Application of composite film for blueberry preservation and freshness

Yating Zhang[1], Siyu Long[1], Lulu Wang[1], Wanjiao Zhu[1], Shujuan Ji[1], Xin Zhou[1], Baodong Wei[1], Qian Zhou[1,*], and Siyao Wang[2,*]

[1]College of Food, Shenyang Agricultural University, Shenyang, China

[2]School of Public Health, Shenyang Medical College, Shenyang, China

Correspondence to: Qian Zhou, College of Food, Shenyang Agricultural University, Dongling Road, Shenhe District, Shenyang110866, China. E-mail: 66zhouqian@syau.edu.cn; Siyao Wang, School of Public Health, Shenyang Medical College, Shenyang 110034, China. E-mail: 2417018005@qq.com

Abstract

In this study, a carvacrol-allicin-anthocyanin composite film was fabricated for the preservation of postharvest blueberries and the application of freshness monitoring. In order to explore the effect of the composite film on the storage quality of postharvest blueberry fruits, 18 quality and physiological indexes of postharvest blueberry fruits were analyzed. The effect of the composite film on the freshness monitoring of blueberry fruit was investigated, and the color difference of the composite film was analyzed. The results indicated that the changes in nutrients and other indicators of blueberries during storage demonstrated that their preservation quality was maintained.. In addition, it results in

significant discoloration of three different packages of blueberry fruits commonly found on the market during storage. This study provides a theoretical basis for the postharvest preservation of blueberry fruits, and alsoprovides technical parameters for monitoring blueberry fruit freshness.

Keywords: Blueberry; Storage; Preservation film; Indicated film.

Introduction

As berries with a unique flavor and high nutritional value, blueberries are deeply loved by consumers (Yang et al.,2022 ; McAnulty et al.,2011 ; Zeng et al.,2021 ; Kalt et al.,2020). Today, the demand for blueberries is increasing as consumers become more aware of their health value (Mustafa et al.,2022 ; Gallardo et al.,2018 ; Trejo-Pech et al.,2024). Due to the high temperature and rainy season of blueberries, coupled with the thin skin and juicy skin, the fruit softens after harvest and ripening at room temperature, resulting in a great decline in quality and a lot of economic losses (Duan et al.,2022 ; Chen et al.,2024). This study provides a method for postharvest preservation and freshness monitoring of blueberries, which belongs to the field of food preservation technology; specifically, this method involves the preparation method and application of a bifunctional composite film for postharvest preservation and freshness monitoring of blueberries.

A large number of studies have shown that most of the essential oils of plants have antioxidant, antiseptic, and anticancer effects (Swamy et al.,2016; Bakkali et al., 2008).

Carvacrol is the main component of volatile organic compounds in plants, such as oregano and thyme, and can volatilize with water vapor (Tópor et al., 2024). Compared with chemical reagents, it has the advantages of low toxicity, no residue, few side effects, and environmental protection, which has a wide range of applications and has been recognized by the U.S. Food and Drug Administration (FDA) (Li et al., 2024). In recent years, carvacrol essential oil has been used in apples (Guo et al., 2023), raspberries (Wang et al.,2012), kiwi (Mi et al.,2023), grapes (Tópor et al.,2024), peaches (Wang et al., 2024), and other fruits and vegetables for preservation. Allicin has great potential as a natural additive to extend the postharvest shelf life of fruits and vegetables, and many studies have confirmed its application in plant disease control and preservation (Peng et al., 2015 ; Borlinghaus et al., 2014 ; Verma et al., 2023).

Anthocyanins are a class of water-soluble pigments that are widely found in a variety of vegetables and fruits (Khoo et al., 2017). As natural color indicators, anthocyanins can present different colors through structural changes in different pH environments due to their pH-responsive color-changing function, which can be used to make smart label packaging (Roy et al., 2021; Alappat et al., 2020; Neves et al.,2022). As shown in Figure 1, CO_2 produced by blueberries during storage can change the pH of the storage environment, resulting in different colors of the anthocyanin-containing composite film, laying the foundation for future hardware development.

The components of carvacrol and allicin in the composite film provided by this study can effectively maintain the freshness of blueberry fruits, while the addition of

anthocyanins can make the composite film reflect the freshness of blueberry fruits, which solves the problem that consumers cannot accurately judge the freshness of blueberries when buying them.

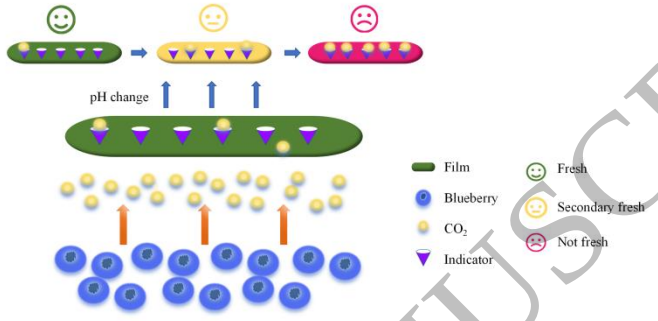


Figure 1. Composite film indicates a color-changing reaction of function

1. Materials and Methods

2.1 Sample processing

The blueberries were harvested from the 'Bluecrop' blueberry base of 'Shi Sheng' in Shenyang City, Liaoning Province. Blueberries are picked at about 80 percent ripeness, when they are usually darker in color, dark purple or blue-black. Blueberries without insect pests and obvious mechanical damage, with moderate ripeness and size, met the experimental conditions, were carefully selected, and then sent back to the laboratory of the College of Food Science, Shenyang Agricultural University, stored for 3–4 h at a temperature of 20 ± 0.5◦C to dissipate the field heat.

All blueberry fruits were stored at room temperature (20 ± 0.5◦C, 80–85 % relative humidity), and three biological replicates were required for each measurement to assess changes in the indicators after 2, 4, 6, 8, and 10 days.

2.2 Fabrication of composite film and determination of apparent properties

2.2.1 Fabrication of composite film

In this study, soybean protein isolate was used as the film-forming agent, glycerin was used as the plasticizer, carvacrol and allicin were used as preservatives, and anthocyanins were used as indicators. The production process is as follows:

(1) A total of 5.994 g of soybean protein isolate and 3 g of glycerin were weighed into a beaker, the volume was adjusted to 100 mL with distilled water, and the mixture was stirred with a magnetic stirrer (600 r/min) for 15 min;

(2) The solution obtained by procedure (1) was adjusted to pH=10 with 1 mol/L sodium hydroxide solution and heated in a water bath at 80 °C for 15 min;

(3) A total of 0.554 g carvacrol, 0.112 g allicin, and 0.1 g anthocyanins were weighed, added to the cooled solution, and homogenized at 10000 r/min for 5min to obtain the liquid for making composite films;

(4) The obtained composite film liquid was poured into a mold and put into an oven at 45 °C to dry, and the freshness and freshness monitoring composite film are obtained after softening and demoulding.

After the composite film was placed inside the blueberry packaging lid, the garlic odor would dissipate within approximately 8 h and would not affect the sensory perception of the blueberry.

2.2.2 Determination of the apparent characteristics of composite membranes

Determination of thickness: Five points were randomly selected on the composite

film and measured separately using a thickness meter, and the average value was taken.

Determination of light transmittance: The film was cut into strips and placed close to one side of the cuvette, and the absorbance was measured at a wavelength of 600 nm, with a blank cuvette as a control.

