

# Cherry tomato preservation using chitosan combined with zinc/cerium ion

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**Abstract:** The effect of 1% chitosan with 0.03% zinc acetate or cerium acetate on the qualitative properties of harvested cherry tomato under ambient temperature was investigated. The results showed that chitosan maintained its crystal structure, but its crystallinity decreased in chitosan-zinc composite coating. While in chitosan-cerium composite coating, the chitosan crystal structure was changed. Two composite coating could inhibit the decrease of cherry tomato firmness and soluble solids, and suppress the increase of weight loss and malondialdehyde content. In addition, the respiratory rate was maintained lower level, and the activities of superoxide dismutase, peroxidase and catalase were maintained higher level during the cherry tomato storage time. Besides, after coating, more vitamin C and lycopene in cherry tomato were reserved compared with control sample.

**Keywords:** Chitosan, Zinc Acetate, Cerium Acetate, Cherry Tomato, Preservation

## 1. Introduction

Chitosan has good film forming performance and excellent biocompatibility, and is also safe and non-toxic. It has broad application in fruit and vegetable preservation [1]. Zinc, the IIB group transition metal element in the fourth cycle, is apt to form coordination bond with hydroxyl functional groups. It is associated with biological auxin synthesis and has important physiological functions to human body [2]. Cerium belonging to rare earth elements has many unique properties in biology, but its safety effect remains to be further studied [3]. Wang etc. had found that chitosan-cerium compound coating could destroy P = O bond and degrade organophosphorus pesticide residues [4].

Cherry tomato is a popular fruit with bright red color and delicious flavor. But cherry tomato is prone to desiccation shrinkage and fruit softening during storage, resulting in the decrease of its commodity value [5]. In this paper, the compound coating of chitosan combined with zinc or cerium was prepared and applied in fresh cherry tomato preservation. The related physiological, biochemical and nutritional parameters of post-harvested cherry tomatoes during storage time were analyzed. We hope explore a new method for fresh cherry tomato preservation.

## 2. Materials and Methods

### 2.1. Materials

Cherry tomatoes were purchased from a vegetable wholesale market in the vicinity of Shanxi Normal University. Those with uniform size, consistent maturity, no deformation, no mechanical damage, no diseases and insect pests were selected as experimental materials.

Water-soluble chitosan with more than 85% deacetylation degree and more than 99% solubility was purchased from AK Biotech Ltd., (Shandong, China); Cerium acetate and zinc acetate with more than 99.9% purity were purchased from Gracian Chemical Technology Ltd., (Chengdu, China); Alfa Aesar Company (Tianjin, China) supplied other reagents, which were all analytical grade.

### 2.2. Preparation of the Coating Solution and Cherry Tomato Treatment

Solutions of (a) 20 mL of 5% chitosan solution, (b) 20 mL of 5% chitosan solution + 40 mL solution containing 0.0075 g of zinc acetate, (c) 20 mL of 5% chitosan solution + 40 mL solution containing 0.0075 g of cerium acetate, were stirred with a magnetic stirrer for 5 h at 85 °C respectively. After

cooling to ambient temperature, above three solutions were all diluted to 1000 mL. Thus, different coating solutions, namely (a) 1% chitosan coating, (b) 1% and 0.03% zinc acetate composite coating, (c) 1% and 0.03% cerium composite coating, were acquired. Besides, 1000 mL of deionized water marked with d was used as control coating. The coating liquid was divided into two parts, one part for filming on a glass slide, then drying in the electrothermal blowing dry oven (Yuejin medical instrument co., Ltd., Shanghai, China) at 35 °C, the membranes were characterized by XRD, FTIR and micrography, and the other part for treating cherry tomatoes.

Cherry tomatoes were washed clean with tap water and dipped into each prepared solution for 3 min. Afterward they were taken out and dried at room temperature. The coated cherry tomatoes were preserved in the crispers for 24d and stored at about 20°C with 85% of relative humidity. The related parameters of cherry tomatoes were determined periodically.

### 2.3. Characterization of Compound Coating

#### 2.3.1. X-Ray Diffraction Analysis (XRD)

The crystallinity of chitosan coating was assayed using X-Ray Diffractometer (type of D8Advance, Bruker Corporation, Germany), which was graphite monochromator, Cu target,  $\lambda(\text{Cu K}\alpha 1)=0.15406\text{nm}$ , 40 kv tube voltage, 40 ma tube current, the scanning speed 4° / min, 5° ~ 40° scan range.

