Hindawi Publishing Corporation
ISRN Agronomy
Volume 2013, Article ID 871396, 7 pages
http://dx.doi.org/10.1155/2013/871396

Research Article

Effect of Carboxymethyl Cellulose and Alginate Coating Combined with Brewer Yeast on Postharvest Grape Preservation

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Received 26 March 2013; Accepted 14 May 2013

Academic Editors: O. Ferrarese-Filho and H. P. Singh

The effect of carboxymethyl cellulose and alginate coating combined with brewer yeast on postharvest grape preservation was investigated. The postharvest grapes were coated with 2% of alginate and 3% of carboxymethyl cellulose combined with 1.5×10^9 CFU/mL of brewer yeast. The combined treatment samples showed good sensory character on day 13 compared with control samples or only coated samples. The increase of weight loss and decrease of total soluble solids of combined treatment grapes were restrained. Furthermore, the protective enzymes including superoxide dismutase, peroxidase, and catalase of combined treatment sample showed higher activities. Accordingly, the increase of malonaldehyde content was also restrained and more vitamin C was preserved in combined treatment samples. At day 13, the weight loss rate and the total soluble solids of grape treated with coating + yeast were 23.6% lower and 20.6% higher than those of control samples, respectively. Coating grapes with 2% of alginate and 3% of carboxymethyl cellulose combined with brewer yeast of 1.5×10^9 CFU/mL was a well-proven method to preserve postharvest grapes.

1. Introduction

In recent years, conventional production systems of fruits and vegetables have been characterized by an excessive use of chemical compounds during pre- and postharvest treatments. The grape is a highly perishable nonclimacteric fruit with reduced shelf-life due to decay, weight loss, and nutrient degradation during the storage time. It is traditionally treated with different chemical products such as SO_2 to control the main postharvest pathogen [1]. Nevertheless, new consumer trends and subsequent legislative changes demand healthier, environmentally friendly food production systems.

Edible films can be used to protect perishable food products from deterioration by retarding dehydration, providing a selective barrier to moisture, oxygen, and carbon dioxide, suppressing respiration, improving textural quality, helping to retain volatile flavor compounds, and reducing microbial growth [2]. Carboxymethyl cellulose (CMC) is the most important water-soluble cellulose derivative, with many applications in the food industry and in cosmetics, pharmaceutcals, detergents, and so forth [3]. Alginate, a polysaccharide derived from marine brown algae, has been preponderant in making edible films due to its unique colloidal properties and its ability to form strong gels or insoluble polymers upon reaction with multivalent metal cations such as calcium [4]. At present, CMC and alginate are used in fruit preservation, such as fresh garlic, processed apples [5, 6].

Postharvest biocontrol is especially feasible for inhibiting postharvest fruit pathogens by the inoculation of antagonists. *Cryptococcus laurentii* has been investigated for the postharvest biological control of gray mold rot of apples, gray mold and blue mold rot of pears [7]. Brewer yeast is cultured from a one-celled fungus called *Saccharomyces cerevisiae* and is applied in beer industry. It also can be grown to make nutritional supplements. Brewer yeast is a rich source of minerals, particularly chromium, an essential trace mineral, which helps the body maintain normal blood sugar levels, selenium, protein, and the B-complex vitamins [8].

The objective of this work was to investigate the effect of carboxymethyl cellulose and alginate coating combined with brewer yeast on postharvest grape preservation during storage under ambient temperature. We tried to explore an integrated strategy to preserve fresh grape and provide reference for other vegetable and fruit preservation.

2. Materials and Methods

2.1. Materials. Grapes (*Vitis labrusca* L. kyoho) were purchased from an orchard in the vicinity of the Shanxi Normal University and picked at a preclimacteric but physiologically mature stage in the noon. Grapes with uniform shape, size, colour, and no defects were selected and quickly transported in open cartons to the laboratory. The grape particles were cut from fruiting pedicel and prepared for the following experiment.

