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Prestorage Heat Treatments Influence Free Sterols and Flavor Volatiles of Tomatoes Stored at Chilling Temperature

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Abstract. **The objective of this study was to determine the effects of prestorage heat treatments on chilling tolerance of tomatoes. Mature-green 'Agriset' tomato fruit (*Lycopersicon esculentum* Mill.), either C_2H_4-treated or not, were immersed in 42C water for 60 min, held in 38C air for 48 hours, or not treated, and then stored at either 2C (chilled) or 13C (nonchilled) for 14 days before ripening at 20C. Heat-treated fruit stored at 2C and transferred to 20C ripened normally while nonheated fruit decayed before reaching red ripe. Color (a*/b* ratio), lycopene content, and internal quality characteristics of fruit were similar at the red-ripe stage irrespective of method of heat treatment. In red-ripe heat-treated fruit, free sterol levels were significantly higher in chilled fruit than in nonchilled fruit. Heating fruit in 38C air resulted in significantly higher levels of some free sterols compared with heating fruit in 42C water. Of the 15 flavor volatiles analyzed, six showed significantly decreased concentrations as a result of C_2H_4-treatment and seven showed decreased concentrations when stored at 2C before ripening. Some volatiles were decreased by the heat treatments. Prestorage short- and long-term heat treatments could allow for storage of mature-green tomatoes at lower temperatures with little loss of their ability to ripen normally.**

High- and low-temperature conditioning treatments have reduced damage to chilling-sensitive horticultural crops during exposure to low temperatures. Prestorage low temperature conditioning, or lowering temperatures to slightly above the chilling temperature for various periods of time, has been reported to reduce chilling injury (CI) in a number of fruit and vegetables (Hatton, 1990). Prestorage high temperature conditioning treatments to reduce CI can be divided into two categories: long term (12 h to 4 days in air) at 38 to 46C and short term (up to 60 min in water) at 45 to 60C (Klein and Lurie, 1992). The amelioration of CI by long- and short-term high-temperature conditioning to various horticultural crops has been reviewed by Paull and McDonald (1994).

Lurie and Klein (1991) found that mature-green tomatoes held 3 days at 36 to 40C before chilling at 2C did not develop CI whereas unheated tomatoes did. The heat-treated tomatoes ripened normally although more slowly than freshly harvested tomatoes. McCollum and McDonald (1993) reported that tomatoes exposed to a short-term heat treatment retained better condition in terms of reduced decay than nontreated fruit.

Although various biochemical and physiological alterations have been associated with heat treatments (Klein and Lurie, 1991), there have been no reports on their effects on flavor volatiles. Even though about 400 volatiles have been identified in tomatoes, apparently only a few are important to fresh tomato flavor (Petro-Turza, 1987). Consequently, it was of interest to determine the effects of C_2H_4, heat treatments, and chilling on these principal flavor volatiles.

Lipid composition changes dramatically during tomato fruit ripening and is affected significantly by chilling before ripening (Whitaker, 1994b). Whitaker (1994b) concluded that chloroplast damage occurs during chilling, but phospholipid-rich cell membranes are not degraded, even upon rewarming. A long-term prestorage heat treatment was found to result in a drop in steryl esters, large increases in phospholipids and cerebrosides, and a decrease in fatty acid unsaturation in galacto- and phospholipids (Whitaker, 1994a). However, Whitaker (1994a) concluded that the heat treatment did not reduce CI of tomato fruit effectively, contrary to the findings of Lurie and Klein (1991). Lurie and Klein (1991) reported a 50% increase in phospholipid content and a 20% decrease in sterol content of heat-treated tomatoes following 3 weeks at 2C, plus 7 days at 20C. The objective of this study was to compare short- and long-term heat treatments on chilling tolerance of tomatoes. Parameters evaluated included chilling injury symptoms, ripening, lipid composition, and flavor volatiles.

