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Research Article

Grape Preservation Using Chitosan Combined with β-Cyclodextrin

Yu Youwei[1,2] and Ren Yinzhe[1]

[1] *College of Chemistry and Materials, Shanxi Normal University, Linfen 041004, China*
[2] *College of Engineering, Shanxi Normal University, Linfen 041004, China*

Correspondence should be addressed to Ren Yinzhe; sxnunew@163.com

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The effect of 1% chitosan combined with 2% β-cyclodextrin to the preservation of fresh grapes under ambient temperature was investigated. The results indicated that the hydrogen bond formed between the hydroxyl group of β-cyclodextrin and the amidogen or hydroxyl group of chitosan and the crystal form of chitosan was also changed when cyclodextrin was doped into chitosan coating. The compound coating could prolong the shelf life of grapes, maintain lower respiration rate and higher activities of superoxide dismutase, peroxidase, and catalase during storage time, and restrain weight loss and malonaldehyde content increase. Coating grapes with chitosan + β-cyclodextrin was a good method in postharvested grape preservation.

1. Introduction

Chitosan is formed by 2-amidogen-2-deoxidize-β-D-glucose. It is a safe, biocompatible, and biodegradable natural alkaline polysaccharide, and can easily form a film on fruit and vegetable surfaces. Chitosan is popular in many fields, such as medicine, environment, chemical industry, and food [1]. β-Cyclodextrin is one of supramolecular compounds and has many particular properties such as emulsion, moisturizing, and inclusion. It is combined by α-1, 4-glucosidic bond with α-D-glucose, and is oligosaccharide of closed loop structure, and the degree of polymerization is 7 unite of glucose. Cyclodextrin demonstrates a high symmetrical characteristic and its spatial structure is cylindrical. Both the cavity depth and internal diameter are 0.7-0.8 nm. The atoms of the glycosidic oxygen are in the same plane. The primary hydroxyl group of residue C6 is located outboard of the ring, and the hydrophobic group of C–H bond is placed inside the cylinder [2].

Grape is one of main fruits in the world. Fresh grape usually possesses good commodity properties and long storage time. However, fresh grape loses water and rots during storage time. Thus, its commercial value decreased [3]. In this paper, the compound coating of chitosan combined with β-cyclodextrin was prepared and applied in fresh grape preservation. There is no report about the edible coating of chitosan combined with β-cyclodextrin at present, and we hope to explore a new method for fresh grape preservation.

2. Materials and Methods

2.1. Materials. Grapes (*Vitis labrusca* L. kyoho) were purchased from an orchard in the vicinity of Shanxi Normal University on August 10, 2011. Grapes with uniform shape, size, color, physiologically mature stage, and no defects were selected and quickly transported in cartons with holes to the laboratory after picked at noon.

Water-soluble chitosan with an 85% deacetyl degree and 99% dissolvability was purchased from AK Biotech Ltd., (Shangdong, China). β-cyclodextrin with a 99.9% purity was purchased from Tianjin Guangfu Fine Chemical Research Institute. The other reagents were analytical grade, which were purchased from Tianjin Municipality kemi'ou Chemical Reagent Co., Ltd.

2.2. Preparation of the Coating Solution and Grape Treatment. Solutions of (a) 20 mL 5% chitosan, (b) 40 mL 5% β-cyclodextrin, and (c) 20 mL 5% chitosan + 40 mL β-cyclodextrin

were diluted to 1000 mL with deionized water, respectively. Each solution was irradiated with 450 W microwave for 4 min.; thus, different coating solutions, namely, (a) 1% chitosan coating, (b) 2% β-cyclodextrin coating, and (c) 1% chitosan and 2% β-cyclodextrin composite coating, were acquired. In this experimental design, the effects of single coating of chitosan or β-cyclodextrin and compound coating of chitosan combined with β-cyclodextrin on grape preservation were investigated, respectively.

Grapes were washed cleanly and dipped into each prepared solution for 3 min. Then they were taken out and a fan generating low speed air was used to hasten the drying of the water on the surface of dipped grape. The samples were placed in plastic bags and stored under ambient temperature (about 20°C) with 90% of relative humidity for 13 days. The related parameters including physiological and biochemical indexes were determined periodically, and each sample was repeated three times.