Brewer yeast was provided by the microbiological laboratory of Northwest Sci-Tech University of Agriculture and Forestry. Carboxymethyl cellulose (food grade) was purchased from Beifang Chemical Limited Company (Renqiu, China). Alginate (food grade) was purchased from Datang Bioengineering Co., Ltd. (Hebei, China). Thiobarbituric acid, methionine, and nitroblue tetrazolium (biochemical reagent) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Alfa Aesar Company (Tianjin, China) supplied other reagents, which were all of analytical grades.

PDA culture medium was prepared as the following method. 10 g of agar, 20 g of glucose, 200 g of peeled potato, and 1000 mL of deionized water were boiled for 30 min. The residual was removed through filtration. Thus, PDA medium was acquired. It was sterilized for 20 min at 121°C with high-pressure steam sterilization. After cooling, the medium in tube is placed into slope. If no bacteria were observed in two days, the medium may be used in the following experiment.

2.2. Fruit Treatment. Brewer yeast was taken out from refrigerator and was placed under ambient temperature for 3 hours. Afterward, it was cultivated with continuous transfers using PDA medium. Then the brewer yeast was diluted with several concentrations through 10 times dilution method with deionized water.

20 g of alginate and 30 g of carboxymethyl cellulose were diluted to 1 liter using deionized water with or without brewer yeast. The grapes were washed clean with tap water and then were dipped into different solutions for 2 min. The only washed grapes were served as control samples. In each treatment, about 350 fruits were coated and each treatment was repeated three times. A fan generating low-speed air was used to hasten the drying. The samples were then placed in plastic bags and stored under ambient temperature with 90% of relative humidity. The related parameters of the grapes were determined periodically.

2.3. Determination of Weight Loss and Total Soluble Solids. The weight loss was determined according to the method of Yu et al. [9]. In each treatment, 30 fruits were selected at random. The weight loss rate was calculated as follows: 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.

Total soluble solids were assayed according to the method of Qiuping and Wenshui with modifications [10]. Tissues (50 g) from six fruits were homogenized and then centrifuged at 8000 ×g for 20 min using an Eppendorf 5417R centrifuge (Germany). The supernatant was collected to measure total solids (Brix) using a refractometer (WYT-II, Qingyang Optical Instrument Co., Ltd., Chengdu, China).

2.4. Determination of Enzyme Activities. SOD (superoxide dismutase) activity was determined using a modified method [11]. About 2 g of fruit tissue from ten fruits was homogenized with 15 mL of 50 mmol/L sodium phosphate buffer (pH 7.8) 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. The reaction mixture (3 mL) containing 0.1 mL of enzyme extracts, 50 mmol/L sodium phosphate buffer (pH 7.8), 13 mmol/L methionine, 75 μmol/L nitroblue tetrazolium (NBT), 10 ηM EDTA, and 20 ηM riboflavin was illuminated using a fluorescent lamp (60 moL m^{-2} s^{-1}) for 20 min. The absorbance at 560 nm was recorded using a UV spectrophotometer (UV-1100, Shanghai Meipuda Instrument Co., Ltd., Shanghai, China). An aliquot of an identical solution was kept in the dark and served as the blank control. One unit of SOD activity was defined as the amount of enzyme that catalyzed a 50% decrease in the SOD-inhibitable NBT reduction.

POD (peroxidase) activity was analyzed using a modified method [12]. The crude enzyme of POD was prepared as the crude SOD enzyme was extracted. The assay mixture contained 1.5 mL of enzyme extract, 2 mL of 50 mmol/L sodium phosphate buffer (pH 7.8), 0.6 mL of 0.04 M guaiacol, and 0.1 mL of 15% H_2O_2. POD activity was measured by an increase in absorbance at 470 nm. One unit of POD activity was defined as a 0.01 increase in absorbance at 470 nm per gram in one minute.