Materials and Methods

Plant material. 'Agriset' tomatoes were grown commercially near Ruskin, Fla. using standard horticultural practices. Large fruit (196 ± 30 g fresh weight) were hand-harvested in May 1994 at the mature-green stage, washed, air dried, and transported the same day to the U.S. Horticultural Research Laboratory at Orlando, Fla., where the fruit were sorted to eliminate defects and establish uniformity of size and color. Fruit were sorted into 12 groups of 75 for treatment and then divided into three replications per treatment

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(25 fruit). A complete factorial combination of treatments included +/– C_2H_4 (100 ppm C_2H_4 at 22C and 90% ± 5% RH for 24 h), heat treatment (none; 42C water for 60 min; 38C air for 48 h), and storage temperature (2 or 13C for 14 days). Preliminary work showed that a 42C water temperature for 60 min resulted in the best protection against chilling injury and lower amounts of fungal infection. At the end of storage, fruit were ripened at 20C and 90% ± 5% RH. Fruit were sampled for condition and chemical analyses at the time of transfer to 20C, after 2 and 7 days at 20C, and after reaching the red-ripe stage. At the time of sampling, tissue samples from five fruit per replication were frozen and stored at –20C until used for chemical analyses.

Fruit color. Color was measured objectively using Commission Internationale de l'Eclairage L*, a*, and b* color space coordinates obtained with a chromameter (model CR-300; Minolta, Ramsey, N.J.). Reported values represent the mean of five fruit per replication with three readings per fruit. The USDA–AMS standard grading system for subjective tomato ripeness was used to assess ripening (U.S. Dept. Agriculture, 1975). Each of the six categories was assigned a value; 1 = green; 2 = breaker; 3 = turning; 4 = pink; 5 = light red; and 6 = red. Reported values represent the mean of at least 15 tomatoes per replication.

Pigments were extracted from 2 g of frozen pericarp with 5 ml *N,N*-dimethylformamide as described by Moran and Porath (1980). Absorbance of the extracts at 455 nm for lycopene and 664 nm for chlorophyll were measured with a spectrophotometer (model UV-160A; Shimadzu, Kyoto, Japan).

Soluble solids and acidity. About 5 g of the frozen tissue were homogenized with a tissue homogenizer (Brinkmann Instruments, Westbury, N.Y.) and then centrifuged at 20,000× *g* for 15 min. Two ml of the supernatant were added to 25 ml H_2O and titrated with 0.01 N NaOH to pH 8.1 to determine acidity. Soluble solids were measured by a refractometer (model MRK II; AO Scientific Instruments, Keene, N.H.).

Fruit firmness. Resistance to compression was measured with a food testing system (model 3200; Instron Inc., Canton, Mass.) fitted with a 9-cm-diameter, round, flat-faced anvil. The amount of force (N) required to compress the radial pericarp surface 3 mm at a constant speed of 5 cm·min^{-1} was recorded. Reported values represent the average of five tomatoes with one measurement per fruit.

Carbon dioxide and C_2H_4 analysis. Whole-fruit respiration and C_2H_4 evolution were determined following 2 and 5 days at 20C and at the red-ripe stage. For gas analysis, a sample of three fruit from each replication per treatment was selected at random and placed into a 3.8-liter glass jar with lid fitted with a rubber serum stopper. Sampling for CO_2 and C_2H_4 was conducted after incubating 1.5 and 2.5 h, respectively. Samples of headspace atmosphere were removed using gas-tight syringes, and the analyses were conducted using standard gas chromatography methods.

Volatiles. Three fruit from each replication per treatment were homogenized in a blender (Waring Products Corp., N.Y.) at high speed for 30 sec. Three minutes after homogenization, 25 ml of the homogenate was blended for 10 sec with 10 ml of saturated $CaCl_2$ to stop enzymatic reactions that can prevent further changes in volatile levels (Buttery et al., 1987). The resulting homogenate was then held at –60C until analyzed for volatiles. Two milliliters of the homogenate was transferred to a 6-ml vial, which was sealed with a crimp-top cap and teflon/silicone septum. Volatile flavor components were analyzed using a gas chromatograph (GC) (model 8500; Perkin Elmer) with a HS-6 headspace sampler, flame ionization detector, and a 30-m × 0.53-mm-i.d. polar stabilwax capillary column (1.0-µm film thickness, Restek Corp., Bellefonte, Pa.) under conditions previously described (Baldwin et al., 1992).

Table 1. Days to red ripe (SE = 0) following C_2H_4 treatment, heat treatments, and 14 days at 2 or 13C.