2.3. Characterization of Compound Coating

2.3.1. X-Ray Diffraction Analysis (XRD). In this assay, we used X-Ray Diffractometer (type of D8 Advance, Bruker Corporation, Germany), which was graphite monochromator, Cu target, λ(Cu Kα1) = 0.15406 nm, 40 kv tube voltage, 40 mA tube current, the scanning speed 4°/min, and 5° ~ 40° scan range.

2.3.2. Characterization Infrared Spectroscope. Infrared spectra was assayed using FTIR meter (Varian 640, Varian Corporation, USA). The resolution is 0.25 cm^{-1}, the optical source is AC Ceramic, the interferometer with 60 degree and 3 sport is laser positioning of dynamic collimation, and the range of spectrum is between 400 and 4000 cm^{-1} (standard configuration). Furthermore, it contains standard check unit.

2.3.3. Micrograph. The surface pattern of the compound coating was observed using a biological microscope (microscope Olympus and camera MC50, Guangzhou Ming-Mei Technology Co., Ltd., China) at 40, 100, and 400 times, respectively.

2.4. Determination of Indexes Related to Grape Preservation

2.4.1. Determination of Decay Incidence and Weight Loss. 60 fruits were statistically analyzed every three days. The decay incidence was calculated with the following formula:

$$\text{Decay incidence (\%)} = \sum \frac{(\text{rank} \times \text{quantity})}{\text{the highest decay order} \times \text{the total fruits}} \times 100\%. \quad (1)$$

Rank: 0, no rot; 1, rotten surface less than 1/4; 2, rotten surface between 1/4 and 1/2; 3, rotten surface more than 1/2.

About 500 g grapes were taken out from each sample and weighed per three days. The weight loss was calculated with the following formula:

$$\text{Weight loss (\%)} = \left[\frac{m_0 - m_1}{m_0}\right] \times 100, \quad (2)$$

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. Approximately 600 g of fruits were sealed in an airtight container under ambient temperature for 1 hour. After 1 hour, the carbon dioxide concentration was assayed using infrared carbon dioxide analyzer (GXH-3010F, Beijing Huayun Analysis Instrument Co., Ltd., Beijing, China). The respiration rate was calculated as the following formula:

$$\text{Respiration rate}\left(\text{mg}CO_2/\text{kg} \cdot \text{h}\right) = \frac{V \times 44 \times C \times 10^3}{22.4 \times \text{Wt} \times h}, \quad (3)$$

where V is the volume of the container, C is volume percentage concentration of CO_2, 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 Activites. SOD (superoxide dismutase) activity was determined using a modified method [4]. 2.0 g samples were homogenized with 15 mL of 50 mmol/L phosphoric acid buffer (pH 7.8) in ice-bath and centrifuged at 8000 g 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 than 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 560 nm. The activity of SOD was calculated with the following formula:

$$\text{SOD activity}\left[\text{U} \cdot \text{g/FW} \cdot \text{h}\right] = \frac{(A0 - As)\,V \times 60}{0.5 \times A0 \times \text{FW} \times a \times t}. \quad (4)$$

In the formula, $A0$ means the absorbance of the controlled samples, As 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).

POD (peroxidase) activity was analyzed using a modified method [5]. 2.0 g samples were homogenized with 15 mL of 50 mmol/L phosphoric acid buffer (pH 7.8) in ice-bath and centrifuged at 8000 g for 15 min at 4°C. The supernatant was collected as a crude enzyme. The assay mixture contained

Table 1: Adding reagents for SOD activity assay.