CAT (catalase) activity was assayed according to the method described by Tejera García et al. [13]. Tissue (2 g) was homogenized with 15 mL of sodium phosphate 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 the crude enzyme of CAT. CAT activity was measured by adding 0.6 mL of enzyme extract to 2 mL of sodium phosphate buffer (pH 7.0) containing 1 mL of 0.03% H_2O_2 as substrate. H_2O_2 decomposition was measured by the reduction in absorbance at 240 nm. One unit was defined as the change 0.1 absorbance per gram in one minute.

2.5. Determination of Malonaldehyde (MDA) Content. MDA was measured as previously described by Xing et al. [12]. Flesh tissue (2.0 g) from 10 fruits was homogenized with 10 mL of 10% trichloroacetic acid containing 0.5% (w/v) thiobarbituric acid. The mixture was then heated at 100°C for 10 min. After the rapid cooling of the sample to room temperature and centrifugation at 4000 g for 15 min at 25°C, the absorbance of the supernatant was measured at both 532 and 600 nm.

Table 1: Sensory character of grape on day 13.

Treatment	Character description of grape particles
Control	No brilliance, dull purple, serious decay, strong bad smell
Coating	No brilliance, dull purple, slight decay, obvious bad smell
Coating + 1.5×10^7 brewer yeast CFU/mL	Weak brilliance, dull purple, slight decay, a little bad smell
Coating + 1.5×10^8 brewer yeast CFU/mL	Weak brilliance, bright purple, slight soft, a little bad smell
Coating + 1.5×10^9 brewer yeast CFU/mL	Brilliance, bright purple, plump, little bad smell
Coating + 1.5×10^{10} brewer yeast CFU/mL	Slight dark, bright purple, slight soft, a little bad smell

Note: coating was 2% of alginate and 3% of carboxymethyl cellulose.

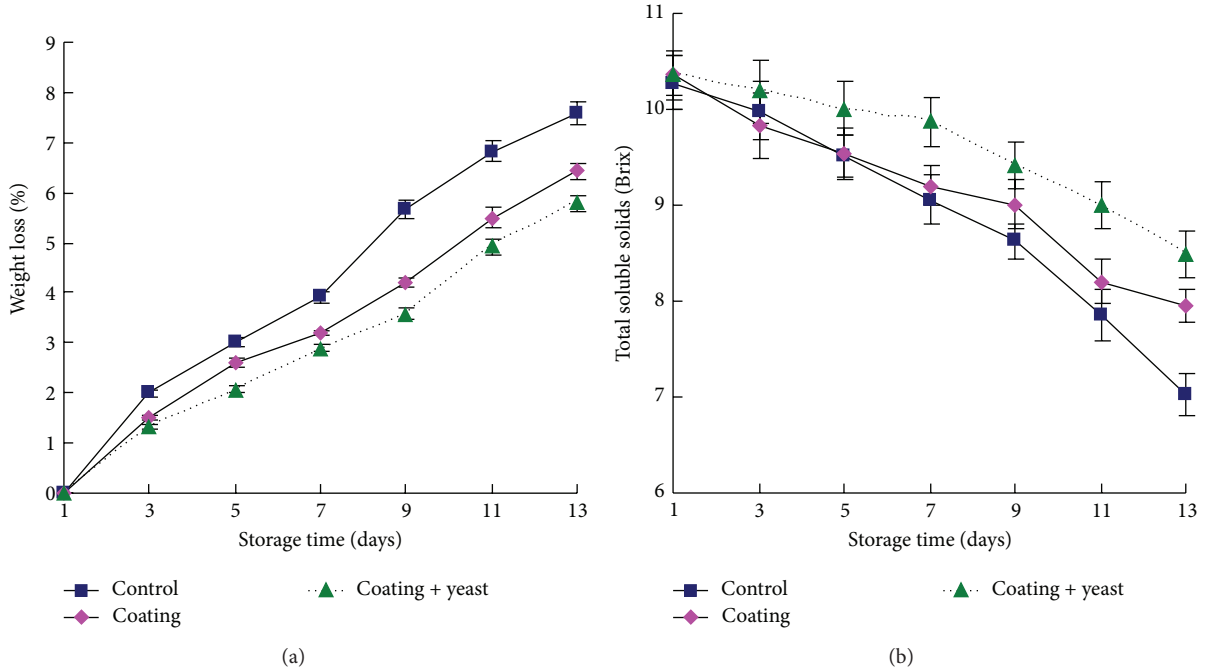

Figure 1: Effects of different treatment on the weight loss (a) and total soluble solids (b) of grape. Each point represents the mean value ± SD.

