant, anticancer, anti-inflammatory, antibacterial and neuroprotective activities. The properties of PVA/CS films can be enhanced by adding Anthocyanins, to manufacture reactive and intelligent packaging. Anthocyanins can interact with PVA/CS film substrates through hydrogen bonding. thus, the mechanical properties of the film are improved. At the same time, Anthocyanins can improve the antioxidant and antibacterial activities of the film. A number of studies have shown that Anthocyanins have antioxidant, antibacterial and bactericidal physiological activities (Mohamed et al., 2019; You et al., 2022). In addition, Anthocyanins have a wide color spectrum (depending on pH changes), besides, it has good water solubility and high stability in temperature and light (Tan et al., 2021). PVA/ CS films contain Anthocyanins, which are sensitive to pH, Anthocyanins from different plants show different color channels due to their unique structure and content. Anthocyanins are made up of several conjugated phenolic rings, as the pH changes, protonation and deprotonation of hydroxyl groups in the phenolic ring of Anthocyanin molecules change the conjugation pattern of the entire molecule, which causes color changes, the composite film can also be used as pH indicator film. Therefore, Anthocyanins can greatly improve the physical and functional properties of PVA/CS films.

However, Anthocyanin-rich PVA/CS films have not been studied. Therefore, Anthocyanin was used as pH response filler in this paper. Anthocyanin/PVA/CS composite films were prepared by solution casting method. In this paper, PVA and CS are selected for material composite. After the reaction of PVA/CS in the coagulation bath, composite films have relatively complete and dense surface structure (Zhao et al., 2020). Hydrogen bonds can be formed between PVA and CS, the blending of the two is expected to improve the degradability of PVA and brittleness of CS (Huo & Wang, 2020). Adding Anthocyanins to PVA/CS composite films, Hoping to take advantage of Anthocyanins' excellent pH sensitivity, further develop the application of environmentally friendly biological materials in the field of food preservation.

2 Materials and methods

2.1 Materials and instruments

Polyvinyl alcohol PVA-124 (Xilong Scientific Co., Ltd.), Chitosan (CS, degree of deacetylation ≥95%, Shanghai Yuanyue Biotechnology Co., LTD.), Ice acetic acid (AAG, ≥99.5%, Tianjin Guangfu Technology Development Co., LTD.), Anhydrous ethanol (Tianjin Guangfu Technology Development Co., LTD.), Peptone (Tianjin Dingsheng Xin Chemical Co., LTD.), AGAR powder (Chengdu Beichen Founder Reagent Factory), Yeast powder (Tianjin Guangfu Technology Development Co., LTD.), Lysozyme (Solarbio), sodium chloride (Tianjin Beichen Founder Reagent Factory), DPPH (Solarbio), ABTS (Solarbio).

2.2 Preparation of anthocyanins

Using purple sweet potatoes as ingredients. Hcl, Ethanol as solvent, Solid-liquid ratio 1:20 Using cellulase as catalyst (enzyme addition amount 54 U/mL), extraction temperature 50 °C, the ultrasonic power was 100W for 30min. Centrifugal rotary evaporation of concentrated liquid, Dry and set aside.

2.3 Preparation of anthocyanin /PVA/CS films

Add PVA, CS, AAG and deionized water according to the ratio in Table 1. Heat to 80 °C and stir for 2 h. After CS and PVA are completely dissolved, the temperature is cooled to 70 °C. Then 0.5 g Anthocyanins were added. Stir at constant temperature for 2 h until the mixture is fully mixed. Take the blend solution and pour it into the glass plate, after uniform flow, stand at room temperature until solidification. After film formation, the Anthocyanin /PVA/CS composite film was prepared by placing it

Table 1. Raw material ratio of composite film.

The name of the film	PVA (g)	CS (g)	AGG (mL)	Anthocyanins (g)	H_2O (mL)
PVA	5	0	0	0	120
PVA/AGG-AGG	5	1.5	1.5	0	120
PVA/CS-AGG/ Anthocyanin	5	1.5	1.5	0.5	120

at room temperature for 24 h. And then transferred to an electric thermostatic drying oven, after 12 h, homogeneous film samples were obtained. The naming and matching of the blending film are shown in Table 1. The film prepared at 25 °C, After 3 days of storage at relative humidity of 40% ± 5%, reanalyze and test each performanc. Figure 1, film pdreparation process.

2.4 Test characterization

Thickness of composite film

Take a complete and uniform composite film, 5 points were randomly selected on the film using an outside diameter micrometer with an accuracy of 0.001 mm. Measure its thickness, calculate the average, the standard deviation.

FT - IR analysis

The prepared film is dried and ground, and the composite film is scanned by Fourier transform infrared spectrometer using potassium bromide tablet method, the scanning range is 4000-400 cm^{-1}.

Mechanical properties test

The film sample was cut to 100 mm, 1.5 mm wide rectangle, Test fixture spacing 50 mm, The tensile speed is 50 mm/min.

SEM analysis

The composite film is brittle broken by freeze drying. Then after spraying gold on the section, the microstructure of brittle section of composite film was analyzed by scanning electron microscope.

Water resistance test

The film sample was cut into 4 cm × 4 cm size and dried to constant weight, so that the dry film mass was W_0, the film is then immersed in deionized water, take it out after 2 hours, after drying the moisture on the surface of the film with filter paper, weigh it to get W_1, the film is then dried to constant weight to obtain a final mass of W_2. Water absorption rate and water solubility rate are calculated according to Equations 1 and 2 (Zhang et al., 2018).

$$Bibulous\, rate\% = \frac{W1 - W0}{W0} \times 100 \tag{1}$$

$$Water\, soluble\, rate(\%) = \frac{W0 - W2}{W2} \times 100 \tag{2}$$

Antibacterial experiment

The antibacterial activity of the film was studied using gram-negative bacteria (E. coli) and gram-positive bacteria (S. aureus). Inhibition zone method, inhibition zone test and growth curve of bacteria were used to determine the antibacterial activity of the film. Soak sterile filter paper with diameter of 5 mm in PVA, PVA/CS and Anthocyanin /PVA/CS film solution for 2h for later use. 100μL bacterial suspension with a concentration of 1.0 × 105CFU/mL was added to the surface of solid medium. Apply evenly with a coater, rest at room temperature for 5min. Then paste the soaked filter paper onto the petri dish. Stick three filters on each petri dish, Culture in 37 °C incubator for 24h, Observe and measure the diameter (mm) of the bacteriostatic zone, the result is expressed as X ± s. Make the film into a square with sides of 2 cm, add each to E. coli (S.areu), the bacterial activity was measured for 24 hours. By measuring its OD value, the bacteriostatic effect was obtained. Inhibition zone experiment the contact antimicrobial or inhibition zone (ZOI) experiment was performed. Put 1.0 × 104 CFU/mL Staphylococcus aureus into the sterilized centrifuge tube. The sample is immersed in it for 2h, then take out 100 μL and put it on a plate and incubate at 37 °C for 24h. Observe the growth of bacteria.