Reagent (mL)	Tube no.			
	1	2	3	4
50 mmol/L phosphoric acid solution	1.5	1.5	1.5	1.5
130 mmol/L methionine	0.4	0.4	0.4	0.4
750 μmol/L NBT solution	0.4	0.4	0.4	0.4
100 μmol/L EDTA-Na2 solution	0.4	0.4	0.4	0.4
200 μmol/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

2 mL of 50 mmol/L phosphoric acid buffer (pH 7.8) and 0.6 mL of 0.04 mol/L guaiacol; after heating and stiring for 10 min, add 1.5 mL enzyme solution. The reaction began with 0.1 mL of 15% H_2O_2. POD activity was measured by an increase in absorbance at 470 nm per min for 3 min in total. The activity of POD was calculated with the following formula:

$$\text{POD activity}\left[\frac{U}{\text{g}\cdot\text{min}}\right] = \frac{\Delta A_{470}\cdot V}{\text{FW}\times a\times 0.01\times t}. \quad (5)$$

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 García et al. [6]. 2.0 g samples were homogenized with 15 mL of phosphoric acid buffer (pH 7.0) containing 1% polyvinyl-polypyrrolidone (PVPP) and centrifuged at 8000 g for 15 min at 4°C. The supernatant was collected as a crude extracting of CAT. The assay mixture contained 2 mL of 50 mmol/L phosphoric acid buffer (pH 7.0), 1 mL distilled water. Afterwards, 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_2O_2 was applied to start reaction. The absorbance was measured at 240 nm per 30s. One unit of CAT activity was defined as a 0.1 decrease in absorbance at A_{240} per min. The activity of the CAT enzyme was calculated as the following formula:

$$\text{CAT activity}\,[\text{U/g}\cdot\text{min}] = \frac{\Delta A_{240}\times V}{0.1\times a\times t\times \text{FW}}, \quad (6)$$

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_{240}, and t is the last reading duration after adding H_2O_2.

2.4.4. Determination of Malonaldehyde (MDA) Content. MDA was measured as previously described by Zhang et al. [7]. 0.5 g samples were homogenized with 10 mL of 50 mmol/L phosphoric acid buffer (pH 7.8) in ice-bath The mixture was then added with 5 mL of 0.5% thiobarbituric acid solution and heated to 100°C for 10 min (determining from the emergence of little bubbles in the tube). After the rapid cooling of the sample to 4°C and centrifugation at 3000 g 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 532 nm, 600 nm, and 450 nm.

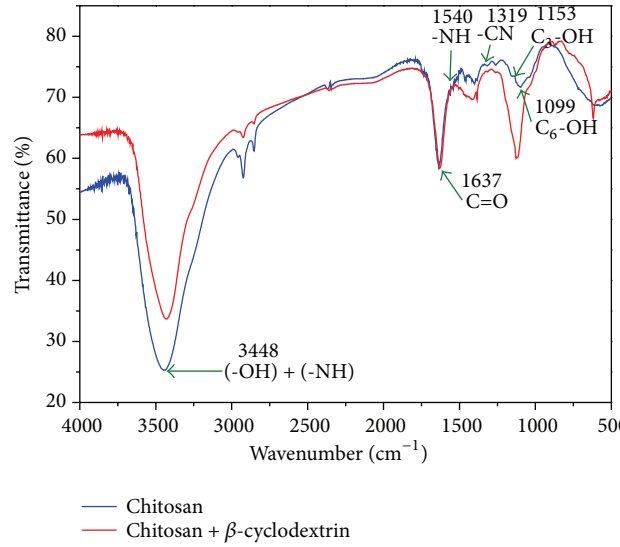

Figure 1: Infrared spectrum of compound coating.

3. Results and Analysis

3.1. Characterization of the Compound Coating with Chitosan + β-Cyclodextrin

3.1.1. Infrared Spectrum of Chitosan Coating. Figure 1 demonstrates the FTIR spectra of water-soluble chitosan and chitosan + β-cyclodextrin coatings. As shown in the spectra, the peak of –NH at 1540 cm^{-1}, –CN at 1319 cm^{-1}, and C=O at 1637 cm^{-1} shifted to lower numbers. The vibrational bands of the secondary hydroxyl group of C_3–OH disappeared. These characteristic bands confirmed that the hydrogen bond was formed by hydroxide radical of chitosan or amidogen and hydroxide radical of β-cyclodextrin. Futhermore, the shifting of hydroxide radical and amidogen at 3448 cm^{-1} to a lower wave number confirmed the reaction. The peak of C_6–OH may be overlapped by the bond C–O and C–O–C of β-cyclodextrin, shifting to a higher wave number in general [8].

