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Physiological responses and quality attributes of table grape fruit to chitosan preharvest spray and postharvest coating during storage

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Abstract

The effects of preharvest chitosan spray (PCS) or/and postharvest chitosan coating (PCC) treatments on the quality and physiological response of table grape fruit stored at 20 or 0 °C was evaluated, respectively. PCS/PCC treatment showed the best control effect on decay. PCC or PCS/PCC treatment significantly decreased the weight loss of fruit stored at 20 °C. Additionally, all chitosan treatments inhibited the increase in rate of soluble solid content to titratable acid in fruit, stored at 20 °C, while enhancing the rate at 0 °C and affecting the content of total phenolic compounds in the fruit. Furthermore, the activities of superoxide dismutase decreased in all chitosan treatments and PCS or/and PCC treatments also changed the activities of polyphenol oxidase, peroxidase and phenylalanine ammonia-lyase. The results indicated the beneficial effect of chitosan by preharvest spray and/or postharvest coating on fruit quality and resistance to fruit decay.

Keywords: Table grape (*Vitis vinifera* L., cv Jingxiu); Pre- and postharvest; Chitosan; Decay; Quality

1. Introduction

Table grape is a highly perishable, non-climacteric fruit. Its shelf life is usually shortened by firmness loss, berry drop, discoloration of the stem, desiccation and fungal rots. The most common commercial method to control decay of the table grape fruit is the use of SO_2 during cold storage, either by fumigation or generators (Crisosto, Palou, Garner, & Armson, 2002; Smilanick et al., 1990). In spite of its excellent effect in controlling decay and preventing stem browning, SO_2 application is becoming restrictive in many countries. SO_2 residues are dangerous to human health and, additionally, SO_2 is highly injurious to most fresh fruits and vegetables and causes phytotoxicity symptoms, including bleaching of the berries and browning of the rachis (Smilanick et al., 1990). As an alternative to SO_2, interest in the exploitation of the modified atmosphere packaging (MAP) technique for table grapes has been reported (Artés-Hernández, Tomás-Barberán, & Artés, 2006). Application of a semi-permeable coating with a modified atmosphere of CO_2/O_2, under small storage environment conditions, has been shown to improve the storability of perishable fruits and vegetables (Del-Valle, Hernández-Muñoz, Guarda, & Galotto, 2005; Hagenmaier, 2005). Chitosan (poly β-(1 → 4) *N*-acetyl-D-glucosamine), a natural polysaccharide with a polycationic nature which has numerous applications in agriculture (Bautista-Baños et al., 2006), is regarded as a promising material for an edible coating in fruit postharvest field (Olivas & Barbosa-Cánovas, 2005).

As chitosan can form a semi-permeable film, a chitosan coating might be expected to modify the internal atmosphere, as well as to decrease transpiration losses and regulate the quality of the fruits (El Ghaouth, Arul, &

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Ponnampalam, 1991; Olivas & Barbosa-Cánovas, 2005). Meanwhile, chitosan has broad-spectrum antimicrobial activity, which has been well documented (Ait Barka, Eullaffroy, Clément, & Vernet, 2004; Plascencia-Jatomea, Viniegra, Olayo, Castillo-Ortega, & Shirai, 2003; Reddy et al., 1998; Sathiyabama & Balasubramanian, 1998) and *in vivo* studies showed that chitosan treatment could control or delay postharvest decay of fruits and vegetables (Bautista-Baños et al., 2006). In addition, chitosan as an exogenous elicitor can affect the activities of several defense-related enzymes and induce the accumulation of special substances in some plants (Cabrera, Messiaen, Cambier, & Van Cutsem, 2006; Trotel-Aziz, Couderchet, Vernet, & Aziz, 2006), which are known to participate in defense mechanisms and prevent pathogen infections.