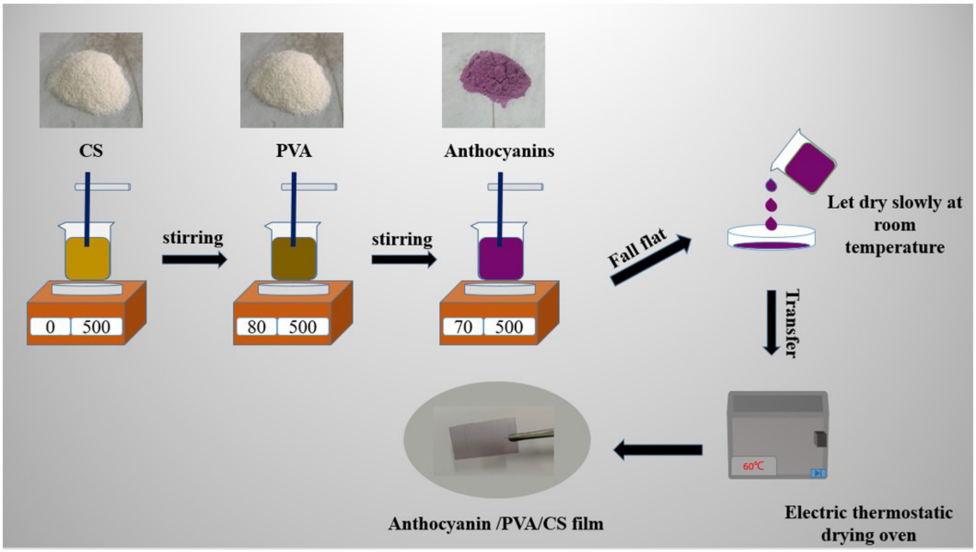

Figure 1. The preparation process of Anthocyanin /PVA/CS film.

Antioxidant test

(1) Determination of ABTS free radical scavenging activity

7 mmol/L ABTS ethanol solution was prepared, add 180 μL solution to a 96-well plate containing 20 μL thin film solution. Shake the 96-well plate oscillator thoroughly, the absorbance of the solution was measured at 734 nm. ABTS original solution plus distilled water as blank control. Free radical clearance rate is calculated according to Formula 3:

$$RABTS(\%) = \left[1 - \frac{As - Ar}{Ao}\right] \times 100 \tag{3}$$

where the symbols in the equation denote the following:

A_s is the subject group, A_r is the control group, A_0 is the blank group

(2) Determination of DPPH free radical scavenging activity

Fully mix 100μ DPPH ethanol solution at a concentration of 1 mmol/L and 100 μ composite film solution in a 90-well plate. Let stand in the dark at room temperature for 30 minutes. Then the absorbance of the mixture was measured at 517 nm with a microplate reader. DPPH free radical scavenging rate R_{DPPH} (%) was calculated according to Formula 4:

$$RDPPH(\%) = \left[1 - \frac{A - A2}{A1}\right] \times 100 \tag{4}$$

Mark: A is the subject group, A_2 is the control group, and A_1 is the blank group

Degradability test

Cut the mixed film into 5cm×5cm squares, bake until constant weight, in saline with and without lysozyme. Stored at 37 °C. Quantitative sampling, washing, after drying to constant weight, degradation percentage was calculated according to Equation 5:

$$Degradation\ percentage = \frac{W1 - W2}{W1} \times 100\% \tag{5}$$

where the symbols in the equation denote the following:

W1 - Mass of film before degradation;

W2 - The quality of the film after degradation

Contact Angle test

Using a contact Angle meter, measure the surface wettability of the film.

Determination of color response of Anthocyanin/ PVA/ CS composite film under different pH environments

(1) pH response of anthocyanin solution

A certain amount of extracted anthocyanin powder was weighed, fully dissolved in glacial acetic acid solution, adjust the pH of the solution with 2 mol/L sodium hydroxide (2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13). Keep the solution of different pH at room temperature, observe and record the color of the solution, scanning the UV-vis spectrum, the scanning wavelength range is 200-800 nm.

(2) pH response of anthocyanin /PVA/CS composite film

Take 50 mL of AAG, adjust the pH of the solution with sodium hydroxide, store 2 mL solution of different pH (2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13) for later use. The Anthocyanin/PVA/CS composite film was cut into a 10 mm×10 mm square. In different pH solutions, observe the color change of the composite film.

2.5 Data processing

All experiments were repeated five times, calculate the mean and standard deviation, the results were expressed as mean ± standard deviation. The data were plotted using Origin 9.0.

