Postharvest Biology and Technology 108 (2015) 28–38

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Effect of methyl salicylate and methyl jasmonate pre-treatment on the volatile profile in tomato fruit subjected to chilling temperature☆

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ARTICLE INFO

Article history:
Received 22 February 2015
Received in revised form 5 May 2015
Accepted 6 May 2015
Available online 27 May 2015

Keywords:
Solanum lycopersicum
Aroma
Ripening
Chilling injury
Sensory

ABSTRACT

Tomato fruits exposed to chilling temperatures suffer aroma loss prior to visual chilling injury (CI) symptoms. Methyl salicylate (MeSA) and methyl jasmonate (MeJA) treatments were reported to alleviate the development of visual CI; however, it is unknown if the treatments alleviate internal CI in the form of aroma loss. In this research, 'FL 47' tomatoes at breaker stage were treated with MeSA or MeJA vapor prior exposure to chilling temperature. The chilling treatment did not result in visual CI; however, for internal CI it generally suppressed production of oxygen-containing heterocyclic compounds, ketones, sulfur- and nitrogen-containing heterocyclic compounds, alcohols, and aldehydes, including 13 important aroma contributors to tomato fruit. MeJA had no impact on sensory evaluation in spite of resulting in slightly altered volatile profile; however, MeSA alleviated the CI-induced reduction of a number of volatile compounds, and thereby enhanced tomato aroma.

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1. Introduction

Chilling injury (CI) in tomato (*Solanum lycopersicum* L.) is a complex syndrome that is detrimental to tomato quality (Sevillano et al., 2009). When mature green tomato is stored at 13 °C for longer than 2 weeks or at 5 °C for longer than 7 d, a battery of physiological and biochemical responses can be activated that damage the fruit during the subsequent ripening at ambient temperature (Suslow and Cantwell, 2006). The most common symptoms include a failure to ripen and to develop full color and flavor, irregular (blotchy) color development, premature softening, water-soaking, surface pitting, browning of seeds, poor appearance, and susceptibility to *Alternaria* rot and decay (Sevillano et al., 2009). Internal CI, such as flavor loss and abnormal ripening, occurs even before visual symptoms (Maul et al., 2000). Our previous research showed that a 4-day exposure of tomato fruit to 5 °C at the mature green stage would impact aroma quality in ripe fruit, although no visual CI symptoms occurred (Wang et al., 2015).

Flavor, a combination of taste and aroma sensations, is an important part of fresh tomato quality, and consumers are willing to pay a premium for full-flavored fruit (Petro-Turza, 1986). More than 400 volatile compounds have been identified in ripened tomato fruit (Petro-Turza, 1986). Of those, 16 have been reported to possess positive log odor units and are likely to contribute to tomato aroma, including *cis*-3-hexenal, β-ionone, β-damascenone, 1-penten-3-one, 2 + 3-methyl-1-butanal, *trans*-2-hexenal, 2-isobutylthiazole, 1-nitro-2-phenylethane, *trans*-2-heptenal, 2-phenylacetaldehyde, 6-methyl-5-hepten-2-one, *cis*-3-hexenol, 2-phenylethanol, 3-methyl-1-butanol, and methyl salicylate (Buttery, 1993). However, compounds with negative odor units may still contribute to the overall flavor of tomato as background notes (Baldwin et al., 2000). Therefore, models based on concentration and odor thresholds of individual volatiles cannot account for synergistic and antagonistic interactions that occur in complex foods such as tomato (Tieman et al., 2012). Over the last 50 years, emphasis on yield, appearance and storability resulted in cheaper, year-round produce availability at the expense of flavor quality (Maul et al., 2000).

Salicylic acid (SA), jasmonic acid (JA) and their methyl esters, MeSA and MeJA respectively, are endogenous signal molecules that play essential roles in regulating abiotic and biotic stress responses in plants (Reymond and Farmer, 1998). Pre-treatments with MeJA/MeSA are a postharvest handling tool used to reduce CI of tomato fruit (Ding et al., 2002; Fung et al., 2006; Zhang et al., 2011).

