RESEARCH ARTICLE

Optimized composite preservation treatment of polysaccharides and bacteriocins to inhibit microbial decay of fresh-cut broccoli and maintain quality

Qianhe Huang[1, 2], Liang Shi[3], Mingxuan Li[2, 4], Ziteng Wang[1, 2], Guiqun Huang[2, 4], Guoxing Hu[1, 2], Li Mi[1, 2], Wenge Yang[5, *], Yonghong Hu[2, 4, *]

[1]College of Biotechnology and Pharmaceutical Engineering, [2]State Key Laboratory of Materials-Oriented Chemical Engineering, Nanjing Tech University, Nanjing, Jiangsu, China. [3]Jiangsu Coastal Agriculture Development Co., Ltd. Rudong Branch, Nantong, Jiangsu, China. [4]College of Food Science and Light Industry, [5]School of Pharmaceutical Sciences, Nanjing Tech University, Nanjing, Jiangsu, China.

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Broccoli is a perennial herbaceous plant with various antioxidants and anticancer bioactive substances. However, slicing and cutting operations will cause tissue destruction and trigger the ease of microbial infection, which can also lead to dehydration, chlorosis, and rotting. This study systematically evaluated the preservation effects of different concentrations of sodium alginate (SA), fucoidan (FUC), chitosan (CTS), nisin, natamycin, and ε-polylysine (ε-PL) on fresh-cut broccoli through single-factor preservation experiments for delaying the yellowing and decay process of broccoli, extending its shelf life, and providing theoretical basis and practical guidance for the storage and transportation of fresh-cut fruits and vegetables. The top three preservatives with the best yellowing inhibition effects were selected and the optimal composite preservative ratio was determined using response surface methodology (RSM). The results showed that 0.46 % SA, 0.25 % FUC, and 0.11% Nisin demonstrated the best preservation effects. The optimized compound preservative (SA-FUC-Nisin) could effectively delay the reduction of chlorophyll content and nutritional components, contributing to maintaining higher contents of total soluble solid (7.60 %), vitamin C (0.32 mg/g), reducing sugar (1.41 mg/g), total phenols (0.57 mg/g) and flavonoids (2.47 mg/g) in fresh-cut broccoli. The compound treatment also effectively inhibited the mutation of titratable acidity and peroxidase activity (POD), the synthesis of carotenoids, and the increase of total bacteria, molds, and yeasts. In addition, the composite preservative showed a good stabilizing effect on the community structure of fresh-cut broccoli and had a bactericidal effect on intestinal pathogens such as *Proteobacteria* (*Escherichia coli*, *etc.*) and *Bacteroidetes*. The results indicated that SA-FUC-Nisin compound preservative could not only inhibit yellowing, maintain higher contents of bioactive compounds in fresh-cut broccoli, but also showed good antibacterial effect, thus provided new guidance for the preservation of fresh-cut foods.

Keywords: fresh-cut broccoli; polysaccharides; bacteriocin; response surface methodology; antibacterial.

[*]**Corresponding authors:** Wenge Yang, School of Pharmaceutical Sciences; Yonghong Hu, College of Food Science and Light Industry, Nanjing Tech University, No. 30 South Puzhu Road, Nanjing 211816, Jiangsu, China. Email: yaochamana6@163.com (Yang W), yonghonghuyg@163.com (Hu Y).

Introduction

Broccoli (*Brassica oleracea* L. var. Italica) is a 1 to 2-year-old perennial herbaceous plant belonging to the cabbage family (*Brassicaceae*) with green flower bulbs containing various anticancer bioactive substances such as sulforaphane and glucosinolates [1, 2]. Broccoli is also rich in

vitamin C (VC) and phenolic compounds, which can enhance the antioxidant capacity of the human body and reduce the incidence of various cardiovascular diseases such as hypertension [3]. In recent years, due to the pursuing of the concept of consumption, convenience, safety, health, and environmental protection, the demand for fresh-cut broccoli has increased greatly among consumers. However, slicing and cutting operations can cause obvious tissue damage, easily leading to microbial infection, dehydration, chlorosis, decay, *etc*. [4], so developing and optimizing new preservation technologies to effectively maintain the quality of fresh-cut broccoli during its shelf life is an urgent task.

Bio-preservation and biocontrol have gradually become a research hotspot both domestically and internationally due to the non-toxic, green, and natural advantages. Numerous studies have shown that the use of bio-preservation is indeed more promising than single traditional physical and chemical preservation methods. Physical preservation such as air-regulation does show high efficiency. However, it greatly increases production costs. Other physical methods such as ultra-low temperature, ultraviolet (UV), or ultrasound require rigorous environmental conditions with low error tolerance and may also run the risk of damaging nutritional content [5]. A variety of chemical preservatives including chlorine dioxide, peracetic acid, and hydrogen peroxide can produce carcinogenic derivatives during the processing [6]. Bio-preservation refers to employing natural extracts such as polysaccharide and antibacterial ingredients to extend the shelf life of products and improve food safety [7]. Fucoidan (FUC) is a kind of antioxidant polysaccharide rich in fucose and sulfate groups, mainly extract from brown algae such as seaweed [8]. Zhang *et al*. treated cold-stored cucumbers with FUC to diminish the occurrence of chilling injury and oxidative aging [9]. FUC treatment could also suppressed degradation of total polyphenol and VC in strawberries during storage, which maintained the antioxidant active ingredients in strawberries [10]. However, there is no report on the use of fucoidan in broccoli preservation. Other environmental-friendly natural polymers including sodium alginate (SA) and chitosan (CTS) are also considered to be the ideal candidates [11]. Other than polysaccharide components, natural antibacterial agents are also efficient and safe ingredients in fresh-cut food preservation. Nisin is a broad-spectrum antimicrobial agent that has been broadly used for dairy products, vegetables, and fruits in more than 50 countries owing to its efficiency against some Gram-positive bacteria [12]. Its analog, natamycin, has also received more attention in the field of preservation due to its antifungal properties. Lin *et al*. found that the combination of freezing point storage and natamycin could delay the reduction of total soluble solid (TSS) and chlorophyll content in broccoli [13]. However, a single component is not sufficient to achieve the desired preservation effect. Therefore, two or more components are usually desirable to be combined to improve preservation performance [14]. Previous study reported that the synergistic use of pectin and SA could form a water locking "egg box" structure, which was helpful to enhance the hydrophobicity and physicochemical properties of compound preservatives [15]. Song *et al*. developed a method by coating chitosan with nisin and ε-polylysine (ε-PL) to retain freshness of fresh-cut carrots, which showed better preservation effect than a single component [16]. Component preservatives can combine the advantages of each component to improve structural performance, thus saving manufacturing costs and achieving better preservation effects [17].

