American Journal of Biochemistry and Biotechnology

Original Research Paper

Preparation of D-Phenyllactic Acid-Chitosan Biodegradable Composite Coating and Its Preservation Effect on Shine-Muscat Grape

[1]Dongxing Zhu, [1]Guanglun Xiong, [1]Wenli Tang, [1]Jin Tang, [1]Jiayang Liu, [1]Zitong Chen, [1]Meiqi Sun, [2]Xiaoyu Fan, [2]Caijun Dong, [2]Yingying Song, [1]Yiyong Chen and [1]Mengqi Zhang

[1]School of Biology and Food Engineering, Changshu Institute of Technology, Changshu, China
[2]School of Environment and Bioengineering, Nantong College of Science and Technology, Nantong, China

Article history
Received: 15-10-2024
Revised: 14-01-2025
Accepted: 16-01-2025

Corresponding Author:
Yiyong Chen and Mengqi Zhang
School of Biology and Food Engineering, Changshu Institute of Technology, Changshu, China
Email: greenpop6688@126.com; mengqizhang@cslg.edu.cn

Abstract: Considering the pollution of plastic packaging and the harm of traditional food containing sulphur to the environment and human health, the development of green and edible coating materials with biodegradable power has become the focus of attention in recent years. In this study, based on a single factor test, D-Phenyl Lactic Acid-Chitosan Biodegradable Composite Coating (PACBC) was prepared by optimizing the formula of D-Phenyllactic Acid (D-PLA), Chitosan (Cs), calcium chloride, Ascorbic Acid (AA) by Response Surface Method (RSM). The preservation effect of PACBC with the optimal formula on the Shine-Muscat grape was also studied. The specific sensory indexes, flavour and nutritional quality during storage were determined. The microscopic morphology of grape peels was observed using scanning electron microscopy (SEM). The results showed that the optimal formula of PACBC was as follows: D-PLA was 0.792%, Cs was 0.484%, calcium chloride was 0.5%, and AA was 0.85%. Compared with the blank control CK1, on the sixth day of storage, PACBC prepared with the optimal formula decreased the decay rate, berry abscission ratio, browning incidence and weight loss of grapes and increased the grape firmness. The microstructure of grape peels was dense and complete. In addition, the grapes were treated with PACBC, and their appearance, colour, and freshness were good. The loss of Titratable Acid (TA), Total Soluble Solids (TSS), AA and Total Phenols (TP) in grapes was delayed. The preservation effect of PACBC with the optimal formula was better than that of the positive control (CK2) without D-PLA. In this study, compared with the traditional sulphur dioxide and plastic packaging for grape preservation, PACBC will provide a potential alternative for grape green preservation.

Keywords: D-Phenyllactic Acid, Chitosan, Composite Coating, Shine-Muscat Grape, Preservation, Preparation

Introduction

Grapes (*Vitis vinifera* L.) are one of the four largest fruit trees in the world and an important economic fruit in China (Liu *et al*., 2020). According to statistics, China's total grape production in 2021 reached 15,0679,900 tons, ranking first in the world, and consumption accounted for about 20% of China's total fruit. Table grapes accounted for more than 80%, with good market prospects in China. In the past 10 years, the Shine-Muscat grape has had excellent quality with an output value of 750,000-1.5 million Yuan/hm^2, which has become the grape variety with the highest output value per unit area in China (Yang *et al*., 2018). However, as a berry, it is susceptible to fungal infection and self-aging (Chervin *et al*., 2004). Improper storage and transportation resulted in annual postharvest losses of more than 20% of table grapes, including the Shine-Muscat grape. In recent years, with the rapid growth of e-commerce sales logistics, grapes are not suitable for centralized batch cold chain storage and transportation. Sulfur dioxide (Romanazzi *et al*., 2012), chlorine dioxide (Zhang *et al*., 2021a) and other chemical preservants were generally used at room temperature combined with Modified Atmosphere Packaging (MAP)

to store and transport grapes. Although sulphur dioxide has been recognized as an effective chemical preservative for grapes for many years (Winkler *et al*., 1925), it has been widely used commercially. As a physical preservation method, MAP can form a suitable environment of low oxygen and high dioxide ponds in breathable plastic packaging, which can inhibit the respiratory decomposition of fruits and vegetables and avoid infection by pathogenic bacteria (JieYu *et al*., 2013). It has become an important means of lowering costs and making the operation of fruits and vegetables easy after harvest preservation (Silva-Sanzana *et al*., 2016). Sulfur residues and refractory packaging plastics (Hillmyer *et al*., 2017; Phelan *et al*., 2020) will cause food and environmental safety issues. Therefore, the development of green technology of sulphur replacement for grape preservation and degradable packaging materials has become the focus of attention (Umaraw *et al*., 2020; Ye *et al*., 2022).

Currently, physics (Artés-Hernández *et al*., 2006) and chemistry (Raban *et al*., 2013) have been applied to the preservation of grapes. Treatment with ethylene receptor inhibitor 1-Methylcyclopropene (1-MCP) can improve the shelf life quality of Shine-Muscat grapes and inhibit the binding of ethylene to the receptor and the expression of key genes of its signalling pathway (Zhang *et al*., 2021a). Inoculation with yeast T-2 can inhibit mould growth and fruit decay of Shine-Muscat grapes (Wu *et al*., 2022). Treatment with chitosan and ozone can regulate gene expression of Shine-Muscat grapes and maintain the content of aromatic substances in fruits. Low-temperature storage is conducive to the preservation of Shine-Muscat grapes. However, it is not conducive to the retention of aromatic components (Zhang *et al*., 2016). The combination of microenvironment air conditioning fresh-keeping tank, biological fresh-keeping agent and 1-MCP treatment is conducive to the retention of aromatic substances and fruit preservation of Shine-Muscat grapes during the cold storage period. In addition, the edible composite coating treatment prepared with natural polysaccharide (konjac glucomannan) as a forming agent improved the postharvest preservation ability of Shine-Muscat grapes (Wu *et al*., 2023). However, as far as we know, there is little research data on other natural edible coatings used in the preservation of Shine-Muscat grapes.

