Innovative Food Science and Emerging Technologies 12 (2011) 310–314

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

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

Combination of salicylic acid and ultrasound to control postharvest blue mold caused by *Penicillium expansum* in peach fruit

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ARTICLE INFO

Article history:
Received 6 January 2011
Accepted 29 April 2011

Editor Proof Received Date 30 May 2011

Keywords:
Peach
Salicylic acid
Ultrasound
Blue mold
Disease resistance

ABSTRACT

The effect of ultrasound (40 kHz, 10 min) and salicylic acid (SA, 0.05 mM) either separately, or combined on blue mold caused by *Penicillium expansum* in peach fruit was investigated. The results showed that the application of SA alone could reduce blue mold, while the use of ultrasound had no effect. Our results also revealed that SA combined with ultrasound treatment was more effective in inhibiting fungal decay during storage than the SA treatment alone. The combined treatment increased the activities of defense enzymes such as chitinase, β-1,3-glucanase, phenylalanine ammonia-lyase, polyphenol oxidase and peroxidase, which were associated with higher disease resistance induced by the combined treatment. Furthermore, the combined treatment did not impair the quality parameters of peach fruit after 6 days of storage at 20 °C. These results suggested that the combination of ultrasound and SA treatment may be a useful technique to reduce blue mold in peach fruit.

Industrial relevance: This paper investigates the effect of ultrasound combined with SA on decay incidence of peach fruit. The results presented demonstrate that the effect of the combined treatment on the disease resistance and fruit quality should be considered by processors prior to its adoption as a preservation technique.

1. Introduction

Peaches are highly susceptible to pathogenic infection and physiological deterioration during storage and ripening at ambient temperature. Blue mold caused by *Penicillium expansum* Link, is one of the most economically significant postharvest pathogen of peach fruit (Zhang, Zheng, & Yu, 2007). The use of synthetic chemical fungicides has been the main method for reducing postharvest disease (Karabulut et al., 2002). However, consumer concern over pesticide residues on foods, along with pathogen resistance to many currently used pesticides, has increased the need to develop new strategies to induce resistance as new technologies for controlling postharvest diseases in peaches (Ragsdale & Sisler, 1994). Recently, biologically active natural products have started to be an effective alternative to synthetic fungicides (Terry & Joyce, 2004).

Salicylic acid (SA) is an endogenous hormone having key roles in different aspects of plant growth and development (Asgharia & Aghdam, 2010; Tian, Qin, Li, Wang, & Meng, 2007). It is also a signaling molecule which induces biosynthesis of defense compounds such as polyphenols or pathogenesis-related proteins (Raskin, 1992; Yao & Tian, 2005). Many researchers have reported SA as a potential tool in inhibiting decay incidence and extending postharvest life of many fresh fruits. The decays caused by *Fusarium oxysporum* in tomato (Mandal, Mallick, & Mitra, 2009), anthracnose disease caused by *Colletotrichum gloeosporioides* in mango (Zainuri, Joyce, Wearing, Coates, & Terry, 2001; Zeng, Cao, & Jiang, 2006), *P. expansum* in sweet cherry fruit (Qin, Tian, Xu, & Wan, 2003; Xu & Tian, 2008; Yao & Tian, 2005) and gray mold decay in peach (Zhang et al., 2008) were effectively controlled by SA treatment.

More recently, application of ultrasonic technology in food processing and preservation attracted widely attentions. Several reviews have summarized the direct food processing improvements of ultrasound such as cleaning surfaces, enhancement of dewatering, drying and filtration, inactivation of microorganisms and enzymes, extraction of enzymes, proteins and antioxidant compounds, disruption of cells, degassing of liquid food and acceleration of heat transfer (Knorr, Zenker, Heinz, & Lee, 2004; Mizrach, 2008; Vilkhu, Mawson, Simons, & Bates, 2008; Zheng & Sun, 2006). However, development of the ultrasound technique as a means of controlling decay incidence and maintaining fruit quality after harvest has not progressed as fast in the fresh fruit industry as in the food industry (Knorr et al., 2004; Mizrach, 2008; Vilkhu et al., 2008; Zheng & Sun, 2006). Recently, we found that postharvest ultrasound treatment was effective in reducing fruit decay and maintaining quality in strawberry fruit (Cao

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doi:10.1016/j.ifset.2011.04.010

et al., 2010; Cao, Hu, & Pang, 2010). However, to our knowledge, there are no reports on the effect of a combination of SA and ultrasound on the control of fungal diseases.

