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Studies on the Aroma of Five Fresh Tomato Cultivars and the Precursors of *cis*- and *trans*-4,5-Epoxy-(*E*)-2-Decenals and Methional

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Three tasty (BR-139, FA-624, and FA-612) and two less tasty (R-144 and R-175) fresh greenhouse tomato cultivars, which significantly differ in their flavor profiles, were screened for potent odorants using aroma extract dilution analysis (AEDA). On the basis of AEDA results, 19 volatiles were selected for quantification in those 5 cultivars using gas chromatography−mass spectrometry (GC-MS). Compounds such as 1-penten-3-one, (*E*,*E*)- and (*E*,*Z*)-2,4-decadienal, and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol) had higher odor units in the more preferred cultivars, whereas methional, phenylacetaldehyde, 2-phenylethanol, or 2-isobutylthiazole had higher odor units in the less preferred cultivars. Simulation of the odor of the selected tomato cultivars by preparation of aroma models and comparison with the corresponding real samples confirmed that all important fresh tomato odorants were identified, that their concentrations were determined correctly in all five cultivars, and that differences in concentration, especially of the compounds mentioned above, make it possible to distinguish between them and are responsible for the differential preference. To help elucidate formation pathways of key odorants, labeled precursors were added to tomatoes. Biogenesis of *cis*- and *trans*-4,5-epoxy-(*E*)-2-decenals from linoleic acid and methional from methionine was confirmed.

KEYWORDS: Fresh tomato; aroma; flavor; odorants; taste; modeling; AEDA; quantification; concentrations; linoleic acid; *cis*- and *trans*-4,5-epoxy-(*E*)-2-decenals; methional; methionine

INTRODUCTION

Tomato flavor has been extensively studied, with over 400 volatiles identified (*1, 2*). Despite these many studies new constituents of sensory importance continue to be characterized. Two isomers of 4,5-epoxy-(*E*)-2-decenal were identified in tomato by Buttery and Ling (*3*), who found a combined concentration of 30 μg/L. Guth and Grosch (*4*) used aroma extract dilution analysis (AEDA) to identify acetic acid, 5-ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone, *trans*-4,5-epoxy-(*E*)-2-decenal, and eugenol as important fresh tomato odorants. Mayer and co-workers (*5*) detected three additional volatiles, (*E*,*Z*)-2,4-decadienal, *cis*-4,5-epoxy-(*E*)-2-decenal, and (*Z*)-1,5-octadien-3-one, as contributors to fresh tomato aroma using AEDA. The latter compound, which possesses a very low odor threshold in water of 0.0012 μg/L (*6*), was identified for the first time in fresh tomatoes. A study of four fresh greenhouse tomato cultivars (FA-624, BR-139, R-144, and R-175) confirmed that (*Z*)-3-hexenal and *trans*-4,5-epoxy-(*E*)-2-decenal were the most potent odorants, with odor units (ratio of the odorant concentration and its odor threshold) ranging from 8400 to 27200 (*7*). It is noteworthy that *trans*-4,5-epoxy-(*E*)-2-decenal had higher odor units than did (*Z*)-3-hexenal in R-144 and R-175, whereas their values were similar in BR-139 and FA-624. *cis*-4,5-Epoxy-(*E*)-2-decenal is also thought to be a significant contributor to fresh tomato aroma, although its concentration was 3.5−4.7 times lower than that of *trans*-4,5-epoxy-(*E*)-2-decenal in the four tomato cultivars (*7*). Whereas the odor threshold of the trans isomer has been determined to be 0.02 μg/L water (*7*) and 0.6–2.5 pg/L air (*8*), the threshold of the cis isomer has not yet been determined. *trans*-4,5-Epoxy-(*E*)-2-decenal has been found to contribute to the green, hay-like off-odor in soybean oil stored in the dark (*9*) and to the warmed-over flavor of stored beef (*10*) and refrigerated beef (*11*). The trans isomer has been reported to be an important odorant in wheat bread crumb (*12*), popcorn (*13*), roasted sesame seeds (*14*), and fresh grapefruit juice (*15*). Kumazawa and co-workers (*16*) investigated black tea (Dimbula) infusion by AEDA and found that both *cis*-4,5-