#### 2.3.2. Characterization Infrared Spectroscopy

Infrared Spectra was assayed using FTIR meter (Varian 640, Varian Corporation, USA). The resolution is 0.25 $\text{cm}^{-1}$ , the optical source is AC Ceramic, the interferometer with 60 degree and 3 sport is laser positioning of dynamic collimation, and the rang of spectrum is between 400 and 4000 $\text{cm}^{-1}$  (standard configuration). In addition, it contains standard check unit.

#### 2.3.3. Micrograph

The surface pattern of the compound coating was observed using biological microscope (microscope Olympus and camera MC50, Guangzhou Ming-Mei Technology Co., Ltd, China ) at 100 and 400 times, respectively.

### 2.4. Determination of Indexes Related to Cherry Tomato Preservation

#### 2.4.1. Determination of Firmness and Weight Loss

The cherry tomato firmness was measured directly from the maximum diameter of fruit surface using a fruit sclerometer (GY-3, Chendu Bsida Instrument Co., Ltd., Chendu, China).

About 500g of cherry tomato was taken out from each samples and weighed per three days. The weight loss was calculated as following formula: weight loss (%) =  $[(m_0 - m_1)/m_0] \times 100$ , where  $m_0$  is the initial weight and  $m_1$  is the weight measured during storage.

#### 2.4.2. Determination of Respiration Rate and Soluble Solids

Respiration rate was assayed using infrared absorption method described by Yu Youwei *et al* [6]. 600 g of cherry

tomatoes was put into the dryer and sealed for 1 h at room temperature. After 1 h, CO<sub>2</sub> concentration in the dryer was measured by infrared carbon dioxide detector (GXH-3010F, Beijing Huayun Analysis Instrument Co., Ltd., Beijing, China). The respiration rate was calculated as following formula: respiration rate (mgCO<sub>2</sub>/kg•h) =  $V \times 44 \times C \times 103 / (22.4 \times Wt \times h)$ , where V is the volume of the container, C is volume percentage concentration of CO<sub>2</sub>, Wt is the weight of sample and h is the determination time.

The soluble solids were determined using a refractometer (WYT-II, Qingyang Optical Instrument Co., Ltd., Chendu, China) and converted to standard value by 20°C.

#### 2.4.3. Determination of Enzyme Activities

SOD (superoxide dismutase) activity was determined using a modified method [7]. 2.0g samples was homogenized with 15 mL of 50 mmol/L phosphoric acid buffer (pH 7.8) in ice-bath, and centrifuged at 8000g for 15 min at 4 °C with an Eppendorf 5417R centrifuge (Germany). The supernatant was collected as a crude enzyme of SOD. Four tubes with good transparency and uniform quality were chosen. The reagents were added into above tubes as table 1. After mixing, No.3 tube was overlapped with bistratal black paper sleeve which is longer to the tube to avoid lighting, and then placed under the light with the other tubes for 20 min. The reaction was terminated by covering with black cloth. The No.3 tube was served as the blank control to set zero. The absorbance of the tubes was separately determined using spectrophotometer at 560nm. The activity of SOD was calculated with the following formula. SOD activity [U·g/(FW·h)] =  $(A_0 - A_s)V \times 60 / (0.5 \times A_0 \times FW \times a \times t)$ . In the formula, A<sub>0</sub> means the absorbance of the controlled samples, A<sub>s</sub> means the absorbance of the treated samples, a means the volume of the solution for determination (mL), FW means the fresh weight(g), V means the total volume of the sample solution (mL), and t means the lighting time (min).

Table 1. Adding reagents for SOD activity assay

Reagent (ml)	Tube No.			
	1	2	3	4
50m mol/L phosphoric acid solution	1.5	1.5	1.5	1.5
130m mol/L methionine	0.4	0.4	0.4	0.4
750 $\mu\text{mol/L}$ NBT solution	0.4	0.4	0.4	0.4
100 $\mu\text{mol/L}$ EDTA-Na <sub>2</sub> solution	0.4	0.4	0.4	0.4
200 $\mu\text{mol/L}$ lactoflavin	0.4	0.4	0.4	0.4
Crude enzyme extracting	0.1	0.1	0	0
Distilled water	0.5	0.5	0.6	0.6