This study employed edible natural polysaccharides (FUC, CTS, and SA) and antibacterial ingredients (nisin, natamycin, and ε-PL) at different concentrations to fabricate an efficient composite preservative to probe the feasibility and superiority of biological packaging on fresh-cut vegetable. The hue angle value (H) in color difference is an important parameter for describing the color change of fruits and vegetables, which can quantify the degree of

yellowing of fruits and vegetables, thereby evaluating the maturity, freshness, and preservation effect of fruits and vegetables. It has been widely used in quality monitoring and preservation research [18]. The concentration of preservatives was optimized with the response surface methodology (RSM) establishing a three-variable, three-level Box-Behnken design, for fresh-cut broccoli. This study innovatively combined nisin with SA-FUC composite coating, significantly delayed the yellowing and nutrient loss of fresh-cut broccoli while exhibiting excellent antibacterial effects. The stabilizing effect of SA-FUC-Nisin treatment on microbial community structure was explored through 16S rRNA amplicon sequencing, providing new insights for the application of natural biological preservatives and promoting the green development of fresh-cut preservation technology.

Materials and methods

Plant material and single factor test

Fresh broccoli with consistent variety, emerald green color, and no obvious damage or pests and diseases were obtained from Pukou Local Farmers' Market (Nanjing, Jiangsu, China) and were immediately transported to the laboratory within 1 hour. The broccoli samples were washed with clean water and drained to remove impurities on the surface before cutting into small florets with 4 - 5 cm in diameter and 2 - 3 cm in length using a sterilized knife. Fresh-cut broccoli was put into distilled water for later use. The fresh-cut broccoli samples were grouped for single factor test experiments with the florets being treated by 0.25%, 0.50%, 0.75%, 1.00% CTS, SA, and FUC solutions and 0.05%, 0.10%, 0.15%, 0.20% nisin, natamycin and ε-PL solutions for 5 min, respectively, while the control group (CK) being soaked in distilled water for 5 min. All food-grade chemicals were purchased from Zhejiang Silver-Elephant Bio-engineering Co., Ltd., Taizhou, Zhejiang, China. All treatments were carried out at room temperature of 23℃ ± 2℃. After soaking treatments, the liquid on sample surface was gently shaken off before the samples were dried in clean plastic wrap and then put into a sealed plastic bag (21 × 20 cm^2) with 10 samples per bag and stored at 4℃. Sampling and observation were conducted every three days.

Chromatic aberration

The superficial color of fresh-cut broccoli flower bulb was measured every 3 days with a 3NH NH310 colorimeter (Guangdong THREENH Technology CO., LTD., Guangzhou, Guangdong, China) for 12 days, while calibrated with a standard white plate. The chromatic aberration values of brightness (L*), redness-greenness (a*), yellowness-blueness (b*) were measured at different positions on each broccoli floret three times each [19]. The mean value was taken as the result. According to the a* and b* values, Hue angle (H) was calculated using the formula below.

$$H = 180° + \arctan\frac{b^*}{a^*} \quad (a^* < 0, b^* > 0) \qquad (1)$$

Response surface methodology (RSM) optimization experiment

SA, FUC, and Nisin were selected as the three factors for response surface optimization based on the single-factor test, and Hue angle value (H) was used as the response value. A three-factor, three-level response surface model was established using Box-Behnken in Design-Expert 8.0.6 (Stat-Ease, Inc., Minneapolis, MN, USA) and schemed to screen the optimal composite coating preservative ratio of fresh-cut broccoli.

Preservation verification experiment

The samples immersed in distilled water were set as the control group (CK), while the compound preservative treatment groups (CP groups) were set up with 0.46% SA + 0.25% FUC + 0.11% nisin. The broccolis were soaked in the treatment solutions for 5 min before being removed and dried in a clean and ventilated area, followed by packing separately in 0.6 mm polyethylene sealed bags. The samples were stored at 4°C, collected every 3 days, and quickly frozen with liquid nitrogen for subsequent indicator testing.

Each experimental group was repeated three times. Frozen samples were stored in -80℃ freezer.

Determination of nutritional indicators

(1) Chlorophyll and carotenoid

Chlorophyll and carotenoid contents were measured by using ethanol extraction method [20]. Briefly, after grinding 0.5 g samples in liquid nitrogen, 10 mL of anhydrous ethanol was added and extracted in dark for 6 h. The supernatants were taken after centrifugation and measured absorbances at 470, 649, and 665 nm with a microplate reader (Multiskan FC, ThermoFisher, Shanghai, China) to determine chlorophyll a (C_a), chlorophyll b (C_b), total chlorophyll (C_{Chl}), and carotenoids contents (mg/g) as follows.

$$C_a = \frac{(13.95A_{665} - 6.88A_{649}) \times V}{m \times 1000} \quad (2)$$

$$C_b = \frac{(24.96A_{649} - 7.32A_{665}) \times V}{m \times 1000} \quad (3)$$

$$C_{Chl} = C_a + C_b \quad (4)$$

$$\text{Carotenoids} = \frac{(1000A_{470} - 2.05C_a - 114.8C_b) \times V}{m \times 245 \times 1000} \quad (5)$$

(2) Total soluble solid, vitamin C, titratable acids and reducing sugar content

Total soluble solid (TSS) content (%) was measured using a PAL-1 handheld refractometer (ATAGO, Tokyo, Japan) after grinding and centrifuging the fresh samples [21]. The VC content (mg/g FW) was determined using 2,6-dichlorophenol indophenol titration method [22]. Titratable acidity (TA) was determined by neutralizing the acid presented in a known quantity (weight or volume) of broccoli sample using a standard base [23]. The end point for titration was the color fading of phenolphthalein. The titration volume of NaOH was recorded along with sampling weight. The TA content (mg/g FW) was then calculated. Reducing sugar content (RSC) (mg/g FW) was determined based on 3,5-dinitrosalicylic acid (DNS) method [24]. Briefly, 2 g of broccoli samples were ground in 5 mL of distilled water before centrifugation. After adding 2 mL of DNS reagent into 2 mL of plant extract, the mixture was heated in a 100℃ water bath for 5 min before cooling down. 25 mL of distilled water was then added to determine the absorbance at 540 nm. RSC was calculated from the D-glucose standard curve.