The objectives of this study were to evaluate (a) the effects of SA and ultrasound used separately or in combination, on controlling postharvest blue mold rot caused by *P. expansum* of peach fruit, (b) the effects of SA and ultrasound, used separately or in combination, on the key enzymes involved in host defense, and (c) the efficacy of SA and ultrasound, separately or in combination, on quality of peach after storage.

2. Materials and methods

2.1. Microorganism, fruit and treatments

The pathogen *P. expansum* was originally isolated from an infected peach fruit and cultured on potato dextrose agar (PDA, containing the extract from 200 g boiled potato, 20 g glucose and 20 g agar in 1 l of distilled water) at 25 °C. Spore suspension was prepared by flooding 14-day-old cultures of *P. expansum* with sterile distilled water. The amount of spores was counted with a hemacytometer and diluted with sterile distilled water as required.

Peach fruit (*Prunus persica Batsch* cv Baifeng) were hand-harvested at firm-mature stage from a commercial orchard in Nanjing, China, and selected for uniform size, color and absence of defects, and then randomly divided into four groups. All the fruit were disinfected with 2% (v/v) sodium hypochlorite for 2 min, washed with tap water, and air dried prior to wounding.

Ultrasound treatment was applied in a water bath (20 °C) with dimension of 500 × 300 × 150 mm in the ultrasonic chamber (SB-500DTY, Ningbo Xinzhi Science and Technology Co.) and treated with 40 kHz frequency operating at power of 350 W for 10 min (8.8 W/L). 0.05 mM SA was chosen as the optimal concentration for our experiment based on previous study (Chan, Qin, Xu, Li, & Tian, 2007). Treatments were applied as follows:

(1) Control: Fruit were immersed into sterile-distilled water at 20 °C for 10 min as control.
(2) SA treatment: Fruit were immersed into solution at 0.05 mM SA at 20 °C for 10 min.
(3) Ultrasound treatment: Fruit were ultrasound treated in an ultrasonic chamber (40 kHz, 350 W) at 20 °C for 10 min.
(4) SA + ultrasound: Fruit were treated with 0.05 mM SA in the ultrasonic chamber at 20 °C for 10 min.

After treatment, all the fruit were wounded with a sterilized nail at 2 points (4 mm deep and 3 mm in diameter) in the equatorial zone and inoculated with 15 μL of *P. expansum* spore suspension (1×10^5 spores/mL). After air-drying, samples were stored in enclosed plastic trays to maintain about 95% of relative humidity and incubated at 20 °C for 6 days. Samples were collected from 5 fruits at 2 day intervals for measurements of fruit decay incidence, lesion diameter, fruit firmness, extractable juice, contents of total soluble solids (TSS), titratable acidity (TA), vitamin C and total phenolics, and activities of chitinase (EC 3.2.1.14), β-1, 3-glucanase (EC 3.2.1.58), phenylalanine ammonia-lyase (PAL, EC 4.3.1.5), polyphenol oxidase (PPO, EC 1.10.3.1) and peroxidase (POD, EC 1.11.1.7). There were three replicates consisting of 15 peaches per replicate and the experiment was conducted twice with similar results.

2.2. Decay evaluation

Fruit decay was visually evaluated during the experiment. When the visible rot zone outside the wounded area on fruit was more than 1 mm wide, it was considered as decay fruit. Decay incidence was defined as decayed fruit/total fruit × 100.