Different from other preservation methods, edible coating is prepared from natural edible materials (polysaccharides, proteins, lipids), plasticizers, crosslinking agents or antibacterial agents and antioxidants, which is a food coating liquid with good safety and preservation performance (Galus *et al*., 2015). As a convenient biological fresh-keeping method, it can replace plastic synthetic packaging and become the focus of fruit and vegetable fresh-keeping research (Galus *et al*., 2015; Ding *et al*., 2023). It extends the storage period of fruits and vegetables by coating the surface of fruits and vegetables with a semi-permeable film that prevents air, moisture and microbial invasion. Among them, Chitosan (CS) is a natural polysaccharide with good film formation, bacteriological inhibition, gas permeability and degradability (Zhang *et al*., 2021b), which has become the focus of attention (Mujtaba *et al*., 2019). Chitosan solution dissolved with acetic acid had a significant inhibitory effect on the grey mould of grapes. Compared with low molecular weight chitosan, coating films prepared from high molecular weight (Molecular weight>100kDa) chitosan have better performance (Zhang *et al*., 2022). It has a better fresh-keeping effect on mangoes (Jongsri *et al*., 2016). However, the combination of chitosan with other antibacterial materials, antioxidants and crosslinking agents can improve the antibacterial function, mechanical strength and other characteristics of the membrane (Ding *et al*., 2023). Green antibacterial edible coating combined with natural antibacterial materials is the direction for future development.

D-phenyllactic acid (D-PLA) is a green broad-spectrum biological antimicrobial preservative discovered in recent years (Singh, 2018). The antibacterial activity of D-PLA is stronger than that of common food chemical preservatives such as sodium benzoate and potassium sorbate (Dieuleveux *et al*., 1998). D-PLA has a wider antibacterial spectrum than nisin, which not only has strong antibacterial and anticorrosive properties on food but also can effectively inhibit the enzymatic browning of fruits and vegetables (Ren *et al*., 2021). D-PLA has a synergistic promotion effect with other bacteriostatic agents and has broad application prospects in the field of food preservation. However, D-PLA has good hydrophilicity and low viscosity of its solution, which makes it difficult to attach to the surface of waxy fruits to form cling film alone (Shan *et al*., 2018). A suitable film coating agent should be selected as the carrier. The combination with film-forming agents is also one of the new directions for the development of natural food preservatives. Ascorbic Acid (AA) and calcium chloride are edible components and are widely used as antioxidants and crosslinkers for edible coatings (Soliva-Fortuny *et al*., 2002). Chitosan film combined with calcium chloride could inhibit the pectin degradation of melon cell wall, maintain fruit hardness and extend shelf life (Chong *et al*., 2015). The composite film prepared by chitosan combined with AA at the appropriate ratio could reduce the permeability and hydrophilicity of the chitosan film-forming agent and improve antioxidant properties (Tan *et al*., 2019). At present, there is no report on the application of edible coating film of D-PLA-CS combined with calcium chloride and AA to the preservation of Shine-Muscat grapes.

On the basis of our previous studies (Wan *et al*., 2018), D-PLA was used as the antibacterial agent. Cs was used as the film-forming agent carrier. AA was used as the antioxidant, and calcium chloride was used as the crosslinking agent. On the basis of a single factor, the formula of D-Phenyllactic Acid-Chitosan Biodegradable Composite Coating (PACBC) was optimized by the Response Surface Method (RSM). The effect of PACBC on grapefruit quality and surface microstructure during the storage period was also explored. The purpose of this study is to find a green preservation technology for Shine-Muscat grapes to replace traditional technology such as sulphur dioxide and plastic packaging.

Materials and Methods

Materials and Chemicals

Shine-Muscat grape was collected from the Changshu grape production base (Changshu, China). Chitosan (relative molecular weight 200kDa) was purchased from Shanghai Macklin Biotechnology Co., Ltd. (Shanghai, China). D-phenyllactic acid was purchased from Shanghai Haohong Biopharmaceutical Technology Co., Ltd (Shanghai, China). L-ascorbic acid was purchased from Shijiazhuang Pharmaceutical Co., Ltd (Hebei, China). Calcium chloride was purchased from Zhejiang Dacheng Calcium Industry Co., Ltd (Zhejiang, China). Other chemical reagents are analytically pure.

Preparation of PACBC and grapes preservation treatment. PACBC was prepared based on the existing methods (Martínez-Camacho *et al*., 2010) with some modifications. The Cs were added into a glacial acetic acid solvent (2%,v/v), heated in a water bath at 50°C and stirred continuously for 4 h to be completely dissolved. After the chitosan solution was centrifuged (4000r/min) for 10 minutes, the supernatant was taken and then cooled to 23±1°C. The CS solution was prepared by adjusting the pH value of the supernatant to about 5.4 with 0.1 mol/L sodium hydroxide solution. D-PLA solution was prepared by weighing a certain mass of D-PLA and dissolving it in a small amount of ethanol. Cs and D-PLA solution were mixed, and then a certain mass of AA and calcium chloride were added to the mixture, respectively, and stirred until completely dissolved. Deionized water was added to the required volume. PACBC with different proportions was obtained.

The harvested grapes were treated with PACBC for each group of nine clusters. After treatment, each of the three clusters was divided into a storage box, and each group was packed in three boxes (three repeats). Each group of grapes was soaked in PACBC with a corresponding concentration ratio for 2 min at room temperature. The normal temperature logistics storage and transportation environment of the e-commerce business was simulated. Each group of grapes was stored in a polystyrene storage box (50×39×28 cm) at 24±2°C and 75±10% relative humidity. On the ^{sixth} day, the firmness, weight loss, decay rate, browning incidence and comprehensive evaluation score of treated grapes of each group were evaluated to investigate the preservation effect of PACBC with different mass concentrations and ratios. The decay rate, berry abscission ratio, browning incidence and weight loss

Determination of Fruit Firmness and Weight Loss

The grape firmness was determined by TA-XT plus texture analyzer (Stable Micro Systems, UK) using the puncture mode of the P/2n needle probe. The probe speed was 2.0 mm/s. The puncture depth was 7 mm, and the minimum perception was 0.049 N. Nine clusters of grapes were measured in parallel in each group. Five grapes were selected at the top of each cluster and the middle of the four directions, respectively. The average value (N) was calculated.

