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Improvement of microwave treatment with immersion of fruit in water to control brown rot in stone fruit

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ABSTRACT

Monilinia spp. are the most important cause of brown rot in stone fruit. Currently, no chemical fungicides are allowed in the European Union to be applied in stone fruit after harvest. Microwave (MW) treatments at 20 kW with fruit immersed in water at 40 °C for 50 or 60 s were selected as effective conditions to control brown rot without affecting the appearance of the fruit. The efficacy of the treatments was analyzed on fruit with different weights and at various infection times and inoculum concentrations. When the MW treatment was applied for 50 s, brown rot control was significantly higher for smaller fruit in comparison with larger fruit and MW efficacy decreased with increasing time between inoculation and treatment from 0 or 24 h to 48 h and inoculum concentration from 10^3 to 10^5 conidia mL^{-1}. When the treatment time was increased to 60 s, a better control of brown rot was observed and, in general, none of the studied factors had a significant effect on the efficacy of the treatment. MW treatments were also evaluated on naturally infected fruit; brown rot incidence was significantly reduced to less than 43% when MW treatment was applied for 50 s and to less than 7% when applied for 60 s.
Industrial relevance: This study demonstrated the efficacy of MW treatment to control brown rot in postharvest of stone fruit. A relationship between brown rot reduction and the applied MW energy was also provided, which could be useful for designing specific equipment to process large quantities of fruit in less time without affecting the efficacy of the treatment or the quality of the fruit.

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 during the growing season when conditions favor disease development. However, postharvest losses are typically more severe than preharvest losses, sometimes reaching high levels (Hong, Holtz, Morgan, & Michailides, 1997). Currently, no chemical fungicides are allowed in the European Union to be applied in stone fruit after harvest. In addition, public demands to reduce pesticide use and improve environmental and human health, as well as the risk of pathogens developing resistance to synthetic fungicides, limit the preharvest application of chemical products in the field. The previous aspects, combined with a lack of effective postharvest treatments against *Monilinia* spp. have increased the need to develop new control methods.

Dielectric heating, including radio frequency (RF) and microwave (MW) heating, can be a potential alternative treatment to control postharvest diseases. Dielectric materials, as most agricultural products, convert electric energy at RF and MW frequencies into heat (Wang et al., 2003). Dielectric properties and particularly the dielectric loss factor (ε'') of a material, influence absorption and attenuation, and describes the ability to dissipate energy in response to an applied electric field, which commonly results in heat generation (Ikediala, Hansen, Tang, Drake, & Wang, 2002). Thus, the magnitude of heat generation is proportional to the value of the loss factor at a given frequency and electric field (Tang, Ikediala, Wang, Hansen, & Cavalieri, 2000).

The use of MW energy has been widely studied in food processes such as pasteurization and sterilization (Chandrasekaran, Ramanathan, & Basak, 2013; Picouet, Landl, Abadias, Castellari, & Viñas, 2009), and for pest control in grain and stored products (Vadivambal, Jayas, & White, 2007). On the other hand, few studies have been published on the use of microwaves to control diseases. The effect of MW treatments to control *Botrytis cinerea*, *Penicillium expansum*, and *Rhizopus stolonifer* in peaches (Karabulut & Baykal, 2002; Zhang et al., 2004) and *P. expansum* in pears (Zhang, Zheng, & Su, 2006) has been assessed in these studies. Moreover, Sisquella, Viñas, Teixidó, Picouet, and Usall (2013) reported a MW treatment to control *Monilinia* spp. in peaches and nectarines; however, the influence of fruit size in the final temperature affected MW effectiveness and internal fruit appearance. Therefore, further experiments should be

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performed to solve the uneven heating and consequently, the negative effect on quality.

Heating uniformity is the most significant problem associated with dielectric heating. Non-uniform temperature distribution and development of hot and cold spots not only can affect the effectiveness of the treatment but also negatively affect fruit quality. Different dielectric properties between food and the surrounding air cause reflection and refraction phenomena of the microwaves at the interface, resulting in non-uniform electric field distribution (Guan, Plotka, Clark, & Tang, 2002). Immersion of fresh fruit in water was suggested by Ikediala et al. (2002) as a means to overcome the problems associated with non-uniform radio frequency heating. Recently, Sisquella, Casals, et al. (2013) reported that RF effectiveness was influenced by fruit size when RF treatment in air was applied at the conditions reported by Casals, Viñas, et al. (2010). However, lower influence of fruit size on the effectiveness of RF treatment to control brown rot in stone fruit was observed when applied to fruit immersed in water at 20 °C for 9 min, mainly due to the similar temperatures reached between the different fruit sizes.

The main objective of this study was to determine the MW power and exposure time with fruit immersed in water at different temperatures needed to reduce brown rot without causing external and/or internal damage to the fruit. The efficacy of the selected MW conditions was also evaluated in: (1) fruit with different weights, (2) fruit artificially inoculated with different inoculum concentrations, (3) fruit inoculated at different times before treatment, and (4) naturally infected fruit. Finally, the effect of the selected MW treatments on fruit quality was also evaluated.