POD (peroxidase) activity was analyzed using a modified method [8]. 2.0g samples was homogenized with 15 mL of 50mmol/L phosphoric acid buffer (PH,7.8) in ice-bath, and centrifuged at 8000g for 15 min at 4°C. The supernatant was collected as a crude enzyme. The assay mixture contained 2 mL of 50 mmol/L phosphoric acid buffer (pH 7.8) and 0.6 mL of 0.04mol /L guaiacol. After heating and stirring for 10 min, 1.5 mL of enzyme solution was added into the mixture. The reaction began with 0.1 mL of 15% H<sub>2</sub>O<sub>2</sub>. POD activity was measured by an increase in absorbance at 470nm per min for 3 min in total. The activity of POD was calculated

with the following formula, POD activity [U/(g·min)] =  $\Delta A_{470} \cdot V / (FW \times a \times 0.01 \times t)$ . In the formula, V means the total volume of the crude enzyme extracting (mL), FW means the fresh weight(g); a means the volume of the crude enzyme extracting for determination (mL), and t means the reaction time(min). One unit of POD activity was defined as a 0.01 increase in absorbance at A470 per min.

CAT (catalase) activity was assayed according to the method described by Garcí'a et al [9]. (2007). 2.0g samples was homogenized with 15 mL of phosphoric acid buffer (pH 7.0) containing 1% polyvinyl-polyrrolidone (PVPP), and centrifuged at 8000g for 15 min at 4 °C. The supernatant was collected as a crude extracting of CAT. The assay mixture contained 2 mL of 50mmol/L phosphoric acid buffer (pH 7.0), 1 mL distilled water. And then the mixture was preheated at 40°C for 10 min, and 0.6 mL crude enzyme was added. After that, 1 mL of 30% H<sub>2</sub>O<sub>2</sub> was applied to start reaction. The absorbance was measured at 240nm per 30s. One unit of CAT activity was defined as a 0.1 decrease in absorbance at A<sub>240</sub> per min. the activity of the CAT enzyme was calculated as the following formula: CAT activity [U/(g·min)] =  $\Delta A_{240} \times V / (0.1 \times a \times t \times FW)$ , where V is the total volume of crude enzyme extracting solution (mL), FW is the weight of fresh samples (g), a is the volume of crude enzyme extracting solution to determine (mL, 0.1 is One unit of CAT activity which was defined as a 0.1 decrease in absorbance at A<sub>240</sub>, and t is the last reading duration after adding H<sub>2</sub>O<sub>2</sub>.

#### 2.4.4. Determination of Malonaldehyde (MDA) Content

MDA was measured as previously described by Xing et al. [10]. 0.5 g samples was homogenized with 10 mL of 50 mmol/L phosphoric acid buffer (pH7.8) in ice-bath, The mixture was then added 5 mL of 0.5% thiobarbituric acid solution and heated to 100°C for 10 min (determinating from the emerge of little bubble in the tube). After the rapid cooling of the sample to 4°C and centrifugation at 3000g for 15 min, the supernatant was collected as a crude extracting of MDA. The absorbance of the MDA solution which was served as the blank control samples was measured separately at 532nm, 600nm and 450nm. Calculating the MDA content via the following formula, MDA concentration =  $6.45(D_{532} - D_{600}) - 0.56D_{450}$ , MDA content (mmol/g) = MDA concentration  $\times V_t / (V_1 \times W)$ . In the formula, V<sub>t</sub> means the volume of extracting solution (mL), V<sub>1</sub> means the volume of extracting solution for determination (mL) and W means the fresh weight of samples (g).

#### 2.4.5. Determination of Ascorbic Acid (VC) and Lycopene Content

The vitamin acid content was measured by 2, 6-dichloroindophenol titration [11]. 2 g of sample was homogenized with 10 mL of 2% oxalic acid solution under ice bath grinding, and then centrifuged at 8000g for 15 min at 4 °C. The supernatant was collected as sample solution. Afterwards, 2 mL of supernatant in 10 mL erlenmeyer flask was titrated to a permanent pink colour using 0.1% 2,6-dichloroindophenol solution, kept the color for 15s, and

recorded the volume of titration solution consumed. The vitamin acid concentration was calculated as follows: Ascorbic acid content ( $\mu\text{g} / 100\text{g}$ ) =  $V_1 \cdot T \cdot V \cdot 100 / FW$ , where V<sub>1</sub> is dye volume consumed (mL); FW is fresh weight of the sample (g); T is milligrams of ascorbic acid required in tests to oxidate 1 mL of dye (mg), which can be calculated by the calibration; V is titrant volume used for measuring (mL)