	–C_2H_4		+C_2H_4	
Treatment	2C	13C	2C	13C
Control	---[z]	11	---	1
HA[y]	19	7	10	0[x]
HW[w]	16	9	9	1

[z]All fruit decayed before reaching the red-ripe stage.
[y]Held 48 h in air at 38C and 92% ± 3% RH.
[x]Fruit ripened before termination of storage.
[w]Held 1 h in 42C water.

Samples were equilibrated in the headspace sampler for 15 min at 80C before injection. Injection parameters for the headspace sampler were 0.5-min vial pressurization time followed by 0.02-min injection time. Column oven temperature programing was held at 40C for 6 min and then raised at 6C/min to 180C. The different volatile components were identified by comparison of retention times with standards and by enrichment of the tissue homogenate with authentic samples. Concentrations of the individual volatile compounds were calculated using regression equations (five concentrations per standard) to obtain a peak height calibration curve as described previously (Nisperos-Carriedo et al., 1990).

Lipid extraction and analysis. Pericarp tissue samples from five fruit (20 g total fresh weight) were lyophilized and stored at –80C before extraction according to Whitaker (1991) with modification. The lyophilized samples (0.5 to 0.6 g dry weight) were homogenized in 25 ml of acidified (0.1% HOAc) $CHCl_3$-MeOH (2:1; v/v) with 3 × 15-sec bursts of a Brinkmann tissue homogenizer. The homogenate was filtered through a sintered glass filter reextracted with 20 ml of $CHCl_3$-MeOH, and the combined extracts were washed sequentially with 0.8% NaCl and MeOH-H_2O(1:1; v/v). The $CHCl_3$ phase containing total lipids was evaporated under a stream of N_2 and lipids dissolved in 2 ml of $CHCl_3$ before silicic acid column chromatography. Neutral lipids were eluted with $CHCl_3$-Me_2CO (20:1; v/v), acylated steryl glycosides (ASG) with $CHCl_3$-Me_2CO (2:1; v/v), steryl glycosides (SG) with Me_2CO and phospholipids (PL) with MeOH-H_2O (10:1; v/v).

An internal standard of 100 µg *n*-heptadecanoic acid was added to the PL fraction and the PL fatty acids were converted to their Me esters (FAME) by adding 10 ml of 14% (w/v) BF_3 in MeOH and heating at 70C for 5 min followed by extraction of FAMEs with hexane. FAMEs were quantified by GC-FID on a 1.8 m × 3.2 mm o.d. stainless-steel column packed with 3% SP-2310/2% SP-2300 on 100/120 chromosorb W (Supelco, Bellefonte, Pa.). The N_2 flow rate was 20 ml·min^{-1}, initial oven temperature 180C, and injector and detector temperatures 200 and 250C, respectively. The oven temperature was programmed to rise 2C/min to 220C and held for 20 min.

Neutral lipids, including steryl esters (SEs) and free sterols

Table 2. Percent decay (±SE) at red-ripe stage following C_2H_4 treatment, heat treatments, and 14 days at 2 or 13C.

	–C_2H_4		+C_2H_4	
Treatment	2C	13C	2C	13C
Control	100[z] ± 0	9 ± 0.9	100[z] ± 0	0 ± 0
HA[y]	0 ± 0	0 ± 0	15.2 ± 0.6	6.3 ± 0.9
HW[w]	2.3 ± 0.3	0 ± 0	12.1 ± 1.2	26.7 ± 0.3

[z]All fruit decayed before reaching the red-ripe stage.
[y]Held 48 h at 38C and 92% ± 3% RH.
[x]Held 1 h in 42C water.