(3) Total phenolic content and flavonoids

The Folin-Ciocalteu method was employed for measuring the total phenolic content [25]. 1.0 g samples were finely crushed in liquid nitrogen and mixed with 10 mL of pre-cooled methanol at 4°C before ground thoroughly under ice bath. The sample was then centrifuged at 12,000 g for 10 min. Total phenolic (TP) contents (mg/g) were measured at 765 nm and calibrated with the gallic acid standard curve. The measurement of flavonoids content (mg/g) was based on the method of Rao *et al*. with a slight modification [26]. Briefly, 2.0 g of broccoli samples were ground evenly with 1% HCl-methanol solution in an ice bath. The mixture was then diluted to 20 mL and extracted for 20 min at 4℃ in dark. The absorbance of supernatant was determined at 324 nm after centrifugation.

Peroxidase (POD) and superoxide dismutase (SOD) activities

POD activity was determined by adopting guaiacol method, while SOD activity was determined by NBT riboflavin microplate method with the test kit (Leagene Biotechnology Co., Ltd, Beijing, China). Each sample was measured three times in parallel, and the unit of enzyme activity was defined as U/g.

Microbiological analysis

(1) Total colony count

Quantitative detection of bacterial colonies in broccoli samples was performed using the PCA agar plate containing 5.0 g/L tryptone, 2.5 g/L yeast extract, 1.0 g/L glucose, and 15.0 g/L agar. 25 g samples were homogenized into 225 mL of sterile saline for 2 min. 1 mL of each homogeneous solution was taken for gradient dilution and then applied onto the plate followed by cultivation at 36℃ ± 1℃ for 48 h before colony forming unit (CFU) counting [27].

(2) 16S rRNA amplicon sequencing

16S rRNA amplicon sequencing was conducted for bacterial strain identification and analysis [28]. The sequences from the amplicon area of 16SV57 were used for the designing of sequencing primers as AAC MGA TTA GAT ACC KG and ACG TCA TCC CCA CCT TCC. The polymerase chain reaction (PCR) was carried out with 15 µL of Phusion® High - Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA), 0.2 µM of each forward and reverse primers, and about 10 ng template DNA in a thermal cycling program of 98℃ for 1 min followed by 30 cycles of 98℃ for 10 s, 50℃ for 30 s, and 72℃ for 30 s before 72℃ for 5 min. The PCR products were examined using electrophoresis and purified using magnetic bead purification method. Sequencing libraries were generated with the added indexes. The library was checked with Qubit and real-time PCR for quantification and bioanalyzer for size distribution detection. Quantified libraries were pooled and sequenced on Illumina platforms (Illumina, San Diego, CA, USA).

Statistical analysis

Microsoft Excel 2016 (Microsoft, Redmond, Washington, USA) and SPSS (IBM, Armonk, New York, USA) were employed for data processing and statistical analysis. The experimental results were expressed as the mean ± standard deviation (SD). t-test was performed to compare the results of different experimental groups. The statistical diagrams were plotted using Origin Pro 2018 (OriginLab Corp., Northampton, MA, USA).

Results and discussion

Chromatic aberration of fresh-cut broccoli with different preservative treatments

The quality of fruits and vegetables can be evaluated through CIELAB (CIE Lab, representing L*, a* and b*) [29], which is an important indicator to reveal the color change of fresh-cut broccoli buds. The L* value represents brightness with the color getting darker as the value downscale. The H value indicates the color saturation, and a smaller value of H indicates a higher degree of yellowing. The changes in L* and H values of fresh-cut broccoli treated with six different concentrations of preservatives during storage at 4°C showed that the L* value demonstrated an upward trend, indicating that the brightness of the broccoli gradually increased and the color became lighter during storage. After 12 days of storage, L* value of all preservative treatment groups was lower than that of control group. The effects of SA, FUC, and nisin treatment groups were more significant, showing better color protection results (Figure 1A). The preservatives delayed the continuously decline of H value, which also confirmed that SA, FUC, and nisin treatment groups had more advantages (Figure 1B). At the end of storage, those three components had lower L* value and higher H value, which could well inhibit the yellows and chlorosis of fresh-cut broccoli. Therefore, SA, FUC, and nisin were preliminarily selected for response surface test. The results of the concentration range selection showed that 0.75% SA treatment had the best color protection effect on fresh-cut broccoli at 12 d with L* and H values of 51.94 and 115.80, respectively, followed by 0.50% and 0.25% SA treatment groups. Therefore, SA at concentrations of 0.25%, 0.50%, and 0.75% were selected as the response surfaces. Similarly, 0.25% FUC had a prominent effect on inhibiting yellowing, and three concentrations of 0.25%, 0.50%, and 1.00% were selected for subsequent experiments. The concentration of nisin was screened, and the L* and H values of 0.10% Nisin treatment on the 12 d were 49.76 and 119.91, respectively, which were the best among all the groups. The preservation effect of each concentration was ranked as 0.10% > 0.15% > 0.05% > 0.20%, and the first three concentrations were selected as the basis of response surface.

Response surface optimization

(1) Box-Behnken experiment

According to the results of single factor tests, SA (A), FUC (B), and Nisin (C) were selected as the three factors for RMS optimization, while the H value of fresh-cut broccoli on 12 d was applied as

A.

B.