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Table 1. Properties of the Investigated Tomato Cultivars

	tomato cultivar				
	R-144	R-175	BR-139	FA-612	FA-624
fruit wt (g)	76–100	51–68	6–8	30–48	48–68
pH	4.5–4.7	4.3–4.5	4.3–4.6	4.3–4.5	4.3–4.5
Brix (early season–late season)	5.4–6.5	4.9–6.6	7.7–10.3	6.4–7.9	6.4–8.5
preference (1 = best, 5 = worst)	4	5	1	3	2

epoxy-(*E*)-2-decenal (0.28 μg/L) and *trans*-4,5-epoxy-(*E*)-2-decenal (0.60 μg/L) were key odorants. *cis*- and *trans*-4,5-epoxy-(*E*)-2-decenal have been described as having fatty and metallic odors (*7, 8*), whereas others researchers have characterized their odors as juicy and sweet with the cis isomer having a fatty note and the trans isomer having a fresh citrus-like note (*16*). The two isomers of 4,5-epoxy-(*E*)-2-heptenal have also been identified in tomato (4 μg/L total; (*3*)), but the odor threshold of the trans isomer is much higher (>1000 ng/L; (*8*)), so these isomers probably do not contribute to tomato aroma.

The first objective of the present study was to determine the most important contributors to fresh tomato aroma. The second objective was to investigate the relationship between sensory perception and instrumental analysis. We selected three highly appetizing and two less appetizing tomato hybrid cultivars and analyzed their volatile flavor compositions to find out if the reason for consumer preference or rejection can be related to particular flavor compounds and their concentrations. Given the importance of the *cis*- and *trans*-4,5-epoxy-(*E*)-2-decenals and methional to fresh tomato aroma, the third objective of this investigation was to clarify the formation mechanism of these isomers using ^{13}C-labeled linoleic acid and ^{13}C-labeled methionine.

MATERIALS AND METHODS

Tomatoes. Tomatoes (greenhouse hybrid cultivars, BR-139, R-144, FA-612, and FA-624 (Hazera Genetics Ltd., Kiriat Gat, Israel) and R-175 (Zeraim Gedera, Gedera, Israel), grown outdoors on a farm in Manteca, CA, in 2000, 2001, and 2002 were picked red-ripe (regularly about every 2 weeks) during the months of July through October and stored at room temperature until used for analysis. The Brix value was determined by filtering blended tomatoes through a Kimwipes wiper (Kimberly-Clark Global Sales, Inc., Roswell, GA) and measuring the refractive index of the juice using a Bellingham+Stanley Inc. (Atlanta, GA) model RFM81 automatic digital refractometer. The properties of the investigated cultivars are detailed in **Table 1**. Hothouse tomatoes, purchased from a local Safeway supermarket, were used for the experiments with ^{13}C-labeled linoleic acid.

Chemicals. All chemicals and reference compounds were obtained commercially or synthesized according to published methods. Calcium chloride, diethyl ether, anethole, 3-hexanone, 2-octanone, 2-undecanone, 3-methylbutanal, 1-penten-3-one, (*E*)-2-hexenal, 2-isobutylthiazole, methional, phenylacetaldehyde, 3-methylbutyric acid, (*E*,*Z*)-/ (*E*,*E*)-2,4-decadienal (isomer mixture), β-ionone, and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone were purchased from Aldrich, Milwaukee, WI. Linoleic acid-^{13}C$_{18}$ (minimum 99 atom % ^{13}C) and L-methionine-^{13}C$_1$ (methyl-^{13}C; minimum 99 atom % ^{13}C) were obtained from Isotec (Miamisburg, OH). Hexanal and (*Z*)-3-hexenal were obtained from Bedoukian Research Inc. (Danbury, CT), 1-octen-3-one was from Lancaster Synthesis, Inc. (Windham, NH), 2-phenylethanol was from Eastman Kodak (Rochester, NY), maltol was from Pfizer (New York, NY), β-damascenone was from Firmenich (Plainsboro, NJ), sodium sulfate and sodium carbonate were from Fisher Scientific (Fair Lawn, NJ), absolute ethanol (200 proof) was from Aaper (Shelbyville, KY), citric acid was from Mallinckrodt-Baker, Inc. (Paris, KY), and *n*-pentane was from Burdick & Jackson (Muskegon, MI). Odorants were purified by preparative gas chromatography (Varian 3700 GC, Walnut Creek, CA) using a glass packed column (250 × 0.5 cm, i.d., packed with 1% Carbowax 20 M on 120–140 mesh Chromosorb G). (*Z*)-1,5-Octadien-3-one was synthesized following procedures described by Swoboda and Peers (*17*). 1-Nitro-2-phenylethane was prepared according to the method of Kornblum et al. (*18*) by reaction of 2-phenylethyl bromide with sodium nitrite in dimethylformamide and urea. Diethyl ether was freshly distilled through a 60 cm long Pyrex column packed with glass helices and stored in the dark after the addition of 1–2 mg/L of antioxidant 330 (1,3,5-trimethyl-2,4,6-tris[3,5-di-*tert*-butyl-4-hydroxybenzyl]benzene; Ethyl Corp., Richmond, VA).