(FSs), were separated by 500-µm-thick thin-layer chromatography (TLC) plates (Merck G 60) according to Wang and Faust (1988) with modification. Before TLC, 100 µg cholesterol and 169 µg cholesteryl palmitate were added to the neutral lipid fraction as internal standards. The plate was developed in a freshly made solvent mix of 80 hexane : 20 ethyl acetate : 2 formic acid (by volume). Spots corresponding to FSs and SEs were visualized by spraying with rhodamine, and those which comigrated with cholesterol and cholesteryl palmitate were removed and eluted with 3 ml $CHCl_3$-MeOH (2:1; v/v) including 4 µg butylated hydroxytoluene/ml. After addition of 1 ml 0.8% NaCl, vortexing under N_2 and centrifugation, the $CHCl_3$ phase containing FSs or SEs was evaporated to dryness under N_2. The FS fractions were resuspended in isooctane before GC analysis. The SE fractions were saponified in 1 ml of 1 M KOH in 85% EtOH for 1 h at 80C (under N_2). After heating, 0.5 ml H_2O was added and the SE and FS fractions were recovered by extraction with 2 ml of hexane. Samples were evaporated to dryness under N_2 and redissolved in 50 µl 100% EtOH. Sterol composition of the FS and SE fractions was determined by GC-FID. The column was 0.9 m glass (2 mm i.d. × 6.4 mm o.d.) packed with 3% SP 2250 on 100/120 mesh supelcoport (Supelco). The N_2 flow rate was 18 ml·min^{-1}, the column oven isothermal at 260C, and the injector and detector temperatures 300 and 350C, respectively. Individual sterols were identified by comparison with authentic standards.

Table 3. Effects of C_2H_4, storage temperature, and heat treatment on red-ripe tomato fruit color, pigment levels, internal quality characteristics, firmness, respiration rate and ethylene evolution rate.

	Ethylene			
	–		+	
	Storage temp (°C)		Storage temp (°C)	
Treatment	2	13	2	13
Objective color (L)*				
Control	---	43.4 a[z]	---	43.3 a
HA[y]	41.8 a	42.1 a	43.3 a	43.7 a
HW[x]	42.8 a	42.4 a	42.8 a	42.8 a
Objective color (a/b*)*				
Control	---	1.10 a	---	1.00 a
HA	1.21 a	1.12 a	1.07 a	1.02 a
HW	1.09 a	1.09 a	0.90 a	1.06 a
Ripeness				
Control	---	5.9 a	---	5.9 a
HA	5.3 a	5.7 a	5.7 a	6.0 a
HW	5.3 a	5.7 a	5.4 a	5.7 a
Lycopene (µg·g^{-1} fresh weight)				
Control	---	15.3 a	---	11.4 a
HA	11.9 a	22.7 a	10.3 a	8.4 a
HW	9.4 a	21.9 a	10.1 a	8.1 a
Chlorophyll (Abs. at 664 nm·g^{-1} fresh weight)				
Control	---	0.059 a	---	0.001 a
HA	0.013 a	0.160 a	0.002 a	0.001 a
HW	0.003 a	0.082 a	0.003 a	0.012 a
Soluble solids content (%)				
Control	---	4.0 a	---	4.3 a
HA	3.8 a	4.1 a	3.9 a	4.2 a
HW	3.9 a	4.2 a	3.9 a	4.3 a
Titrable acidity (%)				
Control	---	0.29 a	---	0.32 a
HA	0.28 a	0.33 a	0.27 a	0.30 a
HW	0.28 a	0.31 a	0.26 a	0.31 a
Solids/acids ratio				
Control	---	13.8 a	---	13.5 a
HA	13.6 a	12.6 a	14.7 a	14.1 a
HW	13.4 a	13.6 a	15.4 a	14.1 a
Firmness (N)				
Control	---	10.2 a	---	12.6 a
HA	6.3 a	10.9 a	7.9 a	12.1 a
HW	8.4 a	8.5 a	7.8 a	12.5 a
Respiration (ml CO_2/kg fresh weight per h)				
Control	---	12.7 a	---	11.7 a
HA	10.1 a	11.9 ab	12.1 a	8.7 a
HW	14.0 a	11.0 b	11.9 a	10.9 a
Ethylene production (nl·g^{-1} fresh weight per h)				
Control	---	20.7 a	---	14.2 a
HA	17.4 a	23.6 a	31.1 a	17.9 a
HW	20.6 a	21.7 a	27.4 a	20.9 a

[z]Means followed by the same letter within columns are nonsignificant at $P = 0.05$ by Duncan's multiple range test.
[y]Held 48h at 38C and 92% ± 3% RH.
[x]Held 1 h in 42C water.

