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Postharvest treatment of table grapes with ultraviolet-C and chitosan coating preserves quality and increases stilbene content

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ABSTRACT

Red table grapes were treated after harvest with ultraviolet-C light (UV-C) and chitosan coating, both alone and in combination. Effect of treatments on quality, fungal decay, and resveratrol content of grapes was studied. In preliminary trials, combination of UV-C with chitosan coating yielded better results compared with the same treatments applied alone. Therefore, further optimization was performed with combinations of the two treatments. Chitosan coating preserved brightness and visual quality of fruit while preventing decay by *Botrytis cinerea*. Furthermore, this coating treatment had no effect on respiration rate or resveratrol content of grapes. UV-C treatment combined with storage at 20 °C for 24 h before refrigerated storage led to increased resveratrol content and had no negative effects on the sensory quality of treated grapes when combined with chitosan coating. In contrast with endogenous resveratrol, the application of resveratrol solutions was effective for the prevention of growth of *B. cinerea*. Use of UV-C combined with chitosan coating followed by incubation for 24 h at 20 °C before refrigerated storage increased the resveratrol content, maintained sensory quality, and reduced fungal decay of red table grapes when compared to control grapes.

1. Introduction

Gray mold caused by *Botrytis cinerea* is the most important postharvest disease of table grapes (Crisosto et al., 1998). Different techniques can be used to prevent this disease, including the application of natural compounds and physical treatments (Romanazzi et al., 2012).

Chitosan is a pseudonatural polymer obtained commercially by deacetylation of chitin from the exoeskeleton of crustaceans (Rinaudo, 2006). Chitosan coating has shown potential for the reduction of fungal rot (Xu et al., 2007) and for preservation of sensory quality of grapes (Ardakani et al., 2010; Gao et al., 2013). Its effect against gray mold has been tested both on preharvest and postharvest applications, and some chitosan-based products are available in the market (Romanazzi et al., 2012).

Resveratrol is a phytoalexin involved in plant defense against pathogens such as *B. cinerea* (Adrian et al., 1997; Romanazzi et al., 2006). Furthermore, its high concentration in grape tissues is correlated with the presence of compounds with even more powerful fungitoxic effect like viniferin (Douillet-Breuil et al., 1999). Apart from its role in plant defense, potential benefits for human health of resveratrol consumption are well established (Das and Das, 2007; Iriti and Faoro, 2009; Xianfeng-Huang and Zhu, 2011). Therefore, induction of resveratrol production is desirable not only from the point of view of postharvest disease control, but also with the aim of increasing nutraceutical properties of grapes. Resveratrol production can be stimulated in grapes by different methods including UV-C irradiation (Třska and Houška, 2012), although different species and varieties of *Vitis* spp. respond differently to this physical treatment (Cantos et al., 2003a).

The combination of preharvest chitosan coating with postharvest UV-C irradiation for the inhibition of gray mold on table grapes during storage has been tested with positive results (Romanazzi et al., 2006). In the present study UV-C and chitosan were both applied at the postharvest level, and their suitability for preservation of table grapes was evaluated and optimized not only taking into account its effect on gray mold but also studying consequences on other quality parameters such as sensory quality, weight loss, titratable acidity (TA), pH, soluble solids content (SSC), resveratrol content, and respiration rate.

2. Materials and methods

2.1. Plant material and processing

The red table grape variety Crimson was harvested at its optimum ripeness between September and November 2007 in Murcia (Spain), transported to the laboratory, and stored overnight

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at 4 °C. The day after, grapes were graded for uniformity of color and size, and grapes without visible damage on their skin or visible microbial infection were selected and sanitized in chlorinated water (80 mg/L free chlorine, adjusted at pH 7.0 with hydrochloric acid) (Panreac, Montcada i Reixac, Barcelona, Spain) for 1 min, rinsed in cold tap water and then drained for 30 s. Sanitized grapes were divided into different groups that were submitted to postharvest treatments as explained in Section 2.2. All the processing was carried out in a fresh-cut produce pilot plant at 6 °C under sanitary conditions.

2.2. Postharvest treatments

For UV-C treatment, the protocol of Allende et al. (2007) was followed. Grapes were placed in a single layer on the illumination vessel for the treatment, and the selected UV-C dose (6.0 ± 0.1 kJ/m^2) was applied by establishing a specific exposure time (two illumination steps of 1 min) at a fixed distance (60 cm). The selection of the UV-C dose was based on previous studies (Allende et al., 2007; Selma et al., 2008). Grapes were turned over after the first illumination step to ensure the total surface exposure to UV-C light. The fluence rate of the lamps at the level of the samples was 2.82 ± 0.44 mW cm^{-2}, measured with a Blak-Ray J-225 photometer (Ultra Violet Products, Inc., San Gabriel, CA). All the treatments were applied in a cold room at 10 °C. UV-C treated grapes were divided in two lots. One lot was stored at 20 °C for 24 h before coating or packaging to enhance the stilbene synthesis (Cantos et al., 2003b; Larrosa et al., 2003), while the second batch of grapes was directly packaged and stored at 4 °C.