However, previous researches which were summarised in the latest review (Bautista-Baños et al., 2006) mainly focused on the control effect by treatment with chitosan inoculation and on the physiological and pathological regulation of the fruit by chitosan coating. There were few studies on the increase of postharvest disease resistance, by preharvest chitosan spray (Reddy, Belkacemi, Corcuff, Castaigne, & Arul, 2000; Romanazzi, Mlikota Galer, & Smilanick, 2006) and no reports about the effect of the combination of pre- and postharvest treatment on the physiological responses and quality during storage, according to our knowledge. In the present experiment, we sprayed chitosan, once at low concentration ($1\ g\ L^{-1}$) before harvest and coated the fruit with a higher concentration ($10\ g\ L^{-1}$) after harvest, to study the effects on decay, weight loss, changes in quality attributes and the activities of pertinent enzymes of the table grape fruit during storage at different temperatures.

2. Materials and methods

2.1. Fruit

Table grapes (*Vitis vinifera* L., cv Jingxiu) were grown according to standard cultural practices in an organic orchard located in the Institute of Botany, Chinese Academy of Sciences in Beijing. No fungicides were applied prior to harvest. Fruit harvested at commercial maturity was immediately transported to the laboratory. The clusters were selected on the basis of uniform colour, size, firmness and the absence of blemishes or disease and were randomly distributed into batches.

2.2. Pre- and postharvest treatments with chitosan

For experimental use, the solution of chitosan was prepared by dissolving, under continuous stirring, the purified chitosan (90–95% of deacetylated degree and 15 cp of viscosity, prepared in our lab) in 0.5% (v/v) acetic acid. When dissolved, the pH value of the chitosan solution was adjusted to 5.6 using $1\ mol\ L^{-1}$ NaOH and 0.05% (w/v) Tween-80 as a surfactant was added to improve the wetting properties of the solution. Then pre- and postharvest treatments were conducted as follows in Table 1. At 10 d before harvest, the chitosan solution ($1\ g\ L^{-1}$) was sprayed on grape clusters once by using a hand-sprayer until clusters were wet to runoff. Additional clusters were sprayed with deionized water at pH 5.6 as the control. At harvest time, all fruit including those treated with chitosan or water before harvest were distributed into groups of five clusters randomly, respectively. Some fruit were dipped in solution containing $10\ g\ L^{-1}$ chitosan prepared as method mentioned above, and others in the deionized water at pH 5.6, accordingly. All treated-fruit were allowed to air-dry for 1 h at 20 °C. One group was regarded as a replicate and three replicates per treatment were conducted in this experiment. The treated and control fruit were packaged in plastic boxes, then overwrapped with plastic bags to maintain the relative humidity (RH) at 90–95%, and finally stored at 20 or 0 °C, respectively.

2.3. Decay assessment

During the storage, the natural incidence was evaluated by means of decay index (DI). Disease severity of a single grape fruit in the bunch was assessed according to the different empirical scales as follows: 0, healthy berry; 1, one lesion lower than 3 mm in diameter; 2, one lesion lower than 10 mm in diameter; 3, several lesions or 25% of berry surface infected; 4, more than 26% of the berry surface infected, sporulation present. The decay index was calculated by the formula, $DI = \sum(df)/ND$, where d is the degrees of rot severity scored on the berry and f is its respective quantity; N is the total number of berries examined and D is the highest degree of disease severity occurring on the scale.

2.4. Determination of weight loss

Weight loss of fresh table grapes in each treatment during storage was measured by monitoring weight changes of the fruit at 7, 16 d at 20 °C and 17, 42 d at 0 °C, respectively and following 3 d shelf life intervals at 20 °C. Weight loss was calculated as percentage loss of initial weight.

2.5. Assay of total phenolic compounds

Total phenolic compounds content was measured according to Zhang and Quantick (1997). Table grape fruit

Table 1
The treatments of grape fruits by chitosan pre- or/and postharvest were conducted in this experiment

Treatment	Preharvest spray		Postharvest coating	
	Water	Chitosan ($1\ g\ L^{-1}$)	Water	Chitosan ($10\ g\ L^{-1}$)
CK	+		+	
PCC	+			+
PCS		+	+	
PCS + PCC		+		+

tissues (10 g) were homogenised in 10 mL 1% HCl–methanol then centrifuged (4 °C, 15,000*g*) for 30 min. The supernatants were collected for assay. Absorption of the diluent was measured at 280 nm. Total phenolic compounds content was expressed as $A_{280\,nm}$ per gram fresh weight. All experiments were performed in triplicate.