Synthesis of *trans*- and *cis*-4,5-Epoxy-(*E*)-2-decenal. The isomer mixture of (*E*,*E*)- and (*E*,*Z*)-2,4-decadienal was separated by preparative HPLC on a silica gel column (Dynamax, 21.4 × 250 mm, 8 μm; Varian, Inc., Walnut Creek, CA). *trans*- and *cis*-4,5-epoxy-(*E*)-2-decenal were synthesized by epoxidation of (*E*,*E*)- and (*E*,*Z*)-2,4-decadienal, respectively, using 3-chloroperbenzoic acid (*19*).

Sensory Evaluation. All sensory evaluations were performed in a specially designed room, which provided space for four panelists at a time in four separate booths divided by vertical walls. Orange lights were used to illuminate the sensory evaluation room to minimize the influence of color on the panelists' perceptions. The samples were presented to the panelists through sliding doors between the sensory preparation room and each of the four booths.

Training of the Sensory Panel. The sensory panel consisted of 16 panelists (6 women and 10 men between the ages of 30 and 70) recruited from the Western Regional Research Center in Albany, CA. Although most assessors had previous sensory panel experience, all panelists were trained during a two-week period in daily sessions at the beginning of each tomato season to familiarize them with different odor qualities and important flavor compounds of fresh tomato. In a preliminary session the panelists were asked to describe the odor of a fresh tomato in their own words using flavor attributes and odor qualities that came into their minds when smelling a fresh tomato sample. Of those odor qualities, the most often mentioned ones were chosen for the following flavor profile analyses: sweet, green/grassy, fruity, floral/flowery, sour/acidic. Aqueous solutions of important fresh tomato odorants at concentrations 100 times above their odor threshold in water were presented together with an odor description to the panelists to acquaint them with the odor qualities expected from a fresh tomato. The sample solutions together with their odor description were presented to the panelists, six at a time, at four sessions on four consecutive days. The panelists had to recognize these odors in the following sessions without being given the odor description. Thereafter, they had to recognize six odors at a time in five sessions on five consecutive days.

Flavor Profile Analysis—Fresh Tomato Samples. After completion of the training, the panelists had to evaluate the flavor profiles of five different freshly picked field tomato cultivars, about six times per season. The panelists were asked to rate the intensities of the odor qualities they had previously chosen (sweet, green/grassy, fruity, floral/flowery, sour/acidic) on a category scale from 0 (not perceptible) to 3 (strongly perceptible) in increments of 0.5. They also had to rank the tomatoes in order of preference from 1 (most preferred) to 5 (least preferred). The tomatoes were freshly cut in eighths immediately prior to evaluation, and four pieces of each cultivar were presented in a glass or styrofoam container covered with aluminum foil. The flavor profiles of the five tomato cultivars were compared for significant differences in each of the five odor qualities by statistical analysis using the SAS GENMOD procedure (*20*).