3 Results and analysis

3.1 FT-IR analysis of composite film

The main absorption peaks of Anthocyanin /PVA/CS films were studied, the characteristic absorption peaks and interactions among the film components were compared, as shown in Figure 2, where, the stretching vibration absorption peak of -OH bond is near 3269 cm^{-1}, this is consistent with the results of hydroxyl rich in film forming materials (polyvinyl alcohol, chitosan, Anthocyanin powder), meanwhile, 2928 cm^{-1} and 1323 cm^{-1} are due to the absorption peaks of C-H symmetric vibration and in-plane bending vibration. There is no characteristic absorption peak of carboxylic acid, it indicates that the unreacted AAG has been volatilized in the film drying process, the characteristic peak at 1636 cm^{-1} in the Anthocyanin powder of raw purple sweet potato was attributed to the bending vibration of C = C aromatic ring, is related to anthocyanin powder belonging to aromatic compounds of purple sweet potato, in addition, the pyran ring segment of flavonoid compounds is located at 1256 cm^{-1}. The absorption band at 1058 cm^{-1} is due to C-H formation of

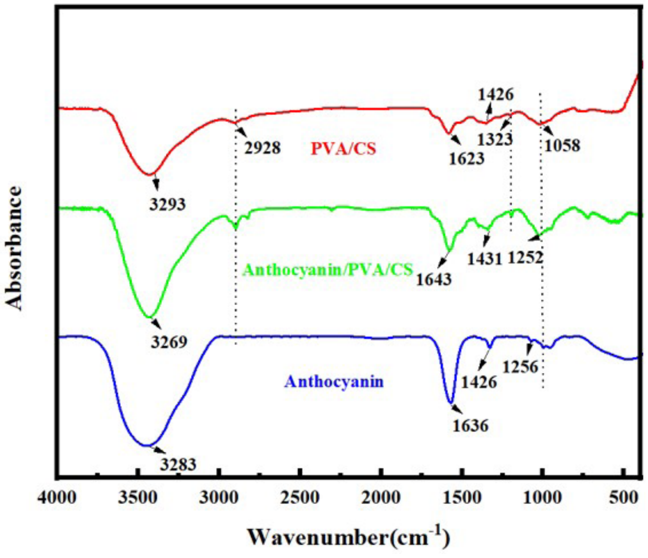

Figure 2. FT-IR spectra of anthocyanins and thin films.

aromatic ring. Because of their chemical environment, the in-plane bending vibration peak of -OH at about 1426 cm^{-1} showed several wave number shifts between different films. The absorption peak shifted from 1636 cm^{-1} to 1643 cm^{-1}, this may be due to the interaction between the Anthocyanin and the raw material, strong intermolecular forces were formed. According to the spectral results, the composition of the raw material has not changed, at the same time, the Anthocyanin powder of purple sweet potato was well embedded in the film forming substrate.

3.2 The morphology of the composite film is microscopic

Figure 2 shows the SEM images of the three films. It can be seen that the PVA film has a tight structure and smooth surface. After adding CS, the surface of CS/PVA film material is relatively smooth. The structure is relatively dense, and the overall morphology of the composite film is uniform and compact, no obvious particulate matter exists, indicating that CS and PVA have good compatibility. As can be seen from Figure 3c, with the addition of anthocyanins, the surface of PVA/CS film material shows obvious network texture. Suggesting that with the increase in anthocyanins, the interaction between Anthocyanins and PVA/CS molecules also gradually increased, Anthocyanins have a certain effect on the structure compactness of PVA/CS thin films.

3.3 Analysis of mechanical properties of thin films

Composite film used for food preservation, etc. It must have excellent mechanical properties. PVA film thickness is 0.118 ± 0.006 mm, PVA/CS film thickness is 0.128 ± 0.007mm, the film thickness of anthocyanin PVA/CS was 0.136 ± 0.011 mm, mechanical properties of PVA/CS composite film and Anthocyanin/PVA/CS composite film were tested. The results are shown in Figures 4 and 5. By comparing the mechanical properties of pure PVA film and PVA/CS composite film, the tensile strength and elastic modulus of PVA/CS films are higher than those of pure PVA films. It shows that PVA/CS film has better mechanical properties. This is because the addition of PVA changes the intermolecular force between the -NH_2 and -OH groups, it is beneficial to improve the mechanical properties of composite films (Abu-Saied et al., 2017). The mechanical properties of Anthocyanin/PVA/CS composite films showed that After the addition of Anthocyanin, the tensile strength of the composite film, the elongation at break and elastic modulus were increased. This is because Anthocyanins were evenly dispersed in PVA/CS blends, improved intermolecular hydrogen bonding of PVA/CS, the interaction between PVA and CS is more intense. Thus, the tensile strength of the composite film is significantly improved.

3.4 Water resistance analysis of film

The water resistance of film is very important for the application of film in preservation. Figure 6 shows the result of water absorption and water solubility of the film. AAG is partially volatilized during the stirring and drying process, the crosslinking is reduced, the hydrogen bond between CS and PVA is weak, therefore, more hydrophilic groups are exposed in the thin film matrix. The water absorption rate of the film is improved (Zeng et al., 2018). Film rupture and dissolution may occur in the experiment; the accumulation of AGG was improved by adding Anthocyanin. To make it easier for water