2.6. Measurement of soluble solids content (SSC) and titratable acid (TA)

Fruit tissues (10 g) from ten grape fruits were suspended in 25 mL of distilled water, homogenised in a grinder, centrifuged (4 °C, 15,000*g*, 30 min) and then the supernatants were analysed immediately. TA content was titrated with phenolphthalein as indicator using 0.1 mol L^{-1} NaOH and expressed as mmol H^+ per 100 g fresh weight. SSC in grape juice was determined by means of an AO MRK II refractometer (AO Scientific Instrument, USA) at 20 °C and expressed as a percentage. All experiments were performed in triplicate.

2.7. Enzyme and protein assay

Fruit flesh (10 g) from six grape fruits was homogenised in 20 mL of ice-cold extraction buffers containing 0.5 g polyvinyl polypyrrolidone (PVPP). For the assay of peroxidase (POD) and polyphenol oxidase (PPO), the extraction buffer was 100 mmol L^{-1} sodium phosphate, pH 6.4, while the 50 mmol L^{-1} sodium borate buffer (pH 8.8, containing 5 mol L^{-1} β-mercaptoethanol), and 100 mmol L^{-1} potassium phosphate (pH 7.8) was used for the phenylalanine ammonia-lyase (PAL) and the superoxide dismutase (SOD) assay, respectively. Homogenates were centrifuged at 15,000*g* for 30 min at 4 °C and the resulting supernatants were used for assay.

For the PPO activity assay (Zhang & Quantick, 1997), 0.5 mL of the crude enzyme extraction solution was incubated with 3 mL of buffered substrate (100 mmol L^{-1} sodium phosphate, pH 6.4 and 500 mmol L^{-1} catechol) and these were monitored by measuring the change of absorbance at 398 nm for 25 s. The specific activity was expressed as U mg^{-1} protein, where one unit was defined as increase one ΔOD_{398} min^{-1} mg^{-1} protein.

The activities of POD and SOD were analysed according to the method of Wang, Tian, and Xu (2005). For POD activity, crude enzyme extraction solution (1.0 mL) was mixed with 2 mL buffered substrate (100 mmol L^{-1} sodium phosphate, pH 6.4 and 0.1% (v/v) guaiacol) and incubated at 30 °C. The absorbance at 460 nm was measured spectrophotometrically for 120 s after adding 1 mL of 0.08% (v/v) H_2O_2. The specific activity was expressed as U mg^{-1} protein, where one unit was defined as increase 0.01 ΔOD_{460} min^{-1} mg^{-1} protein. For SOD activity, the reaction mixture (3 mL) contained 50 mmol L^{-1} sodium phosphate buffer (pH 7.8), 13 mmol L^{-1} methionine, 75 mmol L^{-1} nitroblue tetrazolium (NBT), 10 mmol L^{-1} EDTA, 2 mmol L^{-1} riboflavin and 0.1 mL crude enzyme extraction solution. The mixtures were illuminated by fluorescent lamp (60 mmol m^{-2} s^{-1}) for 10 min and then the absorbance was determined at 560 nm. Identical solutions held in the dark served as blanks. One unit of SOD activity was defined as the amount of enzyme that caused a 50% decrease of the SOD-inhibitable NBT reduction. The specific activity was expressed as U mg^{-1} protein.

PAL activity was assayed following the method of Assis, Maldonado, Munoz, Escribano, and Merodio (2001), with some modifications. One milliliter of crude enzyme extraction solution was incubated with 2 mL of borate buffer (50 mmol L^{-1}, pH 8.8) and 0.5 mL of L-phenylalanine (20 mmol L^{-1}), for 60 min, at 37 °C. The reaction was stopped with 0.1 mL, 6 mol L^{-1} HCl. The activity of PAL was determined by the production of cinnamate, which was measured by the absorbance change at 290 nm. The blank was the crude enzyme preparation mixed with L-phenylalanine, with zero time incubation. The specific activity was expressed as U mg^{-1} protein, where one unit was defined as increase one ΔOD_{290} min^{-1} mg^{-1} protein.

