LWT - Food Science and Technology 43 (2010) 596–601

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Influences of preharvest spraying *Cryptococcus laurentii* combined with postharvest chitosan coating on postharvest diseases and quality of table grapes in storage

Xiang-Hong Meng [a,b], Guo-Zheng Qin [a], Shi-Ping Tian [a,*]

[a] Key laboratory of Photosynthesis and Environmental Molecular Physiology, Chinese Academy of Sciences, Institute of Botany, Nanxincun 20, Xiangshan, Beijing 100093, PR China
[b] College of Marine Life Science, Ocean University of China, Qingdao 266003, PR China

ARTICLE INFO

Article history:
Received 2 April 2009
Received in revised form
22 September 2009
Accepted 7 October 2009

Keywords:
Biocontrol control
Chitosan
Vitis Vinifera L.
Decay
Quality

ABSTRACT

The effects of preharvest spray with *Cryptococcus laurentii* combined with chitosan coating after harvest on decay and quality of table grapes during storage periods were evaluated in the present study. Preharvest spray with *C. laurentii* (PreA) significantly decreased decay index (DI), and postharvest chitosan coating (PCC) enhanced the effectiveness of the pre-harvest spray when fruits were stored at 0 °C. PreA combination with PCC increased the activities of polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) of fruit in storage. PreA + PCC treatment was effective in reducing weight loss of fruits by 85% at 17 d storage and 38% at 42 d storage as compared to PreA alone at the same stage. In addition, PreA enhanced the ratio of soluble solids content (SSC) to titratable acid (TA) by 12% at harvest time, 7% at 17 d storage and 25% at 42 d storage, mainly by increasing SSC and decreasing TA in fruit stored at 0 °C. These results suggested that integration of preharvest spray with *C. laurentii* and postharvest chitosan coating treatment may be a promising management strategy for decay control and quality maintenance of table grapes.

1. Introduction

Table grapes with thin pericarp and succulent flesh is easily damaged and infected by saprophytes and plant pathogens, resulting in a high decay rate during storage. Although low temperature combined with SO_2 fumigation is an effective method for table grape storage (Crisosto, Palou, Garner, & Armson, 2002; Smilanick et al., 1990), SO_2 application usually causes injury to fruits, and is harmful to human health (Taylor, 1993). Moreover, due to increased public concern on food safety, SO_2 as a fungicide is limited to use for grape storage (Taylor, 1993). Therefore, it is necessary to search for some alternatives to SO_2 as disease management strategy for table grape storage.

It has been proved that biological control could be used as an alternative to chemical fungicides in controlling postharvest diseases of fruits and vegetables (Janisiewicz & Korsten, 2002; Tian, 2006). A large number of studies revealed that *Cryptococcus laurentii* had a strong antagonism to *Botrytis cinerea* and also effectively controlled grey mould caused by *B. cinerea* in peach, sweet berry, pear (Lima, De Curtis, Castoria, & De Cicco, 1998; Qin, Tian, & Xu, 2004; Roberts, 1990; Zhang, Zheng, & Yu, 2007), as well as blue mould caused by *Penicillium expansum* (Janisiewicz, Saftner, Conway, & Yoder, 2008; Qin et al., 2004; Zhang, Zheng, & Xi, 2005). However, the widespread commercial application of *C. laurentii* in postharvest disease control of fruits is difficult to be fully realized, similar to other biocontrol agents, because of high applied cost, narrow-spectrum and instability (Fravel, 2005; Tian, 2006). Our previous studies have shown that biocontrol efficacy of *C. laurentii* could be enhanced by combining it with sodium silicate in jujube fruit (Tian, Qin, & Xu, 2005), and sodium bicarbonate in pear fruit (Yao, Tian, & Wang, 2004). The synergistic effects of antagonistic yeasts combined with hot water (Conway, Leverentz, Janisiewicz, Saftner, & Camp, 2005; Spadaro, Garibaldi, & Gullino, 2004), salicylic acid (Zhang et al., 2008), natural compounds (Droby, 2006), food additives (Ippolito, Schena, Pentimone, & Nigro, 2005; Qin, Tian, & Xu, 2006) and low-concentration fungicides (Lima, De Curtis, Piedimonte, Spina, & De Cicco, 2006; Sugar & Basile, 2008) have also been reported in postharvest disease control of fruits and vegetables. Therefore, a combination of biocontrol agent with natural fungicides potentially provides satisfactory effects of decay control as compared with antagonist used alone.