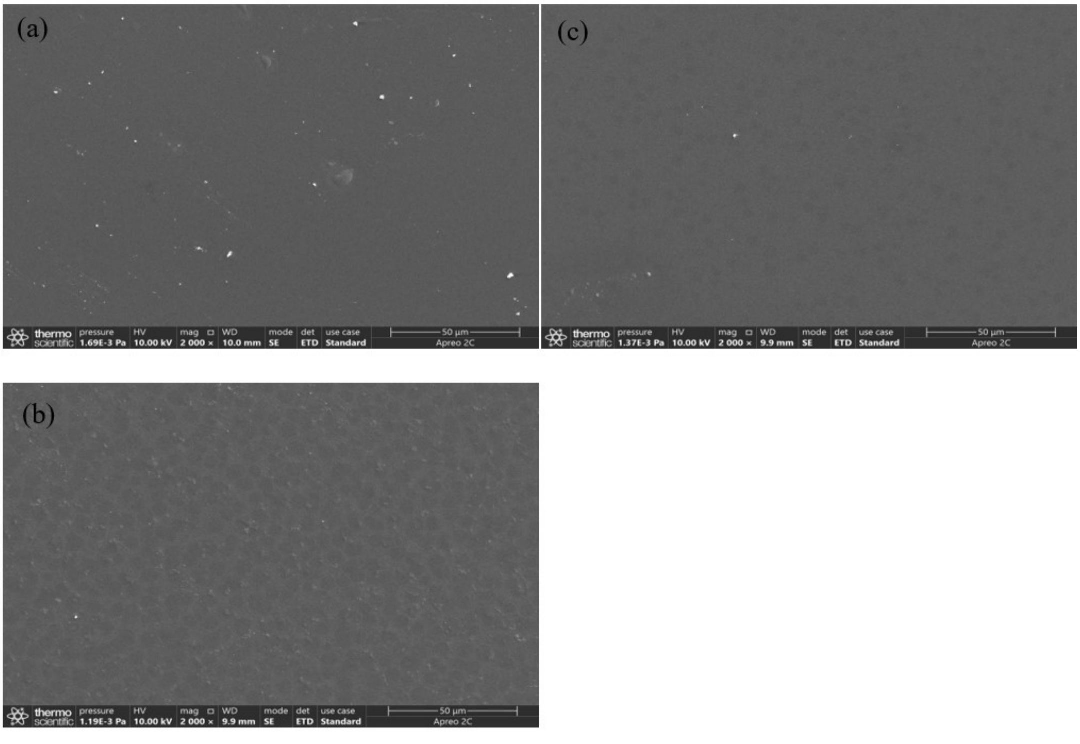

Figure 3. SEM diagram of film surface of different components (a) PVA thin film (b) PVA/CS composite films (c) Anthocyanin /PVA/CS composite films.

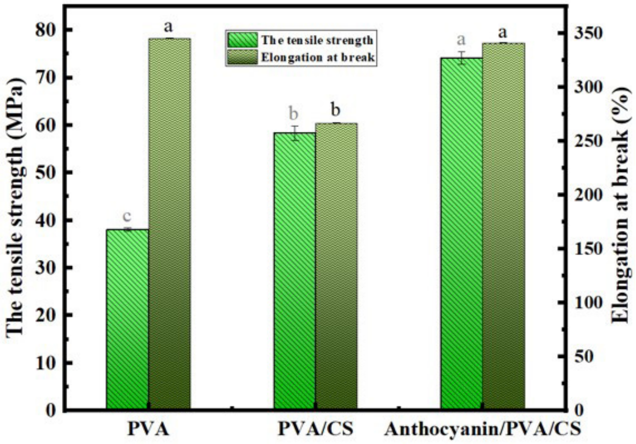

Figure 4. Results of mechanical properties of thin films. infuse: Different lowercase letters indicated significant difference within the group (P < 0.05).

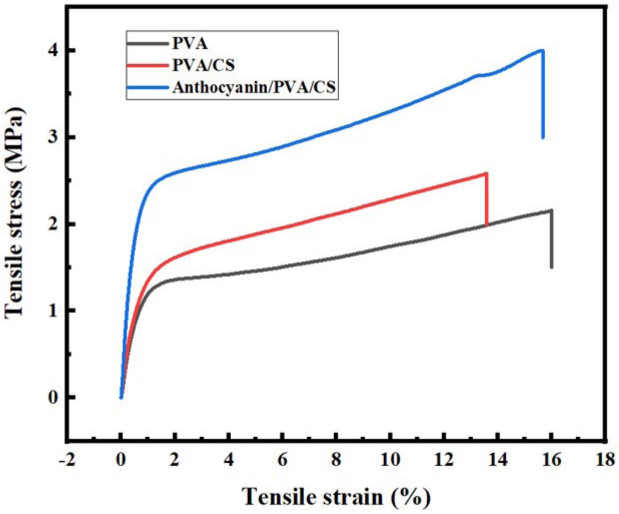

Figure 5. Film tensile stress-strain curve.

molecules to enter, therefore, the water absorption is further strengthened. But the film barely cracked, this is due to the strong hydrogen bond between Anthocyanins and hydrophilic groups in the matrix, The microstructure and mechanical properties of the composite film can also be verified.

3.5 Antibacterial performance analysis of the film

Film used for preservation, need to have good antibacterial activity. In order to investigate the antibacterial activity of composite film, three kinds of antibacterial experiments were carried out respectively. The strength of antibacterial activity can be expressed by the diameter of the inhibition zone around the bacteriostatic tablet (Wang et al., 2022). The results of inhibition zone experiment of several film solutions on E.coli and S. areus are shown in figure. As can be seen from the figure, no inhibition zone was generated in the PVA film solution group, E. coli and S. ureus. In PVA/CS and Anthocyanin/PVA/CS film solutions, both E. coli and S. areus have bacteriostatic zones. And Anthocyanin/PVA/CS film solution has a larger inhibition zone. It shows that the film has stronger antibacterial activity after adding Anthocyanin. Figure 7 is the result of bacteriostatic zone diameter. In general, if the diameter of inhibition zone is less than or equal to 7 mm, there is no inhibition effect; if the diameter of the inhibition zone is between 7 and 10 mm, the inhibition effect is weak; if the diameter of the inhibition zone is 10-20 mm, it has moderate inhibitory effect; if the diameter of inhibition zone is ≥ 20 mm, has a strong inhibitory effect. As can be seen from Table 2, the diameter of the inhibition zone of E. coli by Anthocyanin/PVA/CS film solution was 19 mm. and S. areus with a diameter of 16 mm. It showed that the addition of Anthocyanin did play a significant antibacterial effect. Achieve the desired effect. As shown in Figure 8, the number of bacteria on the AGAR plate of Anthocyanin/PVA/CS film was the least (both kinds of bacteria were present), it can also prove that the addition of Anthocyanins has a strong antibacterial activity. Figure 9, growth curves of the two bacteria It was also obvious that the PVA/CS film system with Anthocyanin did have stronger antibacterial activity.

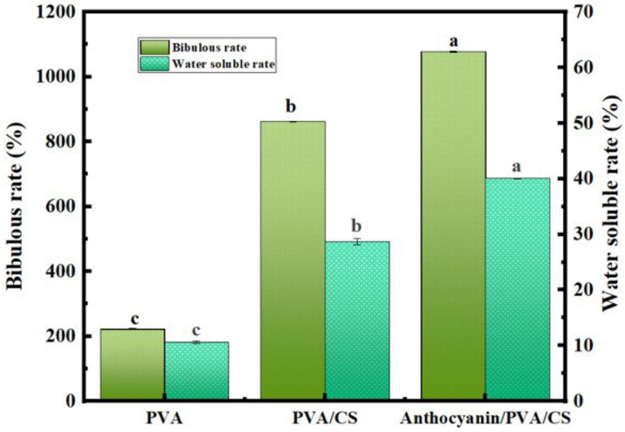

Figure 6. The result of water resistance of the film. infuse: Different lowercase letters indicated significant difference within the group (P < 0.05).