Protein content was measured according to the method of Bradford (1976), using bovine serum albumin (BSA) as the standard protein.

2.8. Statistical analysis

All data were analysed by one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple range test. Differences at $P < 0.05$ were considered as significant.

3. Results

3.1. Weight loss

Weight loss of grape fruit in storage is shown in Fig. 1. The rate of weight loss increased with the storage time at 20 °C. While postharvest chitosan coating (PCC) treatment significantly decreased weight loss and there was no significant difference between preharvest chitosan spray (PCS) + PCC and PCC treatments ($P > 0.05$). Additionally, weight loss of the fruit stored at 0 °C was much lower than that at 20 °C. Treatments with chitosan had no significant difference on weight loss at 17, 42 d in storage at 0 °C. When fruit stored at 0 °C for 42 d were transported to 20 °C for 3 d of shelf life, weight loss of all fruit significantly increased.

3.2. The effects on SSC and TA

SSC increased gradually with maturity of the grape fruit, PCS treatment significantly increased the level of SSC as compared to the control at harvest time ($P < 0.05$) (Fig. 2a). When stored at 20 °C, the fruit treated with PCS/PCC showed an increase and those treated alone with PCS increased more obviously in SSC. After 42 d of

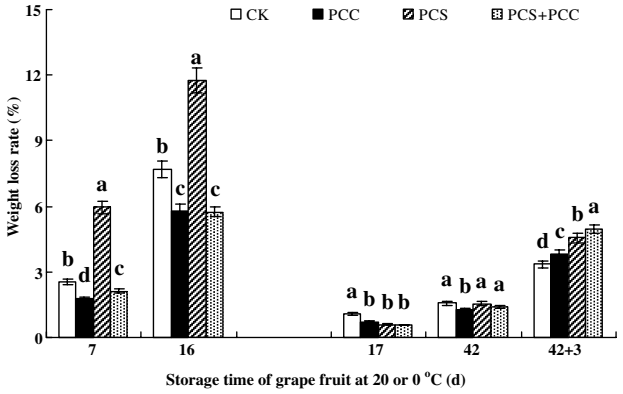

Fig. 1. The effects of chitosan preharvest spray or/and postharvest coating treatment on fruit weight loss of table grapes stored at 20 or 0 °C. The fruit stored at 20 °C for 7 and 16 d (left) and at 0 °C for 17 and 42 d followed 3 d shelf life (right), respectively. CK, control; PCC, postharvest chitosan coating; PCS, preharvest chitosan spray; PCS + PCC, the combination preharvest chitosan spray and postharvest coating. Bars represent standard deviations of the means and values followed by different letters were significant difference according to Duncan's Multiple Range Test at $P < 0.05$.

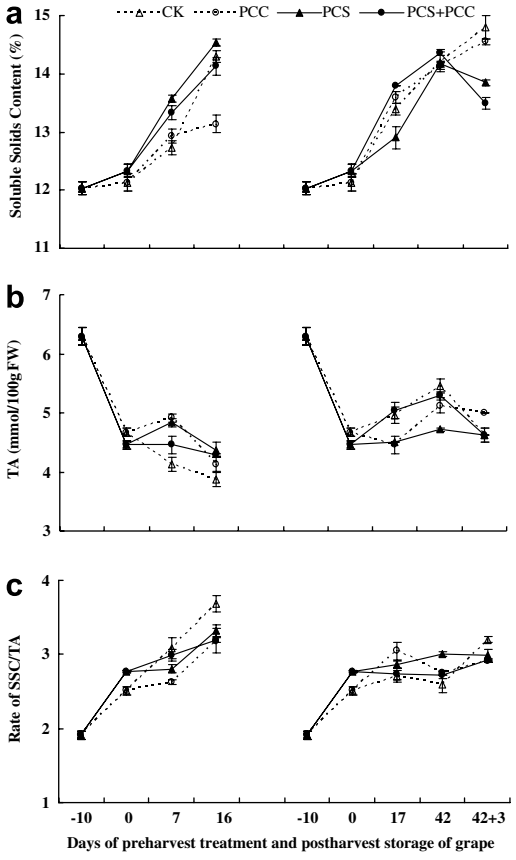

Fig. 2. The changes of SSC (a), TA (b) contents and the rate of SSC/TA (c) in fruit stored at 20 or 0 °C responses to chitosan preharvest spray and/or postharvest coating treatment. Zero and −10 d represent the harvest day and 10 d before harvest, respectively (the same in Figs. 3 and 5). The days of fruit storage at 20 or 0 °C are the same as above. Bars represent standard deviations of the means.