MDA concentration (μmoL g^{-1} fresh weight) was calculated by an extinction coefficient of 155 Mm^{-1} cm^{-1} through the formula $(OD_{532} - OD_{600}) \times 40/(0.155 \times \text{formula weight})$.

2.6. Determination of Vitamin C. The vitamin C content was measured by 2,6-dichloriondophenol titration [14]. Briefly, tissue (2 g) from 10 fruits was immediately homogenized in 10 mL of 2% oxalic acid solution and then centrifuged at 8000 g for 15 min at 4°C. Afterwards, 2 mL of supernatant was titrated to a permanent pink colour using 0.1% of 2,6-dichlorophenolindophenol titration. The vitamin C concentration was calculated according to the titration volume of 2,6-dichloriondophenol.

2.7. Statistical Analysis. Experimental data were analyzed through ANOVA using the DPS7.05 statistical software (Refine Information Tech. Co., Ltd., Hangzhou, China). Experimental data were the means ± SD of three replicates of determinations for each sample. Mean separations were performed via Tukey's test; $P < 0.05$ was considered to indicate statistical significance.

3. Results and Analysis

3.1. Sensory Character. As shown in Table 1, the coating of 2% alginate and 3% carboxymethyl cellulose could increase the quality of postharvest grape during the storage. On day 13, the control sample was seriously rotted, had no brilliance, and gave off strong bad smell, while the coated sample was only slightly rotted, and the bad smell was lower compared with control samples. And brewer yeast was beneficial to grape preservation. With the concentration from 1.5×10^7 CFU/mL to 1.5×10^9 CFU/mL in coating, the quality of grape increased accordingly. On day 13, the sample of coating $+1.5 \times 10^9$ CFU/mL of brewer yeast was brilliant, not rotten, the color was bright purple, and little bad smell was smelt. However, at the concentration of 1.5×10^{10}, sensory character deteriorated. So in the following experiment, the control, coating treatment (2% alginate and 3% carboxymethyl cellulose), and coating + yeast treatment (1.5×10^9) were further investigated.

3.2. Weight Loss and Total Soluble Solids. As shown in Figure 1(a), the weight loss of grape increased during the storage time. Compared with control samples, coating could