3.6 Comparison of antioxidant capacity of thin films

Figure 10 shows the ABTS free radical scavenging rate of different composite films,as can be seen from the figure, the scavenging effect of films without Anthocyanins on free radicals is not more than 50%,the free radical scavenging rate of the film with Anthocyanins was up to 72.18%, indicating that Anthocyanins improved the antioxidant capacity of the film. Results of DPPH free radical scavenging rate of different composite films, It can be seen from Figure 10 that the radical clearance rate is basically consistent with that of ABTS, in addition, the free radical clearance rate of the film with Anthocyanin was up to 96.81%, it is further proved that Anthocyanins do not reduce the antioxidant capacity of the film,Antioxidant capacity is important for food and drug packaging, in the application, Anthocyanin can be added to prepare the required antioxidation composite film. (Gao et al., 2020) proved that Anthocyanins

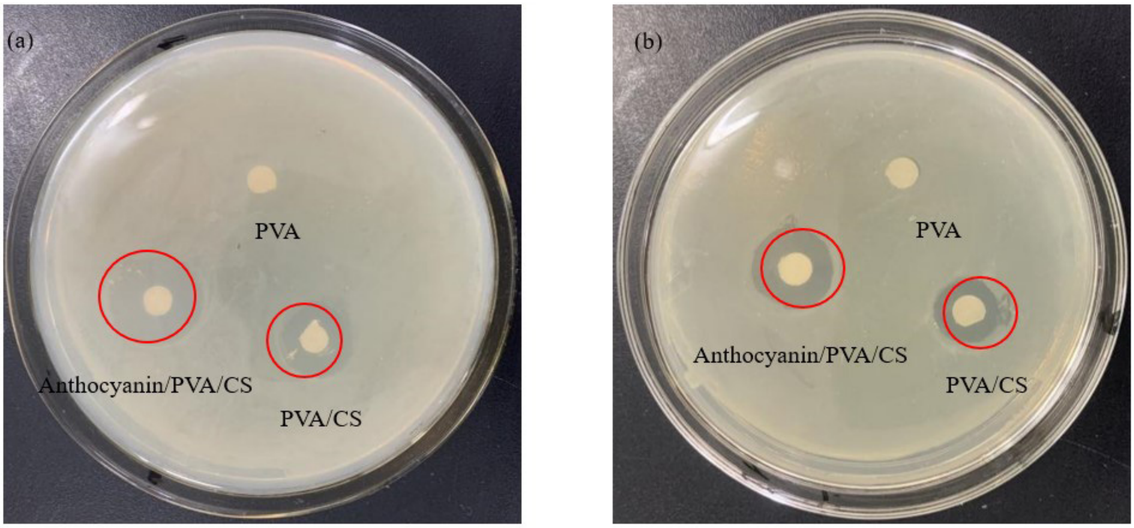

Figure 7. Experiment on bacteriostatic zone of different kinds of thin films. (a) *E.coli* bacteriostatic zone (b) *S.aureus* inhibition zone.

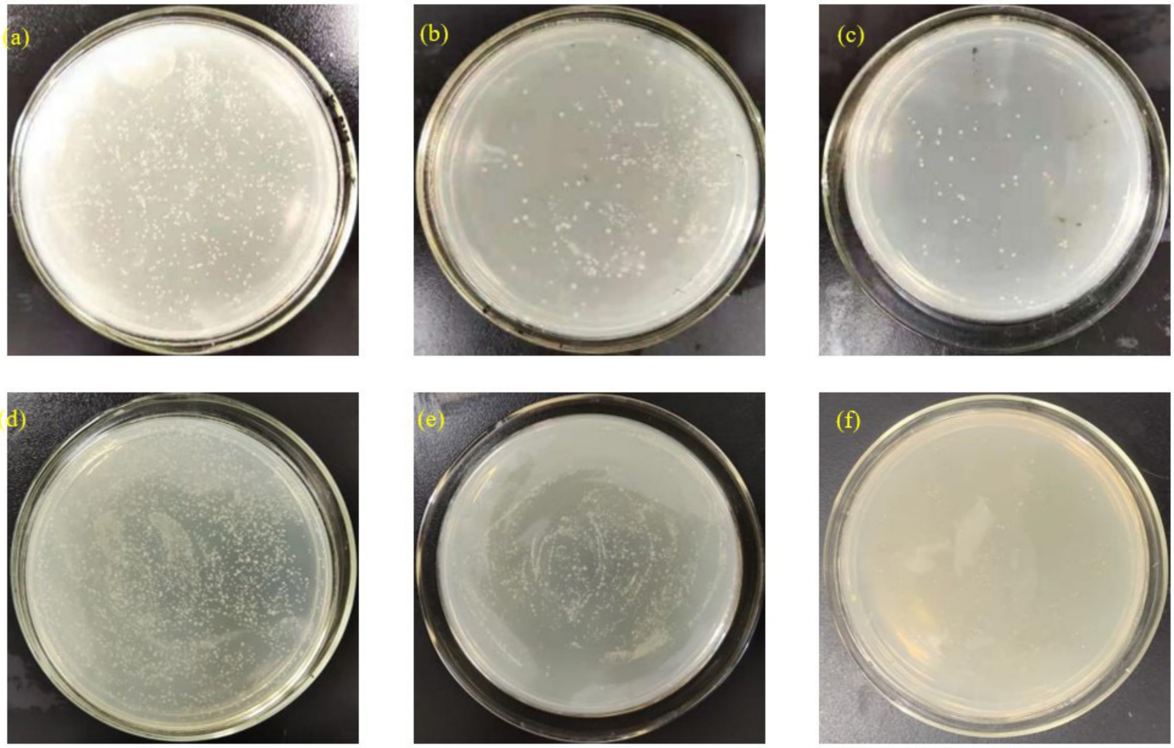

Figure 8. Photo of E. Coli and S. Areus growing on AGAR plates. (a-c) is the photo of PVA film, PVA/CS film, Anthocyanin /PVA/CS film E. Coli immersion solution on AGAR plate, (d-f) is the photo of PVA film, PVA/CS film, Anthocyanin /PVA/CS film S. ureus immersion solution on AGAR plate.