The weighing method was used to determine the daily weight loss value and the percentage of ear weight on the first day of storage (Chen *et al*., 2015; Chiarelli *et al*., 2011). Fruit weight loss was calculated according to the following formula:

$$\text{Weight loss } (\%) = \frac{W_0 - W_n}{W_0} \times 100 \quad (1)$$

where, W_0 is the mass of each ear treated on the postharvest day, *g*; W_n is the mass per ear on the *n* day of storage, *g*.

Determination of Decay Rate and Browning Incidence

Determination of decay rate and browning incidence was performed according to the previous methods (Zhang *et al*., 2021a) with some modifications. The sensory evaluation method was used to record the total fruit and the number of rotten and browned fruit in each treatment group regularly after treatment. When the fruit had a plaque, water, mildew and other conditions, it was identified as rotten fruit. When the diameter of the browning spot on the surface of the fruit was more than 2 mm, it was identified as brown fruit. The decay rate was calculated according to formula (2), and the browning incidence was calculated according to formula (3).

$$Decay\ rate(\%) = (D_n/A_0) \times 100 \quad (2)$$

where, D_n is the number of rotten fruit in each group on the nth day of storage; A_0 is the total number of fruit in each group:

$$Browning\ incidence(\%) = (B_n/A_0) \times 100 \quad (3)$$

where, B_n is the number of browning grains in each group on the n^{th} day of storage; A_0 is the total number of grains in each group.

Comprehensive Score

The comprehensive score was determined according to the reported method with some modifications. In the optimization experiment, the comprehensive score of the fruit decay rate, firmness, weight loss and browning incidence of each treatment group was used as evaluation indexes. The weight coefficients of the four indexes were 0.4, 0.3, 0.15 and 0.15, respectively. The comprehensive score was calculated according to Formula (4):

$$Comprehensive\ score = (100\% - D_i) \times 0.4 + \frac{(F_i - F_{\min})}{(F_{\max} - F_{\min})} \times 0.3 + (100\% - W_i) \times 0.15 + (100\% - B_i) \times 0.15 \quad (4)$$

where, D_i is the measured value of the fruit decay rate of each group, F_i is the measured value of fruit firmness of each group. F_{min} is the minimum value of firmness of 29 groups of *BBD* design. F_{max} is the maximum value of firmness of 29 groups of BBD design. W_i is the measured value of weight loss for each group. B_i is the measured value of fruit browning incidence of each group.

Optimization of Formula of PACBC by RSM

First of all, according to the method of Fan *et al*. (2019), the concentration range of each composite membrane component was determined by single-factor experiments. On the basis of the preliminary single tests, the mass concentration of Cs (X_1), D-PLA (X_2), calcium chloride (X_3) and AA (X_4) were taken as independent variables. The comprehensive score (Y) of the decay rate, weight loss, browning incidence and firmness was taken as the response value. Through the Design Expert V8.0.6.1 software (Stat-Ease, Minneapolis, MN, USA), 29 trials with four factors and three levels were carried out to optimize the formula of PACBC using RSM (Soto-Muñoz *et al*., 2021). The test scheme is shown in Table (1).

Table 1: Independent variables and their levels used for response surface test

Independent variables	Levels		
	-1	0	1
Mass concentration of Cs /% (X_1)	0.05	0.45	0.85
Mass concentration of D-PLA /% (X_2)	0.4	0.8	1.2
Mass concentration of calcium chloride /% (X_3)	0.5	1	1.5
Mass concentration of AA/% (X_4)	0.85	1.35	1.85

Validation of the Preservation Effect of PACBC with Optimal Formula on Grape during Storage

On the basis of the above response surface test, the grapes soaked in PACBC with optimal formula were used as the treatment group (T). The grapes soaked in sterile distilled water for the same time were used as the blank control group (CK1). The grapes soaked in C_S-optimized composite coating (C_S-$CaCl_2$-AA) without D-PLA for the same time were used as the positive control group (CK2). The commercial logistics storage and transportation environment of grapes were simulated at normal temperatures. After treatment, the grapes in each group were stored at 24±2°C and 75±10% relative humidity. The appearance, pericarp microstructure, berry abscission ratio, Total Soluble Solids (TSS), Titratable Acid (TA), AA and Total Phenols (TP) of grapes were determined on the 0^{th}, $^{third\ and\ sixth}$ day of storage to verify the preservation effect of PACBC with optimal formula on grapes during storage.

Determination of TSS

Nine clusters of grapes in each group were measured in parallel. Five grapes were selected at the top and four sides of each cluster. A total of 45 grapes were ground into homogenate in an ice bath and then centrifuged at 10000 rpm for 20 min at 4℃. The supernatant was used to determine TSS and TA. The TSS content was determined with an Abbe refractometer (WAY-2WAJ, Shanghai Precision Scientific Instruments Co., Ltd., China) (Kaewklin *et al*., 2018). The TSS content was expressed as Brix per 100 g fresh weight.

Determination of TA

The determination of TA content was based on the previous method (Yu *et al*., 2020) with minor modifications. 20 mL supernatant diluted 10 times was titrated with 0.1mol/L sodium hydroxide solution to pH 8.1.TA was expressed by the number of grams of tartaric acid per 100 g fresh grapes.

Determination of AA

AA was determined at 244nm by ultraviolet absorption spectrophotometry (Li, 2015) using a UV-visible spectrophotometer (TU-190, Beijing Pu Analysis General Instrument Co., Ltd., China). Grape (30 g) was mixed with 10 mL hydrochloric acid solution (1%) and ground into homogenate in a frozen mortar. The homogenate was centrifuged at 4°C and 10000 r/min for 10 min. The supernatant was diluted 25 times to become the colourimetry sample solution. The absorbance at 244nm was quickly determined under low temperature and dark conditions. By making a standard curve of known AA concentration and absorbance at 244 nm (Y = 0.00586 X-0.0569, R^2 = 0.999), the AA content in the sample could be calculated according to the absorbance at 244 nm. The determination was repeated three times for each treatment group.

