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Decay incidence and quality of different citrus varieties after postharvest heat treatment at laboratory and industrial scale

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ABSTRACT

Mandarins (Fortune, Ortanique, Ellendale, Clemenules and Hernandina) and oranges (Navelate, Navelina, Lanelate, Salustiana and Valencia) were inoculated with the fungi *Penicillium digitatum* and *Penicillium italicum* and then subjected to postharvest heat treatment at laboratory scale by dipping in water at various temperatures for different treatment times before storage. 53 °C (45 °C for varieties showing mottled fruits after heat treatment at 53 °C) and 3 min were found to be the most suitable conditions regarding decay incidence reduction, which were selected for the assays at industrial scale by using a heat water showers system in a commercial orchard. Heat treatment significantly reduced firmness and decay incidence in fruits after 5-day storage at 5 °C and 7 days of shelf life at 20 °C, and induced a delay in the evolution of skin colour. Other main quality parameters, such as juice content, soluble solids, pH, titratable acidity and sensory quality were unaffected. It was demonstrated that hot water postharvest treatments may be implemented in the citrus industry to extend shelf-life of fruits.

1. Introduction

Citrus are one of the most widely produced fruits. They are grown for commercial purposes (mainly as fresh fruit and juice) in more than 137 countries around the world (Ismail and Zhang, 2004). Furthermore, citrus industry, including harvesting, handling, transport, marketing and delivery, is responsible for millions of jobs around the world and provides enormous benefits. Citrus fruits are beneficial for human consumption because of their nutritional and antioxidant properties. However, their higher water content and nutrient composition make them susceptible to infection by microbial pathogens, mainly fungi, from when they are harvested to their consumption (Talibi et al., 2014). This causes considerable losses to citrus producers. *Penicillium digitatum* (green mold) and *Penicillium italicum* (blue mold) are the major pathogens of citrus fruits. Both pathogens can infect citrus in the grove, the packinghouse and during distribution and marketing, the source of fungal inoculum being practically continuous during the season (Palou et al., 2008). Green mold can be reduced by storage at low temperatures. In general, mandarins are stored at 5–8 °C while oranges at 4–8 °C, with relative humidity of 90–95%, during cold storage (Kader, 2002). Decay incidence of citrus also increases with increasing storage period, which could be as high as 50% (Abdel-El-Aziz and Mansoor, 2006).

Fruit decay is nowadays managed principally by the application of chemical fungicides, such as benzimidazole (thiabendazole, benomyl and carbendazim), sterol inhibitors (imazalil, prochloraz and propiconazole), sodium orthophenyl phenate and different mixtures of these compounds (Eckert and Brown, 1986; Eckert, 1990; Palou et al., 2008; Talibi et al., 2014), with the subsequent health hazards and environmental pollution. Besides, continuous use of fungicides may result in increasing fungicide resistance, which further complicates the management of fruit decay. What is more, the increasing spread of high-value markets based on sustainable, environmentally friendly, ecological or green agriculture makes even more necessary the use of alternatives to fungicides for citrus decay control (Palou et al., 2008).

In order to prevent citrus decay and extend their shelf-life, some alternative postharvest treatments have been proposed, including physical treatments (curing, hot water and irradiation treatments), chemical treatments (sodium bicarbonate, calcium polysulfide and ammonium molybdate treatments, borax baths, and addition of natural compounds such as volatiles and essential oils, plant extracts, peptides, proteins, chitosan and chitosan derivatives), biological treatments (utilisation of microbial antagonists, application of naturally derived bioactive compounds and induction of natural resistance) and combinations of the above-mentioned treatments for integrated disease management (Palou et al., 2008;

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Talibi et al., 2014). Among them, heat treatments are regarded as the cheapest and most feasible technologies. Compared to other heat treatments such as curing, hot water treatments are easier, less expensive and more feasible for industrial scale (Palou et al., 2008). Relatively brief immersions (2–5 min) in hot water (45–55 °C) have previously shown a potential in citrus green and blue moulds reduction (Schirra et al., 2004; Erkan et al., 2005; Palou et al., 2008).

Changes in epicuticular wax ultrastructure have been observed on different citrus fruits subjected to postharvest heat treatments. Cuticular cracks disappeared as a consequence of the wax "melting" provoked by the hot water dip (Schirra et al., 2011). This facilitated the closure of this easy path for fruit infection and, consequently, reduced decay incidence. In addition, this improvement of the epithelial coating reduces fruit water loss by evaporation, which contributed to maintain quality traits and health-related properties (Palma et al., 2013; Kaewsuksaeng et al., 2015).

Despite the existence of numerous studies on alternatives to the use of fungicides in postharvest citrus, the control of postharvest losses due to infection by microorganisms in the citrus industry is still based on the application of fungicides. This may be due, first at all, to that such investigations have not achieved a sufficient decay control to compensate their application and replace synthetic fungicides.