2.3. Determination of total phenolic content

One gram of flesh tissue was ground in 5 mL of 80% (v/v) cold acetone and then the mixture was centrifuged at 10,000 × *g* for 20 min at 4 °C. The supernatant was used for the total phenolic measurement. The content was determined according to the Folin–Ciocalteu procedure (Slinkard & Singleton, 1977). The results were expressed as grams of gallic acid equivalent (GAE) per 100 g of fresh weight (FW).

2.4. Enzyme assays

All enzyme extraction procedures were conducted at 4 °C. For chitinase and β-1,3-glucanase, 1 g of tissue sample was ground with 5 mL of 50 mM sodium acetate buffer (pH 5.0). PAL was extracted using 0.2 M sodium borate buffer (pH 8.7) containing 20 mM β-mercaptoethanol. For PPO, 1 g of fruit flesh was ground in 5 mL of 0.2 M sodium phosphate buffer (pH 6.5) containing 1% (w/v) polyvinylpyrrolidone, or with 50 mM sodium borate buffer (pH 8.7) for POD. The extracts were then homogenized and centrifuged at 10,000 × *g* for 20 min at 4 °C. The supernatants were used for the enzyme assays.

Chitinase and β-1,3-glucanase activities were measured according to the method of Abeles, Bosshart, Forrence, and Habig (1971). Chitinase activity was measured by mixing 2 mL of crude enzyme solution with 0.5 mL of 2% (w/v) dye-labeled carboxymethylchitin in 50 mM sodium acetate buffer (pH 5.0). After 2 h of incubation at 40 °C, the reaction was stopped by adding 100 μL of 1.0 M HCl, the reaction mixture was cooled and centrifuged, and the absorbance of the supernatant was measured at 550 nm with a spectrophotometer. One unit of chitinase activity was defined as the amount of enzyme required to catalyze the formation of 1 nmol product/h. β-1,3-Glucanase activity was determined by incubating 1 mL of enzyme preparation for 24 h at 40 °C with 1 mL of 4% (w/v) laminarin (Aldrich, Chemical Company, Wilwaukee, WI, USA). The reaction was terminated by heating the sample in boiling water for 5 min and the amount of reducing sugars was measured spectrophotometrically at 540 nm after reaction with 250 μL of 3,5-dinitrosalicylic reagent (Aldrich, Chemical Company, Wilwaukee, WI, USA). One unit was defined as the amount of enzyme catalyzing the formation of 1 μmol glucose equivalents/h.

PAL was assayed as described by Zucker (1968). The assay medium contained 0.1 mL enzyme extract and 1 mL of L-phenylalanine. After incubation at 40 °C for 1 h, the reaction was stopped by adding 0.2 mL of 6 M HCl. One unit of PAL activity was defined as the amount of enzyme causing an increase in of 0.01 in absorbance per hour at 290 nm.

PPO activity was assayed according to Murr and Morris (1974). One unit of PPO activity was defined as the amount of enzyme causing an increase of 0.01 in absorbance per minute at 410 nm.

POD activity was assayed using guaiacol as a donor and H_2O_2 as a substrate (Kochba, Lavee, & Spiege, 1977). One unit of POD activity was defined as an increase of 0.001 in absorbance per minute at 460 nm.

Protein content in the enzyme extracts was estimated using the Bradford (1976) method, using bovine serum albumin as a standard. Specific activity of the enzymes was expressed as units per milligram protein.

2.5. Determinations of quality parameters

The firmness of the five fruits from each replicate was measured using a FT327 firmness tester (Facchini FG, Alfonsine, Italy) fitted with a 5 mm diameter probe. Extractable juice content was estimated from the weight loss from placental tissue plugs in response to low-speed centrifugation. Four plugs (7 mm wide, 10 mm thick) were placed over sterile cotton rod in a 50 mL centrifuge tube and centrifuged for 10 min at 1700 × *g* at room temperature. The results were expressed as