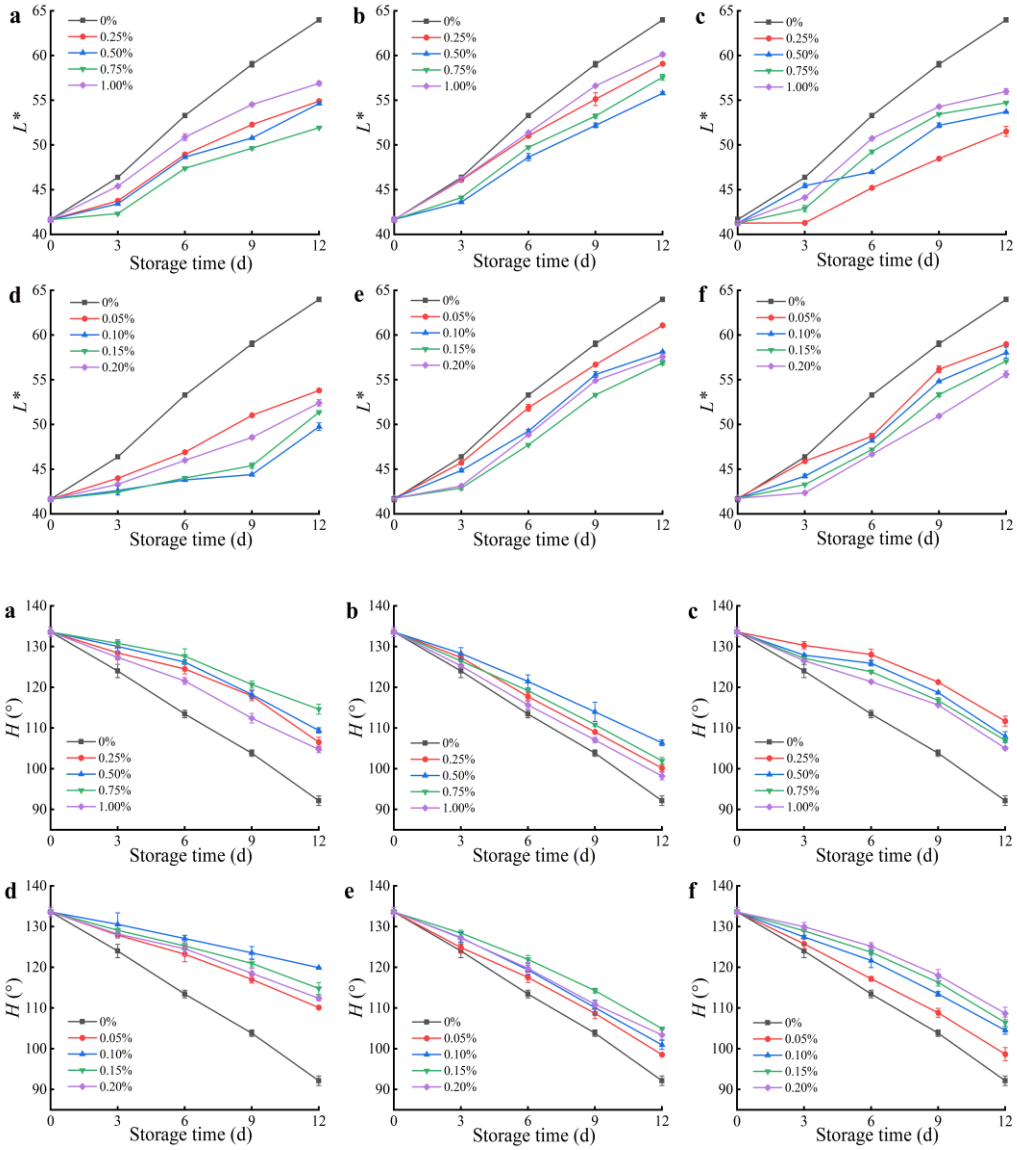

Figure 1. *L** value (**A**) and H value (**B**) of fresh-cut broccoli treated with different concentrations of bio-preservatives. **a**. sodium alginate. **b**. chitosan. **c**. fucoidan. **d**. nisin. **e**. natamycin. **f**. ε-polylysine.

the response value (R1) to establish the 3-factor and 3-level tests. The corresponding factors and levels were shown in Table 1.

Table 1. Design factors and levels by Box-Behnken.

Level	-1	0	1
SA (%)	0.25	0.50	0.75
FUC (%)	0.25	0.50	1.00
Nisin (%)	0.05	0.10	0.15

The results demonstrated that there were in total 17 runs for optimizing the parameters in the current Box-Behnken design (Table 2). After the data analysis, the multiple quadratic regression equation was obtained as follows.

$R_1 = 124.34 + 0.17A - 2.66B + 0.81C + 0.84AB - 0.1AC + 0.41BC - 2.53A^2 + 2.97B^2 - 0.8C^2$

(2) Regression model fitting

Table 2. Experimental design and results for response surface analysis.

Run	A (SA)	B (FUC)	C (Nisin)	R_1 (*H*)
1	0	0	0	124.99
2	-1	1	0	121.60
3	0	0	0	123.89
4	0	1	-1	122.15
5	1	1	0	123.39
6	1	0	1	121.91
7	-1	-1	0	127.85
8	-1	0	-1	119.91
9	0	0	0	124.24
10	1	0	-1	120.67
11	0	1	1	124.77
12	-1	0	1	121.56
13	1	-1	0	126.28
14	0	-1	-1	129.06
15	0	-1	1	130.04
16	0	0	0	123.92
17	0	0	0	124.67

The results showed that, within the range of factors in the design of response surface experiment, the influences of B and C on R1demonstrated significant difference ($P < 0.05$), while A had no significant impact on R1 ($P > 0.05$) (Table 3). Among the interaction items, AB showed a significant effect on R1, while AC and BC were not. The effects of quadratic terms A^2, B^2, and C^2 on R1 were all significant ($P < 0.05$). The coefficient of determination (R^2) of the model was 0.9830, while the R^2_{pred} of 0.8287 was in reasonable agreement with the R^2_{adj} of 0.9611. The model's F-Value was 44.90 with the *P v*alue less than 0.0001, implying that the regression of the model was notable. The "Lack of Fit" had no significant difference compared to the pure error ($P > 0.05$), which suggested that the regression model could meet the analysis and prediction requirements on the chromatic aberration of fresh-cut broccoli. The influencing order of each factor was FUC > Nisin > SA.

(3) Response surface analysis

The response surface plot is a theoretical three-dimensional output of the RSM approach, which reflects the relationship between dependent variable and interactive factors [30]. The contour plot is a two-dimensional screen of surface plot, in which the size of the interaction between factors can be determined by the position and shape of contour lines. The results showed that the response surface slope of the interaction between SA and FUC was steep, and the contour lines were in parabolic or hyperbolic system, indicating that the interaction between these two factors was significant (Figure 2A and 2B). The hue angle H increased first and then reduced later with the increase of SA and nisin concentrations, while the contour plots showed a circle status, suggesting that the interaction between these two factors was weak (Figures 2C and 2D). With the increase of FUC and nisin concentrations, the response surface showed a gradual decreasing trend, indicating that there was a certain interaction between the two factors (Figures 2E and 2F). The results suggested that the optimum compounds of bio-preservatives were 0.46% SA, 0.25% FUC, and 0.11% nisin. The verification experiment results showed that the H value under the SA-FUC-Nisin compound preservative was 129.97 ± 0.84, which was close to the predicted value of 130.06, indicating that this model was precise and feasible for fresh-cut broccoli preservation.