Tensile strength and elongation at break: the membrane wide strip specimen was cut, fixed on a electronic tensile testing machine, and the maximum tensile force and elongation at break of the membrane were measured.

Water vapor transmission rate: Saturated magnesium nitrate solution was added to a dryer to maintain 50% humidity. Anhydrous calcium chloride powder was added to a weighing flask, and the film was covered. The mass of the weighing flask was measured every hour for 6 h.

Water solubility: The film was cut and dried to constant weight to determine the mass of the film as M. Distilled water was added, and after 12 h, the residues were removed and dried to constant weight, and the mass was weighed as m. Solubility of the membrane (S) was calculated as follows:$S=[(M-m)/M]\times100\%$

2.3 Fruit material and treatment.

The blueberry fruit was harvested from the Shisheng Blueberry Base in Shenyang City, Liaoning Province, China. After screening, sample was pre-cooled at 4 °C, and the blueberry variety selected was L11. The harvested blueberry fruits were treated with a composite film, which was placed inside the lid of blueberry box. In the preliminary experiment (Annex 1), with three factors—carvacrol content (A), allicin content (B), and soybean protein isolate content (C)—as influencing variables, the three-factor and three-

level response surface analysis (RSBA) was designed using 15-d stored peel hardness (Y1) and soluble solids (Y2) as response parameters. Design-Except 13 software was employed to optimize the formulation of the carvacrol-allicin-flavonoid composite film. The experimental design is shown in Tables S1 and S2. The proportions of carvacrol, allicin and soybean protein isolate in the composite film were evaluated, and the optimal proportions were determined to be 0.554%, 0.112% and 5.994%, respectively. At 20±0.5 °C, the collected fruits were transferred to a polyethylene box, and the collected fruits were randomly divided into two groups (three replicates per group): the first group was not treated (control group), and the second group was covered with a composite film on the inside of the lid of the packaging box at 20±0.5 °C. The two groups of fruits were collected at 0, 2, 4, 6, 8, and 10 d of storage to determine their basic indicators. Fresh samples frozen in liquid nitrogen are packaged in ziplock bags and stored at -80 °C for additional biochemical index determination.

2.4 Determination of fruit quality indexes

2.4.1 Measurement of firmness

2.4.2 Measurement of total soluble solids

Ten fruits were randomly selected from each group, the juice was squeezed out with a mortar and filtered with four layers of clean gauze. Then, the blueberry juice was dropped into a hand-held refractometer, and the unit of soluble solids was %.

2.4.3 Measurement of total acids

A total of 10 g of fruit was randomly selected from each group, and the juice was squeezed out with a mortar. The total acid (TA) content (%) was determined by titration against NaOH, using phenolphthalein as an indicator.

2.4.4 Measurement of the rate of weight loss

The weight loss rate of blueberry fruits in each group was measured by weighing method, and the weight of the same box of blueberries was measured every 2 d.

2.4.5 Measurement of the rate of decay

One hundred fruits per replication were taken for analysis. The fruits divided into decayed fruits were those with peel rupture, yeast, rot, and loss of edible value.

2.5 Measurement of malondialdehyde (MDA) content

Three blueberry fruits were dissolved in trichloroacetic acid (TCA), centrifuged, and the supernatant was removed and mixed with thiobarbituric acid (TBA). The absorbance was measured after centrifugation.

2.6 Measurement of total phenols, flavonoids, and anthocyanin content

One gram of fruit was weighed, hydrochloric acid-methanol solution was added, the test tube was ground to a fixed volume, the mixture was extracted at 4 °C in the dark for 20 min and filtered, and the filtrate was collected to determine the absorption value.

2.7 Measurement of pectin

Water-soluble polysaccharides (WSP) and protopectin (PP) were determined by the carbazole method. The mixture was extracted with ethanol, distilled water was added, and the mixture was stored in a 50 °C water bath for 30 min by centrifugation. The supernatant was the WSP assay solution. We added the sulfuric acid solution to precipitate, boiled for 1 h, and centrifuged again. The supernatant was the PP assay solution. Absorbance was measured at 530 nm.

2.8 Measurement of cell wall metabolic enzyme activity

Frozen blueberry fruits (10 g) were ground in an ice bath using ethanol (95%) and then centrifuged, after which the supernatant was discarded. Precooled extraction buffer was added to the precipitate, and then centrifuged. The supernatant was crude enzyme solution, which was used to determine the activity of cell wall metabolic enzymes.

Polygalacturonase (PG) content was determined using a colorimetric method. Two test tubes were prepared, and sodium acetate buffer and a 1% polygalacturonic acid solution (w/v, i.e., 1 g of polygalacturonic acid dissolved in 100 mL of solvent) were added to each tube. To determine enzyme activities: Carboxymethyl cellulose (CMC) solution was added to measure the activity of Carboxymethyl cellulase (Cx), while salicin solution (note: "salicinin" may be a typo; corrected to "salicin" as the common substrate for β-Glu) was added to determine β-glucosidase (β-Glu) activity.

For each enzyme assay system: one tube was supplemented with the enzyme extract (experimental group), and the other tube was supplemented with enzyme extract that had been boiled for 5 min (as a blank control to eliminate non-enzymatic reactions). Both tubes were then incubated in a 37 °C thermostatic water bath for 1 h. After incubation, 3,5-dinitrosalicylic acid (DNS) reagent was immediately added to terminate the reaction. The mixture was boiled in boiling water for 5 min, then removed and cooled to room temperature. Finally, the absorbance values of the solutions were measured at a wavelength of 540 nm.

2.9 Measurement of antioxidant enzyme activity

Five grams of blueberry was weighed, extraction buffer was added, and the

supernatant was collected after centrifugation, which was the crude enzyme extract. Then, it was stored at low temperature to determine the activity of antioxidant enzymes.

A test tube was used, and buffer solution and catechol solution were added. Finally, the absorbance value at 420 nm was measured every minute by adding the enzyme extract solution to determine Polyphenol Oxidase (PPO) activity. Guaiacol solution and enzyme extract were added, and the absorbance value at 470 nm was measured every minute after adding H_2O_2 solution to determine Peroxidase (POD) activity. The absorbance value at 240 nm was measured every 30 s after the addition of H_2O_2 solution and extract, which was used to determine Catalase (CAT) activity. The absorbance at 290 nm was measured every 30 s after the addition of reaction buffer and enzyme extraction solution, and then H_2O_2 solution was added to determine Ascorbate Peroxidase (APX) activity.

2.10 Application and measurement of the composite film effect

In this test, three different blueberry packages commonly found on the market were selected for testing. The composite film was placed on the inner lid of the package, and a colorimeter was used to measure it directly on the outer lid to simulate the color change ($\triangle E$) that consumers actually saw. Each group of four parallels, each parallel measures five fixed points per day. The values of L^*(Lightness), a^* (Red-Green Axis) , and b^*(Yellow-Blue Axis) were measured every day, and $\triangle E$ was calculated. $\triangle E$ is calculated as follows:

$$\triangle E=(L^2+a^2+b^2)^{1/2}$$

2.11 Statistical analysis

Excel 2019 software was used for data collation, SPSS software (version 27.0; IBM, Armonk, NY, USA) was used for significance analysis ($P<0.05$), and Origin 2022 software (OriginLab, Northampton, MA, USA) was used for plotting.