Chitosan (poly β-(1 → 4) *N*-acetyl-D-glucosamine) with bio-safe and anti-fungal properties is widely investigated to apply in fields of

* Corresponding author. Tel.: +86 10 62836559; fax: +86 10 82594675.
E-mail address: tsp@ibcas.ac.cn (S.-P. Tian).

0023-6438/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.lwt.2009.10.007

agriculture and food (Rinaudo, 2006). Chitosan and its derivatives are able to form a semi-permeable film on fruit surface, which might be expected to modify the internal atmosphere, as well as to decrease transpiration losses and improve fruit quality (Bai, Huang, & Jiang, 1988; El Ghaouth, Arul, & Ponnampalam, 1991). This coating film could act as a mechanical barrier to protect fruits from pathogen infection and also induce host-defense responses (Chien, Sheu, & Lin, 2007; Meng, Li, Liu, & Tian, 2008; Trotel-Aziz, Couderchet, Vernet, & Aziz, 2006), decreased decay during storage periods. Postharvest application of chitosan and *C. laurentii* has been shown to prevent blue mould caused by *P. expansum* in apple fruit (Yu, Li, & Zheng, 2007). In addition, preharvest spray of biocontrol agents including *C. laurentii* on surface of fruits has been proved to be an effective application technology in decay control (Ippolito et al., 2005; Tian, Qin, & Xu, 2004). The objective of this study was to analyze the integrative effects of *C. laurentii* used at preharvest spray in combination with postharvest chitosan coating on natural decay, physiological properties and quality attributes of table grapes stored at 0 °C during storage periods.

2. Material and methods

2.1. Fruits and treatments

Table grape (*Vitis vinifera* L., cv Jingxiu) were planted in an organic orchard of Institute of Botany, Chinese Academy of Sciences in 1997. *C. laurentii* was isolated from the surfaces of apple fruit with the method of Wilson and Chalutz (1989) and identified by CABI Bioscience Identification Services (International Mycological Institute, UK). The yeast was cultured in nutrient yeast dextrose broth (NYDB: 8 g of nutrient broth, 5 g of yeast extract and 10 g of dextrose in 1000 mL water) for 48 h at 25 °C with a shaker (HZQ-C, Dong Ming Co., China) at 200 rev min^{-1}. Yeast cells were collected by centrifugation at 6000 *g* for 10 min. The concentration of the yeast was measured with a hemacytometer. At 10 d before harvest, a suspension of *C. laurentii* containing 1×10^8 cells/mL in distilled water with 0.05% (w/v) Tween-80 as a surfactant was sprayed on different grape clusters once by using hand-sprayer until all fruit were wet to runoff. Additional clusters were sprayed with deionized water as the control. At harvest time, all preharvest-treated fruit were distributed into groups of five clusters randomly. Chitosan with a deacetylation degree of 90% and viscosity of 15 cp was prepared and dissolved under continuously stirring in 0.5% (v/v) acetic acid. The pH value of 10 g/L chitosan solution was adjusted to 5.6 with 0.01 mol L^{-1} NaOH, and then surfactant Tween-80 was added to a final concentration of 0.05% (w/v). Some Preharvest-treated fruit were dipped in 10 g/L chitosan solution and others in deionized water at pH 5.6 adjusted with acetic acid, accordingly. Then, all treated fruit were allowed to air-dry for 1 h at 20 °C. One replicate of five clusters and three replicates per treatment were conducted and the experiment was repeated twice in this study. The treated and control fruit were packaged in plastic boxes, then overwrapped with plastic bags to maintain a relative humidity (RH) at 90–95%, and finally stored at 0 °C. Fruits stored for 17 or 42 d at 0 °C and then at 20 °C for 3 d were evaluated. All fruit were evaluated according to following methods.