have good antioxidant activity through designed experiments (Yang et al., 2020) extracted Anthocyanins from Auricularia auriculata and proved through experiments that anthocyanins had significantly higher antioxidant activity than ascorbic acid at the same concentration, Liang et al. (2018) proved through experiments that Anthocyanin plays an important role in scavenging free radicals and reactive oxygen species (ROS) to prevent oxidative stress in cells. Rajan et al. (2019) summarized the studies on Anthocyanin structure and antioxidant performance calculation, studies further support that Anthocyanins have good antioxidant properties, Huang et al. (2020) studied the antioxidant component content and antioxidant activity in vitro of purple sweet potato, Anthocyanin components in different varieties of purple sweet potato were analyzed, and its free radical scavenging activity was determined, Anthocyanins showed excellent antioxidant activity and antioxidant activity in vitro.

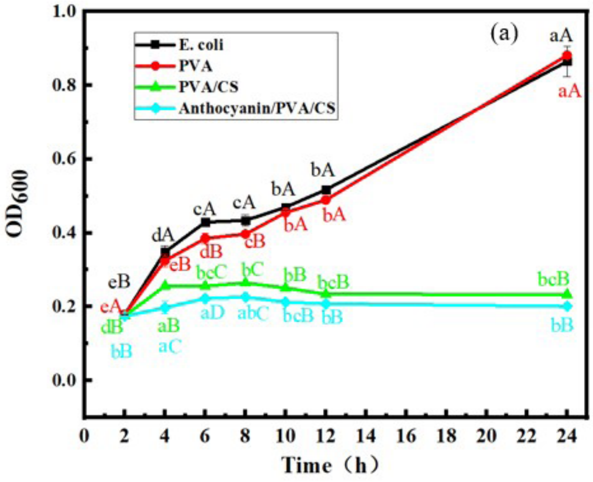

Figure 9. Inhibition growth curves of e. coli and S. ureus by different types of films. (a) E.coli inhibitory growth curve; (b) S. Ureus inhibitory growth curve. infuse: Different lowercase letters indicated significant difference within the group (P < 0.05). Different capital letters indicate significant differences between groups.

Table 2. Test results of the inhibition zone experiment.

Bacterial species	Diameter of bacteriostatic zone (mm)		
	PVA	PVA/CS	Anthocyanin /PVA/CS
E.coli	0	14 ± 0.012	19 ± 0.009
S.aureus	0	11 ± 0.031	16 ± 0.016

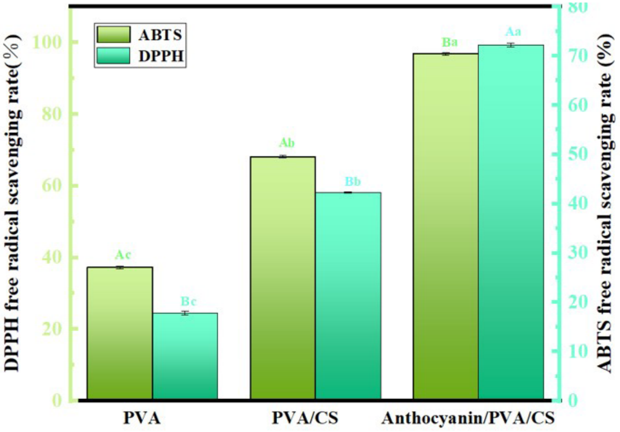

Figure 10. The ability of Anthocyanin/PVA/CS The composite film to scavenging ABTS/DPPH free radicals. ABTS radical scavenging activity diagram and DPPH radical scavenging activity diagram. infuse: Different lowercase letters indicated significant difference within the group (P < 0.05).

3.7 Measurement of contact Angle of composite film

Figure 11 shows contact Angle test images of three different films, the water contact Angle can indicate the wettability of materials, the hydrophilicity of the film surface can be analyzed by measuring the contact Angle (Ismail et al., 2022). As can be seen from Figure 10, the contact Angle of PVA film is (92° ± 0.3°), the contact Angle of PVA/CS film is (71° ± 0.3°), the contact Angle of Anthocyanin/PVA/CS film is (59° ± 0.3°), according to the different proportion of polymer, the contact Angle of the film changes. When Anthocyanins are added, intermolecular hydrogen bonding forces are generated between raw materials. The contact Angle of the film decreases gradually and the hydrophilicity of the sample becomes better and better.

3.8 pH response results and analysis of composite film

(1) UV-VIS spectra of anthocyanins in different pH solutions

The absorption spectra of Anthocyanin solutions in different pH environments are shown in the figure. As can be seen from Figure 12, the color of Anthocyanins in NaAC-HAC solution with ph2-4 gradually changes from light red to light purple, the strongest absorption peak appears at about 525nm, and the intensity increases with the increase of pH, the color changes from light purple to dark purple in pH5~9 solution due to the strengthening of alkaline environment, due to the reaction with hydroxyl groups on the structure of Anthocyanins from purple sweet potato, the strongest absorption peak appears at about 593nm, it turns blue at pH10, in the range of pH10 to 13, the color gradually deepens from blue to dark blue to green and finally to dark brown, and the peak value changes, as shown in Figure 13, the highest absorption peak is around 617nm. The picture shows the color response of Anthocyanins in different pH environments. From the color difference, Anthocyanins can be used as an acid-base indicator for food packaging. Sun et al. (2020) used k-Carrageenan and hydroxypropyl methyl cellulose as substrates, adding the extract of Prunus maackiipomace (EPm), The oxidation resistance film and pH response film were prepared. The pH response of pork showed obvious red to purple variation. Zhu et al. (2019) extracted Anthocyanins from purple sweet potato. The color reaction of anthocyanin solution under different pH values was analyzed. In different pH environment can produce obvious color change, as pH responsive materials, it has great development potential in the field of intelligent packaging. Ebrahimi Tirtashi et al. (2019) used purple potato as a chemical

