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Continuous microwave treatment to control postharvest brown rot in stone fruit

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ABSTRACT

Monilinia spp. are the most important causes of brown rot in stone fruit and no chemical fungicides are allowed in the European Union to be applied to stone fruit after harvest. From preliminary studies, microwave (MW) treatments at 17.5 kW for 50 s and 10 kW for 95 s were selected as effective conditions to control brown rot. Both treatments were investigated to control *Monilinia fructicola* in fruit with different weights and maturity levels and in naturally infected fruit. Fruit weight only had a significant effect on microwave efficacy in 'Placido' peaches treated by MW at 10 kW for 95 s in which better brown rot control was observed in small than large fruit. Maturity level did not have a significant effect on efficacy of MW treatments in any of the varieties evaluated. When both MW treatments were studied in naturally infected peaches and nectarines, brown rot incidence was significantly reduced to less than 14% compared with untreated fruit where brown rot incidence was higher than 45%. The effect of both treatments on fruit quality was also evaluated. Fruit firmness was not negatively affected in the varieties tested and even a delay of fruit softening was observed. However, internal damage around the stone was observed, especially in the smallest fruit in which high temperature is achieved at the end of both MW treatments.

1. Introduction

Brown rot is one of the most important postharvest diseases of stone fruit worldwide and is primarily caused by two species, *Monilinia laxa* (Aderh. et Rulh.) Honey and *Monilinia fructicola* (G. Wint.) Honey. Stone fruit infection by *Monilinia* spp. mainly occurs in the field at bloom, the onset of pit hardening and between 7 and 12 days before harvest (Emery et al., 2000). However, postharvest losses that routinely occur during storage and transport (Hong et al., 1997) are typically more severe than preharvest losses, reaching high values when conditions are favorable for disease development (Larena et al., 2005). No chemical fungicides are registered in the European Union for postharvest treatment of stone fruit, therefore the current methods to control brown rot include preharvest spraying of fungicides at regular intervals, careful handling of harvested fruit to avoid wounding, good preharvest and postharvest sanitation practices, and rapid cooling and storage after harvest. However, all these strategies are not sufficient to control brown rot in stone fruit which has increased the need to develop alternative methods.

Radio frequency (RF) and microwave (MW) heating, also referred to as dielectric heating, could be a potential alternative treatment to control postharvest disease. In the dielectric heating, electromagnetic energy is directly coupled to a dielectric material, such as most agricultural commodities, to generate heat within the material as a result of converting electromagnetic energy into thermal energy. This can significantly increase the heating rates and reduce heating time in comparison with conventional heating (Tang et al., 2000). Dielectric properties and specially the dielectric loss factor (ε'') of a material, influences the conversion of electromagnetic energy into thermal energy, so the amount of heat converted in the food is proportional to the loss factor at a given frequency and electric field (Tang, 2005).

Casals et al. (2010d) have already demonstrated the potential of the use of radio frequency to control brown rot in peaches. The improvement of this treatment by applying the radio frequency with fruit immersed in water was investigated by Sisquella et al. (2013) who reported that RF heating for 4.5 min in fruit immersed in water at 40 °C controlled *M. fructicola* in peach and nectarine. However, shorter exposure times are preferred from the viewpoint of commercial applications. With microwaves, because frequency is much greater than for radio frequency, rapid heating can be achieved with much lower field intensities and the problems of arcing in the product are diminished (Nelson, 1996).

Microwaves have been applied to a wide range of products, however, the most predominant effort of current microwave technology has been to control pests of grain and stored products.

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On the contrary, little information is available about the use of microwaves to control postharvest diseases. Karabulut and Baykal (2002) reported an effective microwave treatment to control *Botrytis cinerea* and *Penicillium expansum* in peaches. Similar results were observed by Zhang et al. (2004) for controlling *Rhizopus stolonifer* in peaches. And Zhang et al. (2006) combined a microwave treatment with a biocontrol agent to control *P. expansum* on pears. Although microwave heating has been investigated to control different diseases on stone fruit, no information is available about the use of microwave heating to control *Monilinia* spp. Furthermore, these previous studies were conducted with a household microwave oven, so information about the use of an industrial microwave tunnel to control postharvest disease would make possible to design continuous equipment to be incorporated in the packinghouse handling procedures and process large quantities of fruit.

The main objective of this study was to determine the microwave conditions in a continuous industrial microwave tunnel that could reduce brown rot without causing damage to peaches and nectarines. We also evaluated the efficacy of the selected microwave conditions (10 kW for 95 s and 17.5 kW for 50 s) in (1) fruit with different weights, (2) fruit with different maturity levels, and (3) fruit with natural infections. Furthermore, the effect of MW treatments selected on fruit quality was also evaluated.

2. Materials and methods

2.1. Fruit

Experiments were conducted with 'Roig d'Albesa' and 'Placido' peaches (*Prunus persica* (L) Batch) and 'Big Top', 'September Red' and 'PP-100′ nectarines (*P. persica* var. Nectarine (Ait.) Maxim.). Fruit were grown in commercial orchards located in Lleida (Catalonia) following standard cultural practices and chemical spray programs in the field for pest and disease control. Fruit free of visible wounds and rots and similar visual maturity were selected by hand from fruit bins immediately after harvest. Fruit not used at the time of harvest were stored at 0 °C for a maximum of 15 days until use.