2. Materials and methods

2.1. Fruit

'Baby Gold 9', 'Sunlate', 'Pollero', 'Roig d'Albesa', and 'Placido' peaches (*Prunus persica* (L) Batch), and 'Red Jim' and 'Autumn Free' nectarines (*P. persica* var. Nectarine (Ait.) Maxim.) were used for the experiments. Fruit were grown in orchards located in Lleida (Catalonia) and no synthetic fungicide against *Monilinia* spp. was used in the field. Fruit free from visible wounds and rots and similar visual maturity were hand-selected immediately after harvest. The fruit not used at the time of harvest were stored at 0 °C for a maximum of 15 days until use.

Fruit weight in all the experiments, except in the analysis of fruit size effect, was 185 ± 10 g for 'Pollero' peaches, 190 ± 10 g for 'Autumn Free' nectarines, 200 ± 10 g for 'Placido' peaches, and 215 ± 10 g for 'Red Jim' nectarines and 'Sunlate' and 'Roig d'Albesa' peaches. In the case of 'Baby Gold 9' peaches, fruit weight was 215 ± 10 g, except in the naturally infected fruit for which the weight was 190 ± 10 g.

2.2. Pathogen culture

The isolate of *M. fructicola* (CPMC1) used in this study was from the collection of the Postharvest Pathology Unit, Fruitcentre IRTA, Lleida, Catalonia. This strain was isolated from an infected stone fruit and was identified by the Department of Plant Protection, INIA, Madrid (Spain). The strain 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. Next, the fruit were incubated at 25 °C and 85% relative humidity (RH) in the dark for 5–7 days. Conidia were scraped from the 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 hemocytometer and the suspension diluted to the desired concentration. For all experiments, fruit were wounded once per fruit with the sterile steel rod and inoculated with 15 μL of the desired conidial suspension.

2.4. Microwave heating system and suitable treatment conditions

An industrial MW tunnel (Synarwave — M.E.S., France) with a frequency of 2450 MHz was used to perform the experiments. The MW tunnel was 6.5-m long with chamber dimensions of 180 cm in length, 70 cm in width, and 45 cm in height. MW energy was delivered by 12 magnetrons well distributed in a compartment on the top of the cavity. Each magnetron had a theoretical output power range of 0.2 to 2.0 kW, so the total MW output power was between 0.2 and 24.0 kW. The continuous conveyer belt was located at 275 mm from the magnetrons in the cavity and belt speed ranged between 40 and 300 cm min^{-1}.

For all experiments, the desired output power level was achieved using 10 magnetrons, each with the same theoretical output power, so the maximum MW power level used in this study was 20 kW. Fruit at room temperature were placed in a container (285 mm × 235 mm × 90 mm) with 2 L of tap water, so that the fruit were completely submerged in the water. The containers were placed on the conveyor belt and the corresponding MW treatment was applied.

In all experiments, the increase of the internal temperature during MW treatment was measured every second with an inside-optical fiber temperature probe (FOT-L/10 m; FISO Technologies Inc., Canada) placed 10 mm inside the fruit. The temperature measuring range was −40 °C to +250 °C with an accuracy of ±0.5 °C. The optical fiber probe was connected to a signal conditioner and data were collected by a FISOCommander software (FISO Technologies Inc., Canada). Moreover, immediately after the MW treatment, the external temperature of eight fruit per treatment was also recorded using a portable infrared thermometer (Testo 831, Testo AG, USA) with a 2-point laser marker and a temperature measuring range of −30 to +210 °C with an accuracy of ±1.5 °C.

For all treatments, external and internal fruit appearance was analyzed. External thermal damages, particularly surface color changes, were evaluated at the end of every MW treatment. Internal thermal damages, such as internal browning, 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. Effect of water temperature on microwave treatment efficacy

'Baby Gold 9' peaches and 'Red Jim' nectarines were artificially inoculated with 10^3 conidia mL^{-1} as described above and incubated at 20 °C and 85% RH for 24 h. Fruit were then immersed in water at 20, 35, 40, or 45 °C and MW treatment was immediately applied at 10 kW for 95 s. A group of artificially inoculated fruit of each variety was immersed in water at 45 °C for 95 s and no MW treatment was applied (controls). After the treatment, fruit were stored for 5 days at 20 °C and 85% RH and then, brown rot incidence was recorded. Each treatment was applied to four replicates of eight fruit each.

2.6. Effect of power level and exposure time on microwave treatment efficacy

2.6.1. Efficacy study

To improve the effectiveness of the microwave treatment with fruit immersed in water at 40 °C, several MW power levels were evaluated in 'Baby Gold 9' peaches and 'Red Jim' nectarines. Fruit were artificially inoculated with 10^3 conidia mL^{-1} as described above and incubated at 20 °C and 85% RH for 24 h. Next, fruit were immersed in water at