Effects of compound preservative on quality indexes of fresh-cut broccoli

(1) Effects on chlorophyll and carotenoids contents

Yellowing and chlorosis involve the coordination metabolize of multi-pathway pigment in plant tissues, and the main metabolic pathways leading to these are chlorophyll degradation and carotenoid synthesis [31]. The contents of chlorophylls in fresh-cut broccoli were continuously reduced during storage, while the treatment with SA-FUC-Nisin compound preservative effectively slowed down this process (Figures 3A to 3C). At the end of storage (12 d), the chlorophyll contents of the CP treatment group were 1.77 (chlorophyll a), 1.32 (chlorophyll b), and 1.66 (total chlorophyll) times more than that of the CK group, respectively. On the contrary, the carotenoids in fresh-cut broccoli increased during the storage period with

Table 3. Regression models and analysis of variance.

Source	SS	DF	MS	F value	*P* value
Model	129.17	9	14.35	44.90	< 0.0001*
A	0.22	1	0.22	0.69	0.4330
B	56.82	1	56.82	177.75	< 0.0001*
C	5.27	1	5.27	16.47	0.0048*
AB	2.82	1	2.82	8.83	0.0208*
AC	0.042	1	0.042	0.13	0.7276
BC	0.67	1	0.67	2.10	0.1902
A^2	26.89	1	26.89	84.13	< 0.0001*
B^2	37.02	1	37.02	115.82	< 0.0001*
C^2	2.71	1	2.71	8.48	0.0226*
Residual	2.24	7	0.32		
Lack of fit	1.32	3	0.44	1.91	0.2696
Pure error	0.92	4	0.23		
Cor total	131.40	16			

Note: *significant difference ($P < 0.05$).

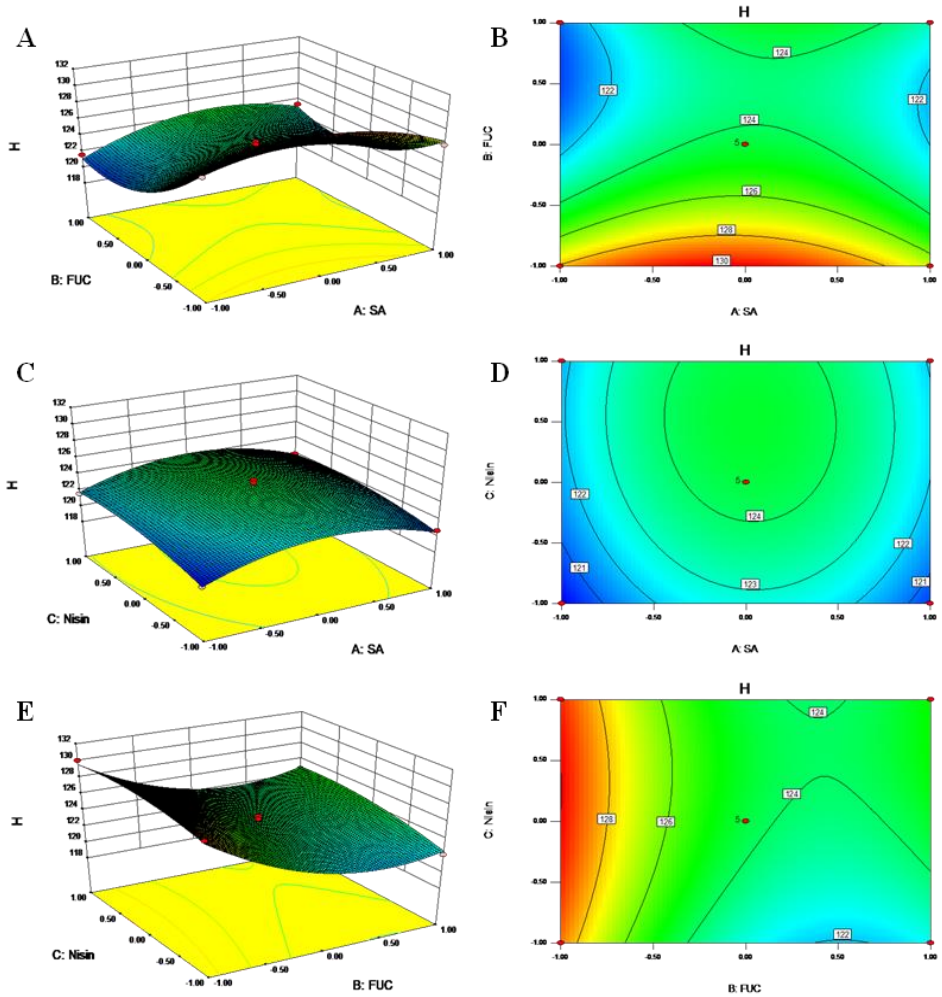

Figure 2. Interactive effects of SA, FUC, and nisin on the H value of fresh-cut broccoli. **A.** 3D surface of SA and FUC. **B.** contour of SA and FUC. **C.** 3D surface of SA and nisin. **D.** contour of SA and nisin. **E.** 3D surface of FUC and nisin. **F.** contour of FUC and nisin.

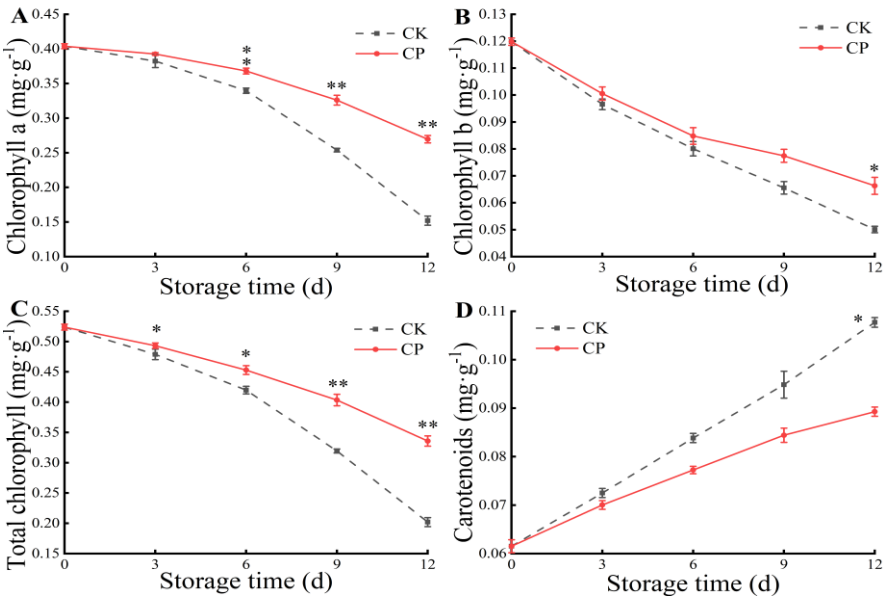

Figure 3. Effect of compound preservative treatment on the chlorophyll a (**A**), chlorophyll b (**B**), total chlorophyll (**C**), and carotenoids (**D**) in fresh-cut broccoli.

