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Effect of preharvest application of tea tree oil on strawberry fruit quality parameters and possible disease resistance mechanisms

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ABSTRACT

Strawberries were sprayed with 1.4 mL L^{-1} of tea tree oil (TTO) 6, 3, and 0 d before harvest to investigate the effects of preharvest TTO treatment on fruit quality parameters. The results showed that TTO treatment reduces the incidence of natural decay and delays the loss of firmness during storage at 20 °C for 5 d. TTO treatment has no effect on total soluble solids content or titratable acidity. Preharvest treatment with TTO reduces the number of microorganisms on the surface of fruit at harvest, reduces H_2O_2 accumulation by increasing the activities of catalase and ascorbate peroxidase, and increases β-1,3-glucanase activity by the first day postharvest. Treated fruits also accumulate more phenolics as a result of increased phenylalanine ammonia-lyase activity. A total of 188 differentially expressed proteins were identified by comparing untreated and TTO-treated fruit using isobaric tags for relative and absolute quantification (iTRAQ). Of these, 29 were more abundant and 159 were less abundant in TTO-treated fruit. Three proteins related to cell wall metabolism were down-regulated by TTO treatment, while 4 proteins involved in stress response were up-regulated. Therefore, TTO treatment prior to harvest effectively controls postharvest decay in strawberries by reducing microorganisms at harvest, delaying fruit senescence, and inducing defense responses.

1. Introduction

Strawberries (*Fragaria* × *ananassa*) are one of the most popular fruits in the world because of their delicious flavor, health benefits, and exceptional nutritional value (Mikulic-Petkovsek et al., 2013). The fruit is rich in vitamin C, phenolics, flavonoids, and anthocyanin, which have antioxidant effect. The suitable storage routine of strawberries is refrigerated storage, but there is no complete cold chain for strawberries in China. In China, strawberries are stored and transported at room temperature, which are highly perishable due to fungal attack. Although most postharvest diseases appear in the packinghouse, infection by pathogens often occurs in the field prior to harvest (Cai et al., 2015; Zheng et al., 2011). Strawberries are a non-climacteric fruit and do not ripen after harvest. They must therefore be harvested at almost full ripeness when they are extremely delicate and subject to rapid senescence. For these reasons, preharvest treatment is the preferred approach for controlling postharvest disease of strawberry (Feliziani et al., 2015; Wei et al., 2014). Preharvest spraying with chemical fungicides such as captan, thiram, iprodione and procymidone has been widely used to control postharvest decay (Blacharski et al., 2001), but increases in regulation, along with consumer concerns about residues, have stimulated a search for alternatives.

Preharvest application of biocontrol or natural agents has recently attracted interest as a potential strategy for disease control in fruits and vegetables. In postharvest disease control, biological control is an ecofriendly means using antagonists to prevent postharvest pathogen. Natural agents are obtained or extracted from various plants, animals and microbes, which may have antifungal activity and have preservation effect. Preharvest treatment with antagonist yeast (Cai et al., 2015; Lu et al., 2013; Wei et al., 2014), chitosan (Bhaskara Reddy et al., 2000; Feliziani et al., 2015; Yan et al., 2012), essential oils (Goñi et al., 2013, 2014; Washington et al., 1999), and other fungicide alternatives, such as laminarin and fir extract (Feliziani et al., 2015) has been reported to control postharvest decay. Among these agents, essential oils have received attention as inhibitors of a wide variety of phytopathogenic fungi, and are generally recognized as safe at the doses typically used in foods (Guerra et al., 2015; Tao et al., 2014).

Tea tree oil (TTO), the volatile essential oil with a pleasant odor derived mainly from the Australian native plant *Melaleuca alternifolia* (Washington et al., 1999), has been used as an alternative antimicrobial, antifungal and antioxidant agent (Li et al., 2016b; Shao et al., 2013a). It has been reported that 5% TTO did not cause allergic reactions (Hammer et al., 2006), 2.5% TTO did not display dermal toxicity, and 2% TTO did not produce liver toxicity (Lee et al., 2013).

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Therefore, drugs and care products containing TTO are frequently used to treat skin, oral, vaginal and airway infections (Hammer, 2015). TTO has been studied in different ways on horticulture products. When TTO was applied to lettuce plants before harvest, it effectively reduced microbial counts after storage without affecting sensory quality (Goñi et al., 2013, 2014). It has been demonstrated that preharvest foliar spraying of strawberries with TTO controlled leather rot and anthracnose in field, although failed to control grey mold or increase fruit yield (Washington et al., 1999). In our previous report, postharvest TTO vapor treatment has been proved to be an effective method for reducing gray mold and soft rot in strawberries and maintaining fresh quality of strawberries such as firmness, soluble solids content and titrated acid content during storage (Shao et al., 2013b). However, it is not known whether preharvest application of TTO could control postharvest decay and affect fruit quality of strawberries.

The objectives of this study were to (1) investigate the effects of preharvest treatment with TTO on natural decay and fruit quality of strawberry during storage; (2) explore the possible mechanisms including the effect of preharvest TTO treatment on microorganism on the strawberry surface, iTRAQ-based protein profiling of TTO-treated and untreated strawberry fruit, and the defense responses elicited by preharvest TTO treatment; (3) evaluate whether preharverst application of TTO could be used as an alternative of chemical fungicides to control postharvest decay of strawberry.