3.1.2. XRD Diffraction Pattern. As shown in Figure 2, water-soluble chitosan had two characteristics of diffraction peaks at 15.4° and 22.6°. Cyclodextrin powder has strong characteristics of the diffraction peaks at 10.6° and 12.3° and 17.6° and 19.4°, according to the research by He et al. [9]. The characteristics of diffraction peaks at 9.4° and 15.4° of compound coating may have formed after the chitosan crystal

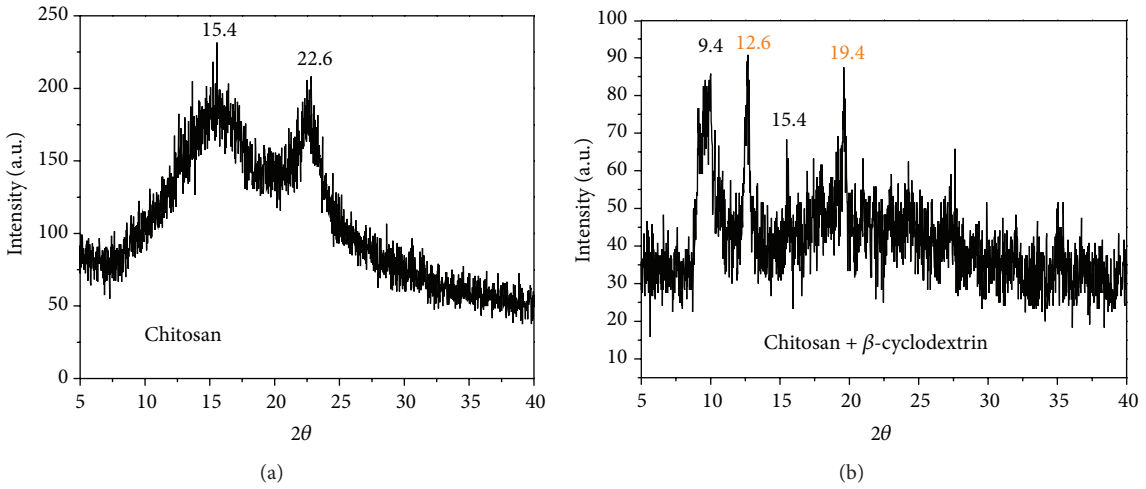

Figure 2: XRD diffraction pattern of compound coating.