Determination of TP

The TP was determined according to the Folin-ciocalteu reagent method (Abdipour *et al*., 2020). The fruit extract was prepared with a hydrochloric acid-methanol solution (1%). The standard gallic acid curve (Y = 0.0326 X-0.0594, R^2 = 0.9992) was obtained. The results were expressed as mg gallic acid equiv per 100 g of fresh grapes.

Determination of Berry Abscission Ratio of Grapes

On the third and sixth days after storage, the total grape number and grape dropping number for each treatment were recorded. The whole cluster of grapes was removed from the storage box and gently shaken. The grape number in the box and the grape number under the shaking were counted and divided by the total number of grapes in each group to obtain the berry abscission ratio as shown in Formula (5):

$$Berry\ absvission\ ratio(\%) = (A_n/A_0) \times 100 \quad (5)$$

where, A_n is the number of falling grapes in each group on the *n* day of storage; A_0 is the total number of grapes in each group.

Microstructure Scanning of the Grape Peels

Scanning Electron Microscopy (SEM) was used to observe the microstructure of the grape peels. The peels at the equator of grapes were cut into small pieces (5×5 mm) and fixed in glutaraldehyde solution (4%) for more than 12 h, then rinsed with 0.1 mol/L phosphate buffer (pH 7.2) for 3 times for 15 min each time. Dehydration was carried out with ethanol in a series of concentrations (30, 50, 70, 80, 90 and 95%) for 15 minutes step by step. Then dehydration was carried out twice with anhydrous ethanol for 30 min each time and finally replaced with acetone. The slices were transferred to an ultra-low temperature refrigerator (-80°C) for pre-cooling and dried in a vacuum freeze dryer (ALPHA 1-2 LD plus, Christ, Germany) for 24 h. The dried sample was attached to the sample table for gold-spraying treatment by scanning electron microscopy (Regulus 8100 Cold Field Emission, Hitachi, Japan). The surface morphology of grapes was observed.

Data Processing and Analysis

Design Expert V8.0.6 software was used to optimize the formula of PACBC and analyze the variance. Each experiment was done in triplicate, and the average value was taken. The data results were expressed as mean ± standard deviation (x ± s).

Results

Statistical Analysis and the Model Building

According to the result of single-factor tests, the Box-Behnken design response surface optimization test was conducted with the mass concentration of Cs (X_1), D-PLA (X_2), calcium chloride (X_3) and AA (X_4) as independent variables and the comprehensive score (Y) of the decay rate, weight loss, browning incidence and firmness as response values. Results of response surface analysis of the variation of the comprehensive score with the mass concentration of Cs (X_1), D-PLA (X_2), calcium chloride (X_3) and AA (X_4) were shown in Table (2).

Multiple lincluster regression fitting analysis was performed based on the results in Table (2). The quadratic regression equation of the comprehensive score and the concentration of each component in the composite coating agent was obtained as follows:

$$Y = 0.606961 + 0.718437X_1 + 0.51776X_2 - 0.142125X_3 + 0.024792X_4 - 0.296875.X_1X_2 + 0.0125X_1X_3 - 0.0125X_1X_4 + 0.0875X_2X_3 + 0.05X_2X_4 + 0.09X_3X_4 - 0.494792X_1^2 - 0.236979.X_2^2 - 0.046667.X_3^2 - 0.041667.X_4^2$$

The results of variance analysis of the regression model are shown in Table (3). The model had a very high F value (F = 35.52) and a very low P value (p<0.0001), indicating that the model was highly significant. The mismatch item (p = 0.3467) was not significant, indicating that the experimental results were in good agreement with the model. The model could be used to analyze and predict the comprehensive preservation score of PACBC.

The variance analysis of each item in the model showed that the concentration of chitosan (X_1), calcium chloride (X_3) and AA (X_4) had a very significant effect on the comprehensive score (p<0.01). The concentration of D-PLA (X_2) had a significant effect on the comprehensive score (p<0.05). The interaction terms of X_1X_2 (p<0.01), X_3X_4 (p<0.01) and X_2X_3 (p<0.05) were significant, indicating that the interaction between chitosan and D-PLA, calcium chloride and AA, D-PLA and calcium chloride significantly affected the comprehensive score.

Table 2: Results of response surface analysis of the variation of the comprehensive score with the mass concentration of Cs (X_1), D-PLA (X_2), calcium chloride (X_3) and AA (X_4)

Run	X_1/%	X_2/%	X_3/%	X_4/%	Y Comprehensive score
1	1	1	0	0	0.75
2	0	1	1	0	0.85
3	-1	0	0	1	0.77
4	0	0	0	0	0.88
5	0	0	0	0	0.9
6	0	1	0	1	0.81
7	0	-1	0	-1	0.86
8	1	0	0	-1	0.84
9	-1	-1	0	0	0.69
10	0	1	-1	0	0.86
11	0	0	0	0	0.9
12	-1	1	0	0	0.81
13	-1	0	1	0	0.78

14	0	0	-1	1	0.84
15	0	-1	-1	0	0.87
16	1	0	1	0	0.79
17	0	0	1	-1	0.84
18	1	0	0	1	0.8
19	0	-1	0	1	0.82
20	0	0	0	0	0.88
21	0	0	-1	-1	0.92
22	0	0	1	1	0.85
23	1	-1	0	0	0.82
24	0	0	0	0	0.89
25	0	-1	1	0	0.79
26	1	0	-1	0	0.82
27	-1	0	0	-1	0.8
28	0	1	0	-1	0.89
29	-1	0	-1	0	0.82

