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Suppression of volatile production in tomato fruit exposed to chilling temperature and alleviation of chilling injury by a pre-chilling heat treatment

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ABSTRACT

Chilling exposure of tomato fruit to 5 °C for less than 5 days at mature green stage does not cause visual symptom of chilling injury (CI), however, it is unknown whether such conditions would impact flavour quality (internal CI) after ripening, and if a pre-chilling heat treatment could alleviate internal CI. In this experiment, mature green 'FL 47' tomatoes were gassed with ethylene to initiate ripening before heating and/or chilling treatment, and fruits were ripened at 20 °C after short exposure to the high or low temperature. Volatile analysis of the fruits were conducted after ripening. Chilling treatment generally suppressed production of aldehyde, alcohol, ketone, ester, acid and terpene volatile compounds, including the following abundant and/or important volatiles: hexanal, *trans*-2-hexenal, 6-methyl-5-hepten-2-one, β-ionone, 2-methylbutanal, 2-phenylethanol, guaiacol and 2-isobutylthiazole. Heat treatment alone did not affect most volatile levels after ripening. Heat treatment prior to chilling exposure alleviated the reduction of some key volatile compounds caused by chilling exposure, which agreed with sensory panel results in that panellists perceived more tomato flavour in "heating + chilling" treated fruit than fruit that were chilled only.

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1. Introduction

Flavour is a critical quality characteristic for acceptance of fresh tomatoes (*Solanum lycopersicum* L.) and consumers are willing to pay a premium for full-flavoured fruit (Tandon, Baldwin, & Shewfelt, 2000; Yilmaz, Tandon, Scott, Baldwin, & Shewfelt, 2001). More than 400 volatile compounds have been identified in the ripening tomato fruit (Baldwin, Nisperos-Carriedo, & Moshonas, 1991). Of those, only around 15–20 are reported to impact flavour (Buttery, 1993; Klee, 2010). Over the last 50 years, much effort has been focused on yield, appearance and storability, which has resulted in cheaper, year-round produce availability, however, less attention has been paid to flavour quality. Consumers have noticed a significant decline in tomato flavour quality over the years and tomato and other produce flavour is a major source of consumer complaints (Klee, 2010).

Inappropriate postharvest conditions, such as temperature, humidity, and atmosphere cause flavour loss in tomato fruit. Like many other tropical and subtropical horticultural crops, tomato fruit are sensitive to low temperature, which can cause chilling injury (CI) (Soto-Zamora, Yahia, Brecht, & Gardea, 2005). Decreased aromatic volatile production is one of the many signs of chilling damage (Baldwin, 2004; Boukobza & Taylor, 2002; Maul et al., 2000). On the other hand, while heat treatment of tomatoes has been shown to be an effective means to sanitize the fruit surface by reducing microbes, disinfect insects, delay ripening, and alleviate pathological and physiological disorders (McDonald, McCollum, & Baldwin, 1999), a disadvantage of heat treatment can be loss of volatile production (Baldwin, 2004; McDonald, McCollum, & Baldwin, 1996). Nevertheless, pre-treatments with heat is a postharvest handling tool used to reduce CI of tomato fruit. Tomatoes pre-treated at the mature green stage with either hot water (42 °C for 1 h) or hot air (38 °C for 48 h), did not suffer external chilling

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injury after exposure to cold temperature (McDonald et al., 1996), and had less volatile flavour loss (McDonald et al., 1999) at 2 °C storage than without pre-chilling heat treatment. Our previous research shows that both chilling and heating treatments at the red stage suppressed production of fatty acid-derived volatiles, which did not fully recover after transferring tomatoes to 20 °C for four days (Bai et al., 2011).

As previously reported, mature green tomatoes exposed to 10 °C or lower temperature for longer than 2 weeks or at 5 °C for longer than 6–8 days suffered surface pitting, irregular (blotchy) colour development and other surface symptoms of CI during the ripening process (Suslow & Cantwell, 2014). But few studies focused on the effect of short-term exposure to chilling temperatures on tomato flavour components, although no visible CI symptoms occur. The objectives of this study were to investigate whether a 4-day exposure of tomato fruit to 5 °C at the mature green stage would impact flavour quality (internal CI) after ripening at 20 °C, and if a pre-chilling heat treatment could alleviate the internal CI. Heat treatment condition was 52 °C for 5 min, which was reported to improve sensory quality in tomatoes (Loayza, Brecht, Plotto, Baldwin, & Bai, 2012).