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http://dx.doi.org/10.1016/j.postharvbio.2015.05.005
0925-5214/Published by Elsevier B.V.

Tomatoes incubated at breaker stage in 0.01 mM of MeSA or MeJA for 16 h at 23 °C showed less severity of CI symptom (surface pitting) after 5 °C storage for more than 2 weeks, than fruits without pre-treatment (Ding et al., 2002; Ding et al., 2001). Mitigation of CI in tomato by MeJA/MeSA could be attributed to: (1) enhancement of heat shock protein (HSP) gene expression (Ding et al., 2001); (2) enhancement of antioxidant system activity, such as alternative oxidase (AOX) (Fung et al., 2006); (3) enhancement of pathogenesis-related (PR) protein gene expression (Ding et al., 2002); and (4) enhancement of the arginine pathway, such as polyamines, nitric oxide, and proline, which lead to the accumulation of signaling molecules with pivotal roles in improving chilling tolerance such as polyamines, nitric oxide, and proline (Zhang et al., 2011, 2012). However, little is known about the effect of MeSA or MeJA on aroma loss caused by CI.

The objectives of this study were to investigate (1) the impact of a 9-day exposure of tomato fruit at the breaker stage to 5 °C on visual quality, sensory (smell) score and volatile profile, as well as (2) whether a pre-chilling MeJA/MeSA incubation could alleviate CI-caused aroma loss.

2. Materials and methods

2.1. Plant materials

Mature green 'FL 47' tomatoes were harvested from a commercial field in Fort Pierce, FL, on May 7, 2014. Uniform and defect-free fruits, 300, with an average weight of 265 g were exposed to 80 μL L^{-1} of ethylene for 48 h at 20 °C to initiate and synchronize ripening. After removing immature (remaining at green stage) and over-developed fruits, 240 tomatoes at breaker stage (a^* = −7.3 ± 0.93; red area less than 10% of the whole surface) (USDA, 1997) were then selected and divided into three lots of 80 fruits each for H_2O (control), MeSA or MeJA vapor treatment. For each lot, 80 fruits were placed in two 45-L airtight glass containers with 40 fruits each. A 7-cm diameter filter paper disc soaked in one of following agents, 31.0 μL of DI water, 222.9 μL of MeSA or 374.6 μL of MeJA was suspended from the top of the glass container with the fruits for 24 h at 20 °C. The final chemical vapor concentration in the containers was 0.05 mM. After fumigation, the containers were opened, and ventilated for 12 h, 80 fruits in each treatment were divided into two groups: one group was directly placed at 20 °C for ripening, while the other was transferred to 5 °C for 9 d before ripening at 20 °C. Overall, this was a two-factor combination experiment with 3 chemical fumigations × 2 storage temperatures, and each combination (treatment) contained 40 fruits.

For non-chilled treatments, samples were taken on the same day when the color of the control reached a plateau (red) after a 7-day storage, the a^* values were 20.9 ± 0.8, 19.5 ± 1.2, and 19.1 ± 0.6 for control, MeJA and MeSA, respectively. For chilled treatments, ripening of fruits was delayed by 14 d (9 d at 5 °C + 5 d at 20 °C), and the samples were taken when color reached a plateau with an a^* value of 18.6 ± 0.6, 20.2 ± 1.1 and 20.7 ± 0.9 for water + chilling (chilling control), MeJA + chilling and MeSA + chilling, respectively. Twenty four out of a total 40 fruits per treatment were selected for volatile analysis with three fruit per replicate × eight replicates. The remaining fruits were used for sensory panel.