Table 3: Variance analysis of the regression model

Source	Sum of Squares	Df	Mean Square	F-value	p-value	significant
Model	0.0707	14	0.0051	35.52	< 0.0001	**
X_1	0.0019	1	0.0019	13.18	0.0027	**
X_2	0.0012	1	0.0012	8.44	0.0115	*
X_3	0.0044	1	0.0044	30.99	< 0.0001	**
X_4	0.0056	1	0.0056	39.6	< 0.0001	**
X_1 X_2	0.009	1	0.009	63.44	< 0.0001	**
X_1 X_3	2.5×10^{-5}	1	2.5×10^{-5}	0.1757	0.6814	
X_1X_4	2.5×10^{-5}	1	2.5×10^{-5}	0.1757	0.6814	
X_2X_3	0.0012	1	0.0012	8.61	0.0109	*
X_2X_4	4×10^{-4}	1	4×10^{-4}	2.81	0.1158	
X_3 X_4	0.002	1	0.002	14.23	0.0021	**
X_1^2	0.0407	1	0.0407	285.76	< 0.0001	**
X_2^2	0.0093	1	0.0093	65.55	< 0.0001	**
X_3^2	8.83×10^{-4}	1	8.83×10^{-4}	6.21	0.0259	*
X_4^2	7.04×10^{-4}	1	7.04×10^{-4}	4.95	0.0431	*
Residual	0.002	14	1.42×10^{-4}			
Lack of Fit	0.0016	10	1.59×10^{-4}	1.59	0.3467	
Pure Error	0.0004	4	0.0001			
Cor Total	0.0727	28				
$R^2 = 0.9726$; CV = 1.43% ; Adeq Precision = 26.6182 Note: * means significant difference ($p<0.05$). ** means a very significant difference ($p<0.01$)						

The three-dimensional surface map and contour map of the response surface also reflect the significant interaction of these factors. The steeper the slope of the three-dimensional surface of the response surface formed by the two factors and the more elliptic the contour lines are, the stronger the interaction is, while the other way around, the weaker the interaction is (Wang *et al*., 2012).

As can be seen from Fig. (1), the three groups of contours between the above two related factors were all ellipses, and the response surface was a relatively steep surface, indicating that the interaction between the two components of the coating film was strong and had a significant impact on the comprehensive score, which was consistent with the regression analysis of Table (3).

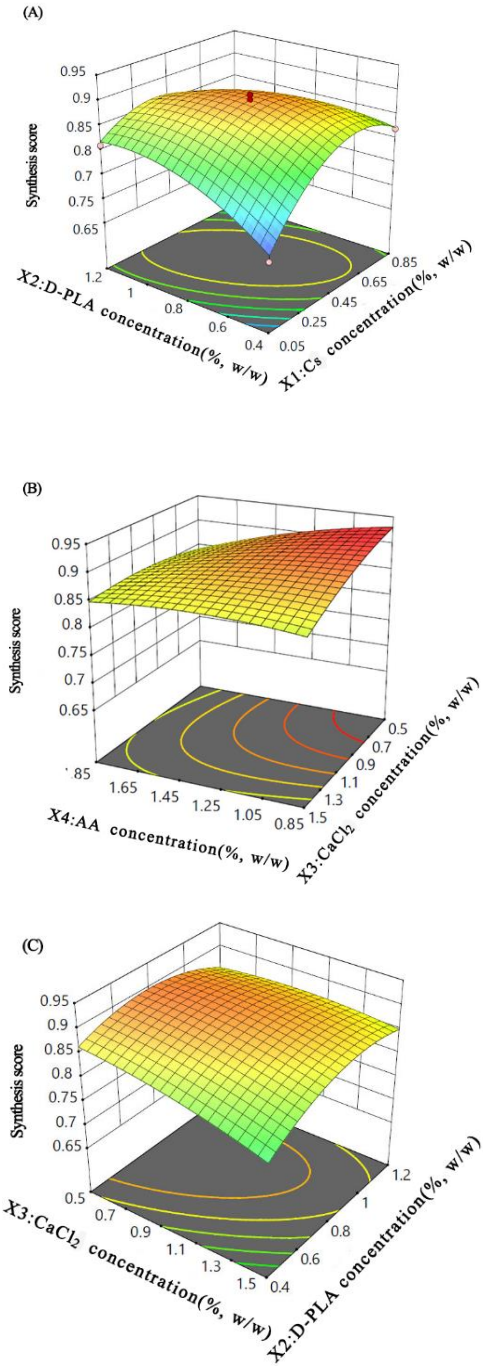


Fig. 1: Response surface and contour plots showing the interaction effects on the comprehensive score

Optimization of PACBC formula and model verification

Based on the established model, the optimal formula of PACBC was obtained as follows. The mass concentration of D-PLA, Cs, calcium chloride and AA was 0.792, 0.484, 0.5 and 0.85%, respectively. Under this optimal formula of PACBC, the comprehensive score of decay rate, weight loss, browning incidence and firmness of grapes was predicted to be 0.932.

According to the optimal formula, PACBC was prepared. To verify the adequacy of the model equations, three parallel preservation tests were carried out at room temperature for 6 days. The result showed that the decay rate was 4.06%, weight loss was 0.52%, browning incidence was 1.90%, firmness of grapes was 0.72N, and the comprehensive score was 0.905, which was 2.90% (less than ±5%) error with the theoretical predicted value, which verified the validity of the model.

Effect of PACBC with Optimal Formula on Decay Rate, Berry Abscission Ratio and Browning Incidence of Grapes

The preservation effect of PACBC with optimal formula on decay rate, berry abscission ratio and browning incidence of grapes during storage was presented in Table (4). As shown in Table (4), the decay rate, berry abscission ratio and browning incidence of grapes increased with the extension of storage time. Compared with the blank control (CK1), the decay rate and browning incidence in the chitosan composite coating group (CK2) were lower than those in the CK1 group ($p<0.05$) on the sixth day. However, compared with CK1 and CK2, the decay rate, berry abscission ratio and browning incidence of grapes in the T group decreased significantly on the sixth day ($p<0.05$). The results showed that PACBC with optimal formula had a good preservation effect on grapes. The reason may be the bacteriostatic effect of D-PLA in combination with Cs in the composite film and the microenvironmental gas regulation, which can form a low-oxygen and high-carbon dioxide microenvironment on the surface of fruits to reduce respiratory consumption, thereby preventing pathological rot and seed fall (Shan *et al*., 2018). Meanwhile, AA in the composite coating as an antioxidant can indirectly improve the antioxidant enzyme activity of fruits (Yuan *et al*., 2020) or directly remove reactive oxygen species in plant cells (Smirnoff & Wheeler, 2000).