3 Results

3.1 Apparent properties of composite membrane

Table 1. Measurement results of various indexes of composite membranes

Index	Unit	Result
Thickness	mm	0.12±0.01
Light transmittance	%	76.26±1.53
Tensile strength	MPa	2.32±0.21
Elongation at break	%	121.91±6.75
Water vapor transmission rate	%	0.46±0.12
Water solubility	%	46.64±3.6

The thickness of the film agent affects the mechanical properties and barrier properties of the film agent; the better the transparency of the film is, the more it can reflect the original color and appearance of the food to be preserved, and the more conducive it is to the sales of food. Materials with good mechanical properties have high tensile strength. The larger the elongation at break is, the greater the elongation of the film when it is subjected to external force, and it will not break immediately, which also reflects the flexible nature of the film from the side. Water vapor permeability and water solubility can reflect the water resistance of packaging materials, which is one of the important indicators to evaluate the water

resistance of packaging film. As can be seen in Table 1, the thickness of this composite film is 0.12 mm, the light transmittance is 76.26%, the tensile strength and elongation at break are 2.32 MPa and 121.91%, respectively, and the water vapor transmittance and water solubility are 0.46% and 46.64%, respectively. In the subsequent freshness test and freshness indication test, the film performed well, the preservation effect and indication effect were obvious, and the impact on future market sales and transportation was not found.

3.2 Effect of composite film treatment on fruit quality indexes during blueberry shelf life

Hardness is the most intuitive indicator to evaluate the quality of the fruit. As shown in Figures 2A and 2B, the peel firmness and pulp firmness of the two groups of blueberries decreased during storage, but the composite film treatment showed a positive effect on slowing the decline of blueberry fruit and peel firmness. Compared with the control group, the peel hardness of blueberries in the treatment group was significantly higher on the 8th day of storage ($P<0.05$), and the pulp hardness on the 6, 8, and 10 d of storage was significantly higher ($P<0.05$). This indicates that the composite membrane treatment can maintain the hardness of blueberry fruit during storage at room temperature.

In general, the soluble solids content can represent the sugar content of blueberry fruit, with higher sugar content indicating sweeter blueberry fruit. As can be seen from

Figure 2C, the soluble solids content of blueberry fruits decreased, increased, and then decreased gradually with the extension of the test period. On the 6 d of the experiment, the soluble solids content of blueberry fruits in the treatment group and the control group increased to the maximum value, and the soluble solids content of blueberry fruits in the treatment group was 0.89% higher than that in the control group (P<0.05). The soluble solids content of blueberry fruits in the treatment group was higher than that in the control group from 6 to 8 d of storage. The results showed that the composite membrane treatment could effectively delay the reduction in soluble solids and prolong the storage period of blueberries under normal temperature storage conditions.

Titratable acid content can directly affect the flavor of blueberry fruits, and it is also one of the main factors affecting shelf ability. Figure 2D shows that the titratable acid content of blueberry fruit first decreased but then increased with the extension of storage time at room temperature. On the 2 d of the experimental period, the titratable acid content of blueberry fruits in the control group reached a peak of 0.74%, and then decreased steadily. On the 4 d of the experimental period, the titratable acid content of blueberry fruits in the treatment group reached a peak of 0.77%, and on the 4 d and 6 d of storage, the titratable acid content of the treatment group was higher than that of the control group, with a significant difference (P<0.05), indicating that the composite film delayed the decline of titratable acid content and maintained the taste of blueberry fruits to a certain extent.

The loss of moisture and quality of blueberries is directly related to decay, and this

phenomenon is very likely to occur during storage. It can be seen from Figure 2E that the weight loss rate of blueberry fruits in the control group and the treatment group increased with the extension of storage time, and the weight loss rate of blueberry fruit in the control group and the treatment group was always higher than that in the composite film treatment group. On the 4, 6, 8, and 10 d of storage, the weight loss rate of blueberry fruits in the control group was significantly lower than that in the treatment group (P<0.05), and at 8 d, the weight loss rate of blueberry fruits in the treatment group was 1.12% lower than that in the control group. The difference between the weight loss rate of the control group and the treatment group reached a maximum, indicating that this composite film treatment could effectively inhibit the weight loss of blueberry fruits.

The rot rate can reflect the degree of disease of blueberries during storage, and is the most intuitive indicator to evaluate the storage quality of fruits. It can be seen from Figure 2F that the fruit decay rate of the two groups increased gradually, but the decay rate of the treatment group was always lower than that of the control group. On the 8 d and 10 d of storage, the decay rate of blueberry fruits in the treatment group was significantly lower than that in the control group (P<0.05), especially on the 10 d of storage, the decay rate of blueberry fruits in the treatment group was 7.34% lower than that of the control group, indicating that this composite film could reduce the decay rate of blueberry fruits.