storage at 0 °C, all fruit were kept at 20 °C for 3 d of shelf life, SSC increased in the control (CK) and PCC treated fruit, but decreased dramatically in PCS and PCS/PCC treated fruit.

TA content of the grape fruit decreased with increase in maturity and was not significantly affected by PCS treatment (Fig. 2b). Compared with CK at 20 °C, all treatments almost significantly increased TA content ($P < 0.05$), reaching a peak at 7 d, but the contrary effect of all chitosan treatment on TA content of the fruit stored at 0 °C compared to 20 °C was found.

The rate of SSC/TA of the fruit in the CK increased gradually with increasing maturity and storage time at 20 °C (Fig. 3c). PCS treatment enhanced the rate of SSC/TA at harvest, but as compared to CK, all chitosan treatments inhibited the increase of SSC/TA when fruits were stored at 20 °C. When stored at 0 °C, the rate of SSC/TA in CK fruit was relatively stable and all chitosan treatments enhanced it. In the following shelf life after 42 d storage at 0 °C, the rate in all treatments increased and it did so most significantly in the CK.

3.3. Total phenolic compounds content

The changes in the content of total phenolic compounds are shown in Fig. 3. When the fruits were stored at 20 °C, the content of total phenolic compounds decreased with the storage time and PCC treatment significantly inhibited the decrease trend ($P < 0.05$). Total phenolic compounds content in PCS treated fruit decreased but then increased, at the end of the storage period. At 0 °C of storage and in the shelf life, the content of total phenolic compounds in all treatments decreased (17 d) and then increased gradually, PCS treatment enhanced it compared to the CK.

3.4. Decay index of fruit

As shown in Fig. 4, the decay index of grape fruit in the CK was higher than those treated with chiotsan when stored for 16 d at 20 °C or 42 d at 0 °C, respectively. All treatments with chitosan markedly decreased decay index

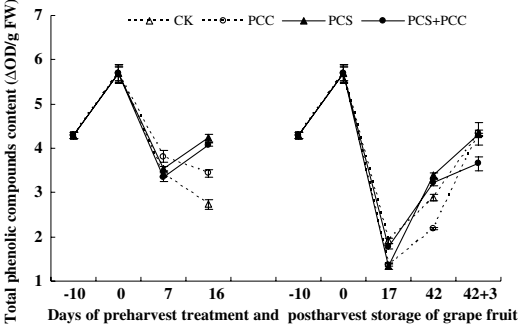

Fig. 3. The effects of chitosan preharvest spray or/and postharvest coating treatment on the content of total phenolic compounds of table grape fruit stored at 20 or 0 °C. Bars represent standard deviations of the means.

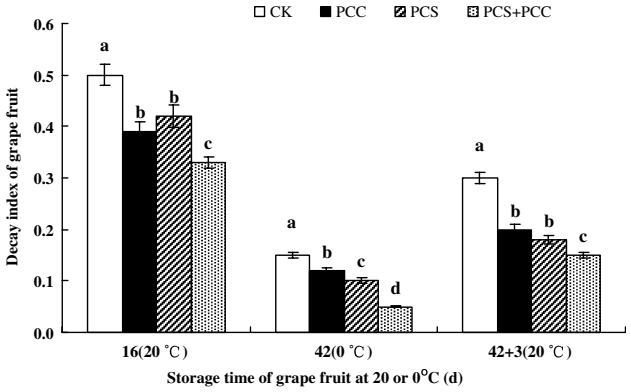

Fig. 4. The decay index of table grape fruit stored at 20 or 0 °C after chitosan preharvest spray and postharvest coating. Bars represented standard deviations of the means and values followed by different letters were significant difference according to Duncan's Multiple Range Test at $P < 0.05$.