2.2. Volatile analysis

Pericarp tissue was quickly collected from three fruits per replicate with a sharp stainless steel knife, immersed in liquid N_2, crushed to roughly 0.5-cm pieces by mortar and pestle and then stored at −80 °C until analysis. Frozen pericarp tissue, removed from frozen storage, was further ground to powder under liquid nitrogen using mortar and pestle and 4.3 g of powder, together with 1.7 mL of saturated $CaCl_2$ solution were transferred to a 20-mL vial sealed with Teflon-lined septa (Gerstel Inc., Linthicum, MD).

Volatiles were analyzed by a headspace, solid-phase microextraction, and gas chromatography–mass spectrometry (HS-SPME-GC–MS) system following Bai et al. (2011)'s method with modifications. The sample vials were thawed under tap water, vortexed for 30 s, and loaded onto an autosampler (Model MPS2, Gerstel Inc.) equipped with a cooling tray (Laird Tech, Sweden) controlled by a Peltier Thermostat (CTC Analytics AG, Switzerland) with temperature setting at 4 °C, and held until headspace analysis. For headspace analysis, the sample vials were incubated for 30 min at 40 °C before a 2-cm solid phase microextraction (SPME) fiber (50/30 μm DVB/Carboxen/PDMS; Supelco, Bellefonte, PA) was exposed to the headspace for another 30 min at 40 °C. After exposure, the SPME fiber was inserted into the injector of a GC–MS (Model 6890, Agilent, Santa Clara, CA) to desorb the extract for 15 min at 250 °C. The GC–MS equipment and settings were: DB-5 column (60 m length, 0.25 mm i.d., 1.00 μm film thickness; J&W Scientific, Folsom, CA), coupled with a 5973 N MS detector (Agilent Technologies). The column oven was programmed to increase at 4 °C min^{-1} from the initial 40 °C to 230 °C, then ramped at 100 °C min^{-1} to 260 °C and held for 11.70 min for a total run time of 60 min. Helium was used as carrier gas at flow rate of 1.5 mL min^{-1}. Inlet, ionizing source and transfer line were kept at 250, 230, and 280 °C, respectively. Mass units were monitored from 30 to 250 m/z and ionized at 70 eV. Data were collected using the ChemStation G1701 AA data system (Hewlett–Packard, Palo Alto, CA). A mixture of C-5 to C-18 *n*-alkanes was run at the beginning of each day to calculate retention indices (RIs). Volatile compounds were identified by comparison of their mass spectra with library entries (NIST/EPA/NIH Mass Spectral Library, version 2.0d; National Institute of Standards and Technology, Gaithersburg, MA), as well as by comparing RI with authorized standard aroma compounds purchased from Sigma–Aldrich (St. Louis, MO) or Fluka Chemical Corporation (Buchs, Switzerland).

Quantification was conducted by using a peak size vs. concentration curve built by serially diluting five point standard solutions (Bai et al., 2002). Briefly, a standard compound was dissolved in pure methanol and the mixture was then introduced into a deodorized tomato homogenate. The concentrations in the standard curve for each compound covered the concentration range found in the samples.

2.3. Sensory evaluation

Paired-comparison tests (Meilgaard et al., 1999) were performed comparing either MeSA or MeJA treatment with water control, separately. Because of the differences in ripening time, panel evaluation for the chilled treatments was run separately from the non-chilled treatments, and there was no chilling vs. non-chilling comparison. Each sensory panel was carried out by 30 members for overall aroma evaluation. Tomatoes were cut into ~2 cm^3 wedges, and three wedges (about 35 g) were placed in 4-oz (118 mL) plastic souffle' cups (Solo® Cups Co., Lake Forest, IL) with lids and labeled with three-digit coded numbers. Panelists were presented the two coded samples with an alternated order of presentation. They were asked to open the lids, smell the samples and indicate which one had the most tomato odor. The time between cutting fruit and sensory evaluation was less than one hour. All panel members, untrained for the specific evaluation of tomato aroma, were familiar with sensory evaluation of other fruit and fruit products, and had evaluated tomato aroma previously.