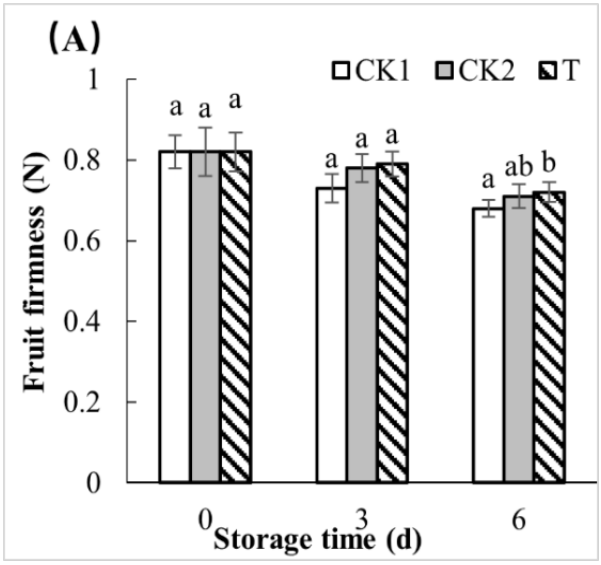
Effect of PACBC with Optimal Formula on Grape Firmness and Weight Loss

Water transpiration, degradation of cell walls and other substances and respiratory consumption of postharvest fruits can lead to a decrease in fruit hardness (Yu *et al*., 2020), increased mass loss and decreased freshness, thus affecting the sensory quality of fruits (Wei *et al*., 2019). As shown in Fig. (2A-B), PACBC effectively inhibited the decline of firmness and weight loss during storage, and the inhibition effect was better than that of CK2. Thus, the firmness of grapes in the T group was higher than that of grapes in the CK1 group and CK2 group during the same period. The weight loss of grapes in the T group was lower than that of grapes in the CK1 group and CK2 group during the same period. By the sixth day of storage, the firmness of grapes in the T group increased by 5.88%, and the weight loss decreased by 60.61% compared with that of grapes in CK1. The reason is that chitosan composite membranes inhibit water transpiration and respiratory gas exchange (Zainal *et al*., 2019). In addition, D-PLA also inhibited pathological softening and mass loss caused by microorganisms (Saichao and Jing, 2022).

Table 4: The decay rate, berry abscission and browning incidence of grapes subjected to different treatments during E-commerce logistics

Groups	Decay rate /%		Berry abscission ratio /%		Browning incidence /%	
	3d	6d	3d	6d	3d	6d
CK 1	5.98± 3.07^{a}	15.08± 1.61^{a}	7.50± 2.14^{a}	18.23± 4.51^{a}	2.34±0 .18^{a}	4.77± 0.20^{a}
CK 2	5.28± 1.05^{a}	10.03± 1.49^{b}	5.00± 0.95^{a}	11.20± 4.03^{a}	1.87±0 .24^{ab}	2.74± 0.07^{b}
T	2.72± 2.38^{a}	4.06±0. 68^{c}	1.36± 1.19^{a}	2.88±1. 85^{b}	1.70±0 .13^{b}	1.90± 0.21^{c}

Note: Mean values with different letters (a, b, c) in the same column are significantly different from each other (Fisher's LSD test, $p<0.05$)



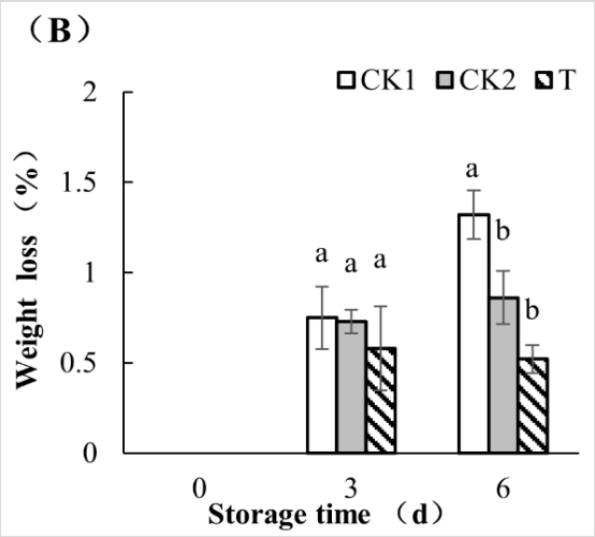


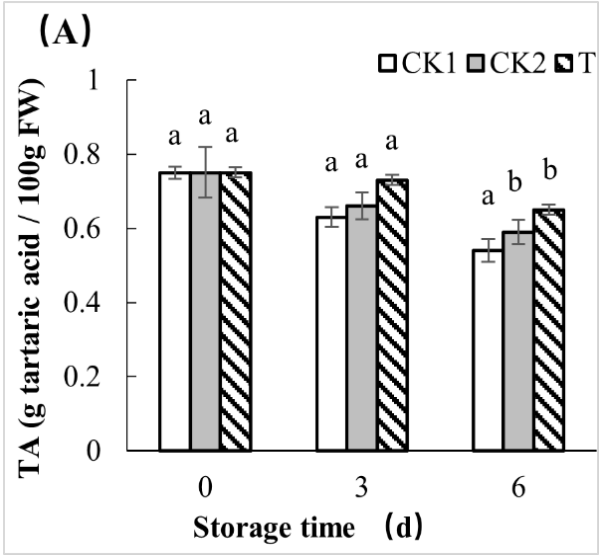
Fig. 2: The change of grapefruit firmness (A) and weight loss (B) with different treatments; Note: Mean values with different letters are significantly different from each other (Fisher's LSD test, $p<0.05$)