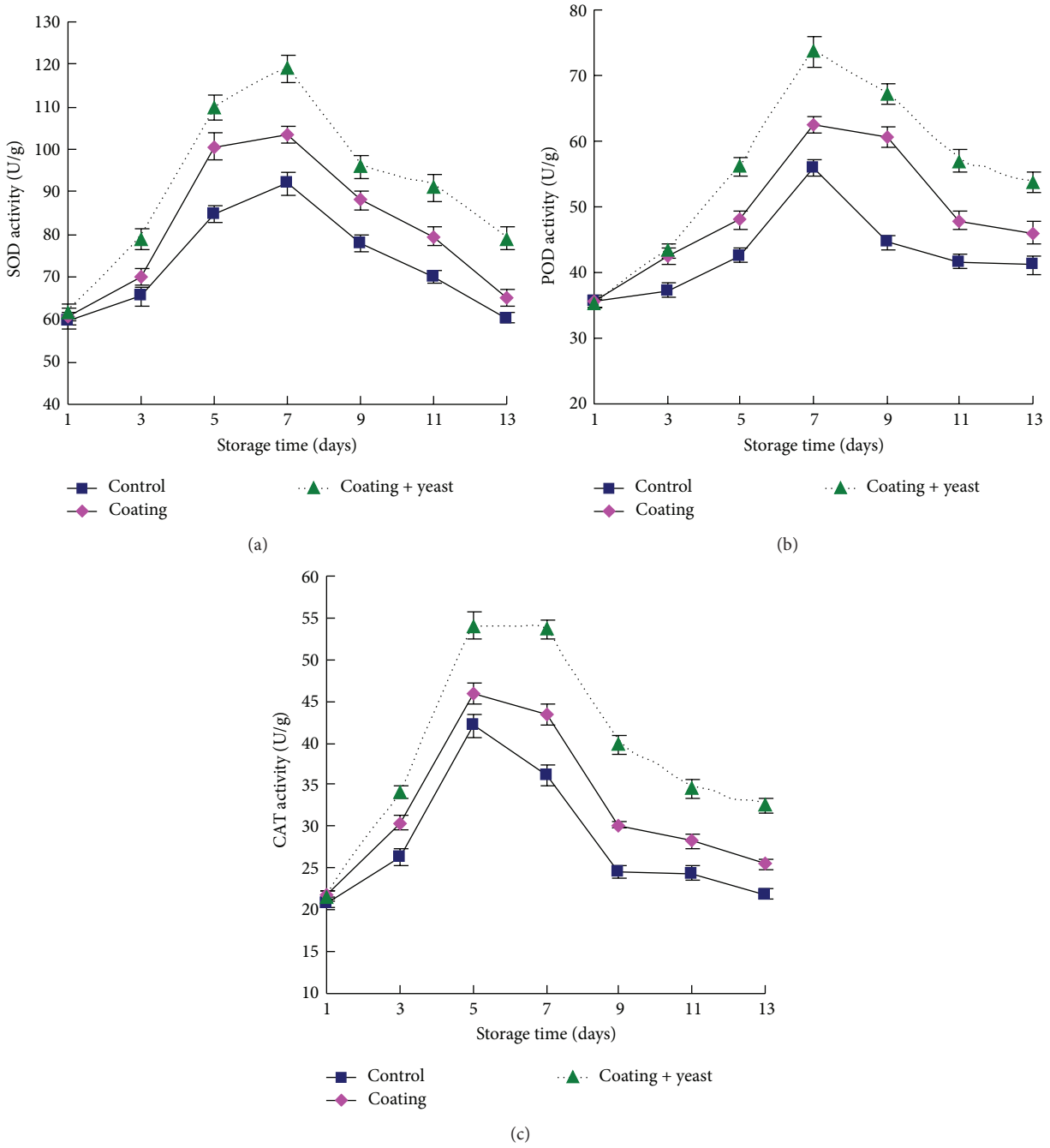

Figure 2: Effects of different treatments on the SOD (a), POD (b), and CAT (c) activities of grape. Each point represents the mean value ± SD.

restrain the increase of weight loss. Particularly the grape treated with coating + yeast demonstrated the lowest increase. At day 13, the weight loss rate of grape treated with coating + yeast was 5.79%, which was 23.6% lower than that of control samples. And there was significant difference between them ($P < 0.05$). The total soluble solids decreased in all samples (Figure 1(b)). The grape treated with coating + yeast showed the lowest decrease, the coated grape showed the lower decrease, and the control sample decreased the fast. On day 13, the total soluble solids of grape treated with coating + yeast was 20.6% higher than that of control samples ($P < 0.05$).