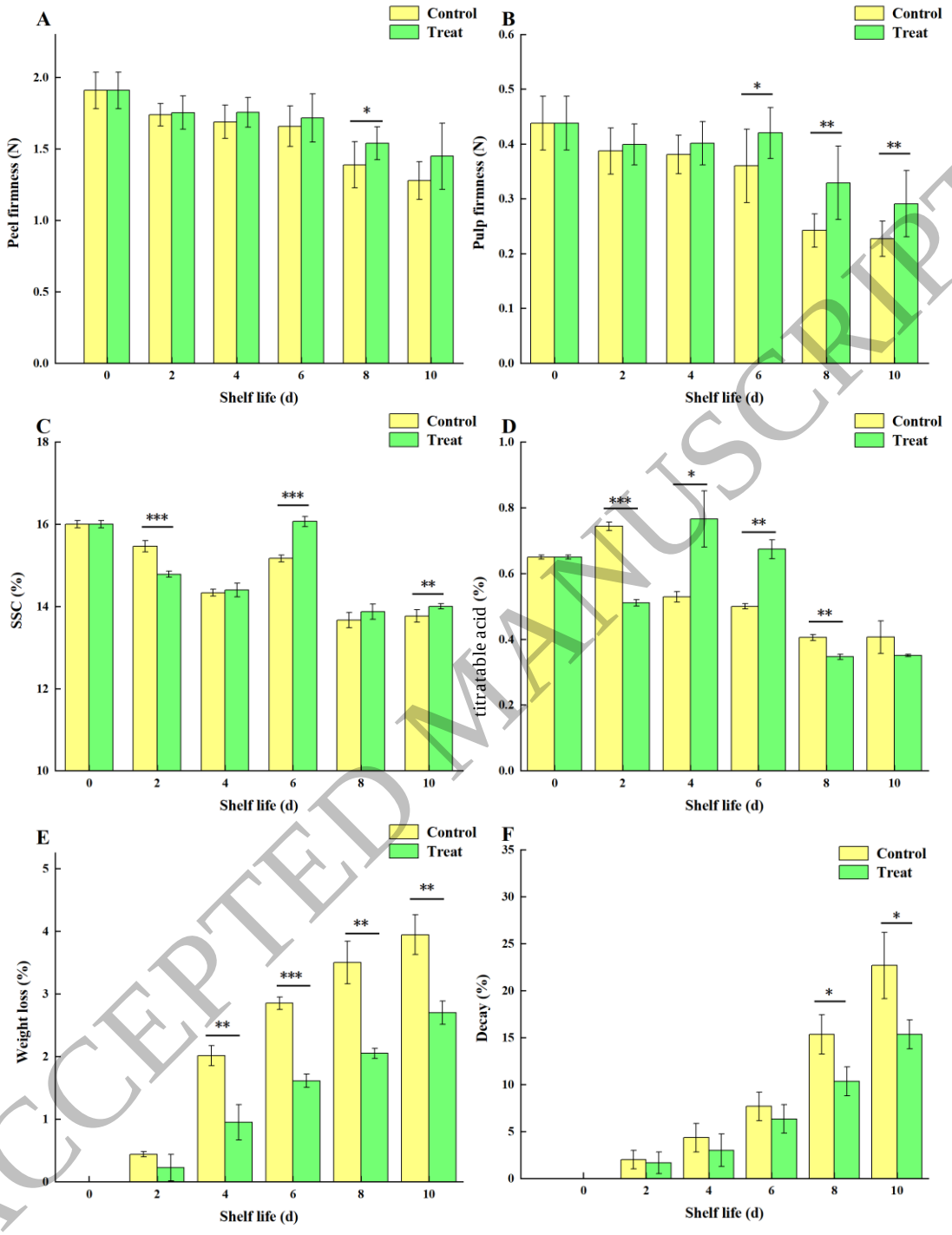


Figure 2. Peel firmness (A), pulp firmness (B), soluble solids content (SSC) (C), titratable acid (D), weight loss (E) and decay (F) of blueberry fruit during shelf storage. *P < 0.05, **P < 0.01, ***P < 0.001

3.3 Effect of composite film treatment on MDA content during blueberry shelf life

The accumulation of MDA can often be used to reflect the degree of damage to fruit by stress, as well as the potential antioxidant capacity of the fruit, and the accumulation of MDA can cause certain damage to the cytoplasmic membrane and organelles of fruits and vegetables (Ibáñez et al., 2011; Shaikh et al.,2024). It can be seen from Figure 3A that the MDA content in the control group first increased but then decreased, and that in the treatment group showed a fluctuant trend. The content of MDA in the control group was significantly higher than that in the treatment group at 2, 4, 6, and 8 d (P<0.05). In particular, on the 4th day, the MDA content in the control group peaked at 0.461 μmol/g fresh weight (FW), while that in the composite membrane treatment group was only 0.350 μmol/g FW. This also indicates that composite film treatment is less likely to cause membrane lipid peroxidation during fruit storage. In conclusion, the antioxidant capacity of blueberry fruits treated with composite film was relatively good, thereby delaying the deterioration of the quality of blueberry fruits.

3.4 Effect of composite film treatment on total phenols, flavonoids, and anthocyanins in blueberry fruit during shelf life

There are a large number of metabolites such as phenols, flavonoids, and anthocyanins in fruit and vegetable tissues, which are closely related to fruit quality and flavor, and have an important impact on the browning and anti-stress activity of fruit and vegetable tissues.

As shown in Figures 3B, 3C, and 3D, the trends of total phenols, flavonoids, and anthocyanins were similar during storage, which first decreased, then increased, and finally decreased. As shown in Figure 3B, the increase trend of the total phenolic content in the control group occurred between 6 d and 8 d, while that in the treatment group was between 2 d and 8 d. The total phenolic content in the treatment group was significantly higher than that in the control group at 4, 6, and 8 d (P<0.05). Figure 3C shows that there were significant differences in total phenolic content on the 4, 6, and 8 d of storage (P<0.05). The total phenolic contents in the treatment group were 54.73%, 91.30%, and 22.59% higher than those in the control group at 4, 6, and 8 d, respectively. Figure 3D shows that the anthocyanin content of blueberry fruits in the composite film treatment group was significantly higher than that in the control group at 4, 6, and 8 d (P<0.05). The anthocyanin content in the control group reached a peak of 1.182 (OD_{530} and OD_{600}) g/FW on the 8 d of storage, while the anthocyanin content in the treatment group reached a peak of 2.133 (OD_{530} and OD_{600}) g/FW at 8 d, which was 80.46% higher than that of the control group. In conclusion, blueberry fruit treated with composite membrane delayed the decline of total phenols, flavonoids and anthocyanins and accumulated more nutrients to ensure the quality of blueberry fruit.

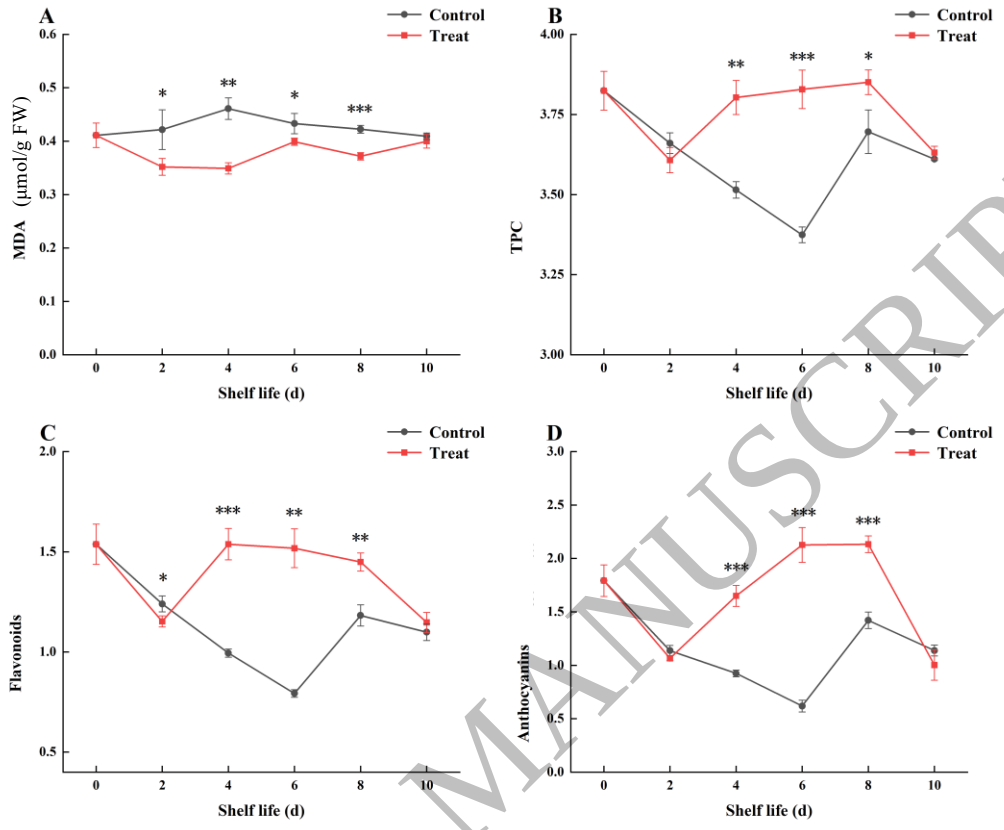


Figure 3. MDA content (A), total phenolic content (TPC) (B), flavonoid content (C), and anthocyanin content (D) of blueberry fruit during shelf storage.*P < 0.05, **P < 0.01, ***P < 0.001

3.5 Effect of composite film treatment on fruit pectin in blueberry during shelf life

When the fruit is not ripe, pectin substance combines with cellulose in the form of protopectin, and the presence of protopectin makes the fruit firm; however, with the ripening of the fruit, the protopectin gradually transforms like soluble pectin, which makes the fruit become soft and the hardness and quality decrease.