The cherry tomato lycopene was measured according to the method of GB/T14215-2008 [12]. The standard curve was drawn as followed. 0.025 g of Sudan red I pigment was dissolved with a small amount of anhydrous acetone, and then diluted to 50 mL with anhydrous acetone. 0.26, 0.52, 0.78, 1.04, 1.30 mL of diluted solution were respectively transferred to 50 mL of volumetric flask and diluted to the 50 mL, which were equivalent to 0.5, 1.0, 1.5, 2.0, 2.5  $\mu\text{g}/\text{mL}$  lycopene standard solution. Then, the absorbance values were measured at 485 nm and repeated 3 times for each solution. Fresh tissue (1.5 g) from cherry tomatoes was homogenized with 15 mL of acetone under ice bath and centrifuged at 8000g for 15 min at 4 °C, the supernatant was collected as lycopene extract. The absorbance was measured at 485 nm. Lycopene content was calculated with the following formula:  $X = 5 \cdot C / FW$ , where X is lycopene content in the sample (mg/g); FW is sample fresh weight (g); C is lycopene concentration in the sample extract ( $\mu\text{g}/\text{mL}$ ).

### 3. Results and Analysis

#### 3.1. Characterization of the Compound Coating with Chitosan+ Acetic Zinc / Cerium Ion

##### 3.1.1. Infra-Red Spectrum

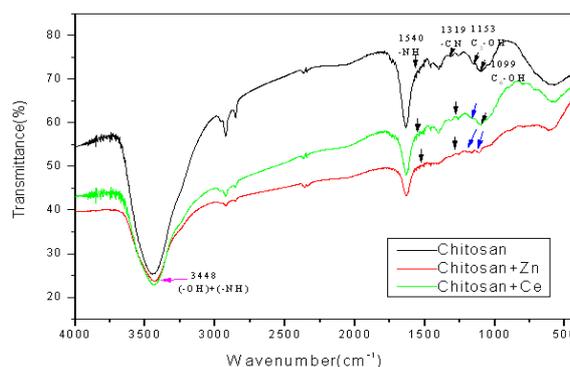


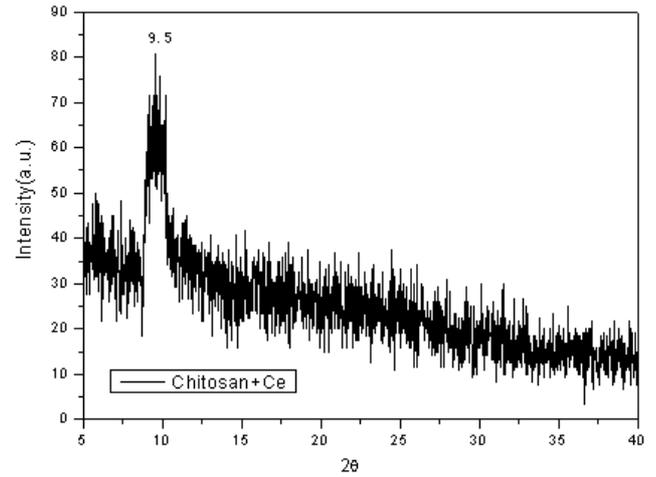
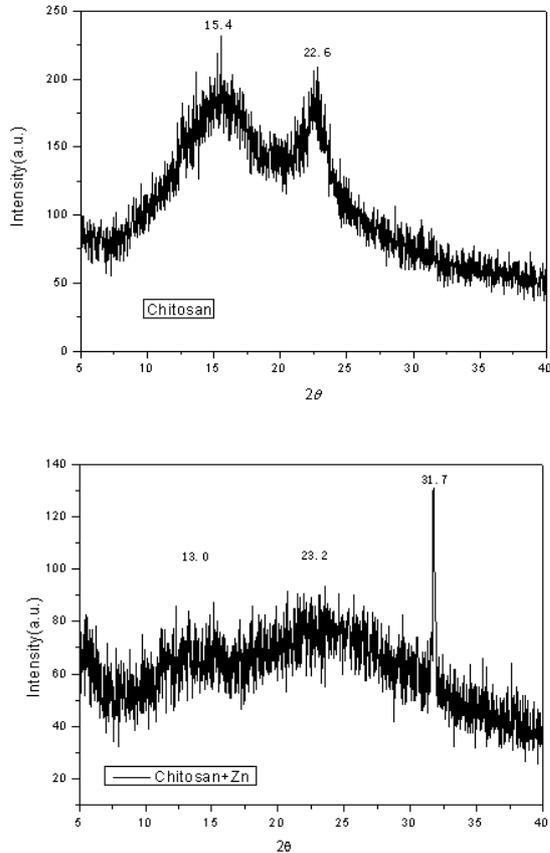
Fig 1. Infrared spectra of composite coatings of chitosan acetic zinc / cerium ion