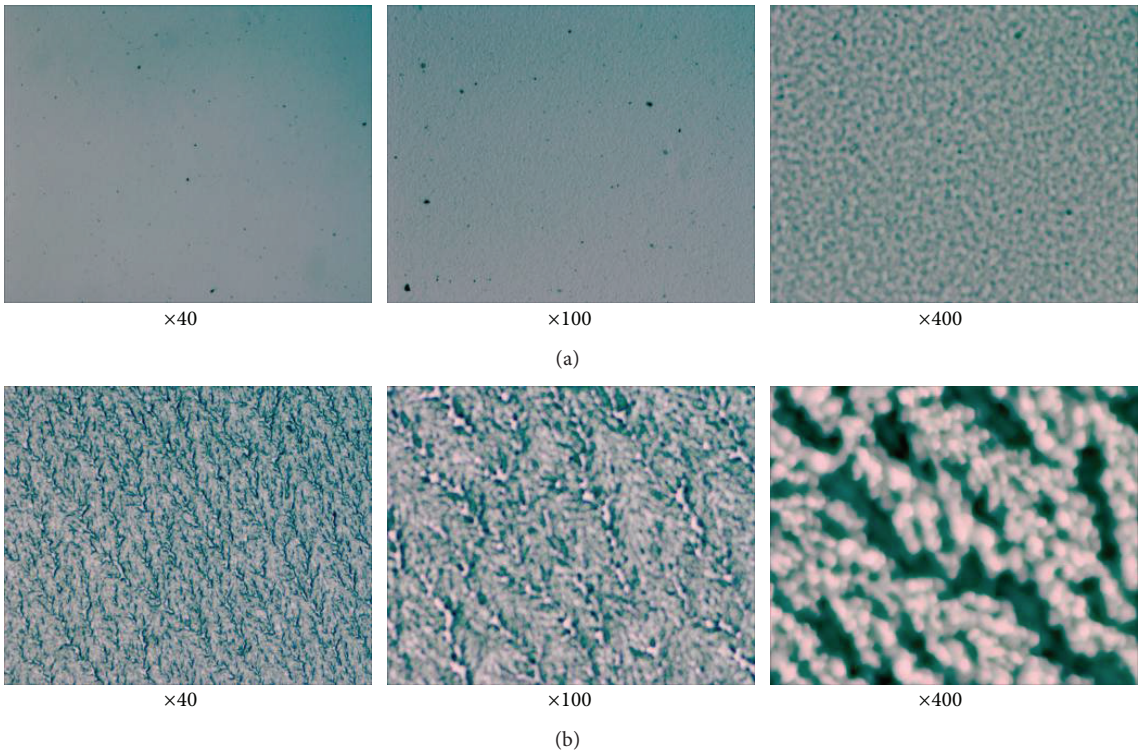

Figure 3: The coating micrograph of (a) chitosan and (b) chitosan + β-cyclodextrin.

type transformed, or formed through superposition of β-cyclodextrin and chitosan. In summary, after β-cyclodextrin was doped into composite membrane, the original crystal form of chitosan changed.

3.1.3. Micrograph. As shown in Figure 3, single-water-soluble chitosan coating was more compact and uniform; the morphology structure of composite coating was similar to the ears of wheat. The composite gathered into a tight cluster structure at each ear of wheat. There was a bigger gap among clusters. Tight fasciculate cluster and broad gap formed a picturesque disorder feature. The –OH of β-cyclodextrin was at the outer edge of the drum molecules, and the C–H was located in the interior. Hydroxyl group on the β-cyclodextrin and hydroxyl group on the chitosan or amino interact when the compounds of β-cyclodextrin and chitosan were formed. Thus, the particular structure similar to wheat ear formed, owing to β-cyclodextrin regulation.

3.2. Effect of Compound Coating on Grape Preservation

3.2.1. Decay Incidence and Weight Loss. As shown in Figure 4(a), the decay incidence of grapes increased with the storage time extension. The control samples began to rot after

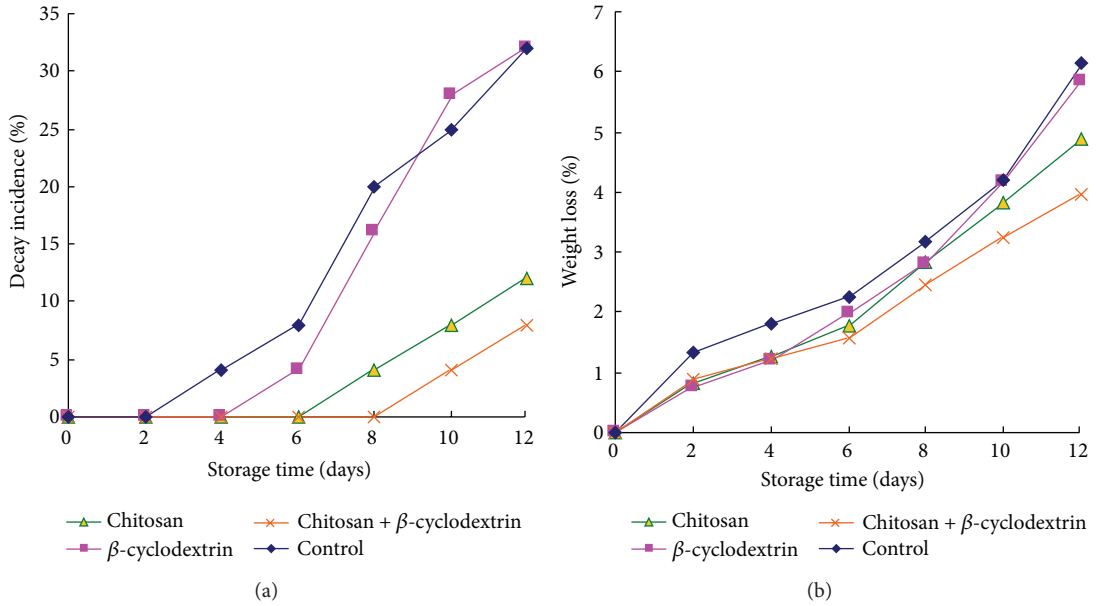

Figure 4: Effects of different coatings on decay incidence (a) and weight loss (b) of grape.