As shown in Figure 4A, the content of protopectin in the control group and the treatment group first increased but then decreased during storage, and the changes in the

composite film treatment group were particularly obvious compared with those in the control group. The content of protopectin in blueberry fruits treated with composite film was significantly higher than that in the control group at 2, 4, 6, 8, and 10 d (P<0.05). At 4 d, the protopectin content of blueberry fruits in the two groups reached the peak, and the protopectin content of the composite film treatment group was 0.122%, which was 69.44% higher than that of the control group. It was proved that the composite film treatment inhibited the hydrolysis of protopectin, thereby ensuring the stability of the protopectin content. It can be seen from Figure 4B that soluble pectin first decreased but then increased during blueberry fruit storage. During storage, the content of soluble pectin in the control group was 13.95%, 20.45%, and 15.15% higher than that in the treatment group at 4, 6, and 8 d, respectively, and the differences were significant. In summary, blueberry fruits treated with the composite film effectively inhibited the hydrolysis of the protopectin content and slowed the rise of soluble pectin to better ensure the quality of blueberries after harvest.

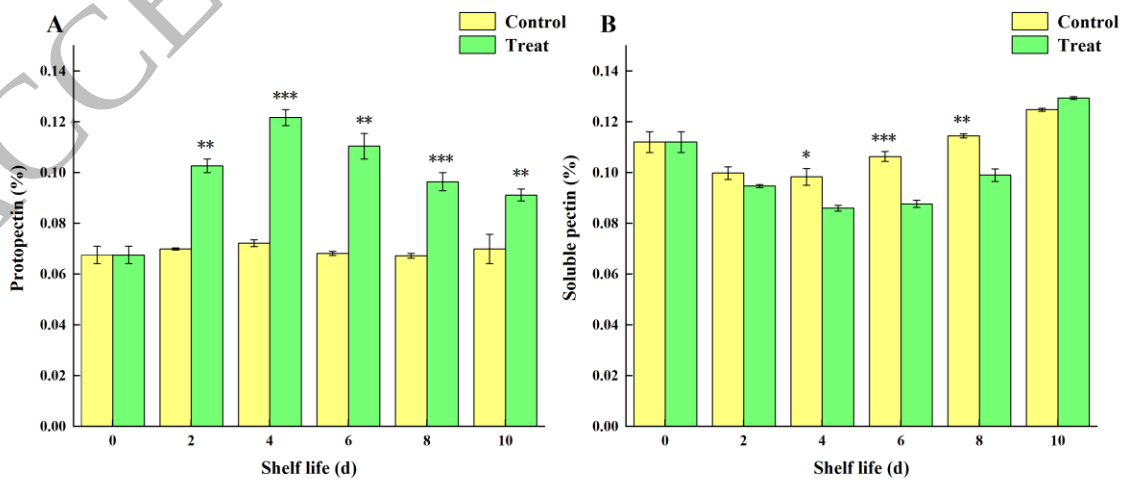


Figure 4. Protopectin (A) and soluble pectin (B) contents of blueberry fruit during shelf storage.*P < 0.05, **P < 0.01, ***P < 0.001

3.6 Effect of composite membrane treatment on cell wall degradation enzyme activity of blueberry fruit during shelf life

Figure 5A shows that the PG activity of blueberry fruits in the control group experienced fluctuant trend, while the trend in the composite film treatment group was the opposite. The trends of the control group at 0, 2, 4, 6, and 8 d were the same as those of the treatment group, and we predicted that the hydrolysis of PG was inhibited in the blueberry fruits in the composite film treatment group. The PG activity of the control group was as high as 365.758 μg/(h·g) on the 8 d of the end of the shelf stage, while the PG activity of the treatment group was 44.946 μg/(h·g) and there were significant differences at 2 d and 8 d (P<0.05).

As the main component of plant cell walls, cellulose plays an important role in supporting and protecting cells. The cellulose content in fruits and vegetables increases with the aging of fruits and vegetables, and Cx and β-Glu are gradually hydrolyzed by the cell wall, which affects the quality of fruits and vegetables. Figure 5B shows that the Cx activity of blueberry fruits in the two groups had the similar trend in the early stage of storage, and there was no significant difference. On day 4, the activity of the control group was 4323.594 μg/(h·g), while that of the treatment group was only 2130.289 μg/(h·g), with a significant difference (P<0.05). Overall, blueberry fruits in the treatment group were more stable than those in the control group were during the whole storage

period.

As shown in Figure 5C, the activity of β-Glu experienced a downward trend at the beginning of storage, and then began to increase at 6 d, followed by a decrease from 8 to 10 d. On the 10 d of storage, the activity of β-Glu in the control group was 4035.548 μg/(h·g), while that in the composite film treatment group was 4903.097 μg/(h·g), and there was a significant difference (P<0.05). In addition, the activity of the treatment group was significantly higher than that of the control group during storage (P<0.05), indicating that the composite film treatment could effectively inhibit the activity of β-Glu.

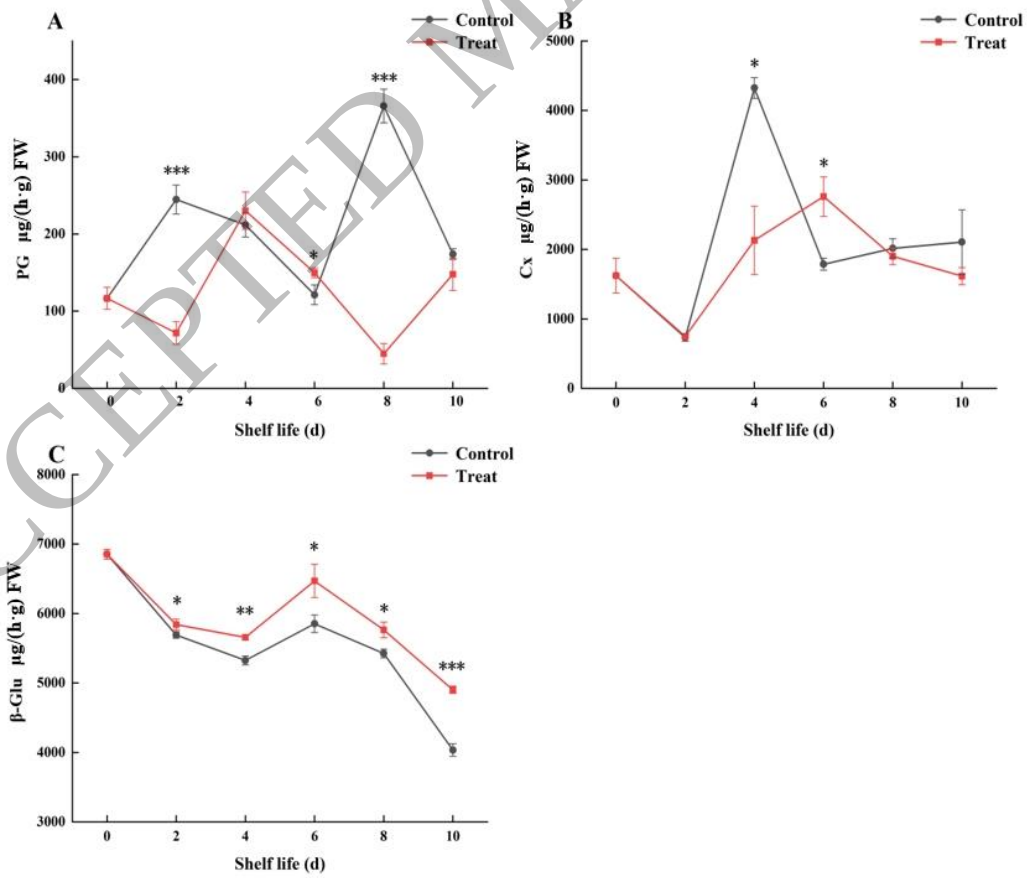


Figure 5. PG (A), Cx (B) and β-Glu (C) of blueberry fruit during shelf life.*P < 0.05, **P < 0.01, ***P < 0.001