Fig 1 demonstrates the FTIR spectra of composite coatings of chitosan acetic zinc / cerium. In comparison with the IR spectra of chitosan, -OH and -NH overlapping peaks of chitosan-zinc composite membrane at 3448  $\text{cm}^{-1}$  shifted to lower wave numbers, showing that the atoms of the chitosan coordinated with  $\text{Zn}^{2+}$ . -NH absorption peak at 1540  $\text{cm}^{-1}$  and -CN absorption peak at 1319  $\text{cm}^{-1}$  had not shifted. -C<sub>3</sub>OH absorption peak at 1153  $\text{cm}^{-1}$  and -C<sub>6</sub>OH absorption peak at 1099  $\text{cm}^{-1}$  of Chitosan-zinc composite

membrane shifted to higher wave numbers, indicating  $Zn^{2+}$  coordinated with hydroxyl. The  $-C_3OH$  absorption peak at  $1153\text{ cm}^{-1}$  in chitosan-cerium acetate composite coating shifted to higher wave numbers, indicating  $Ce^{3+}$  mainly coordinated with secondary hydroxyl, which was consistent with research results by Wang *et al* [13]. The reason is probably that  $-NH_2$  is border alkaline,  $Ce^{3+}$  is hard acid, and hydroxyl is hard base; according to the reaction rule of the hard and soft of acids and bases,  $-OH$  of chitosan molecules will coordinate with  $Ce^{3+}$  primarily [14].

### 3.1.2. XRD Diffraction Pattern

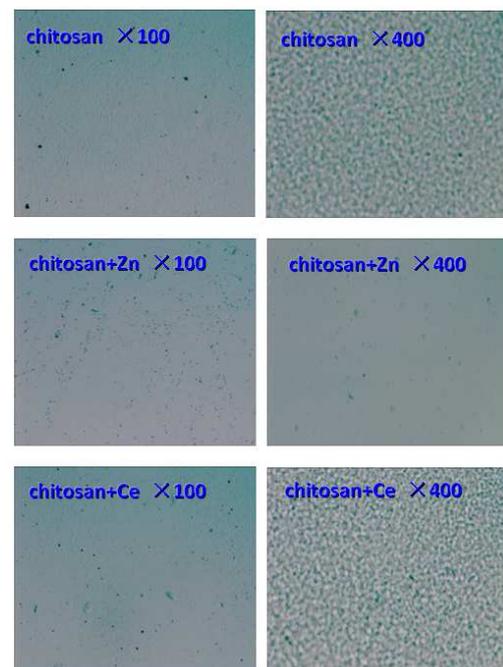
As shown in Fig 2, water-soluble chitosan appeared two characteristic diffraction peaks in the vicinity of  $15.4^\circ$  and  $23.6^\circ$ . And chitosan-zinc composite membrane still had two diffraction peaks in the vicinity of  $13.0^\circ$  and  $23.2^\circ$ , but compared with single chitosan membranes, the peaks widened, indicating that the crystallization of chitosan decreased. In chitosan-zinc acetate composite membrane, there was a sharp diffraction peak at about  $31.7^\circ$ , which might be characteristic peak of zinc acetate [15]. In chitosan-cerium composite membrane, the diffraction peaks of chitosan in the vicinity of  $15.4^\circ$  and  $23.6^\circ$  disappeared completely, and a new diffraction peak emerged near  $9.5^\circ$ . Because of no characteristic peak for cerium acetate below  $10^\circ$ , these characteristics showed that the crystal form of chitosan in chitosan-cerium composite membrane changed [16].