2. Materials and methods

2.1. Plant materials

Mature green 'FL 47' tomatoes (120), defect free with an average size of 151 g, were harvested from a commercial field in Fort Pierce, FL, on December 3, 2013 and then were exposed to 80 μl L^{-1} of ethylene for 40 h at 20 °C to initiate and synchronize ripening. The fruits were then divided into the following four treatments: 1) heat treated in 52 °C hot water for 5 min, then exposed to 5 °C for 4 days before being transferred to 20 °C for ripening, or 2) heat treated then placed directly at 20 °C without chilling, 3) chilled at 5 °C for 4 days without heating then transferred to 20 °C, and 4) untreated control, continuously stored and ripened at 20 °C. Fruit samples were taken at the red stage (colour a* value ≈ 20), thus sampling dates were varied. Nine out of a total of 30 fruit per treatment at the red-ripe stage were selected for volatile analysis (three fruit per replicate × five replicates). For sampling, pericarp tissue from the three fruit composite replicates were quickly removed from the fruit with a sharp stainless steel knife, immersed in liquid N_2, fractured to roughly 0.5 cm pieces and then stored at −80 °C until analysed.

2.2. Volatile analysis

Volatile analysis was by Headspace, Solid-Phase-Microextraction, and Gas Chromatography–Mass Spectrometry System (HS-SPME-GC-MS). HS-SPME-GC-MS analysis was applied following Bai, Baldwin, Hearn, Driggers, and Stover (2014)'s methods with modifications. Frozen pericarp tissue was ground to powder under liquid nitrogen and 4.3 g of powder, together with 1.7 mL of saturated $CaCl_2$ solution were transferred to a 20-mL vial and sealed with Teflon-lined septa. For headspace analysis, the homogenized samples were incubated for 30 min at 40 °C, after which time a 2-cm solid phase microextraction (SPME) fibre (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 fibre 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 (60 m length, 0.25 mm i.d., 1.00 μm film thickness; J&W Scientific, Folsom, CA) columns, 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–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 diluted five point standard solutions (Bai et al., 2014). Briefly, a standard compound was dissolved in pure methanol and the mixture was then introduced into a soluble solids equivalent deodourized tomato homogenize. The range of concentrations in the standard curve for each compound covers the concentrations found in the samples.

2.3. Sensory evaluation

Paired-comparison tests (Meilgaard, Civille, & Carr, 1999) were performed comparing heat-treated fruit with control, and heat-treated then chilled fruit with chilled only fruit. The two taste panels had to be performed at one week intervals because of the delayed ripening in the chilling involved fruit (making it impossible to compare with controls). Sensory evaluation was carried out by a panel of 21 members for overall flavour. Tomatoes were cut in ~2 cm^3 pieces, and two or three pieces were placed in 4-oz (118 mL) plastic soufflé cups (Solo® Cups Co., Lake Forest, IL) with lids and labelled with three-digit coded numbers. Panellists 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 odour. 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 flavour in the past.

2.4. Statistical analysis

Data presented were the mean values of five biological replicates for volatile compounds. SAS Version 9.1 (SAS Institute, Gary, NC) was used to analyse the data, using analysis of variance (PROC ANOVA). Mean separation was determined by Duncan's multiple range test at the 5% level. For multivariate statistical analyses, principal component analysis (PCA) and average linkage cluster analysis were performed using JMP (SAS Institute). For panel data, the number of samples that were marked as having more tomato flavour was compiled and compared with the critical number of correct responses in a two-sided directional difference test (Meilgaard et al., 1999).

3. Results

3.1. Volatile components detected in full ripe 'FL 47' tomatoes by HS-SPME-GC-MS

44 compounds were detected after HS-SPME-GC-MS analysis, including 16 aldehydes (in order of abundance, *trans*-2-hexenal, hexanal, 2-methylbutanal, isopentanal, *trans, trans*-2, 4-hexadienal,