Although the experiment was initiated as a complete factorial combination of treatments, no nonheated chilled fruit reached the red-ripe stage and therefore the data could not be analyzed as such. Instead, the data were analyzed as two separate complete factorials. The first factorial tested the effects of C_2H_4 and heat treatments on nonchilled tomatoes. The second factorial tested the effects of C_2H_4, method of heat treatment, and storage temperature and did not include nonheated fruit. For discussion purposes, only data for red-ripe fruit are presented from the latter factorial.

Results

All nonheated control fruit stored at 2C, C_2H_4-treated and not C_2H_4-treated, decayed before reaching red ripe (Table 1). However, heat treating the fruit with 38C air or 42C water before storage enabled their eventual ripening. Fruit that had been treated with C_2H_4 reached red ripe before those that were not C_2H_4-treated. There was more decay in fruit that had been C_2H_4-treated compared with those that had not been treated irrespective of the method of heat treatment (Table 2). Both heat treatments had a positive effect on reducing decay, particularly in chilled fruit. In nonchilled fruit (stored at 13C), heat treatment resulted in less decay in fruit that were not C_2H_4-treated but resulted in more decay in C_2H_4-treated fruit.

Table 4. Analysis of variance for the effects of C_2H_4, heat treatment method, and storage temperature on free sterol content of tomato pericarp at the red-ripe stage.

		Mean square and significance		
Source	df	Campesterol	Sigmasterol	Sitosterol
Ethylene (E)	1	147	73,103	449,456
Heat treatment (HT)	1	6,297**	378,984**	158,113
Storage temp (ST)	1	32,500***	1,447,958***	1,755,085**
E × HT	1	2,662	103,791	117,230
E × ST	1	4,412*	4,569	0
HT × ST	1	68	10,628	31,564
E × HT × ST	1	5,284*	136,215	156,927
Residual	16	661	36,321	121,074

*,**,***Significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

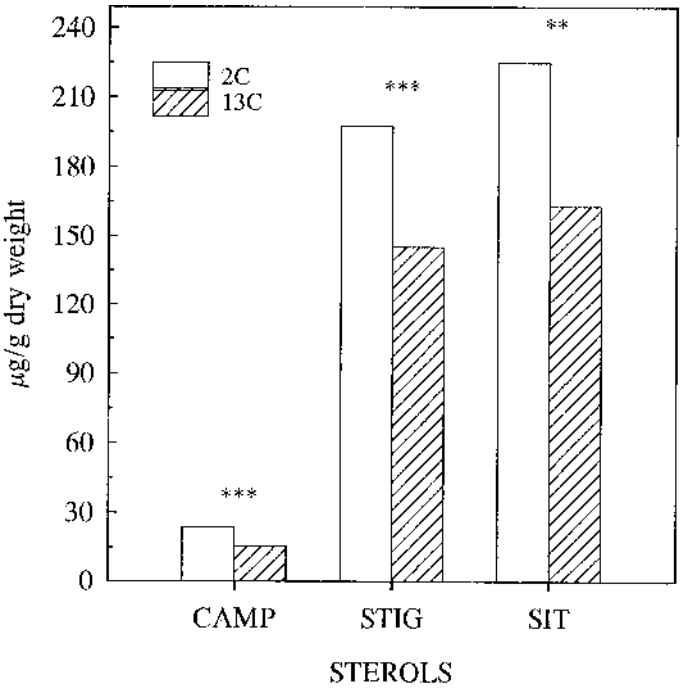

Fig. 1. Main effects of storage temperature on free sterols in tomato fruit pericarp that had been heat-treated (38C air or 42C water) before storage for 14 days at 2 or 13C and ripened to red ripe at 20C (storage temperature ×treatment interaction not significant). CAMP = campesterol; STIG = stigmasterol; SIT = sitosterol.

There were no significant treatment effects on color (L* and a*/b* values), ripeness, lycopene, chlorophyll, percent soluble solids, percent titratable acidity, solids/acid ratio, fruit firmness, or C_2H_4 evolution rate in red-ripe fruit (Table 3). Respiration rate was not affected by heat treatments with the exception that it was lower in the 42C water heat-treated fruit that was not C_2H_4-treated and stored at 13C compared with nonheated fruit.