**Fig 2.** XRD spectra of composite coatings of chitosan acetic zinc / cerium

### 3.1.3. Micrograph

Composite membrane formed by chitosan and zinc acetate presented uniform film, while single chitosan membrane or chitosan-cerium acetate composite membrane was similar to particles which were evenly distributed (Fig.3). Chitosan-cerium composite membrane particles were more compact than that of single chitosan membrane. The crystallinity of chitosan in chitosan-zinc acetate composite membrane was lower, so the composite coating tended to distribute evenly during film forming, forming uniform film. In contrast, the crystallinity of chitosan was higher in chitosan-cerium composite membrane and single chitosan membrane, and it might be that the crystalline and amorphous particles strewn at random, thus forming particle-shaped film.



**Fig 3.** Photomicrograph of composite coatings of chitosan acetic zinc / cerium

### 3.2. Effect of Compound Coating on Cherry Tomato Preservation

#### 3.2.1. Firmness and Weight Loss

Cherry tomato firmness presented downtrend with the extension of storage days (Fig.4a). Coated cherry tomato kept higher firmness during storage. In the first 8 d, the firmness of cherry tomato with chitosan coating alone was the highest. Between 12 and 20 d, the firmness of that with chitosan-zinc and chitosan-cerium compound coating were higher than that of the single coating. The weight loss of cherry tomato increased with the storage time extension (Fig.4b). During the whole storage, the weight loss of cherry tomato coated with chitosan-zinc composite was the lowest, followed by that coated with chitosan-cerium composite, and that of the control was the highest. Throughout the storage period, preserving effect of cherry tomato coated with composite was superior to that of cherry tomato coated with chitosan composite alone.

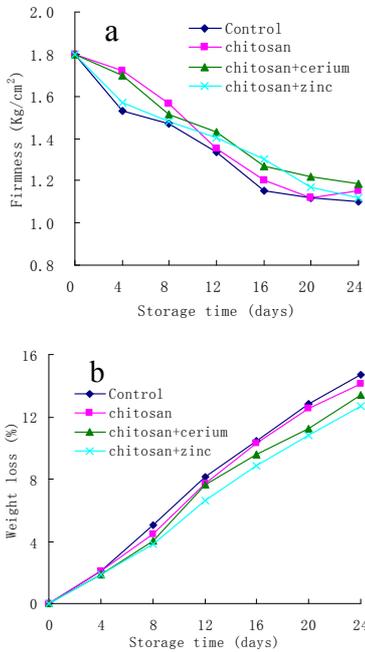


Fig 4. Effects of different coatings on firmness (a) and weight loss rate (b) of cherry tomato

#### 3.2.2. Respiration Rate and Soluble Solids

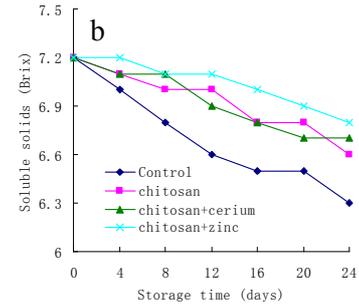
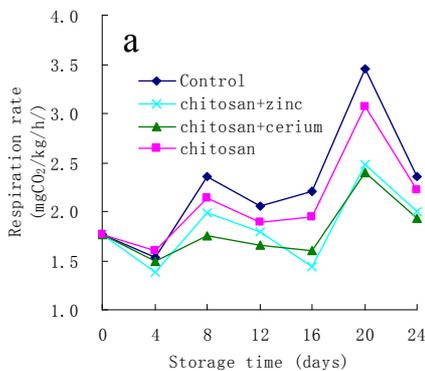
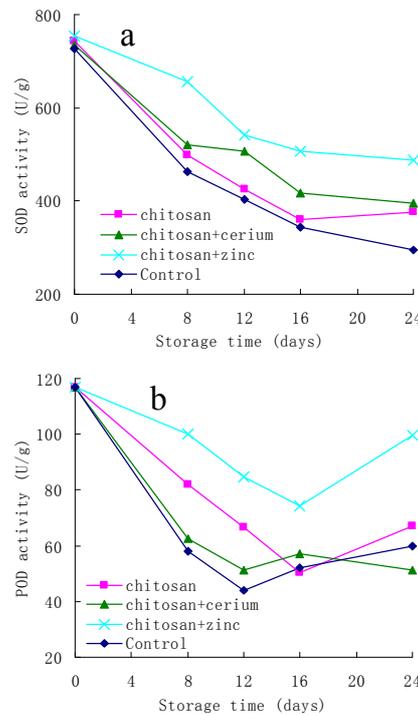
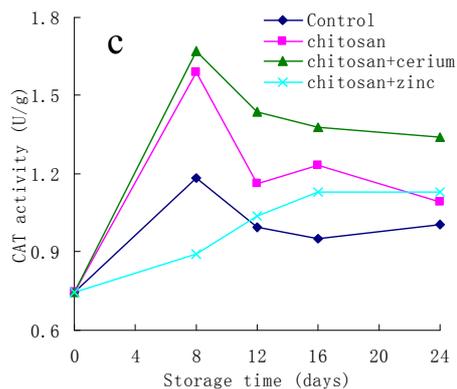