3.3. SOD, POD, and CAT Activities. The SOD activities of all sample grapes increased before 7 days and then decreased from 7 to 13 days (Figure 2(a)). Compared with control samples, grape treated with coating + yeast showed the highest SOD activity, and the coated sample showed higher SOD activity. The peak value of control samples was 7.3% and

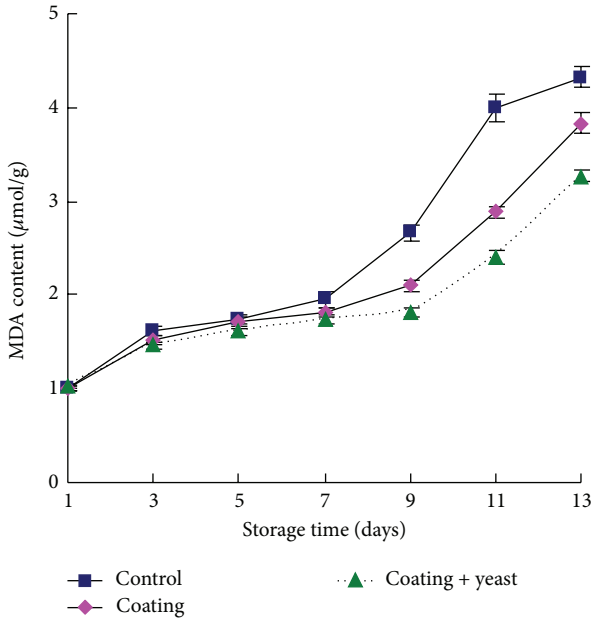

Figure 3: Effects of different treatments on the MDA content of grape. Each point represents the mean value ± SD.

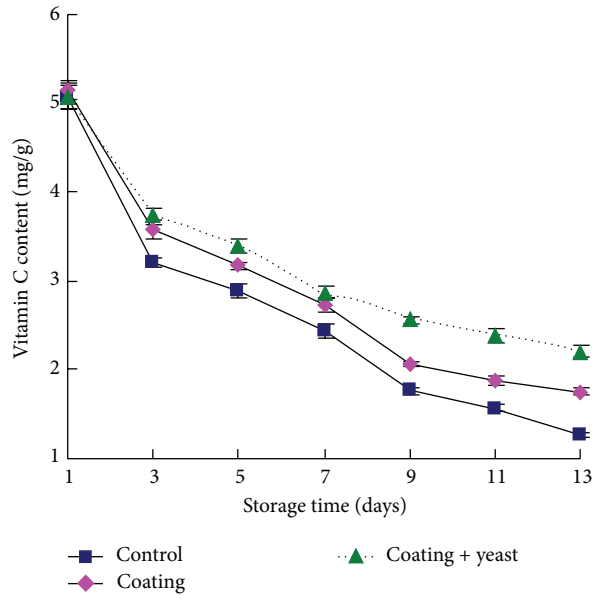

Figure 4: Effects of different treatments on the vitamin C content of grape. Each point represents the mean value ± SD.

23.6% lower than that of coated sample or grape treated with coating + yeast, respectively.

As shown in Figure 2(b), the POD activities of all sample grapes firstly increased and then decreased during the storage time. The coating or coating + yeast could maintain a higher level of the POD activity. And during the whole storage time, the grape treated with coating + yeast demonstrated the highest POD activity.

Similar to SOD activity or POD activity, the CAT activities of grapes also firstly increased and then decreased during the storage time (Figure 2(c)). The grape treated with coating + yeast showed the highest CAT activity, and the control sample showed the lowest activity during the whole storage. On day 13, the POD activity of grape treated with coating + yeast was 32.5 U/g, which was 48.4% and 27.5% higher than that of control sample or coated sample, respectively ($P < 0.05$).

3.4. MDA Content. The MDA content of all grapes increased during the storage time (Figure 3). Before 7 days, the MDA content of all samples slowly increases, and there were no differences among the treatments ($P > 0.05$). However, from 7 to 13 days, the MDA content of control sample fast increased. Though the MDA content of coated sample or grape treated with coating + yeast also increased from 7 to 13 days, they were lower than that of control sample. The grape treated with coating + yeast showed the lowest MDA content among all samples. At day 13, the MDA content of grape treated with coating + yeast was only 3.27 μmol/g, which was 24.3% lower than that of control samples ($P < 0.05$).