CP group consistently lower than CK group (Figure 3D), which might be because, in the etiolated sepals, the plastids in the chloroplast development process gradually moved from the storage site of chlorophyll to the storage site of carotenoids [32]. Compared with control group, SA-FUC-Nisin composite treatment gained a positive impact on chlorophyll retention and could significantly inhibit carotenoid synthesis during the storage period of fresh-cut broccoli, showing a good color protection effect.

(2) Effects on TA, TSS, VC, and RSC of fresh-cut broccoli

TA refers to the collection of organic acids found in fruits and vegetables. The types and contents of these acids significantly impact the flavor, pH value, and storability of garden stuff. TA content in untreated fresh-cut broccoli showed a repeated trend of decreasing then increasing during storage, while that in CP group showed a relatively stable state (Figure 4A). This phenomenon might be attributed to the fact that broccoli, even after fresh cutting, continued to perform metabolic activities, consuming a significant number of organic acids in the respiratory process [33]. Simultaneously, oxidative decomposition of reducing sugars could replenish the continuously consumed organic acids [34]. The result indicated that the CP treatment could stabilize the synthesis and accumulation of organic acids in fresh-cut broccoli, maintain its internal acid-base balance, and prevent sourness or flavor change. TSS contains water-soluble organic substances such as acids and sugars, and their contents can reflect the flavor and nutritional variations in fruits and vegetables. TSS may be used in the respiration process and be affected by the presence of metabolic activity [35]. The results showed that the TSS of fresh-cut broccoli increased first and then decreased during storage, and the advantages of the composite preservative treatment became more and more obvious over time. TSS content of untreated broccoli was initially 8.17% and increased to 8.67% after 3 days, then decreased to 6.17% after 12 days of storage. A similar tendency during storage was noticed in CP treated samples as well, and the TSS mass fraction of which was 17.33% higher than that in CK group at the end of storage (Figure 4B). VC is an antioxidant nutrient widely present in fruit and vegetable tissues, and its content can be used as one of the evaluation indicators to

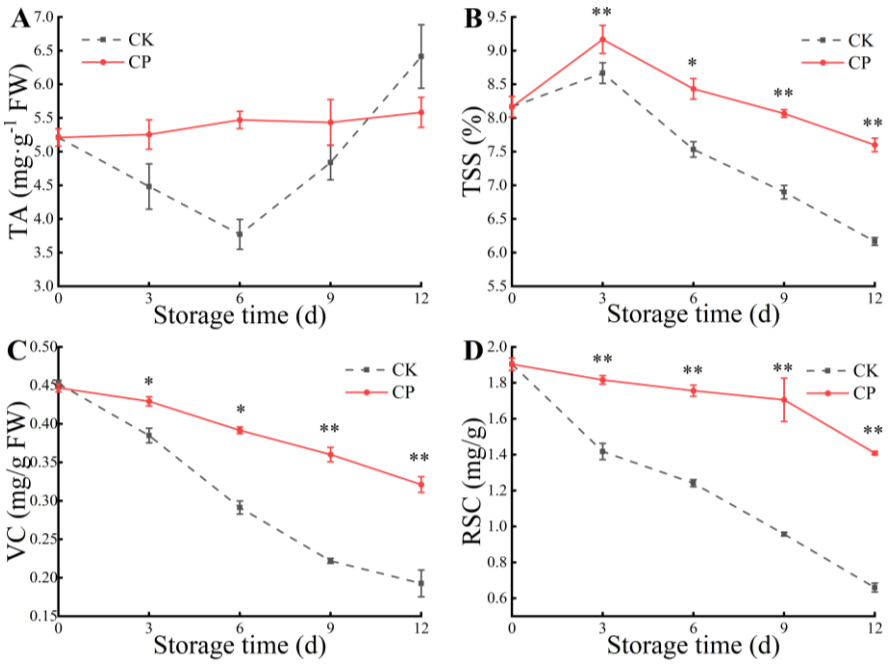

Figure 4. Effect of compound preservative treatment on TA (**A**), VC (**B**), TSS (**C**), and RSC (**D**) in fresh-cut broccoli.

measure the nutritive quality and stash effect of fresh-cut foods. VC content in fresh-cut broccoli continuously decreased as the storage period progressed. This decline was slower in SA-FUC-Nisin treated broccolis. After 12 days of storage, the VC content of CP group was 0.32 ± 0.01 mg/g, obviously higher than that in CK group of 0.19 ± 0.02 mg/g (Figure 4C), indicating that the compound preservative treatment could delay the aging process of fresh-cut broccoli and maintain its antioxidant capacity. Reducing sugar is another nutrient that can preserve the sensory quality of vegetables and fruits, and its content can measure the flavor of fresh-cut broccoli, which also related to respiratory metabolic intensity as well as degree of decay [36]. The RSC of fresh-cut broccoli decreased significantly in CK group than that in CP group (Figure 4D). RSC of the CK group changed dramatically during storage, and the minimum content at 12 d of storage was 0.66 ± 0.02 mg/g, which only 34.65% of the initial level. Meanwhile, compound preservative treatment effectively inhibited the mutation of RSC with the content of 1.41 ± 0.01 mg/g at the end of storage, which was possibly because of the inhibitory effects of SA-FUC-Nisin compound preservative on respiratory intensity, thereby reducing the degradation of RSC.