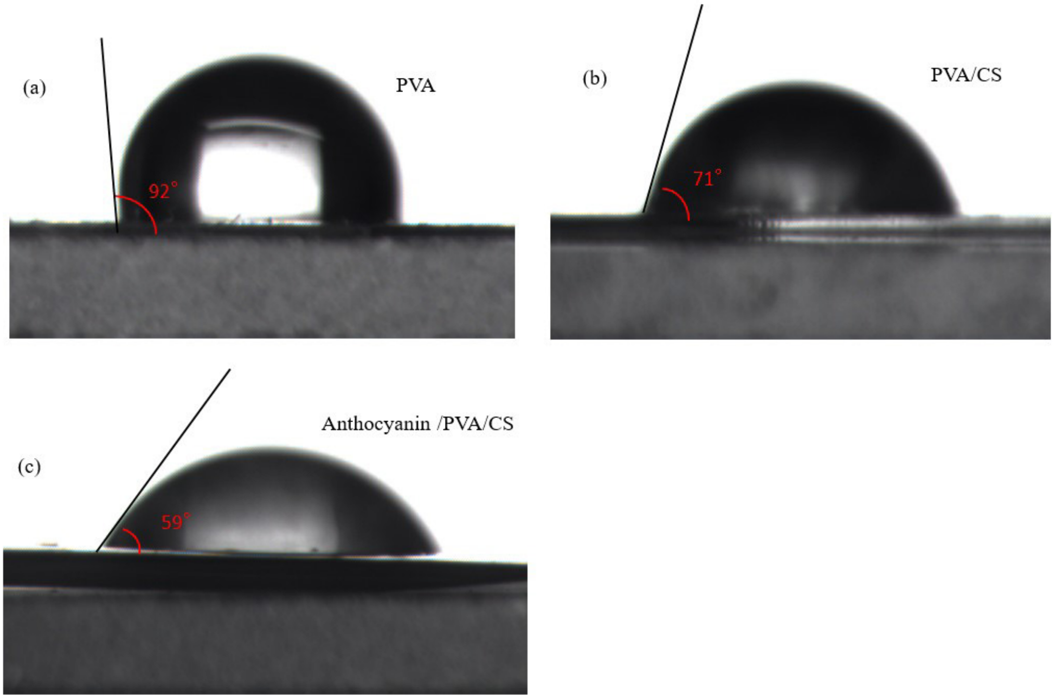

Figure 11. Images of water contact Angle of three kinds of films. (a) PVA film (b) PVA/CS film (c) Anthocyanin/PVA/CS film

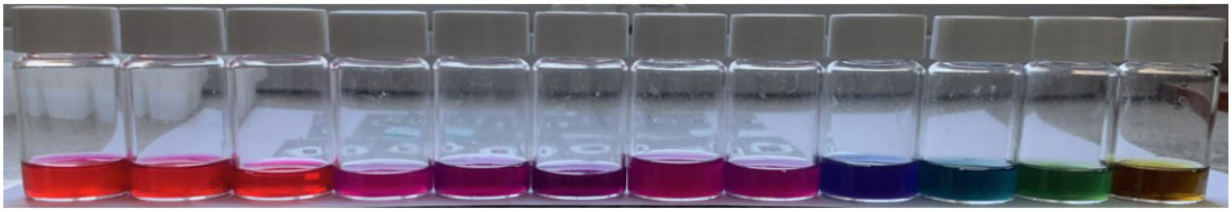

Figure 12. pH response of Anthocyanins. (2) pH response of Anthocyanins /PVA/CS

reactive dye, a colorimetric pH indicator is manufactured in a cellulose-chitosan matrix, at different pH values (pH 2-11), colorimetric pH indicators vary significantly in color from pink to khaki. (Jiang et al., 2019) purple sweet potato anthocyanin (P) as freshness indicator, prepare intelligent indicator film, Anthocyanins show different colors at different pH values, the development of stable indicator film using purple sweet potato Anthocyanin has a good application prospect.

The pH sensitivity and color of Anthocyanin/PVA/CS composite film were observed in the environment of different pH solutions, the results are shown in Figure 14. Chitosan is an alkaline polysaccharide, The Anthocyanins are purplish red in color, the film samples are placed in buffer solutions with different pH values, within a short period of time (about 3 min), the color of the film shows a significant change, and Anthocyanin in different pH environment color change rule is almost the same. After color comparison, it was found that with the increase of pH (from pH2 to pH7), the color gradually became lighter, from dark purple to pale, as pH increases (from pH8 to pH13), the composite film changes from mauve to dark, then blue, then light green, and finally appears dark green in the buffer solution of pH13. When evaluating indicator film, the change in ΔE must be significant enough so that the consumer can easily see the color change and obtain information about the condition of the product. As shown in Table 3, ΔE of Anthocyanin /PVA/CS

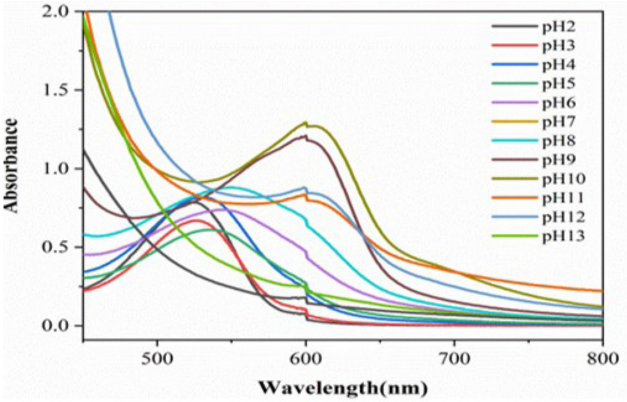

Figure 13. Absorption spectra of anthocyanin solution.

Figure 14. Color response of PVA/CS/ anthocyanin composite films under different pH conditions.