Flavor Profile Analysis—Aroma Model—Fresh Tomato Comparison. Aroma models of all five tomato cultivars were prepared by dissolving determined volumes of concentrated stock solutions of the odorants in 0.5 L of water and adjusting the pH to 4.3 by the addition of citric acid/sodium carbonate. The concentration of 1-penten-3-one and β-damascenone added to the different aroma models was reduced to 50% compared to the concentration determined, because the odor of those two compounds was perceived as too dominant in the models. This was done because the models lacked a matrix, and therefore the vapor pressures of some of the odorants in the aqueous models are probably different from the vapor pressures in a real tomato. For those two compounds the vapor pressures are probably higher in the model than in a tomato, so they are perceived more intensely in the model. To compensate for the lack of a matrix, the concentrations of those two compounds in the models were reduced to 50% of the determined concentrations. All stock solutions were prepared in absolute ethanol (200 proof) with the exceptions of (*Z*)-3-hexenal, which was dissolved in pentane, and the 4,5-epoxy-(*E*)-2-decenal isomers, which were dissolved in pentane/diethyl ether 50:50. When the models were prepared, the odorants dissolved in pentane [the 4,5-epoxy-(*E*)-2-decenal isomers and (*Z*)-3-hexenal] were added first into the container, and then the solvent was evaporated very carefully at 38 °C. Water was added, followed by all of the other odorants (which were dissolved in small amounts of ethanol). The aroma models were compared to their corresponding real tomato sample by presenting 2 mL of the model solution and 2 mL of tomato juice (prepared by squeezing tomato pieces and recovering the juice plus pieces of pulp; the samples were prepared immediately prior to sensory evaluation) in two red tubes. The panelists were asked to dip an aroma-testing paper strip (Measureline, 6 × 0.25 in., Orlandi Inc., Farmingdale, NY) into each tube, smell the paper strip, and score the intensities of the chosen odor qualities, that is, sweet, green/grassy, fruity, floral/flowery, sour/acidic, on a category scale from 0 (not perceptible) to 3 (strongly perceptible) in increments of 0.5. The intensity scores of all odor qualities were compared between the model and the real tomato for significant differences using the *F* test and paired *t* test.

Sample Preparation. Sample preparation was carried out using three previously described methods (*5, 7, 21–23*). Four tomatoes of each cultivar were cut in eighths, and one-eighth of each tomato was randomly sampled and weighed. The total amount of the four tomato pieces for analysis was 30–35 g. The number of tomato pieces sampled sometimes varied from four depending on the tomato size. For example, BR-139 is a cherry tomato, and more pieces were needed for the 30–35 g sample size. The tomato pieces were blended for 30 s in a Waring blender. The blended tomatoes were allowed to stand at room temperature for 3 min to allow enzymatic generation of flavor constituents. After 3 min, the enzyme activity was stopped by the addition of saturated calcium chloride solution [volume (mL) = weight of tomato sample (g)] and blending for 10 s (calcium chloride and SAFE method, see below) or by the addition of 240 g of anhydrous sodium sulfate and thorough mixing by blending and by stirring with a glass rod (sodium sulfate method, see below). One milliliter of an aqueous solution of 3-hexanone, 2-octanone, anethole, and maltol (internal standards) in a concentration range between 10 and 100 mg/L was added to the mixture, which was then blended again for 10 s.

*Dynamic Headspace Calcium Chloride Method (**5, 7, 21**).* The mixture of blended tomatoes, saturated calcium chloride solution, and internal standards was added to a 1 L round-bottom flask. A Tenax trap (10 g of Tenax in a glass column 14 × 2.2 cm) was attached to the flask, and an all-Teflon diaphragm pump (model UN726 FTP, KNF Neuberger, Inc., Trenton, NJ) was connected (via Teflon tubing) after the trap. The system was flushed with nitrogen for 2 min, and then the loop was closed by connecting the outlet of the pump to the 1 L flask. The pump circulated nitrogen at a flow rate of ~6 L/min through the system for 3 h. The Tenax trap was removed and eluted with 60 mL of diethyl ether. The eluate was concentrated to a final volume of about 100 μL using a Vigreux column (15 × 1 cm) and water bath at 40 °C. The extract was analyzed by GC-O and GC-MS.