3.5. Vitamin C Content. As shown in Figure 4, the vitamin C content of grapes decreased during the storage time. The control sample demonstrated the fastest decrease in vitamin C content, the coated sample showed the much faster decrease, and the grape treated with coating + yeast showed the slowest decrease. On day 13, the vitamin C content of control sample, coated sample, and grape treated with coating + yeast was 1.26, 1.75, and 2.21 mg/g, respectively. There were significant differences among them ($P < 0.05$).

4. Discussion

Postharvest grape is an active organism, undertaking metabolism ceaselessly. During the process, the inner substances of fruit were gradually exhausted. Thus, the weight loss and the total soluble solids decreased [7]. After treatment with coating + yeast, the increase of weight loss and the decrease of total soluble solids were partly restrained. All this suggested that coating+ yeast was beneficial to postharvest grape preservation.

Harvested grape generates free radicals, such as $O_2^{\bullet -}$ and H_2O_2, because of biochemical reactions. Free radicals can oxidize and destroy the cytoplasmic membrane, thereby accelerating senescence. The harm induced by free radicals is resisted by defence enzyme systems [15]. SOD can change $O_2^{\bullet -}$ into H_2O_2, and POD or CAT can eliminate H_2O_2. The united action of these three enzymes can reduce the harm to the cytoplasmic membrane [16, 17]. The SOD, POD, and CAT activities of the grape with coating + yeast demonstrated higher activities (Figure 2), which could efficiently eliminate $O_2^{\bullet -}$ and H_2O_2.

MDA originated from cytoplasmic membrane oxidation, and it may indicate the degree of cell consenescence [18]. The MDA content of the grape treated with coating + yeast was the lowest (Figure 3), and the reason was probably that higher activities of SOD, POD, and CAT could quickly eliminate

the free radical. Thus, the harm to the cytoplasmic membrane by the free radical was minimized to the least degree.

Ascorbic acid of grape is an important nutrient as well as antioxidant to eliminate the active oxygen of fruit tissue. It has certain function to postpone the senescence of harvested fruit. The ascorbic acid content of grapes gradually decreased during storage (Figure 4). The reactive oxygen species of fruit tissue oxidizes ascorbic acid into MDHA (monodehydroascorbic acid) or DHAA (dehydroascorbic acid). Defense enzymes including SOD, CAT, and POD can protect ascorbic acid from degradation. Coating + yeast could enhance activities of SOD, CAT, and POD, which was advantageous to eliminate reactive oxygen rapidly. Thus, the ascorbic acid was retained due to reactive oxygen elimination [19].

The characteristics of the grapes treated with coating + yeast were superior to those with coating alone. Such characteristics include sensory character, weight loss, the activities of defense enzymes, and vitamin C content. Similar results were observed; TU Kang [20], who sprayed strawberry with antagonistic yeast before harvest, and found that the weight loss increase and vitamin C decrease of strawberry were significantly reduced.

5. Conclusion

The postharvest grape coated with 2% of alginate and 3% of carboxymethyl cellulose combined with brewer yeast of 1.5×10^9 CFU/mL showed good sensory character on day 13 compared with control sample or only coated sample. The increase of weight loss and decrease of total soluble solids of grape treated with coating + yeast were restrained. Furthermore, the defence enzymes including SOD, POD, and CAT of grape treated with coating + yeast showed higher activities. Accordingly, the increase of MDA content was also restrained and more vitamin C was preserved in grape treated with coating + yeast. At day 13, the weight loss rate and the total soluble solids of grape treated with coating + yeast were 23.6% lower and 20.6% higher than those of control samples, respectively. Coating grapes with 2% of alginate and 3% of carboxymethyl cellulose combined with brewer yeast of 1.5×10^9 CFU/mL was a well-proven method to preserve postharvest grapes.

Acknowledgments

This work was supported by the project of the National Natural Science Foundation of China under Grant no. 31101359 and was also supported by Program for the Innovative Talents of Higher Learning Institutions of Shanxi (2012) as well as by project of Natural Science Foundation of Shanxi under Grant no. 2012021025-3.

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