Two coating solutions were tested containing 10 mL/L of yam starch, 10 mL/L of glycerol, 5 mL/L of glacial acetic acid, and 5 mL/L chitosan (low molecular weight, 75–85% deacetylated, dissolved in acetic acid) in the case of the 0.5% chitosan solution and 10 mL/L of chitosan in the case of the 1% chitosan solution (Durango et al., 2006). All chemicals used were provided by Sigma–Aldrich (St. Louis, USA). Grapes were submerged in these solutions for 1 min and dried under forced air for up to 2.5 h at 6 °C and 90% relative humidity (RH) before packaging.

First, a preliminary trial was performed in order to select optimal postharvest treatments. A total of nine treatments were evaluated in the preliminary trial: untreated sanitized grapes stored at 4 °C (control), UV-C treated grapes stored at 4 °C (UV-C), UV-C treated grapes stored 1 day at 20 °C + 8 days at 4 °C (UV-C + 20 °C/24 h), chitosan-coated grapes (0.5%) stored at 4 °C (CHT 0.5%), chitosan-coated grapes (1%) stored at 4 °C (CHT 1%), UV-C treated grapes coated with 0.5% of chitosan solution and stored at 4 °C (UV-C + CHT 0.5%), UV-C treated grapes coated with 0.5% of chitosan solution and stored 1 day at 20 °C + 8 days at 4 °C (UV-C + CHT 0.5% + 20 °C/24 h), UV-C treated grapes coated with 1% of chitosan solution and stored at 4 °C (UV-C + CHT 1%), and UV-C treated grapes coated with 1% of chitosan solution and stored 1 day at 20 °C + 8 days at 4 °C (UV-C + CHT 1% + 20 °C/24 h). In this preliminary trial resveratrol content and sensory quality were determined and results of these analyses were used as selection criteria to choose the best treatments for detailed study in the main experiment. In the main experiment, selected treatments were compared by studying the effect on resveratrol content, respiration rate, quality of grapes, and decay susceptibility. The main experiment was repeated three times.

2.3. Packaging and storage

After postharvest treatment, grape samples of 250 g each were packaged in polypropylene (PP) trays (185 mm × 138 mm × 32 mm), sealed in air conditions with a perforated plastic film (OPP multilayer films, 1255 perforations/m^2, 35 μm thickness) and stored at 4 °C and 90% RH to avoid water loss for up to 9 days.

2.4. Respiration rate

For respiration rate measurements, grapes (100 g) were placed in a 1 L glass jar at 4 °C and 95% RH. A continuous humidified air flow was pumped into the jars to avoid dehydration and excessive CO_2 accumulation. The respiration rate as CO_2 production was monitored every day for up to 9 days. On each sampling date, three jars per treatment were evaluated. Samples of 1 mL of headspace gas were taken from each jar with a calibrated syringe and CO_2 production monitored in an infrared gas analyzer (Horiba Via 510, Horiba Instruments Co., Irvine, CA, USA).

2.5. Quality indexes

Weight loss, TA, pH and SSC of the samples were evaluated as quality indexes after 9 days of storage. Weight loss during storage was determined comparing the weight of the samples before and after storage using a laboratory scale. The TA was determined by titrating 10 mL of juice using NaOH 0.1 mol/L to pH 8.1 (AOAC, 1984). The pH was measured using a pH meter (Crison, Barcelona, Spain) and SSC by a digital temperature compensated hand-held refractometer (Atago, Tokio, Japan). Sensory quality was evaluated at the end of the storage by a six member panel, trained to score the quality attributes of grapes. Samples were scored for overall visual quality using a 9 point interval hedonic scale as previously described (Allende et al., 2008). Brightness and flavor of the product was evaluated in a 5 point scale, where: 1 = dislike extremely, no characteristic of the product; 3 = neither like nor dislike, limit of acceptance from the consumers point of view; and 5 = like extremely, very characteristic of the product. Defects of the product such as dehydration of the grapes were evaluated as follows: 5 = severe; 3 = moderate, limit of acceptance from the consumer's point of view; and 1 = absence. All quality evaluations were performed in a sensory room, equipped with individual cabinets with white and red lights. Flavor was evaluated under red light, while whiteness and overall visual quality were evaluated under white light.

2.6. Resveratrol content

Resveratrol content was measured after 9 days of storage in the preliminary trial, and after 5 and 9 days in the main experiment. Grape extracts were obtained and analyzed by HPLC as previously described (Selma et al., 2008). Pure resveratrol was used as stilbene standard and quantified at 320 nm (Cantos et al., 2003b). *Trans*-resveratrol content was expressed as mg/kg of fresh weight by considering the amount of these compounds in both supernatant and pellet. Identification and quantification of *trans*-resveratrol was carried out following the protocols described in Cantos et al. (2003b) and Selma et al. (2008).

2.7. Decay susceptibility

To determine the effect of postharvest treatments in grape susceptibility to decay, 4 kg of sanitized grapes were separated into two groups. In one group, each grape was five times punctured with a sterile needle (damaged grapes), while grapes of the other group were left undamaged. Grapes were artificially infected with *B. cinerea* (CECT 2100), a fungus that causes a quality defect in grapes commonly known as bunch rot. Conidia suspension was prepared by flooding three plates (6 days old) of PDA (Scharlau Chemie S.A.) with 9 mL of sterile nanopure water containing 0.05% Tween 80 (Fluka Biochemika, Steinheim,