Table 3. CIELab color model chromaticity values of PVA/CS/ anthocyanin composite films under different pH conditions.

pH	L	a	b	ΔE
2	39.11 ± 0.233	14.09 ± 0.021	1.12 ± 0.012	30.42 ± 0.026
3	35.56 ± 0.568	6.07 ± 0.02	1.98 ± 0.116	30.41 ± 0.034
4	44.53 ± 0.205	4.36 ± 0.012	0.36 ± 0.014	21.46 ± 0.013
5	42.75 ± 0.541	6.84 ± 0.464	-1.03 ± 0.120	23.31 ± 0.015
6	40.8 ± 0.556	5.54 ± 0.177	-1.10 ± 0.066	24.54 ± 0.048
7	45.08 ± 0.502	2.57 ± 0.005	0.33 ± 0.008	20.48 ± 0.014
8	37.54 ± 0.339	4.47 ± 0.005	0.46 ± 0.005	29.69 ± 0.003
9	34.40 ± 0.347	5.56 ± 0.008	0.24 ± 0.005	30.77 ± 0.006
10	36.94 ± 0.213	4.21 ± 0.182	1.24 ± 0.554	28.76 ± 0.028
11	41.17 ± 0.339	-3.73 ± 0.025	0.74 ± 0.005	23.65 ± 0.009
12	51.34 ± 0.361	-11.63 ± 0.233	16.66 ± 0.257	29.51 ± 0.013
13	45.4 ± 0.173	-10.26 ± 0.191	17.80 ± 0.069	32.82 ± 0.037
blank	62.71 ± 0.615	-2.27 ± 0.278	-8.91 ± 0.556	

Table 4. Film degradation experiment.

methods	Thin film	Degradability time(day) and Percentage degradability (%)						
		1	2	3	4	5	6	7
Physiological saline(lysozyme)	A	15.00	25.70	31.67	43.24	49.17	51.53	56.02
	B	14.29	22.86	28.57	40	42.86	48.57	51.43
	C	34.38	43.75	46.88	50	53.13	59.38	68.75
Physiological saline	A	10.96	22.86	29.74	31.85	35.72	41.79	47.37
	B	8.33	21.11	25	27.78	33.33	41.67	44.44
	C	15.38	23.08	34.62	38.46	38.46	42.31	50

Where the symbols in the equation denote the following: A is PVA film, B is PVA/CS film, and C is PVA/CS/ anthocyanin composite film.

composite films does not change significantly with pH = 2-3, pH = 4-6 and pH = 8-10, but changes dramatically with pH = 3-4, pH = 7-8 and pH = 10-11. It is generally believed that the color change can be read by the naked eye when the color difference value ΔE is greater than 5 (Ezati & Rhim, 2020). ΔE values greater than 12 indicate an absolute color difference that even untrained group members can notice (Ju et al., 2022). From this indicator film ΔE index can also indicate that the film can be used for food preservation and other fields to do pH indicator film.

3.9 Degradability of composite film

Lysozyme, also known as muramidase, it is an alkaline enzyme that can hydrolyze mucopolysaccharide from bacteria. Can be used to accelerate the degradation of thin films of acidic systems, in this experiment, physiological saline containing lysozyme and without lysozyme were tested respectively. In vitro degradation of the mixed film was studied and compared with that of PVA film. The results are shown in Table 4. According to the data in Table 4, the degradation rate of mixed films containing Anthocyanins is the fastest, and the degradation rate is more than 2/3 in 7 days. PVA pure film followed, and PVA/CS composite film degradation rate was the slowest; in addition, it was proved that lysozyme could promote the biodegradation of the film. It can be seen from the figure that, without the addition of lysozyme, the three films all have certain degradation ability. When lysozyme was added, the degradation rate of the three films increased, and the film containing Anthocyanins had the highest degradation rate, therefore, it can be concluded that PVA/CS film has a certain degradation ability, and the addition of Anthocyanin can enhance the degradation ability of the film. Figure 15 is the film degradation curve

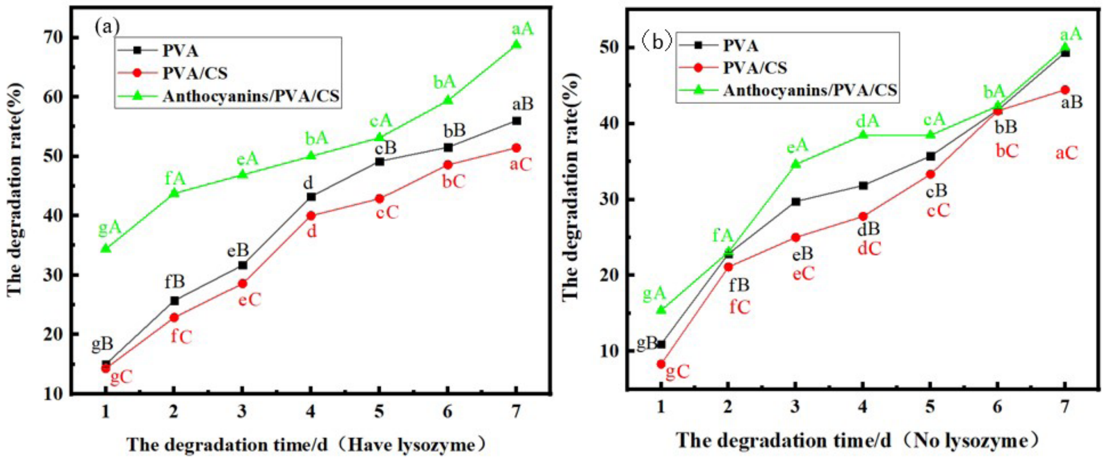

Figure 15. Film degradation diagram of different components. (a) without lysozyme(b) Including lysozyme. infuse: Different lowercase letters indicated significant difference within the group (P < 0.05). Different capital letters indicate significant differences between groups.

4 Conclusion

(1) In this paper, Anthocyanin was prepared by enzyme-ultrasonic assisted method as raw material, and the Anthocyanin/PVA/CS composite film was successfully prepared by solution casting method and Anthocyanins are mainly bonded with PVA and CS components by hydrogen bonding, and anthocyanins are well embedded in the film forming substrate, and the compatibility between components is good, and Anthocyanins are evenly distributed on the surface of the film;

(2) Anthocyanins further give the film a sensitive pH response. Composite films show different color changes in different pH environments. Generally, Anthocyanins are red at low pH, blue/purple at neutral pH, and green/yellow at high pH;

(3) After the addition of Anthocyanin, the water resistance of the film is greatly improved, and has mechanical properties comparable to pure PVA film, and has good water resistance, antibacterial, antioxidant and degradability.

Conflict of interest statement

The authors declare no conflicts of interest regarding this article.

Date availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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