The C_2H_4 treatment had no significant effect on any of the FSs (Table 4). However, FSs were significantly affected by storage temperature. Campesterol, stigmasterol and sitosterol levels were significantly higher in chilled compared with nonchilled fruit after ripening at 20C to red ripe (Fig. 1). Heating fruit in 38C air resulted in significantly higher levels of stigmasterol (186 $\mu g \cdot g^{-1}$) than did heating fruit in 42C water (157 $\mu g \cdot g^{-1}$). The three-way interaction for C_2H_4, heat treatment and storage temperature for campesterol was significant. Campesterol levels were increased by 2C storage except that there was no change with C_2H_4- and water heat-treated fruit. The heat treatments had no significant effects on levels of FAME from the PL fraction or SEs from the neutral lipid fraction.

Fifteen tomato volatiles were identified and quantified by headspace analysis on the GC (Table 5). Treatment with C_2H_4 significantly reduced the levels of hexanal, 6-methyl-5-hepten-2-one, geranylacetone, methanol, 2-isobutylthiazole, and 1-nitro-2-phenylethane. Heat-treatment in water significantly reduced levels of 1-nitro-2-phenylethane compared with heat-treating in air. Storage at 2C significantly reduced levels of hexanal, *trans*-2-hexenal, geranylacetone, 1-penten-3-one, methanol, 2+3-methylbutanol, and 1-nitro-2-phenylethane.

The two-way interaction between heat treatment and storage temperature for 6-methyl-5-hepten-2-one and 2-isobutylthiazole was significant. Heat-treatment in water reduced 6-methyl-5-hepten-2-one more in chilling storage than heat-treatment in air. Heat-treatment in air reduced, while heat-treatment in water increased 2-isobutylthiazole in chilling storage. There was a significant interaction between C_2H_4 and heat treatment for 1-penten-3-one. Fruit that were non-C_2H_4- and water heat-treated had lower levels of 1-penten-3-one than those air heat-treated while fruit that were C_2H_4- and air heat-treated had lower levels than those that were water heat-treated.

There were two significant three-way interactions among C_2H_4,

Table 5. Effect of C_2H_4, heat treatment method, and storage temperature on tomato fruit flavor volatiles[z] at the red-ripe stage.

	Heat	Storage	Aldehydes			Ketones	
C_2H_4	treatment	temp	HEX	CHEX	THEX	METH	GERN
–	Air	2	7.4	8.3	10.1	0.56	0.73
		13	13.6	16.6	15.8	0.74	0.73
	H_2O	2	6.2	5.9	7.3	0.54	0.49
		13	14.1	6.7	12.2	0.58	1.07
+	Air	2	5.6	5.7	8.8	0.35	0.29
		13	6.1	12.2	10.5	0.51	0.40
	H_2O	2	4.5	4.8	6.7	0.40	0.22
		13	9.4	12.5	12.3	0.45	0.43
ANOVA							
Ethylene (E)			0.0178[y]	0.5122	0.0982	0.0001	0.0007
Heat treatment (HT)			0.8277	0.0027	0.1170	0.0390	0.8834
Storage temp (ST)			0.0046	0.0001	0.0004	0.0002	0.0413
E × HT			0.6352	0.0051	0.1477	0.0600	0.7326
E × ST			0.1580	0.1862	0.4304	0.9412	0.5594
HT × ST			0.3273	0.1027	0.4462	0.0136	0.1106
E × HT × ST			0.6533	0.0316	0.2646	0.7682	0.2506

[z]Data expressed in ppm. Three aldehyde volatiles were quantified: hexanal (HEX), *cis*-3-hexenal (CHEX), and *trans*-2-hexenal (THEX); six ketone flavor volatiles were quantified: 6-methyl-5-hepten-2-one (METH), geranylacetone (GERN), 1-penten-3-one (PENT), acetone (ACE), ß-ionone (IONE), and *trans*-2-heptenone (HEP); four alcohol flavor volatiles were quantified: methanol (MEOH), ethanol (ETOH), 2+3-methylbutanol (MEBU), and *cis*-3-hexenol (HEOL); and two miscellaneous flavor volatiles were quantified: 2-isobutylthiazole (ISOB) and 1-nitro-2-phenylethane (PHEN).
[y]*P* value.

heat treatment and storage temperature. *cis*-3-Hexenal levels were reduced by chilling except in non-C_2H_4- and water heat-treated fruit where levels were the same at both storage temperatures. Acetone levels were greater in non-C_2H_4- and air heat-treated fruit stored at 2C compared with other main effects combinations.