(3) Effects on total phenolic and flavonoid contents

Vegetables contain abundant phenolic compounds, flavonoids, and other plant secondary metabolites in their tissues, which are closely linked to the overall antioxidant activities including coloration and browning. Total phenolic (TP) and flavonoid content of fresh-cut samples were significantly affected by compound preservative and storage time. The total phenol content of fresh-cut broccoli decreased after rapid growth with storage time prolonging, and the content of CP group remained at a relatively high level. At the end of storage (12 d), the TP content of broccoli treated with compound preservative was 10.87% higher than that of controls (Figure 5A). The content of flavonoid in fresh-cut broccoli increased firstly and then decreased with prolonged storage (Figure 5B). The content reached the peak on the 6th day of storage with the contents in the CP group and CK group being 2.68 ± 0.02 mg/g and 2.21 ± 0.01 mg/g, respectively. The flavonoid content in CP

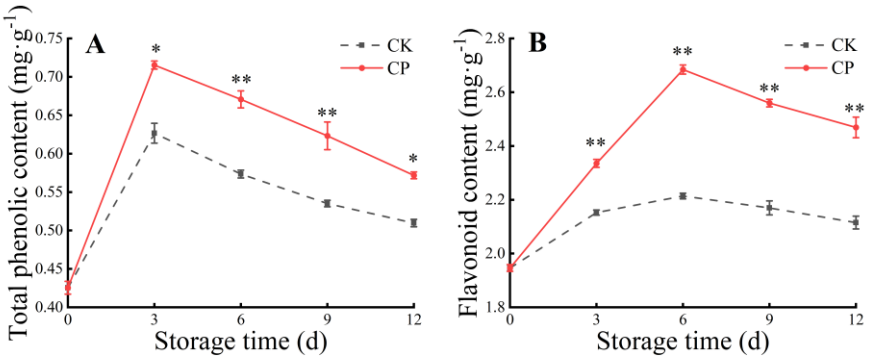

Figure 5. Effect of compound preservative treatment on total phenolic content (**A**) and flavonoid content (**B**) in fresh-cut broccoli.

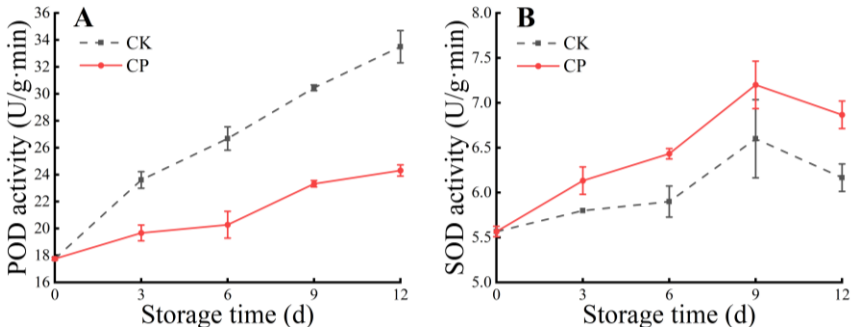

Figure 6. Effects of compound preservative on POD activity (**A**) and SOD activity (**B**) of fresh-cut broccoli.

group was 14.3 % higher than that in CK group at 12 d of storage. These results indicated that SA-FUC-Nisin combined treatment could promote the synthesis of total phenols and flavonoids in fresh-cut broccoli and maintain the strong antioxidant capacity of the samples during storage.

(4) Effects on POD and SOD activities

POD and SOD are antioxidant enzymes closely related to aging and yellowing of plants. They can interact with each other to effectively defend plant membrane against the damage of reactive oxygen species (ROS) and other superoxide radicals, thereby reducing ROS produced during metabolic process in plants and delaying plant senescence [37, 38]. As storage time increased, POD activity of fresh-cut broccoli showed an overall increasing trend with the treatment group remarkable lower than the control group (Figure 6A). After 12 days of storage, POD activities in CK and CP groups reached 1.92 times and 1.37 times of their initial values (0 d), respectively, indicating that compound preservative treatment could significantly inhibit the mutation of POD activity. The results further showed that the activity of SOD tendency was activated first and then inhibited during storage, and that of CP treatment group was consistently higher than CK group. After a massive rise in SOD activity to 6.6 ± 0.44 U/g in CK group and 7.2 ± 0.26 U/g in CP group on the 9th day, there was a sudden slump at the end of storage with the SOD activity of untreated broccoli being 89.81% of compound preservative group (Figure 6B). The results suggested that the SA-FUC-Nisin compound preservative could dramatically increase and maintain the SOD activity of fresh-cut broccoli.

Analysis of antibacterial effect of compound preservative

(1) Microbiological quality

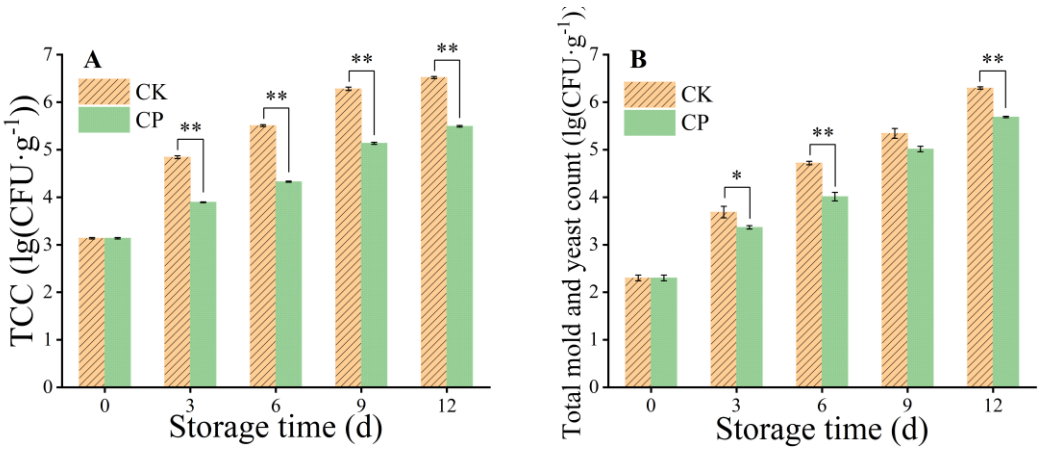

Figure 7. Effect of compound preservative treatment on TCC (**A**), total mold and yeast count (**B**) in fresh-cut broccoli.

Table 4. Alpha diversity of fresh-cut broccoli before and after storage.