($P < 0.05$) and PCS/PCC treatment had the best effect on decay control. At the 3 d shelf life, following 42 d storage at 0 °C, the decay index obviously increased.

3.5. The effects on enzymes activities

PCS treatment significantly inhibited PPO activity at harvest time ($P < 0.05$). When the fruits were stored at 20 °C, PPO activity in the CK fruit reached the highest value at 7 d (Fig. 5a). PCS treatment stimulated the increase of PPO activity but PCC treatment showed a contrary effect. At 0 °C, PPO activity increased in the early period of storage (17 d) and there was no significant difference among all treatments ($P > 0.05$). At the end of storage (42 d), PPO activity decreased in the CK and PCC treatment, but significantly increased in PCS/PCC treated fruit ($P < 0.05$). During the shelf life, PPO activity increased in all treatments.

At 20 °C, POD activity in all treatments increased gradually (Fig. 5b). The PCS treatment significantly increased the POD activity of the grape fruit ($P < 0.05$), while PCC and PCS/PCC treatments inhibited the increase of POD activity. When the fruits were stored at 0 °C, POD activity in all treatments dramatically increased ($P < 0.05$) and POD activities in PCS and PCS/PCC treatments were higher than those in the CK and PCC treatment. During

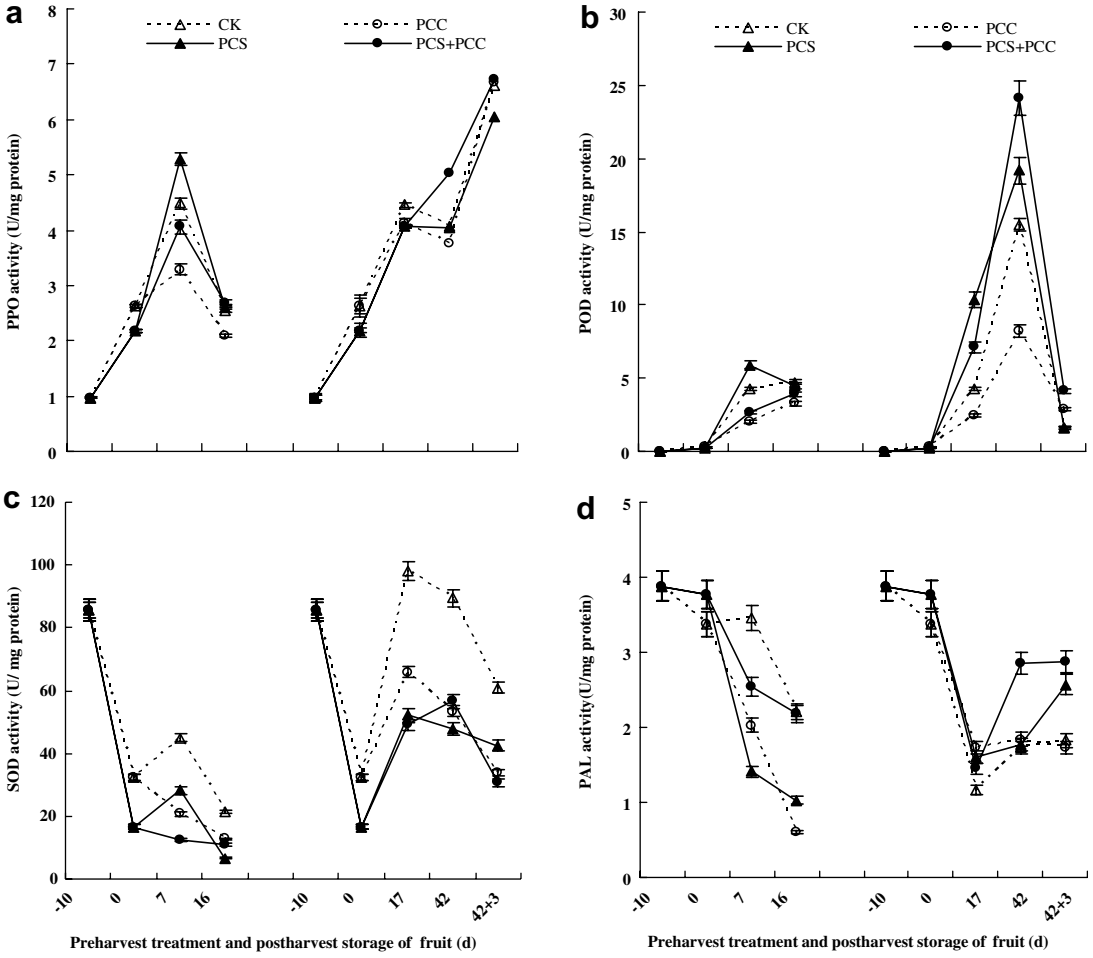

Fig. 5. The effects of chitosan pre- and postharvest treatments on the activities of PPO (a), POD (b), SOD (c) and PAL (d) in table grape fruit during mature and storage time. Bars represented standard deviations of the means.

the shelf life, POD activity of the grape fruits decreased quickly.

The SOD activity of grape fruit at harvest time was obviously lower than that at 10 d before harvest in the CK and PCS treatment decreased the activity of SOD ($P < 0.05$) (Fig. 5c). At 20 °C, SOD activity of the grape fruits in CK and PCS treatment peaked at 7 d, while the activity decreased gradually in PCC and PCS/PCC treatments (Fig. 5c). At 0 °C, SOD activity exhibited a similar trend with those at 20 °C but was higher than those at 20 °C. When the fruits were transported to 20 °C for shelf life, SOD activity of the fruits decreased ($P < 0.05$).

Grape fruit had a low PAL activity at harvest time and the PCS treatment maintained the high activity of PAL, as that of preharvest 10 d. (Fig. 5d). At 20 °C, PAL activity of the grape fruits in all treatments decreased gradually and PCS treatment quickened the decrease in PAL activity, while PCS/PCC treatment could inhibit the change trend. PAL activity in fruit stored at 0 °C reached the lowest level at 17 d, then increased, especially that in the fruits of PCS/PCC. After 3 d of shelf life, there was no obviously difference in PAL activity in the CK and PCC treatment, but significant increase could be found in PCS and PCS/PCC treated fruit ($P < 0.05$).

4. Discussion