3.7 Effect of composite film treatment on antioxidant enzyme activity of blueberry fruit during shelf life

During the postharvest ripening and senescence of fruits and vegetables, tissue browning is closely related to PPO activity. In Figure 6A, the PPO activity of the control group and the treatment group first increased but then decreased, after which increased throughout the remaining storage period. The PPO activity of the control group was higher than that of the blueberry fruit treated with the composite film throughout the storage period, and the difference was significant at 8 d and 10 d (P<0.05).

As shown in Figure 6B, the POD activity of blueberry fruits in the two groups first increased but then continued to decrease during storage, but the peak POD activity of the control group was 0.237 U/g at the 2 d, while that of the composite film treatment group was 0.263 U/g at 4 d, indicating that the composite film treatment could effectively delay the decline of POD activity, and there was a significant difference between the control group and the treatment group at 10 d of storage (P<0.05). An increase in POD can improve the ability of fruits and vegetables to resist stress, thus, it can be concluded that the composite film treatment could delay the senescence of blueberry fruits.

In Figure 6C, it was found that the CAT activity of the two groups of blueberry fruits first increased but then decreased during storage. This may be due to the fact that during the early stages of storage, the fruit maintains its own activity by increasing CAT activity

to break down hydrogen peroxide. With prolonged storage, the ability to maintain high CAT activity gradually decreased, resulting in a continuous decrease in CAT activity. On the 4 d of storage, the CAT activity of the composite film treatment group was twice that of the control group. The CAT activity in the treatment group on the 4, 6, 8, and 10 d of storage was higher than that in the control group, and there was a significant difference (P<0.05). It was proved that the treatment of composite film improved the CAT activity of blueberry under normal temperature storage conditions.

The change in APX activity is shown in Figure 6D. The APX activity in the control group is generally in a trend of first increase but then decrease, while the composite film treatment group is generally in a state of continuous increase. During the whole storage period, the APX activity of blueberry fruit in the treatment group was significantly higher than that in the control group (P<0.05).

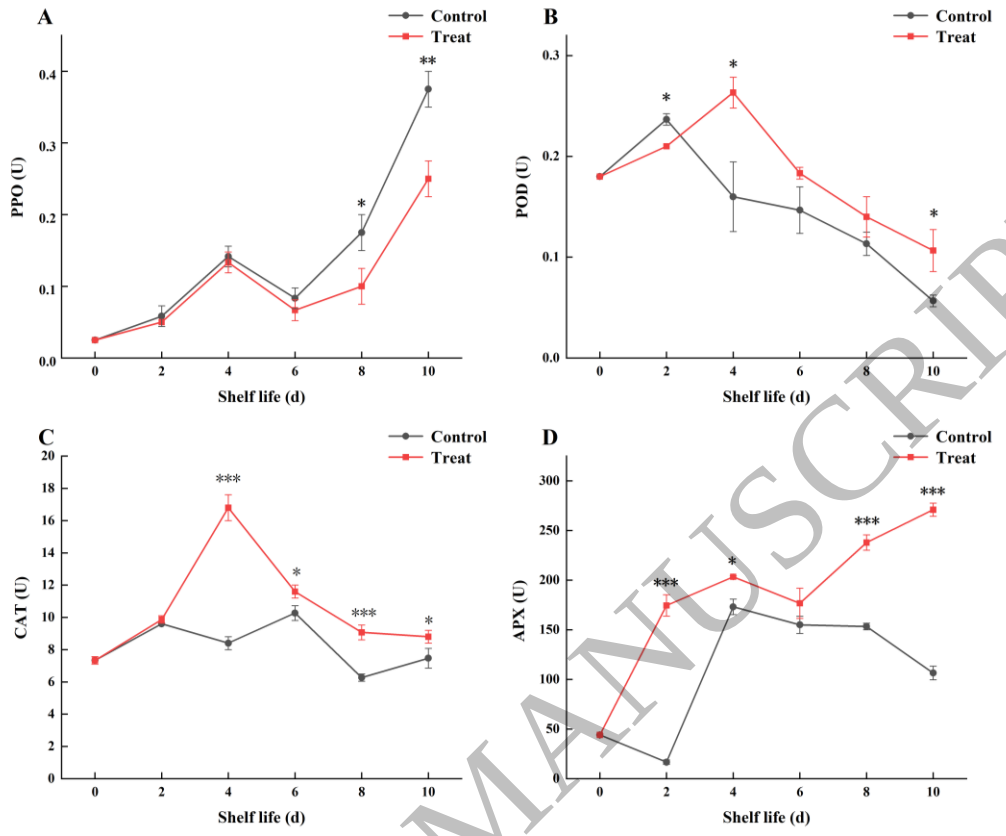


Figure 6. PPO (A), POD (B), CAT (C) and APX (D) activities of blueberry fruit during shelf life. *P < 0.05, **P < 0.01, ***P < 0.001

3.8 Effect of composite film treatment on discoloration of blueberry fruit during shelf life

From left to right, Figure 7A shows the discoloration of the composite films placed in the square box, round box, and honeycomb box, with each composite film exhibiting two colors. Since honeycomb box is evenly distributed in blueberries, and consumers can see that it is the background color of blueberries in some places and the color of the film itself in some places, both images were shot and recorded in this experiment. It can be seen that the square box, round box, and honeycomb box have obvious changes with the

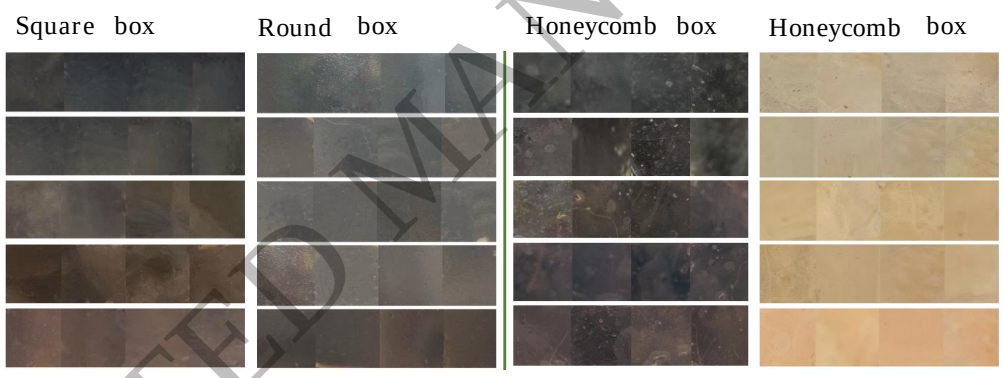
naked eye after 5 d of storage at room temperature, and the background color of the blueberries gradually turned red during storage. Combined with the data in Table 2, during the period when blueberries do not begin to decay over a large area, the composite film simulated by consumers has obvious discoloration, indicating that this composite film has a color development effect on common blueberry packaging on the market when consumers cannot see decay and can be used to monitor the freshness of blueberries.