Fig 5. Effects of different coatings on respiration rate (a) and soluble solid substance (b) of cherry tomato

As shown in figure 5a, the respiratory rate of cherry tomato in the first 20 d presented a rising trend overall, and then began to decrease after 20 d. The change trend was consistent with the general rule of respiration climacteric fruit. During storage, the respiratory rate of control sample was the highest, and that of cherry tomato with the composite coating was the lowest. On the 20th day, the respiratory rate reached to peak value, and the rates of fruits with chitosan-cerium or chitosan-zinc composite coating were 30.6% and 28.3% lower than that of control samples, respectively. As shown in figure 5b, the soluble solids of cherry tomato gradually reduced during storage, that of chitosan-zinc composite membrane declined the slowest, and that of the control decreased the most. After 24 d, the soluble solids of cherry tomato coated with chitosan-cerium/zinc compound coating were 6.3% and 7.9% higher than that of the control sample, respectively.

#### 3.2.3. SOD, POD and CAT Activities

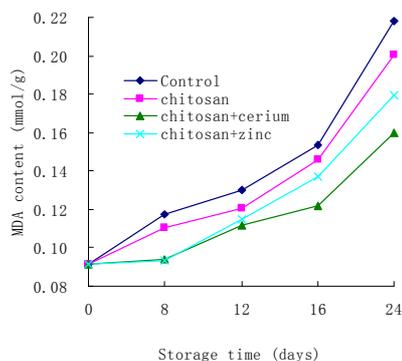




**Fig. 6.** Effects of different coatings on the activities of SOD (a), POD (b) and CAT (c) of cherry tomato

The SOD activity of cherry tomato appeared a downtrend during storage (Fig.6a). Cherry tomato with Chitosan-zinc composite coating had the highest SOD activity, followed by that with chitosan-cerium composite coating, and the SOD activity of the control was the lowest. The POD activity of cherry tomato decreased at first, and then overall increased in the course of storage (Fig.6b). Chitosan-zinc composite coating kept the highest activity, those with chitosan coating alone and chitosan-cerium composite coating were also higher. From figure 6c, the CAT activity of cherry tomato first increased, and then decreased overall. Among them, chitosan-cerium composite coating had the highest activity, followed by single chitosan coating. The CAT activity of cherry tomato with chitosan-zinc coating was lower in the first 12 d, but 12 d later, it was still higher than the control.