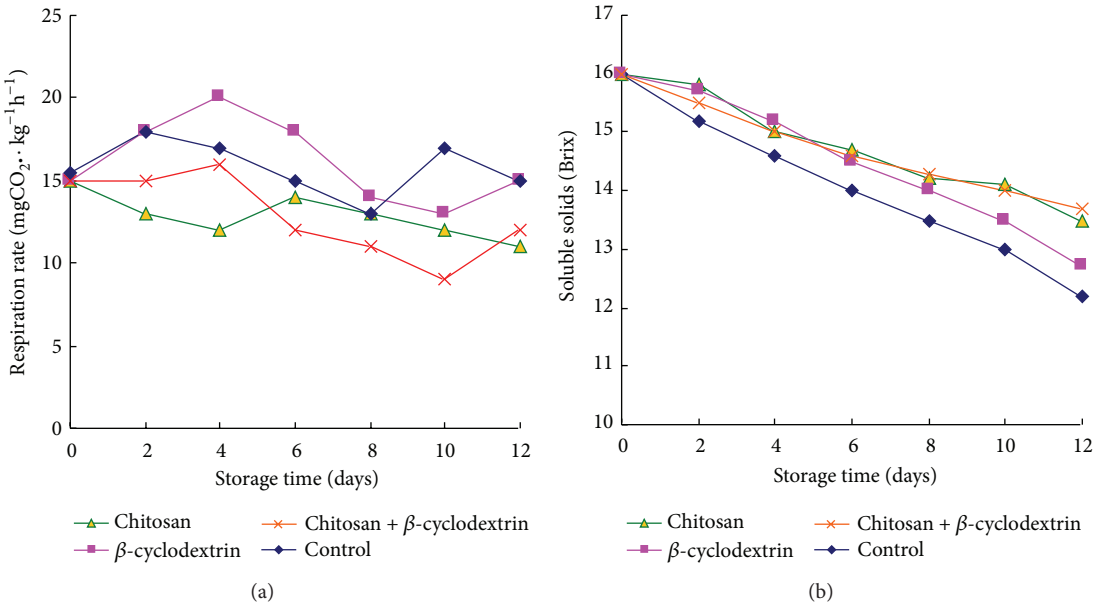

Figure 5: Effects of different coatings on respiration rate (a) and total soluble solids (b) of grape.

2 days, the grapes coated with chitosan began to rot after 6 days, and those coated with chitosan + β-cyclodextrin began to rot after 8 days. After 12 days, the decay incidence of control samples was 32%; those coated with chitosan + β-cyclodextrin was 8%, and just was 1/4 of the control samples. As shown in Figure 4(b), the weight loss of grapes increased over time during storage. The weight loss of the grapes coated with chitosan + β-cyclodextrin was the lowest, and those of control samples were the highest. The weight loss of the control samples was about 1.5 times compared to that of grapes coated with chitosan + β-cyclodextrin after 12 days.

3.2.2. Respiration Rate and Soluble Solids. As shown in Figure 5(a), the respiration rate of the grapes firstly increased and then decreased with the storage time extension in general. The respiration rate of the grapes coated with β-cyclodextrin was the highest, the next was the control samples, and those coated with chitosan + β-cyclodextrin or chitosan was the lowest. In the first 6 days, the respiration rate of those coated with chitosan was lower than that of those coated with chitosan + β-cyclodextrin. The respiration rate of those coated with chitosan + β-cyclodextrin was lower than that of those coated with chitosan after 6 days. Considering