Chitosan, as a natural fungicide, is a polysaccharide. Previous studies showed that chitosan could inhibit directly spore germination, germ tube elongation and mycelial growth of many phytopathogens (Ben-Shalom, Ardi, Pinto, Aki, & Fallik, 2003; El Ghaouth, Arul, Grenier, & Asselin, 1992; Xu, Zhao, Han, & Du, 2006) and decrease decay incidences and prolong the storage time of fruits (Bautista-Baños et al., 2006; Ben-Shalom et al., 2003; Reddy et al., 2000). The mechanism by which chitosan affects the growth of pathogens may be related to the ability of chitosan to interfere with the negatively charged residues of macromolecules exposed on the fungal cell surface, resulting in the leakage of intracellular electrolytes and proteinaceous constituents (Leuba & Stossel, 1986; Xu et al., 2006). Another mechanism may be the interaction of diffused hydrolysis products with microbial DNA, which affected mRNA and protein synthesis (Hadwiger, Kendra, Fristensky, & Wagoner, 1986; Zakrzewska, Boorsma, Brul, Hellingwerf, & Klis, 2005). It is well known that grey mould caused by *Botrytis cinerea* is the major postharvest disease of grape fruit. In the previous experiment, Liu, Tian, Meng, and Xu (2007) found that chitosan could significantly inhibit spore germination, germ tube elongation and mycelial growth of *B. cinerea in vitro* due to damaging the plasma membrane of the spore and it effectively decreased the incidence of grey mould in tomato fruit. The result of this experiment suggested that chitosan, with such molecular characteristics significantly reduced the natural decay incidence of grape fruit during storage ($P < 0.05$), and PCS/PCC treatment showed the best control effect (Fig. 4). The data in our experiment also revealed that preharvest chitosan spray induced the activities of defense-related enzymes (PPO and PAL), thus promoted protection of latent infection of pathogens of grape fruit (Fig. 5a and d), which was consistent with the previous studies on the induced resistance of some fruits, vegetables and other crops (Ben-Shalom et al., 2003; Cabrera et al., 2006; Romanazzi et al., 2006; Trotel-Aziz et al., 2006). So with its antifungal activity and elicitation potential, preharvest chitosan spray is promising to partially substitute the utilisation of synthetic fungicides in preventing the latent disease. Moreover, postharvest chitosan coating can form a film on the fruit surface, which acts as a mechanical barrier to protect the fruit from pathogen infection, contributing to decrease decay during storage periods (Butler, Vergano, Testin, Bunn, & Wiles, 1996; Chien, Sheu, & Lin, 2007). In general, low temperature storage allows the fruit to have a slower physiological process and pathogens have a weaker pathogenicity, resulting in a relative lower decay incidence as compared to room temperature storage. However, when the fruits were stored at room temperature for shelf life, the decay incidence increased rapidly. Our results indicated that pre- and postharvest chitosan treatment significantly decreased decay incidence ($P < 0.05$) (Fig. 4).