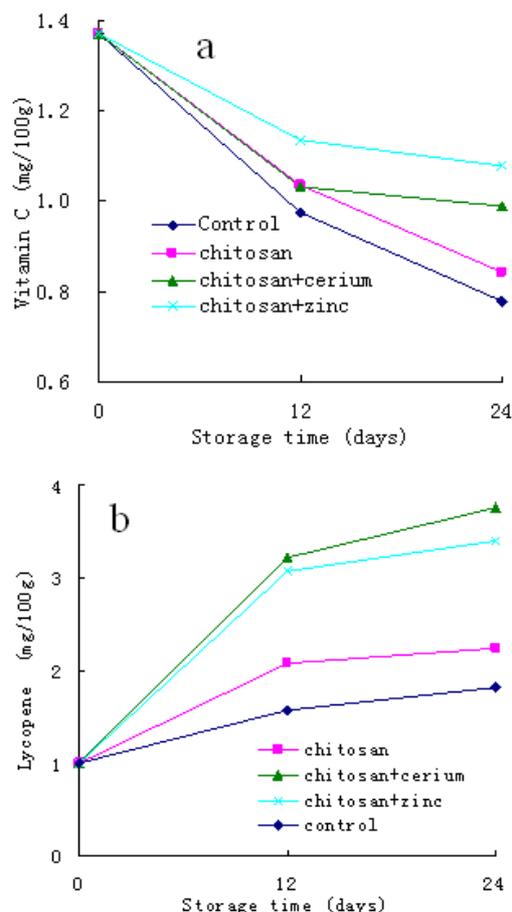
### 3.2.4. MDA Content



**Fig. 7.** Effect of different coatings on MDA content of cherry tomato

Malondialdehyde (MDA) is the product of lipid membrane peroxidation. The higher MDA content is, the more serious the fruit cell membrane is damaged during storage. From Fig 7, the MDA content of cherry tomato gradually increased as time went by. The control sample increased the most, and the composite coating was the least. The increase extent of single chitosan coating fell in between the control and composite coating. After 24 d, MDA content of cherry tomato with chitosan-cerium/zinc composite coating was respectively 26.6% and 17.4% lower than that of the control.

### 3.2.5. Vitamin C and Lycopene Content



**Fig. 8.** Effects of different coatings on vitamin C (a) and lycopene (b) content of cherry tomato

Vitamin C and lycopene have antioxidant effect and are important functional ingredients of cherry tomatoes. As shown in Fig. 8a, vitamin C content of cherry tomato decreased during storage and Coating cherry tomato could retain more Vitamin C. chitosan-zinc composite coating could preserve the most Vitamin C among all treatment. On the 24th day of the storage, vitamin C contents of cherry tomato fruit with chitosan-zinc/ cerium composite coating were 38.2% and 26.7% higher than that of the control, respectively. As shown in Fig. 8b, lycopene content of cherry tomato overall showed a trend of increase during storage. Among them, the lycopene of cherry tomato with chitosan-cerium coating retained the most, followed by that with chitosan- zinc composite coating. After 24 d, lycopene content of the control was 46.4% lower than that of the chitosan-cerium coating.

## 4. Discussion

As Zinc acetate or cerium acetate form film with chitosan, the morphology and crystal form of the composite membrane had some certain change compared with single chitosan film. The crystallinity of chitosan- zinc acetate

composite film decreased, and the crystal form of chitosan-cerium acetate also had changed. All these changes were likely to improve gas and moisture permeability of the membrane, thus keeping cherry tomato good commodity characters during storage. The respiratory rate of cherry tomatoes with composite coating remained at lower levels, which helped reduce the loss of the soluble solids and the decrease of fruit firmness.

The composite coating maintained the SOD, POD and CAT activities higher level, which was conducive to eliminate free radicals produced during storage and reduce damage to cell membrane [17]. Thereby, MDA content rise of the coated cherry tomato was restrained; vitamin C and other antioxidant nutrients in cherry tomato were more reserved. The cerium or zinc composite coating all showed certain positive preserving effect to cherry tomato compared with single chitosan coating. As for the soluble solids, SOD and POD activities and the preservation of vitamin C, the effect of chitosan-zinc acetate composite coating was slightly superior to that of chitosan-cerium acetate composite coating.

## 5. Conclusion

Chitosan-zinc/cerium composite coating could restrict the conescence of post-harvested cherry tomato. After post-harvested cherry tomatoes were coated with composite coating, their physiological, biochemical and nutritional parameters were effectively improved. In chitosan-zinc or chitosan-cerium composite coating, zinc or cerium chelated with hydroxyl group of the chitosan. Chitosan-zinc composite kept the crystal structure of the coating, but the crystallinity of chitosan declined, and the formed film was uniform. For chitosan-cerium composite coating, the crystal structure of chitosan was changed, the morphology of membrane seemed like gathering of small particles, but more compact than chitosan membrane. Two composite coating could inhibit the reduction of cherry tomato firmness and soluble solids, and suppress the increase of weight loss rate and MDA content. Composite coating maintained the cherry tomato lower respiratory rate, higher SOD, POD and CAT activity, more vitamin C and lycopene during storage. In next research work, how composite coating influences the related enzyme activities of cherry tomato should be further investigated, especially the relationship between the enzyme activities and their gene expressing contents. In addition, the changing reason of ascorbic acid and lycopene content should be deliberately explored as well.

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