The aim of this research was to develop an industrial-scale hot water system for postharvest decay control without affecting the quality of fruits. To this end, 9 citrus varieties (5 mandarins and 4 oranges) were treated in a hot water showers system in a citrus industry, and decay incidence and quality parameters were monitored during storage and shelf life. Prior to this, the same varieties were treated in a hot water bath at laboratory to determine the most suitable treatment times and temperatures for each variety. The final objective was to effectively reduce citrus decay incidence at industrial facilities, without affecting fruit quality, and assess the shelf life of citrus.

2. Materials and methods

2.1. Citrus

Citrus used throughout this research were grown in the commercial orchard "The Zumajo" located in Rio Tinto (Huelva, Spain) by the company Rio Tinto Fruit S.A. Citrus varieties used for the tests were mandarins (Fortune, Ortanique, Ellendale, Clemenules, and Hernandina) and oranges (Navelate, Lanelate, Salustiana and Valencia).

2.2. Pathogen microorganisms

The fungi *P. digitatum* and *P. italicum* were obtained from the Spanish Type Culture Collection and maintained on potato dextrose agar plates.

Conidia of a 7–12 days culture grown at 25 °C were suspended in 100 mL sterile distilled water with two drops of Tween 80. The suspension was adjusted to 10^6 conidia/mL, using a haemacitometer. Fruits were wounded and inoculated on their flavedo using a 1.4 mm diameter steel rod, previously immersed in the conidia suspension. This concentration of pathogen was chosen because it has been verified that this amount is enough to provoke complete decay of citrus (Eckert and Brown, 1986) and it has been already successfully tested in a previous work (Nunes et al., 2007).

2.3. Heat treatments at laboratory scale

After harvesting, citrus were let stand for one night at room temperature. Afterwards, 4 groups of 20 fruits each per citrus variety and pathogen were inoculated (i.e. 4 replicates per experiment). Two hours after the inoculation they were submerged for 30, 60, 90, 180 and 300 s at 40, 43, 45, 50, 48, and 53 °C in thermostatically controlled water baths for heat treatments. At the same time, control was formed by another 4 groups with the same number of inoculated fruits per citrus variety and pathogen, which were not subjected to heat treatments. Finally, they were stored at 5 °C for 5 days and then at 20 °C for 7 days.

During storage both losses by decay or physiological damage, and quality parameters of the fruits were monitored. These determinations were performed just after the heat treatment, after cold storage and after the period of shelf life.

For non-destructive qualitative determinations (colour, firmness and weight loss) 20 fruits randomly taken from each treatment were selected at the beginning of the experiment, and the same fruits were measured in each sampling. For the destructive parameters (juice content, soluble solids, acidity and pH) 4 groups of 5 fruits per treatment and sampling were used, and the juice obtained from each group was considered as a replicate.

2.4. Heat treatments at industrial scale

Industrial-scale heat treatments were carried out at the facilities of the company Rio Tinto Fruit S.A. (Rio Tinto, Huelva, Spain). A batch shower system was used which was fed by a 5000-l thermostatic bath water by means of a pump with a flow capacity of 200 L/min. Pathogen inoculation was not performed in the industrial scale tests, and 2 water temperatures were assayed: 45 and 53 °C. The procedure for each citrus variety was as follows: 16 packages of 20 kg fruit (not previously treated with fungicides) each one was simultaneously treated in the shower system with hot water for 180 s. Then they were stored for 5 days at 6 °C and then an exhibition for sale at 20 °C for a period of seven days was simulated. Losses due to decay and qualitative parameters of the fruits were monitored during both storage and shelf life periods. The determinations were performed at the beginning, at the exit of the cold room and after 7 days at 20 °C. In each sampling 4 packages were extracted to assess the incidence of decay and the percentage of physiological damage, each package being a replicate. Complete decay of fruit was considered when there was visible growth of fungal mycelium. 5 fruits were randomly taken from each 20-kg package (i.e. 20 fruits per sampling since 4 replicates were performed) for destructive determinations. Another 5 random fruits per package were marked before heat treatment to monitor the non-destructive qualitative parameters during the whole process.

2.5. Quality parameters

Decay incidence was quantified by calculating the percentage of rotten citrus (fruits with visible mycelial growth) independently in each treatment.

Colour index (CI), strongly related to degreening and ripening stage of citrus (Jiménez-Cuesta et al., 1981), was calculated from the CIELab parameters obtained by using a Minolta CR-200 handheld chroma meter (Konica Minolta Inc.) as follows:

$$CI = 1000 \times a^*/L^* \times b^*$$

Fruit firmness (N/cm^2) was measured by a Zwick 3300 hand densimeter (Zwick GMBH & Company, Ulm, Germany) and expressed in percentage of softening referred to fruit firmness before heat treatment. Similarly, weight losses were approximated as percent of original weight.