Figures 7B, 7C, 7D, and 7E show the L^*, a, b, and $\triangle E$ values of the three packages, respectively, which show that these three indicators are in a continuous upward trend during the storage of blueberry fruits and vegetables. In Figure 7B, the L^* value of the honeycomb box was significantly different from those of the square box and the round box during the whole storage period (P<0.05). In Figure 7C, the round box and the honeycomb box had significant discoloration, and there were significant differences between the round box and the honeycomb box during storage (P<0.05). Figure 7D shows the change trend of b value during storage of the three packages, and it can be found that the change in the b value of the square box is not significant; on the contrary, the change trend of b value of the honeycomb box is the most significant, and there is a significant difference between the other two groups at the end of the storage period on the 6 d of the storage period (P<0.05). For the analysis of the change in $\triangle E$, as shown in Figure 7E, the change in the $\triangle E$ value of the square box and the honeycomb box is more significant than that of the round box. To sum up, the comprehensive analysis revealed that the composite film is the most prominent in the honeycomb box packaging of these

three blueberry fruit packages, and this test also provides an effective reference value for the technology of freshness monitoring of composite film in later stages.

Table 2. Decay of blueberry fruit in different packaging during shelf life

Storage period (d)	Square box	Round box	Honeycomb box
1	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{b}
2	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{b}
3	0.50±0.50^{c}	0.83±0.83^{c}	0.00±0.00^{b}
4	3.50±0.50^{b}	4.17±0.83^{b}	0.00±0.00^{b}
5	6.50±0.50^{a}	5.83±1.60^{ab}	2.50±1.44^{a}



A

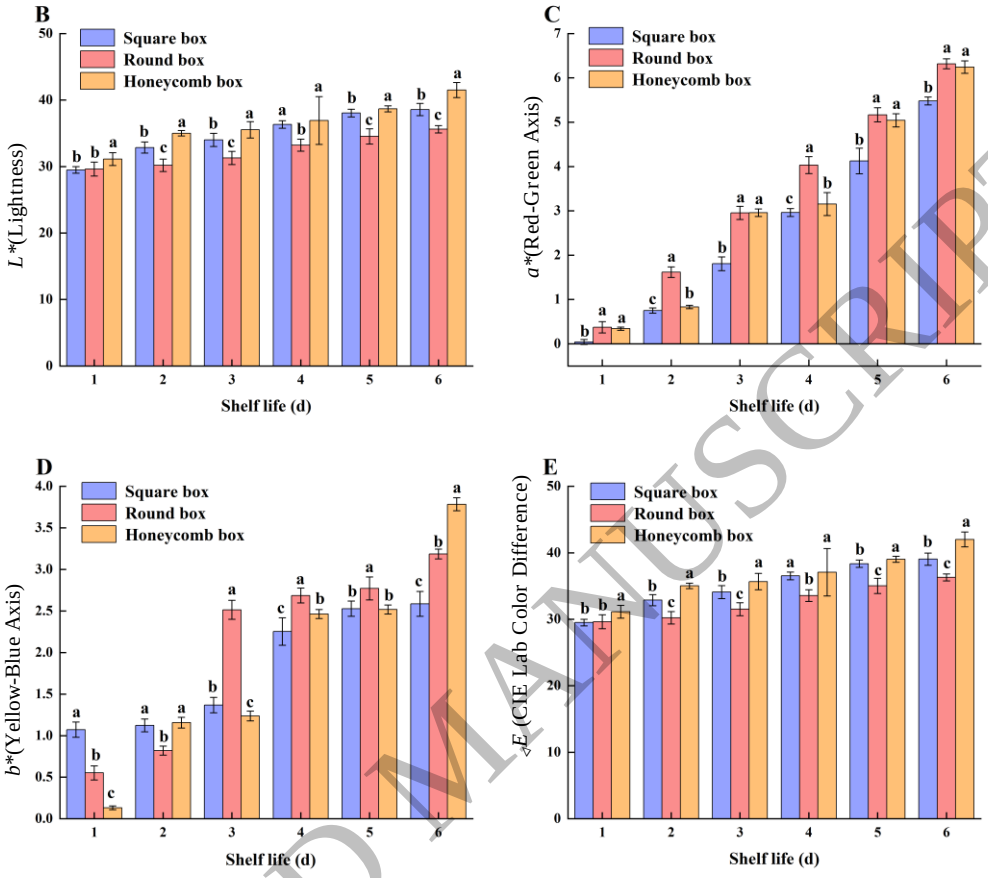


Figure 7. Discoloration condition (A), L^* (B), a (C), b (D) and $\triangle E$ (E) of blueberry fruit in different packaging during shelf life.

Discussion

Blueberries are sweet and sour and are rich in nutrients. However, blueberry fruits are very susceptible to external factors after harvest and quickly lose water, soften, and rot, leading to quality deterioration, and in severe cases, shortening their shelf life, which results in a reduction in both edible value and commercial value. In addition, due to factors such as temperature, transportation, and other force majeure, when purchasing blueberries, the freshness of blueberries cannot be monitored solely based on the

production date, which may affect consumers' judgment of freshness. Therefore, this study aims to extend the storage period of blueberry fruits and provide a theoretical basis for monitoring freshness. To this end, a two-in-one composite film was fabricated with soybean protein isolate as the film-forming agent, glycerol as the plasticizer, carvacrol and allicin as preservatives, and anthocyanin as the freshness indicator for freshness monitoring and color development. The composite film was applied to the inside of the blueberry fruit packaging box, and the changes in various indexes of blueberry fruit and the discoloration of the composite film were observed during storage at room temperature (20±0.5 °C).

The change in fruit texture is closely associated with the hardness index. For blueberries, hardness is not only one of the main factors limiting their postharvest shelf life (Angeletti et al., 2010) but also affects their resistance to postharvest transportation stress and physiological diseases—ultimately reducing their commercial value (Chen et al., 2015). Consistently, a decrease in hardness during storage has been observed in various fruits, including blueberries (Casorzo et al., 2024), bananas (Deng et al., 2017), strawberries (Nasrin et al., 2017), and peaches (Allegra et al., 2013).The results showed that the peel and pulp hardness of the two groups of blueberry fruits decreased during storage, but the flavor of the blueberry fruits was maintained well from 0 to 4 d in the early stage of storage, which greatly preserved the good taste of the blueberries. On the 6th to 8th days of storage, the peel hardness of the untreated blueberries decreased greatly, the fruit began to soften, and the titratable acids and soluble solids also decreased

significantly. Meanwhile, the hardness of the blueberries in the composite film treatment group decreased, but the fruit firmness remained stable and retained good flavor. From the 8th to the 10th days of storage, compared with the treatment group, the control group began to experience more severe large-scale decay of blueberry fruits, and the epidermis began to shrink due to water loss, which greatly affected the nutritional value and commercial value of the fruits.