Effect of PACBC with Optimal Formula on the Content of TA and TSS in Grapes

TSS mainly contains soluble substances such as sugars, organic acids, minerals and vitamins. TA is mainly composed of organic acids in fruits (Reyes-Atrizco *et al*., 2019). TA and TSS are important indicators affecting the sweet and sour flavour and freshness of fruits (Li *et al*., 2023). As can be seen from Fig. (3A-B), the content of TA and TSS in each group decreased during storage, and the decline rate of TA and TSS in the T group treated with PACBC was slower than that of TA and TSS in CK1 and CK2. The content of TA and TSS in grapes in the T group was significantly higher than that in CK1 and CK2. The difference was significant compared with CK1 on the sixth day ($p<0.05$), indicating that the PACBC could effectively reduce the consumption rate of the grape nutrient substrate and maintain the flavour and freshness of the grapes. The reason may be that the microenvironment gas regulation of the coating film can form a low-oxygen and high-carbon dioxide microenvironment on the surface of fruits, which reduces respiratory consumption, gas exchange and production of reactive oxygen species and inhibits the oxidative consumption of these substances by respiration and reactive oxygen metabolism (Lin *et al*., 2020b; Yuan *et al*., 2020). In addition, AA and D-PLA in the film are conducive to the protection of nutrients (Lo'ay and Dawood, 2017; Ren *et al*., 2021).

Effect of PACBC with Optimal Formula on the Content of AA and TP in Grapes

AA and TP are important endogenous antioxidants in plant tissues like total flavonoids and saponins, which can inhibit membrane lipid peroxidation and reduce the occurrence of browning and aging (Gao *et al*., 2017). AA and TP are important indicators for judging the storage quality of fruit (García-Betanzos *et al*., 2017). AA and TP are reduced by metabolic consumption during the antioxidant process (Singh *et al*., 2005). As shown in Fig. 4A-B), the content of AA and TP in grapes decreased during the storage. Compared with CK1 and CK2, the content of AA and TP in grapes treated with PACBC (Tgroup) decreased more slowly. By the sixth day of storage and transportation, the content of AA and TP in grapes of the T group was significantly higher than those of the CK1 and CK2 groups ($p<0.05$). The results indicated that PACBC can effectively prevent the loss of AA and TP and maintain the high nutritional quality of grapes. The possible reason is that the composite film gas-regulated microenvironment forms an environment of low oxygen and high carbon dioxide on the surface of fruits, thus reducing the contact with oxygen, inhibiting respiration, reactive oxygen species and other aerobic metabolism (Chen *et al*., 2021). Chitosan can induce the activity of fruit disease-resistant enzymes and the transcriptional levels of their genes (Liu *et al*., 2016). Enhancement of free radical scavenging ability is related (Han *et al*., 2014; Lin *et al*., 2020a). In addition, chitosan can induce the activity of fruit disease-resistant enzymes (Liu *et al*., 2016) and enhance free radical scavenging ability (Han *et al*., 2014; Lin *et al*., 2020a).



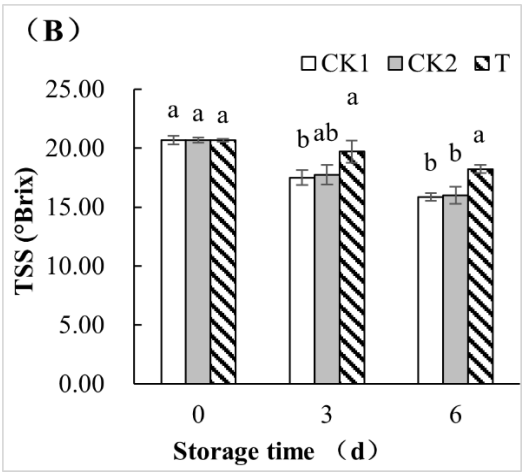


Fig. 3: The change of TA (A) and TSS (B) contents in grapes with different treatments. Note: Mean values with different letters are significantly different from each other (Fisher's LSD test, $p<0.05$)

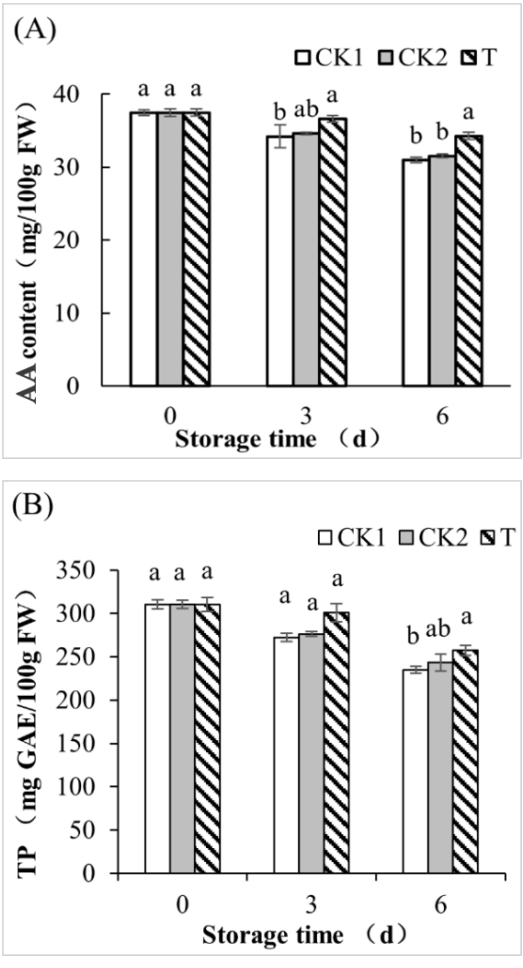


Fig. 4: The change of V_C (A) and TP (B) content in grapes with different treatments Note: Mean values with different letters are significantly different from each other (Fisher's LSD test, $p<0.05$)