Dynamic Headspace Sodium Sulfate Method (22). The mixture of the blended tomatoes, sodium sulfate, and internal standards was added to a glass column (30 × 3 cm). A Tenax trap (10 g of Tenax in a glass column 14 × 2.2 cm) was attached, and an all-Teflon diaphragm pump (same as above) was connected (via Teflon tubing) after the trap. The system was flushed with nitrogen for 2 min, and then the loop was closed by connecting the outlet of the pump to the other end of the glass column. Nitrogen, at a flow rate of ~6 L/min, was pumped through the system for 3 h. The Tenax trap was removed and eluted with diethyl ether (60 mL). The eluate was concentrated to a final volume of about 100 μL using a Vigreux column (15 × 1 cm) and water bath at 40 °C. The extract was then subjected to GC-O and GC-MS analyses.

Solvent-Assisted Flavor Evaporation [SAFE (23)]. The SAFE apparatus was heated to 40 °C with a circulating water bath, and the mixture of blended tomatoes, saturated calcium chloride solution, and internal standards was added to the dropping funnel of the apparatus. The distillation flask (500 mL) was heated to 40 °C in a water bath. The receiving flask for the distillate as well as the safety-cooling trap of the SAFE apparatus was cooled with liquid nitrogen. The SAFE apparatus was connected to a high-vacuum pump (<0.01 Pa), and then the mixture in the dropping funnel was added in small aliquots into the distillation flask over 20 min. The distillate was thawed at room temperature and then extracted with diethyl ether (2 × 30 mL). After the addition of brine (25 mL), the distillate was again extracted with diethyl ether (2 × 30 mL). The combined ether extract was dried over anhydrous sodium sulfate and then concentrated to about 100 μL using a Vigreux column (15 × 1 cm) and water bath at 40 °C. The extract was used for GC-O and GC-MS analyses.

Addition of Labeled Linoleic Acid-$^{13}C_{18}$ to Tomatoes. Hothouse tomatoes purchased from a local supermarket (Safeway) were prepared according to the procedures described under Sample Preparation. Labeled linoleic acid-$^{13}C_{18}$ (4.5 mg; minimum 99 atom %^{13}C) and internal standards were added to the tomato sample (30 g) prior to blending. In contrast to the previous experiments, no saturated calcium chloride solution was added and the mixture was subjected to the SAFE method.

Addition of Labeled L-Methionine-$^{13}C_1$ to Tomatoes. R 144 tomatoes were prepared according to the procedures described under Sample Preparation. Labeled L-methionine-$^{13}C_1$ (12 mg; methyl-^{13}C; minimum 99 atom % ^{13}C) was added to the tomato sample (35 g) prior to blending. The mixture was blended for 30 s. After 5 min, enzyme activity was stopped by the addition of saturated calcium chloride solution. After the addition of internal standards, the mixture was subjected to the SAFE method.

Aroma Extract Dilution Analysis [AEDA (*24*)]. An aliquot of the extract obtained from each cultivar (prepared by SAFE method without the addition of the internal standards) was analyzed by GC-O. The extract was then diluted to twice its starting volume with diethyl ether and analyzed again by GC-O. This procedure was repeated until no odor active compounds could be detected. GC-O analysis was performed on DB-Wax and DB-1 fused silica capillary columns [60 m × 0.32 mm (i.d.), d_f = 0.25 μm; J&W Scientific, Folsom, CA] installed into two HP 5890 gas chromatographs. At the outlet of the capillary column the effluent was split 1:1 between a flame ionization detector (FID) and a sniffing port using a fused silica "Y" connector (Supelco, Bellefonte, PA) and deactivated fused silica capillary tubing [30 cm × 0.25 mm (i.d.), J&W Scientific]. The temperature program for GC-O on the DB-Wax column was as follows: the temperature was programmed from 30 °C (4 min isothermal) to 170 at 8 °C/min (held for 25 min), increased by 10 °C/min to 210 °C, and held for 10 min. For GC-O on the DB-1 column the GC oven was programmed from 30 °C (4 min isothermal) to 230 at 6 °C/min (final hold = 10 min).