Some nutrients in fruits gradually decline with the extension of storage time, and a large number of phenolic substances, flavonoids and anthocyanins in fruits and vegetables have an important impact on the color, quality, flavor, and tissue browning of fruits and vegetables. However, phenols have strong antioxidant functions and can delay browning and lipid peroxidation, which are associated with tissue browning of blueberry fruits (Yan et al.,2020). Experimental results revealed that the total phenolic content of blueberry fruits in the two groups decreased, increased, and then increased, and this trend was similar to that of Wang et al. (2023). The results indicated that the composite membrane treatment could greatly stimulate the accumulation of total phenols (P<0.05), indicating that it could effectively alleviate the browning of blueberries. Similarly, the combination of 1-Methylcyclopropene (1-MCP) and refrigeration was found to be effective in delaying the decline of total phenols in apples (Hoang et al., 2011). With the extension of the storage period, we found that the flavonoids and anthocyanins of blueberry fruits treated with composite film were higher than those of untreated blueberries and the composite film could effectively delay the decline in flavonoids and

anthocyanins of blueberry fruits, so as to maintain higher nutritional quality of the fruits. We also found that total phenols, flavonoids, and anthocyanins showed similar trends during storage, which may be due to the consistent trend of nutrients in blueberry fruits during storage. In addition, flavonoids play an important role in the prevention of cardiovascular disease (Ciumărnean et al.,2020). Anthocyanins, on the other hand, account for 60%-70% of the total phenols in blueberries and are pigments that give the surface of the fruit its blue color (Yan et al.,2023).

The senescence of fruits is related to the balance of reactive oxygen species metabolism (Shang et al.,2021). Reactive oxygen species are by-products of various plant metabolic pathways, and the fruit itself produces some reactive oxygen species under certain physiological metabolism conditions (Fang et al.,2021). However, because of the existence of reactive oxygen species scavenging system in fruits and vegetables, mainly including antioxidant enzymes to weaken the ability of fruits to produce reactive oxygen species or offset the excessive production of reactive oxygen species (Apel et al., 2004), fruits can delay aging by increasing their own antioxidant enzyme activity. PPO, as a copper-based enzyme, can catalyze the formation of quinones from a variety of phenolic compounds, which are closely related to tissue browning due to aging during the storage of fruits and vegetables (Hu et al.,2015). The results of this study revealed that the surface composite film treatment could effectively reduce the PPO activity, thereby delaying fruit browning and senescence.

As a dynamic structure, cell wall can generally determine the outcome of the

interaction between plants and pathogens; thus, when pathogens are in the early stages of infection, the integrity of the plant cell wall is disrupted (Bellincampi et al.,2014). Some cell wall degradation enzymes (eg. PG, β-Glu, and Cx) catalyze changes in cell wall polysaccharides (Chen et al.,2015), and the degradation of cell wall polysaccharides leads to softening in most fruits, including blueberries (Liu et al.,2019). Our enzyme assay results showed that the activity of cell wall degrading enzymes in the treated blueberry fruit was significantly lower than that of the cell wall degrading enzymes in the untreated blueberry fruit. The components of the cell wall, including pectin, lead to a decrease in the storage period and fruit quality (Wei et al., 2015). As pectin dissolves, the structure of the cell wall changes (Sun et al., 2015). In conclusion, blueberries treated with composite membrane effectively inhibited the increase of soluble pectin content and effectively maintained the accumulation and preservation of protopectin compared with the untreated blueberries, which helped to inhibit the dissolution of cell wall polysaccharides, maintain the hardness of blueberries, and delay the softening of fruits.

In recent years, with the increasing attention of consumers to food safety, research on food packaging related to freshness monitoring has become popular. Smart packaging for freshness indication is actually a simple and scientific way to directly determine the freshness of food (Shao et al., 2021). Under the action of microorganisms, with the extension of food storage, food will produce some characteristic releases with the external environment, and these releases are used as the basis for monitoring the freshness of food. At present, the market is mainly based on carbon dioxide, volatile

alkaline nitrogen, sulfide, and some other characteristic substances. For example, a freshness indicator label with a ratio of methyl red to bromocresol blue was used to determine the freshness of freshly cut green peppers (Chen et al.,2018); composite indicator labels based on bromothymol blue and methyl red were used to detect the freshness of pork (Chen et al.,2019); and durian freshness monitoring was carried out by volatile sulfides using a novel starch-chitosan complex pH dye (Niponsak et al.,2016). Anthocyanins, the discoloration indicator used in this study, are safer and healthier than some chemical reagents such as methyl red, and the results show that the discoloration range of anthocyanins can be distinguished under naked eye observation. Total color difference (ΔE) can be used as a parameter to distinguish color difference between images. When ΔE>5, the color difference can be distinguished by the naked eye (Li et al. 2023). Therefore, it is feasible to apply anthocyanins to the technology of freshness monitoring.

Conclusions

In conclusion, the composite film containing carvacrol and allicin can reduce the weight loss rate and decay rate of blueberry fruit at room temperature, and a decrease in hardness also has a positive slowing effect. Studies have shown that this is achieved by the accumulation of pectin in blueberries, the stability of stress resistance-related enzyme activity, and the maintenance of cell wall-associated enzyme activity. In addition, due to the addition of anthocyanins in the composite film, consumers can rely on the degree of discoloration of the composite film to identify freshness when purchasing fruits, which

also provides relevant theoretical support for future blueberry fruit freshness monitoring technology.

CRediT authorship contribution statement

Yating Zhang: Writing - Original Draft, Data Curation, Writing - Review & Editing, Conceptualization. **Siyu Long:** Writing - Review & Editing, Validation, Formal analysis. **Lulu Wang:** Writing - Review & Editing, Validation, Formal analysis. **Wanjiao Zhu:**Visualization, Methodology.**Shujuan Ji:**Validation, Visualization. **Xin Zhou:**Visualization. **Baodong Wei:** Visualization. **Qian Zhou:**Writing - Review & Editing, Funding acquistion, Project administration, Resources.**Siyao Wang:**Visualization, Methodology,Funding acquistion

Declaration 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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Appendix

Schedule 1 Factor levels of RSD

level	A carvacrolcontent （%）	B Garlic content （%）	C SPI content （%）
-1	0.4	0.1	5
0	0.5	0.15	6
1	0.6	0.2	7

Schedule 2 Design and results of RSD test

number	factor A Carvacrol content %	B Garlic content %	C SPI content %	Y1 Peel firmness N	Y2 Soluble solids %
1	0.4	0.1	6	2.71	13.42
2	0.6	0.1	6	2.76	13.91
3	0.4	0.2	6	2.71	13.66
4	0.6	0.2	6	2.68	14.02

5	0.4	0.15	5	2.65	14.56
6	0.6	0.15	5	2.76	14.42
7	0.4	0.15	7	2.73	14.02
8	0.6	0.15	7	2.69	14.75
9	0.5	0.1	5	2.73	13.48
10	0.5	0.2	5	2.76	14.10
11	0.5	0.1	7	2.77	13.83
12	0.5	0.2	7	2.73	13.28
13	0.5	0.15	6	2.87	15.36
14	0.5	0.15	6	2.84	15.4
15	0.5	0.15	6	2.87	15.22
16	0.5	0.15	6	2.87	15.47
17	0.5	0.15	6	2.86	15.29

Highlights

- Carvacrol-allicin-anthocyanin complex film can significantly improve the nutrients of blueberry fruit
- The carvacrol-allicin-anthocyanin composite film can also monitor the freshness of blueberry fruit during no rot rate
- Carvacrol-allicin-anthocyanin composite film can achieve the dual function of preserving freshness and monitoring freshnes