Fruit weights in all the experiments except in the study of the effect of fruit size were 170 ± 10 g in 'Big Top' nectarines, 210 ± 10 g in 'September Red' nectarines, 215 ± 10 g in 'Placido' peaches and 'PP-100' nectarines and 245 ± 10 g in 'Roig d'Albesa' peaches.

2.2. Pathogen culture

The isolate of *M. fructicola* (CPMC1) used was from the collection of the Postharvest Pathology Unit, Centre IRTA, Lleida, Catalonia. This strain was isolated from an infected stone fruit and was identified by the Department of Plant Protection, INIA, Madrid (Spain). Then, the isolate was maintained on potato dextrose agar (PDA) medium (Biokar Diagnostics, 39 g L^{-1}) amended with acetone (J.T. Baker, 1%) at 4 °C in the dark.

2.3. Pathogen production and inoculation methodology

The isolate of *M. fructicola* (CPMC1) was subcultured onto PDA amended with acetone (J.T. Baker, 1%) and incubated in the dark at 25 °C for approximately two weeks. The isolate was then inoculated onto peaches or nectarines by wounding the fruit with a sterilized steel rod (1 mm wide and 2 mm long) and transferring conidia and mycelium from the PDA culture to the wound site with a sterile pipette tip. Fruit were then incubated at 25 °C and 85% RH in the dark for 5–7 days. Conidia were scraped from infected fruit using a sterile loop and transferred to a test tube containing 5 mL of sterile distilled water and a drop of Tween-80 per liter. Conidia concentration was measured with a haemocytometer and the suspension diluted to 10^3 conidia mL^{-1}. Then the fruit were wounded once per fruit with the sterile steel rod and inoculated with 15 μL of conidial suspension. All microwave treatments were performed with artificially inoculated fruit incubated at 20 °C and 85% RH for 24 h before the treatment.

2.4. Microwave heating system and suitable treatment conditions

An industrial microwave tunnel (Synarwave, France) at 2450 MHz was used to perform the experiments. The microwave tunnel is provided with 12 magnetrons which theoretical power of each one range from 200 to 2000 W, so the generator can provide microwave power from 0.2 to 24 kW. The speed of the continuous conveyer belt ranged from 40 to 300 cm min^{-1}. For all the experiments, the desired power was achieved using 10 magnetrons with the same theoretical power each one, so maximum microwave power used in this study was 20 kW.

In all the experiments, the increase in internal temperature during MW treatment was measured with inside-optical fiber temperature probes (FOT-L/10 m; FISO Technologies Inc., Canada) with an accuracy of ±0.5 °C. Temperature sensor was placed 10 mm inside the fruit. Immediately after MW treatment the external temperature of eight fruit was recorded by a portable infrared thermometer (Testo 831, Testo AG, USA) with an accuracy of ±1.5 °C.

In addition, in all the experiments, external and internal fruit appearance was observed. External thermal damages, specifically changes in the color of the surface, were evaluated at the end of MW heating. Internal thermal damages, mainly flesh browning and development of internal cavities, were determined cutting each fruit in half after 5 days of incubation at 20 °C and 85% RH once brown rot incidence was recorded.

2.5. Treatment conditions affecting microwave efficacy

'Big Top' nectarines artificially inoculated as described above were used to perform the experiment to investigate the belt speed related to the exposure time at different powers that could reduce brown rot without affecting visual fruit quality. In a first experiment, microwave powers evaluated were 5, 10, 15 and 17.5 kW and treatment time ranged between 34 and 120 s depending on the power. In a second experiment, 10, 15, 17.5 and 20 kW of microwave powers were evaluated and exposure time ranged between 40 and 100 s. A set of artificially inoculated fruit of each experiment was not treated and was used as a control. All treatments were conducted with four replicates and eight fruit per replicate. After 5 days of incubation at 20 °C and 85% RH, brown rot incidence was recorded.

2.6. Effect of fruit size on microwave efficacy

The effect of fruit size on the efficacy of microwave treatments was evaluated in fruit with different weights artificially inoculated as described above. 'Roig d'Albesa' peaches with 180 ± 10, 245 ±10 and 310 ± 10 g of weight were treated by MW at 17.5 kW for 50 s. 'Placido' peaches and 'PP-100' nectarines, both with 170 ± 10, 215 ± 10 and 260 ± 10 g of weight, were treated by MW at 10 kW for 95 s. A set of artificially inoculated fruit of each weight was not treated and was used as a control. All treatments were conducted with four replicates and eight fruit per replicate. After 5 days of incubation at 20 °C and 85% RH, brown rot incidence was recorded.

2.7. Effect of fruit maturity on microwave efficacy

In order to achieve different maturity levels, after harvest and before treatment, fruit were maintained for 24, 48 or 72 h at 20 °C and 85% RH. After this time, fruit were artificially inoculated as