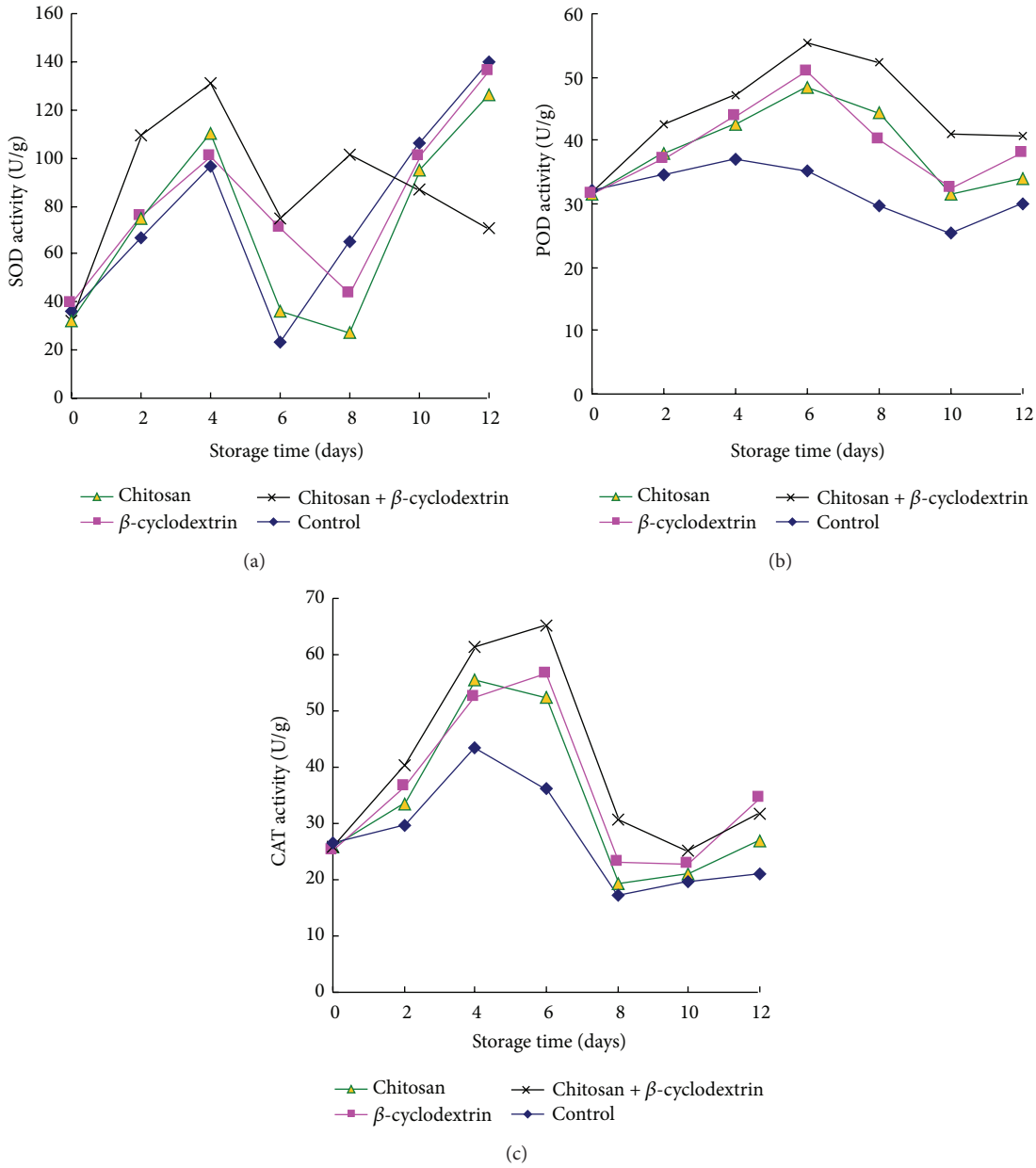

Figure 6: Effects of different coatings on the SOD (a), POD (b), and CAT (c) activities of grape.

that respiration would consume the nutritional ingredient of the grapes, a low respiration rate was beneficial to fruit preservation. As shown in Figure 5(b), the total soluble solids of grapes remarkably decreased during the storage time. The control samples decreased most rapidly, the next was those coated with β-cyclodextrin, and those coated with chitosan + β-cyclodextrin or single chitosan showed the slowest.