2. Materials and methods

2.1. TTO

TTO (Fuzhou Melalyn Biotechnology Co., Ltd., Fujian, China) mainly contained terpinen-4-ol (37.11%), cterpinene (20.65%), a-terpinene (10.05%), 1, 8-cineole (4.97%), terpinolene (3.55%), q-cymene (2.14%), and a-terpineol (3.82%), as specified by the supplier, and consistent with ISO 4730 standards and the European Pharmacopoeia. TTO solution (1.4 mL L^{-1}) was prepared by dispersing TTO in distilled water containing 1% (v/v) Tween-80 as a surfactant. TTO concentration and spraying intervals were based on our preliminary field experiments, which could reduce postharvest decay and have no negative effect on the growth and quality of strawberry. TTO solution was prepared immediately before application to prevent vaporization of volatile compounds.

2.2. Plant material and preharvest spray experiment

Strawberry (*Fragaria* × *ananassa*) cv. 'Hongyan' were grown in a greenhouse located in Ningbo (latitude: 29°96′N, longitude: 121°72′E), Zhejiang province, China. The experiments were performed from December 2015 to March 2016, and plants were grown in the greenhouse with natural light, and temperature 15–25 °C. No fungicides were applied during the whole plant growth period. At 6, 3, and 0 d before harvest, strawberry plants were sprayed with 1.4 mL L^{-1} of TTO solution or 1% (v/v) Tween-80 diluted in water (control) until the surfaces of the fruits and leaves were dripping wet. Each treatment consisted of 3 replicates, and each replicate consisted of a row of plants 50 m in length. In each spray, 4 L of TTO solution (1.4 mL L^{-1}) was used in each row with the length of 50 m. Approximately 200 fruits selected for uniform maturity and size, and without visible injuries, were picked from each row, and then stored in polyethylene plastic boxes at the simulated shelf-life temperature (20 ± 1 °C) in 90–95 % relative humidity for 5 d. Strawberries at harvest (day 0) were used to determine microbial counts on fruit surfaces and for proteomic analyses. Decay incidence, quality parameters including fruit firmness, total soluble solids (TSS), titratable acid (TA), and physiological and biochemical changes including hydrogen peroxide (H_2O_2) content, activities of antioxidant and defense-related enzymes, and total phenolics content were determined daily during storage. Each treatment consisted of 3 replicates, and 15 strawberry fruits per replicate from each treatment were assessed every day for decay incidence. Twenty-five strawberries per replicate from each treatment were analyzed daily for fruit firmness, TSS, TA, H_2O_2 content, activities of antioxidant and defense-related enzymes, and total phenolics content.

2.3. Microbiological evaluations of fresh strawberry fruit at harvest

To evaluate the effects of preharvest TTO treatment on fruit surface microflora, 10 strawberries were randomly selected from each replicate immediately after harvest. About 25 g of flesh were macerated in 225 mL sterile saline solution and were homogenized using a Stomacher paddle blender (Seward Ltd., West Sussex, UK). The homogenized samples were serially diluted and 0.1 mL from each dilution was spread onto solid media. The enumeration of microbial populations was performed using the following culture media and culture conditions : bacteria on nutrient agar medium at 37 °C for 24 h; molds and yeast on Rose Bengal agar medium at 25 °C for 3 d. All culture mediums were purchased from Land Bridge, Beijing, China. Colonies were counted and the results expressed as $\log_{10}$ CFU kg^{-1}.

2.4. Measurement of natural decay incidence, fruit firmness, TSS, and TA

The effects of applying TTO before harvest on postharvest decay and fruit quality were assessed as follows. Natural decay incidence was evaluated according to the method described by Wei et al. (2014), and the percentage of decayed fruit was recorded. Fruit firmness was measured using a GY-4 portable firmness tester (Zhiqu Precision Instrument Ltd., Dongguan, China) and recorded in Newtons (N). TSS content was determined by measuring the refractive index of strawberry fruit juice using a PAL-1 pocket refractometer (Atago, Tokyo, Japan), and the results were expressed as %. TA was determined by titrating 50 mL of fruit juice to pH 8.1 with 0.1 N NaOH and calculated as % citric acid.

2.5. Measurement of H_2O_2 content and determination of catalase and ascorbate peroxidase activities

H_2O_2 content was measured using the procedure described by Shao et al. (2013b). Briefly, 1 g of strawberry flesh was homogenized in 5 mL of chilled acetone. H_2O_2 content was determined using the assay kit available from Nanjing Jiancheng Institute of Bioengineering (Nanjing, China), following the manufacturer's instructions. Results were expressed as mol kg^{-1} on a fresh weight basis.

For catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.11) assays, fruit tissue (1 g) was homogenized in 5 mL of 50 mM sodium phosphate buffer (pH 7.0) and then centrifuged at 10,000 × *g* at 4 °C for 20 min. The supernatant was assayed for CAT and APX activities. CAT activity was determined by adding 0.2 mL of the enzyme preparation to 3 mL of sodium phosphate buffer containing 0.2 mL of H_2O_2 as a substrate, as described by Wang et al. (2015). One unit of CAT activity is defined as the amount of enzyme that decomposes 1 μmol of H_2O_2 per second at 30 °C. APX activity was determined by the method of Zhang et al. (2013) with slight modification. The reaction mixture included 2.7 mL of 50 mM sodium phosphate buffer, 0.1 mL of the crude enzyme extract, 0.1 mL of 5 mM ascorbic acid, and 0.1 mL of 0.75% (v/v) H_2O_2. One unit of APX is defined as the amount of enzyme that oxidizes 1 μmol ascorbate per second at 30 °C.

2.6. Measurement of defense-related enzyme activities and total phenolics content

Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity was analyzed using the method described by Zhao et al. (2009). Strawberry tissue (2 g) was extracted with 5 mL of 100 mM phosphate buffer solution (pH 8.8) containing 5 mM β-mercaptoethanol and 2% (w/v)