Sample	Chao 1	Dominance	Goods coverage	Observed features	Pielou e	Shannon	Simpson
0 d	167.76	0.19	1.00	118	0.43	2.93	0.73
CK12	289.62	0.30	1.00	265	0.28	2.25	0.49
CP12	138.96	0.20	1.00	106	0.38	2.59	0.70

Another important factor to measure the quality of fresh-cut foods is the number of microorganisms. Cutting processing destroyed the cellular structure of fruits and vegetables, causing juice leakage, which could easily lead to the proliferation of microorganisms in the cut area, affecting the safety of fresh-cut foods for consumption [39]. Bacteria, mold, and yeast are the main microorganisms that cause the spoilage of fresh-cut foods. The inhibitory effects of compound preservative on different microbial populations in fresh-cut broccoli showed that the total colony count (TCC) in fresh-cut broccoli continuously increased during storage (Figure 7A). When stored for 12 days, TCC of the control group exceeded 10^6 CFU/g, which seriously affected the quality and safety of consumption. In contrast, the treatment with SA-FUC-Nisin compound preservative could inhibit bacterial growth to a large degree. In addition, the changing trends of total mold and yeast counts were similar to those of TCC, and the compound preservative could also inhibit their growth rates (Figure 7B).

(2) Alpha diversity

Alpha diversity can be a promising method for analyzing the richness and diversity of microbial community in samples [40]. Chao1 and observed features are commonly used to evaluate the abundance of community distribution, where higher values indicate greater species. Shannon, Simpson, and Pielou e can reflect the evenness and diversity of species distribution in community. The species distribute more uniformly and have richer diversity as Shannon/Simpson rise [41]. The results showed that the coverage values of all three samples were 1.00, expressing good sequencing depth and broad reach. From day 0 to day 12, the microbial abundance of the CK group significantly increased while the CP group slightly decreased. On the 12^{th} day, the bacterial richness of the CK group was prominently exceeded that of CP group, and Shannon indices of both groups were lower than those on 0 d as well as Simpson (Table 4). The CP group showed the smallest changes in various indices compared to 0 d, indicating that the compound preservative could stabilize the

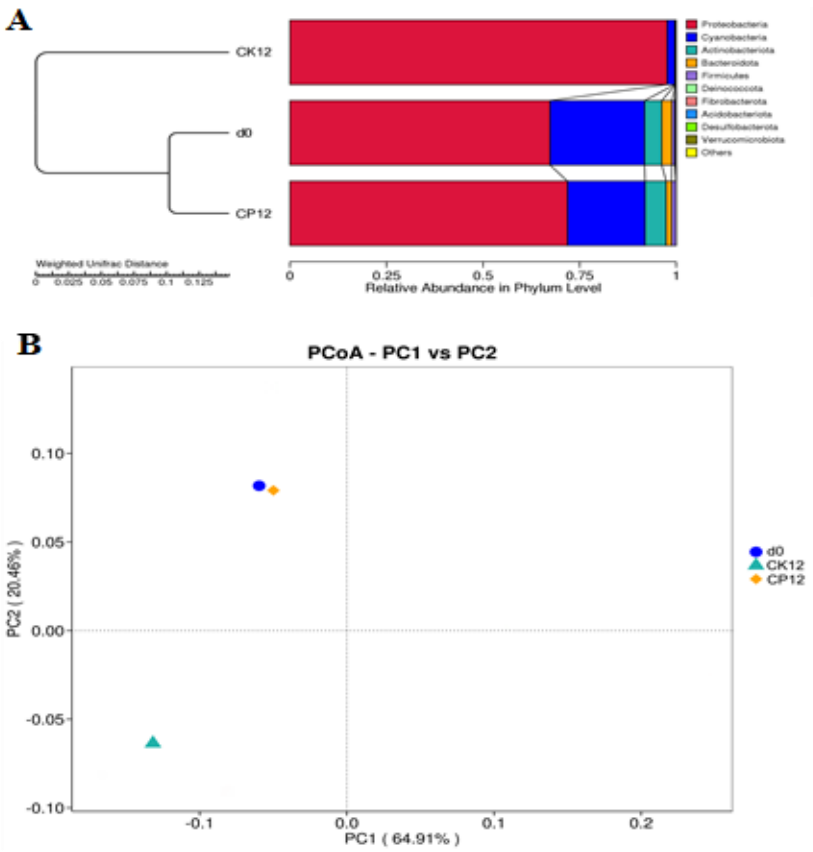

Figure 8. UPGMA analysis (**A**) and PCoA (**B**) of bacterial communities of fresh-cut broccoli before and after storage with different treatments.

diversity and partly inhibit community enrichment.

(3) Unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis

A UPGMA tree diagram constructed through clustering analysis based on the similarity between different samples demonstrated that the difference in bacterial colonies between fresh-cut broccoli on day 0 and the one treated with compound preservative on day 12 were the smallest (Figure 8A). Compared with CK group, CP treatment resulted in a bactericidal effect on intestinal pathogens such as *Proteobacteria* (*E. coli*, *etc.*) and *Bacteroidota*. Meanwhile, principal co-ordinates analysis (PCoA) was conducted and the results showed that the closer the two sampling points were, the more similar the species composition of the two samples was (Figure 8B). The results indicated that the colony difference between fresh-cut broccoli on day 0 and those treated with SA-FUC-Nisin on the day 12 were minimal. The results were consistent with the total bacterial count, indicating that the composite coating effectively inhibited the growth of microorganisms.

Conclusion

This study used SA, FUC, and Nisin as base materials to prepare the composite preservative according to the color protection effects on fresh-cut broccoli. Through the optimization by RSM, the optimal concentrations of each component in the composite preservative were 0.46% SA, 0.25% FUC, 0.11% Nisin, respectively. The results showed that, through the management of composite preservatives, the degradation of chlorophyll and nutrients in fresh-cut broccoli during storage at 4°C was effectively slowed down, no quality decline or odor

occurred, and the quality level was improved compared with the control group. Further, the results also demonstrated that the use of SA-FUC-Nisin composite preservative was a feasible alternative solution for controlling the microbiota structure in fresh-cut broccoli. The positive effects exerted by composite preservative on fresh-cut broccoli were significant, respect to the stability and uniformity of species distribution. The total bacterial count as well as mold and yeast were considerably depressed during the whole storage period owing to the application of the composite preservative. Besides, the compound preservative treatment resulted in a bactericidal effect on intestinal pathogens such as *Proteobacteria* (*E. coli*, *etc*.) and *Bacteroidota*. This research provided data basis for the exploitation of edible polysaccharides combined with natural antibacterial agents. The combination of those components as bio-preservative offers greater potential for inhibiting chlorosis, spoilage, and nutrient loss of fresh-cut vegetables, thereby prolonging their shelf-life.

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