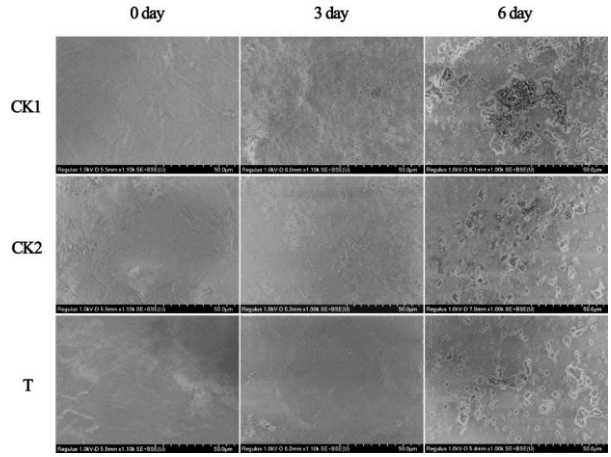


Fig. 5: SEM image of grape peel morphology during storage

Effect of PACBC with Optimal Formula on Microstructure of Grape Peels

The change in cell wall structure and the degradation of components are the main reasons for the texture softening of fruits (Zhang *et al*., 2018). In the process of grape autolysis softening, not only the external morphology but also the internal cell microstructure will change significantly.SEM image of grape peel morphology during storage was shown in Fig. (5). As can be seen from Fig. (5), on day 0, the surface of grape in each group was dense, uniform, smooth and without cracks and shrinkage. With the extension of storage time, the fruit surface of the CK1 group deteriorated. There were obvious micropores in the peel on the third day of storage and large cracks in the peel on the sixth day of storage. The peel structure was seriously damaged, which was also confirmed by other studies on the change of grape peel microstructure (Zhang *et al*., 2018).

Compared with the CK1 and CK2 groups, grape skins treated with PACBC (T group) had the least pores and cracks. These results indicated that the composite membrane microenvironment gas-regulating barrier with chitosan as the carrier could inhibit the degradation of epidermal structure caused by respiratory consumption. D-PLA, as a synergistic bacteriostatic agent, could also prevent peel rot caused by pathogen infection.

Discussion

Chitosan and D-PLA are new green biological preservatives discovered in recent years, which have a significant effect on film forming and antibacterial properties (Van Den Broek *et al*., 2015; Muzzarelli *et al*., 2012), as well as inhibiting postharvest aging of fruits (Ren *et al*., 2021). The combination of AA, calcium ion preservatives and other ingredients to exert their synergistic effect is the development trend in this field

(Shan *et al*., 2018; Ding *et al*., 2023). The fresh-cut Gala apples treated with a composite coating of 5 g/L calcium chloride, 20 g/L ascorbic acid and 10 g/L carboxymethyl cellulose can better maintain the quality of fruit slices for 12 days (Koushesh Saba and Sogvar, 2016). Fresh-cut Fuji apples are treated with a composite coating of 10 g/L chitosan, 10 g/L pullulan and 8 g/L glutathione, which can extend the shelf life to 10 days (Wu and Chen, 2013). The concentration ratio of the composite coating preservative with good effect on mango preservation is 10 g/L ascorbic acid, 10 g/L citric acid and 5 g/L calcium chloride (Siddiq *et al*., 2013).

In this study, CS, AA, calcium chloride and D-PLA were selected as independent variables. A four-factor and three-level test was carried out through RSM to optimize the PACBC formula based on comprehensive sensory scores such as decay rate, weight loss, browning incidence and firmness. Based on the best-predicted formula, the actual sensory scores of the grapes treated by PACBC were almost equal to the predicted values, indicating that RSM is a feasible method to predict the formula of PACBC. The optimized PACBC was applied to the postharvest preservation of Shine-Muscat grapes, which could significantly improve the internal and external quality of the grapes during storage, delay the decline of texture hardness and the destruction of the microstructure of the peel. The preservation effect of D-PLA combined with chitosan optimal composite membrane treatment was better than that of chitosan composite membrane treatment without D-PLA.

An analysis of variance on the comprehensive score of composite film preservation showed that the various components of the coating agent synergistically affected the grape preservation effect (Table 3), which was consistent with the results on other fruits and vegetables (Chong *et al*., 2015; Tan *et al*., 2019). In this study, the PBABC achieved a synergistic effect through the subtle combination of multiple ingredients and played a superposition effect on the preservation of grapes, thereby maintaining the freshness and internal quality of fruits during storage.

Conclusion

This study provides compelling evidence that sodium chloride (NaCl) significantly influences the stability and binding affinity of the Ab3 lipase-LEA K complex with olive oil. Molecular docking simulations demonstrated that NaCl enhances the binding affinity by promoting the formation of additional molecular interactions. Specifically, NaCl facilitates the establishment of stronger hydrogen bonds and hydrophobic interactions, which are critical for maintaining the structural integrity and functionality of the enzyme complex. These interactions stabilize the lipase-LEA K complex, counteracting the destabilizing effects typically associated with saline environments.

The findings underscore the dual role of NaCl: While high salt concentrations generally disrupt enzyme activity, the presence of NaCl in the context of the LEA K-stabilized complex enhances the enzyme's performance. By enabling the lipase to remain stable and active in high-salinity conditions, NaCl plays a pivotal role in extending the applicability of Ab3 lipase for industrial processes that operate under such challenging conditions. This study provides mechanistic insights into how NaCl interacts with the lipase-LEA K complex and highlights the potential of leveraging NaCl and coexpression strategies to engineer enzymes with enhanced salt tolerance. These insights pave the way for designing more robust biocatalysts suitable for industrial applications where high salinity is a common stressor.

Acknowledgement

The authors would like to thank Dr. Huang Youru and Dr. Zhang Yang from School of Biology and Food Engineering, Changshu Institute of Technology and teachers Xu Bing, Zheng Lixue, Han Xiaolei and Pan Hongying from the Central Laboratory of School of Biology and Food Engineering, Changshu Institute of Technology for their help in the research process.

Funding Information

This research was funded by the Science and Technology Support Plan (Agriculture) of Zhangjiagang Science and Technology Bureau (ZKN1914).

Author's Contributions

Zhu Dongxing: Project administration, conceptualization, formal analysis, and writing of the original draft.

Xiong Guanglun: Investigation, Methodology, Software, Resources.

Tang Wenli, Tang Jin and Liu Jiayang: Investigation, validation, methodology.

Chen Zitong: Methodology and data curation.

Fan Xiaoyu, Caijun Dong and Song Yingying: Methodology and resources.

Chen Yiyong and Zhang Mengqi: Funding acquisition, Supervision, Project administration, writing review and editing.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

Declaration of Competing Interest

The authors declare that there are no competing financial or personal relationships that could inappropriately influence the work reported in this study.

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