Discussion

Treatment of tomatoes in 42C water for 60 min or 38C air for 48 h before storage at a chilling temperature (2C) for 14 days reduced CI and subsequent decay upon rewarming. This confirms our earlier report of a short-term heat treatment being beneficial for maintaining tomato fruit quality (McCollum and McDonald, 1993). Lurie and Klein (1991) previously demonstrated that a long-term (36 to 40C for 3 days) heat treatment conferred tolerance to low-temperature stress to tomato fruit.

Lurie and Klein (1992) found that a 3-day heat treatment at 38C inhibited ripening of mature green tomatoes. They concluded that the inhibition of ripening was maintained at 2.5C, but was removed when the fruit were transferred to 20C. Our results with two heat treatments support the finding that recovery from the heat-induced inhibition occurred during the time the fruit were stored at 13C, but not when they were stored at 2C. We found that the time to ripen after transfer from 13C to 20C, whether C_2H_4-treated or not, was approximately equal for both heat treatments when one considers the fruit subjected to the 38C 2-day air treatment were chronologically 2 days older than the 42C water-treated fruit. However, when stored at 2C before ripening at 20C, the fruit heated in 42C water for 60 min ripened faster than those heated in 38C air for 2 days whether C_2H_4-treated or not C_2H_4-treated.

Chlorophyll degradation and lycopene synthesis were not inhibited following either heat treatment. At the red-ripe stage, the heat-treated tomatoes held at 13C for 2 weeks before ripening did not have significantly different levels of chlorophyll or lycopene compared with control fruit. Pigment levels at the red-ripe stage in heat-treated fruit stored at 2C were not different from those stored at 13C. The data on lycopene synthesis are in agreement, while the data on chlorophyll degradation are not in agreement with those of Lurie and Klein (1992). The difference in chlorophyll levels in the two studies may be explained by the fact that in the previous study fruit were ripened for only 5 days at 20C whereas we held fruit at 20C until they were red ripe. At the full ripe stage, chlorophyll differences among treatments may not be large enough to detect.

The higher levels of FSs in fruit subjected to a chilling temperature (2C) compared with fruit stored at a nonchilling temperature (13C) support the findings of Whitaker (1991). Campesterol and stigmasterol levels were elevated in the 38C air treatment compared with the 42C water treatment. These differences could be important, as FSs are involved in controlling membrane stability and membrane permeability (Kuiper, 1975). Correlations have been found in many plant tissues between CI and increased electrolyte leakage (Murata and Tatsumi, 1979). Elevated levels of FS could be an indication of alterations in membrane properties.

While Whitaker (1994b) found the sterol isofucosterol in tomato pericarp tissue, we did not find this sterol in our study. Perhaps, the GC conditions used in our study did not permit its quantification.

Of the 15 volatiles analyzed in this study, six showed decreased concentrations in C_2H_4-treated fruit. The changes were significant for hexanal, 6-methyl-5-hepten-2-one, geranylacetone, methanol, 2-isobutylthiazole, and 1-nitro-2-phenylethane. To our knowledge, and based on our assessment of ripeness, this is the first report to show C_2H_4 treatment of fruit reduces volatile levels. However, Kader et al. (1978) reported that C_2H_4 did not influence tomato fruit flavor as determined by a taste panel.

Comparing the two heat treatments, some of the 15 volatiles showed decreased levels in fruit exposed to air heat-treatment while other volatiles showed decreased levels in fruit exposed to water heat-treatment. 1-Nitro-2-phenylethane levels were significantly lower in the water-heated fruit.