2.2. Decay determination

During storage, natural decay incidence was evaluated using a decay index (DI). Disease severity of every grapes in each replicate was seriatim assessed according to the different empirical scales as follows: 0 = healthy berry; 1 = one lesion less than 2 mm in diameter; 2 = one lesion less than 5 mm in diameter; 3 = several lesions or 25% of berry surface infected; 4 = more than 26% of the berry surface infected, sporulation present. Decay index was calculated by the formula, $DI = \sum (d \times f)/N/D$, where d is the degrees of rot severity scored on berry and f is its respective quantity, N is the total number of examined berries and D is the highest degree of disease severity occurring on the scale.

2.3. Assay activities of enzymes

Fruit flesh sampled (10 g) randomly from 10 grapes of a replicate was homogenized in 20 mL ice-cold extraction buffer containing 0.5 g polyvinyl polypyrrolidone (PVPP). For POD (peroxidase) and PPO (polyphenol oxidase) activities, extraction buffer was 100 mmol L^{-1} sodium phosphate (pH 6.4). To measure PAL (phenylalanine ammonia-lyase), 50 mmol L^{-1} sodium borate buffer containing 5 mol L^{-1} β-mercaptoethanol (pH 8.8) was used, and 100 mmol L^{-1} potassium phosphate (pH 7.8) for SOD (superoxide dismutase) activity. Homogenates were centrifuged at 15,000 *g* for 30 min at 4 °C, and the supernatants, as crude enzyme extract kept on ice for assay.

Activity of enzymes was assayed according to the methods of Meng et al. (2008). Specific activity of polyphenol oxidase (PPO) was expressed as U mg^{-1} protein, where one unit was defined as ΔOD_{398} min^{-1} mg^{-1} protein. Specific activities of peroxidase (POD) and superoxide dismutase (SOD) were expressed as U mg^{-1} protein, where one unit was defined as 1% of ΔOD_{460} min^{-1} mg^{-1} protein for POD, and SOD as the amount of enzyme that caused a 50% decrease of SOD-inhibitable NBT (nitroblue tetrazolium) reduction. Specific activity of phenylalanine ammonia-lyase (PAL) was expressed as U mg^{-1} protein, where one unit was defined as increase of 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 standard protein. All measurements above were performed in triplicate and three times for one replicate.

2.4. Rate of weight loss

Weight loss of fresh table grapes in each treatment during storage was measured by monitoring weight change of fruit of every storage period as compared to initial weight of fruits before storage.

2.5. Measurement content of total phenolic compounds

Content of total phenolic compounds was measured according to Zhang and Quantick (1997). Fruit flesh (10 g) in 10 mL 1% HCl-methanol was homogenized, then centrifuged at 4 °C for 50 min at 15,000 *g*. The supernatants were collected and absorbance was measured at 280 nm. Content of total phenolic compounds was expressed as A_{280} g^{-1} FW (fresh weight). All assays were performed in triplicate.

2.6. Soluble solids content (SSC) and titration acid (TA)

Fruit flesh samples (10 g) obtained randomly from 10 grape berries were homogenized in 25 mL of distilled water and centrifuged at 15,000 *g* for 30 min at 4 °C, then the supernatants were collected. SSC was assayed by means of an AO MRK II refractometer (AO Scientific Instrument, USA) at 20 °C and expressed as °Brix. TA was determined by titrating with phenolphthalein as indicator using 0.01 mol L^{-1} NaOH to pH 8.2 and expressed as mmol H^+ per 100 g fresh weight. All assays were performed in triplicate.

2.7. Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA). Mean separations were obtained by Duncan's Multiple Range Test. Differences at $P < 0.05$ were considered as significant.