The quality of the grape fruits is also an important index evaluating the storage effect. Previous studies revealed that the chitosan coating functioned as a self control atmosphere and selectively permeated C_2H_4, CO_2 and O_2 inside and out of the fruit, thus reducing fruit respiration metabolism (Bai, Huang, & Jiang, 1988; El Ghaouth et al., 1991; Hagenmaier, 2005). In this experiment we found that at 20 °C all chitosan treatments, especially PCC and PCS/PCC, decreased the rate of SSC/TA of the grape fruits (Fig. 2). This may be related to the inhibition of fruit respiration by chitosan coating. At 0 °C, the respiration metabolism of the grape fruits was relatively weak. Chitosan treatment increased the rate of SSC/TA (Fig. 2), which perhaps availed increasing cytoplasm osmotic potential to some extent, thereby enhancing the adaptability to low temperature. The results of this experiment suggested that PCC and PCS/PCC treatments significantly decreased weight loss of grape fruit at 20 °C ($P < 0.05$), while there were no significant differences at 0 °C ($P > 0.05$), being consistent with the previous reports (Bautista-Baños et al., 2006; El Ghaouth et al., 1991). When grape fruits stored at 0 °C for 42 d were transferred to 20 °C for 3 d, weight loss of the fruit was significantly enhanced ($P < 0.05$) (Fig. 1). Such phenomenon may be due to the temperature increase, which results in an increase of respiration metabolism of the fruit and loss of water absorbed by the chitosan film on the fruit surface. The property that the film formed by chitosan could absorb moisture has been previously reported (Olivas & Barbosa-Cánovas, 2005; Wiles, Vergano, Barron, Bunn, & Testin, 2000). Meanwhile, the effect of coating on preventing the water loss from fruit was related to the composition of film-form-

ing solution and hydrophobe adding was helpful (Butler et al., 1996; Olivas & Barbosa-Cánovas, 2005). Therefore, to prevent water loss it is suggestible to consider the addition of some edible lipid to the chitosan film-forming solution but the film's selective permeability of CO_2/O_2 also needs to be taken into account at the same time.

In order to apply chitosan commercially in postharvest field, the following questions should be considered. The molecular weight, structure, application concentration and solvent of chitosan will affect its antifungal ability on pathogenicity of pathogens and its exogenous elicitor activity on fruits (Bautista-Baños et al., 2006; Liu et al., 2006). The property of film formed by chitosan, including water permeability and selective permeability of gas, are related to its molecular characteristic and concentration (Park, Marsh, & Rhim, 2002; Wiles et al., 2000). There are some latent diseases in fruits, which usually occur in postharvest storage (Zahavi et al., 2000). Therefore, further studies will be focused on the preparation of different molecular characteristic chitosan, the physical property of chitosan film, the antimicrobial activity of different chitosans on the major fungal pathogens of fruits and the technology of preharvest application in our lab.

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