Ketones				Alcohols				Misc.	
PENT	ACE	IONE	HEPT	MEOH	ETOH	MEBU	HEOL	ISOB	PHEN
0.38	0.73	0.14	0.05	199	21.0	0.84	0.23	0.06	0.10
0.53	0.31	0.17	0.06	230	18.4	1.51	0.11	0.07	0.11
0.29	0.32	0.13	0.04	165	15.9	1.12	0.10	0.09	0.09
0.38	0.34	0.13	0.05	254	18.6	1.43	0.09	0.07	0.09
0.29	0.29	0.10	0.04	165	15.6	0.78	0.07	0.04	0.08
0.34	0.36	0.04	0.05	183	15.7	1.67	0.08	0.05	0.09
0.33	0.33	0.23	0.06	167	19.6	1.07	0.28	0.06	0.07
0.37	0.30	0.21	0.05	193	19.2	1.23	0.10	0.05	0.08
0.0270	0.0216	0.8028	0.6003	0.0348	0.3237	0.0842	0.9843	0.0010	0.0019
0.1173	0.0320	0.0554	1.0000	0.9690	0.4742	0.1346	0.6810	0.0463	0.0309
0.0062	0.0471	0.8886	0.4344	0.0171	0.9631	0.0005	0.0884	0.5458	0.0309
0.0082	0.0471	0.0110	0.1284	0.7519	0.0040	0.6708	0.3870	0.5458	0.3256
0.1818	0.0199	0.3535	0.7927	0.2366	0.9193	0.2369	0.7688	0.3683	0.7397
0.5461	0.0636	0.9347	0.2001	0.2991	0.2213	0.1105	0.6810	0.0463	0.7397
0.5887	0.0064	0.5940	0.7927	0.4395	0.1350	0.6667	0.0823	1.0000	0.7397

Ethylene and heat treatments had a tendency to reduce the levels of some flavor volatiles. All volatiles measured are reported to contribute to tomato flavor based on odor threshold studies (Buttery et al., 1989) with the exception of methanol and ethanol. The latter two volatiles have been associated with anaerobic conditions and off-flavor in citrus (Baldwin, 1995; Cohen et al., 1990). Although no differences in flavor were detected in informal taste tests, sensory analysis would need to be conducted on a larger scale to determine the effect of these treatments on tomato flavor. Some lipids, principally unsaturated fatty acids, are thought to be precursors of hexanal, *cis*-3-hexenal, *trans*-2-hexenal, and 1-penten-3-one, while carotenoid related volatiles in this study are 6-methyl-5-hepten-2-one, geranylacetone, and β-ionone (Buttery and Ling, 1993). However, neither heat treatment affected the PL fraction, chlorophyll degradation, or lycopene synthesis. It therefore appears that changes in levels of these volatiles were not related to changes in PL or pigment precursors, but perhaps due to enzyme synthesis that resulted in enzymatic degradation of the precursors upon homogenation.

Storage temperature had a greater effect on volatile levels than did C_2H_4 or heat treatment. Storage at chilling temperature significantly reduced the levels of seven volatiles including hexanal, *trans*-2-hexenal, geranylacetone, 1-penten-3-one, methanol, 2+3-methylbutanol, and 1-nitro-2-phenylethane. Decreased flavor volatile profile levels were also observed by Kader et al. (1978) when mature green fruit were held 7 days at 5C before ripening at 20C. Buttery et al. (1987) reported that refrigeration (2C) of ripe tomato fruit generally resulted in decreased volatile levels. In this case, chilling fruit at 2C before ripening had a similar effect.

The treatment combination that resulted in the highest volatile levels at the red-ripe stage was no C_2H_4, treatment with air at 38C, and storage at 13C before ripening. Five of the 15 volatiles showed highest levels with this combination (*cis*-3-hexenal, *trans*-2-hexenal, 6-methyl-5-hepten-2-one, 1-penten-3-one, and 1-nitro-2-phenylethane). The level of *trans*-2-heptenone was not affected by any treatment.

The data presented here indicate that prestorage short- and long-term heat-treated tomatoes can be successfully stored at chilling temperatures. Postharvest treatments that extend storage life of fruits and vegetables can result in altered flavor volatile profiles compared with naturally-ripened fruits and vegetables. Selecting for treatments that result in extended storage life with the least alteration of the volatile profiles may result in better flavor quality of the ripened product. Heat-treated tomatoes appeared to ripen normally, and the magnitude of changes in FSs and flavor volatiles were apparently not detrimental to overall quality as far as could be determined without conducting sensory analysis.

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