3.2.3. SOD, POD, and CAT Activites. The SOD activity of grape coated with chitosan + β-cyclodextrin increased and then decreased with storage time extension (Figure 6(a)). It reached the bottom after 6 days and then increased until the 8th day. It decreased again between 8 and 12 days; while the SOD activity of grapes coated with single chitosan or cyclodextrin demonstrated an increase after 6d. Before 8d, the SOD activity of grapes coated with compound coating maintained the highest activity. Compared with the control and other treated grapes, the peak value of SOD activity of grapes coated with compound coating was the highest (131.4 U/g∗min), which was 36.0% higher than that of the control samples. As shown in Figure 6(b), the POD activity of grapes increased and then decreased during the storage time. The POD activity of grapes coated with compound coatings was the highest and that of the control was the lowest. The CAT activity variation of postharvested grapes was similar to the POD activity (Figure 6(c)). The CAT activity of grapes coated with chitosan and the control increased to the peak after 4 days, while the CAT activity of grapes coated with

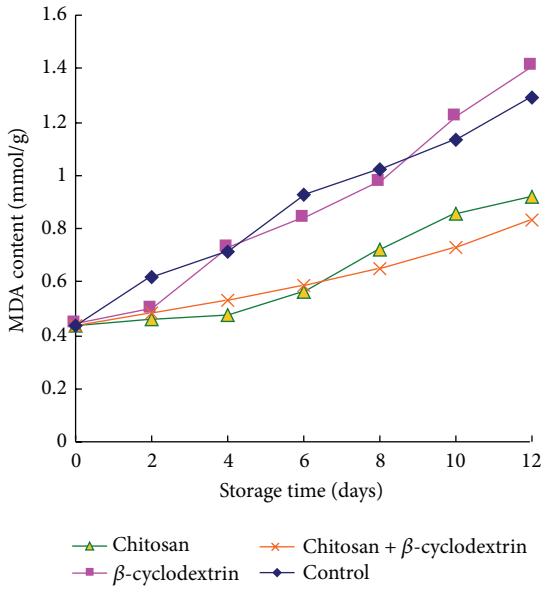

FIGURE 7: Effects of different coatings on the MDA content of grapes.

compound coatings and single β-cyclodextrin increased to the peak at the 6th day.

3.2.4. MDA Content. Malondialdehyde (MDA) is one of the peroxidation productions of cell membranes owing to free radical oxidation, and it may indicate the degree of cell consenescence [10]. As shown in Figure 7, the MDA content of harvested grapes increased with the storage time extention. The MDA content of control samples and those coated with β-cyclodextrin increased more than that of those coated with chitosan or chitosan + β-cyclodextrin. After 12 days, the MDA content of those coated with chitosan + β-cyclodextrin was 0.83 mmol/g, 35.7% lower than that of the control samples.

4. Discussion

After the grapes were coated with chitosan + β-cyclidextrin, a coating with an orderly arranged structure similar to the ear of wheat formed on the surface of grapes. Moreover, the compound coating crystal was different than that of single chitosan coating. That structure maybe relates to the hydrogen bond forming between hydroxide radical of chitosan and the hydroxide radical of β-cyclodextrin.

The SOD, POD, and CAT activities of grapes coated with compound coating maintained a higher level during storage time, so the free radicals produced by metabolism were effectively cleaned. Thus, the damage to cell membranes was probably reduced. Low MDA content of grapes coated with compound coating may serve as verification [11].

Coating on the surface of fruit may adjust CO_2 and O_2 permeability. Grapes coated with compound coating maintained a lower respiratory rate, and nutrients consume such as soluble solids was reduced [12]. Accordingly, the weight loss was also reduced. The cell of grapes remained in relatively good condition grapes coated with compound coating demonstrated better physiological status in storage time. Thus, disease resistance was also enhanced. The grapes of control samples began to rot after two days, but those coated with chitosan + β-cyclidextrin began to rot at the 8th day.

5. Conclusion

After β-cyclodextrin was dosed into chitosan coating, a hydrogen bond formed between the amino or hydroxyl of chitosan and hydroxyl of β-cyclodextrin. The crystal type of chitosan membrane changed, and a structure similar to the ears of wheat showed. The compound coating could prolong the shelf life of grapes, maintain lower respiration rate and higher activities of superoxide dismutase, peroxidase, and catalase during storage time, and restrain weight loss and malonaldehyde content increase. Coating grapes with chitosan + β-cyclodextrin was a good method in postharvested grape preservation.

Acknowledgments

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