Quantification of Fresh Tomato Odorants by GC-MS. *Instrumentation.* Fresh tomato odorants in the aroma extracts were quantified by GC-MS analyses. Two different GC-MS systems were used. The first system consisted of an HP 6890 gas chromatograph coupled to an HP 5973 MSD (Hewlett-Packard, Avondale, PA). A 60 m × 0.25 mm (i.d.) DB-1 fused silica capillary column (d_f = 0.25 μm) was employed. The temperature program for the GC oven was 30 °C (4 min isothermal) to 230 at 3 °C/min (final hold = 10 min). The second system consisted of an Agilent Technologies 6890 gas chromatograph coupled to an Agilent Technologies 5973 Network MSD (Agilent Technologies, Palo Alto, CA). A 60 m × 0.25 mm (i.d.) DB-Wax fused silica capillary column was used (d_f = 0.25 μm). The GC oven was programmed from 30 °C (4 min isothermal) to 170 at 2 °C/min (hold for 25 min) and then raised to 210 at 10 °C/min (final hold = 10 min). Both systems utilized helium as the carrier gas. The concentration of each odorant was calculated by comparing the areas of certain characteristic fragment ions of the odorants with that of a certain internal standard (*5, 7*). The following internal standards were used: (i) 3-hexanone was used for the quantification of the C_4, C_5, and C_6 compounds; (ii) 2-octanone was used for 1-octen-3-one, methional, and 2-isobutylthiazole; (iii) maltol was used for the determination of the concentration of 4-hydroxy-2,5-dimethyl-3-(2*H*)-furanone; and (iv) anethole was used for all of the other compounds. MS data were recorded in the electron impact mode with an ionization voltage of 70 eV. The differences in the ion intensities of the odorants and internal standards were corrected by determining MS response factors. Therefore, known amounts of the odorants and internal standards were mixed and injected into the GC-MS systems. The response factors were calculated by comparing the area of the fragment ion of the odorant with the area of the fragment ion of the standard with their concentrations.

Recovery Values. Recovery values of the fresh tomato odorants and internal standards were determined by adding known amounts of all of the compounds to blended green tomatoes and preparing this sample the same way as the fresh tomato samples. The concentrations of all odorants and internal standards were determined against 2-undecanone, which was added at the end of sample preparation just before GC-MS analysis. Quantification was done by comparing the areas of certain characteristic mass fragment ions of the compounds (*5*) with that of 2-undecanone (*m*/*z* 170) including correction with the MS response factors between the compound ion and the 2-undecanone ion.

A background concentration of the odorants contained in green tomatoes was also determined by analyzing the same green tomato mix without the addition of odorants and standards. The concentrations were also calculated against 2-undecanone, which was added to the aroma extract at the end of sample preparation. These background concentrations were taken into consideration in the calculation of the recovery values by subtracting the background level from the concentration found after the addition of the compounds. The recovery values were determined in this way for all odorants and internal standards for all three sample preparation methods (*5*).

RESULTS AND DISCUSSION

After an independent tomato flavor testing with 30 consumers, five different fresh tomato cultivars, three tasty (BR-139, FA-612 and FA-624) and two less tasty (R-144 and R-175), were chosen for detailed investigation to get an insight as to whether differences in odorant composition and concentration can be related to preference ranking. Hedonic acceptance tests for overall flavor (odor, taste, texture, etc.) done on these cultivars with human subjects indicated that tomato cultivars BR-139, FA-612, and FA-624 are more preferred than cultivars R-144 and R-175 (Ben-Oliel et al., unpublished data). All five cultivars were grown outdoors on a research farm in Manteca, CA, during the years of 2000−2002 and provided fruits from July until late October. Before starting with chemical and instrumental analysis, sensory evaluation of the five cultivars was repeated with a trained sensory panel at our laboratory. Our panel's preference ranking was identical to the consumers' ranking: BR-139 > FA-624 > FA-612 > R-144 > R-175.

Although 95% of ripe tomato fruit is water (*25*), there is considerable flexibility available to manipulate the major components that affect the taste of the fruit. Eight percent of the dry matter is minerals, and >50% of the difference is reducing sugars (glucose and fructose) and about 12% is organic acids (*26*). The less preferred tomato cultivars, R-144 and R-175, had lower dry matter contents and less sugars (**Table 1**).

Our panel scored the intensities of certain odor qualities, typical for fresh tomato flavor, to get flavor profiles of the five different fresh tomato cultivars (see Materials and Methods). The flavor profiles are shown in **Figure 1**. Significant differences in certain odor qualities among the five cultivars (according to the SAS GENMOD procedure) are detailed in **Table 2**.

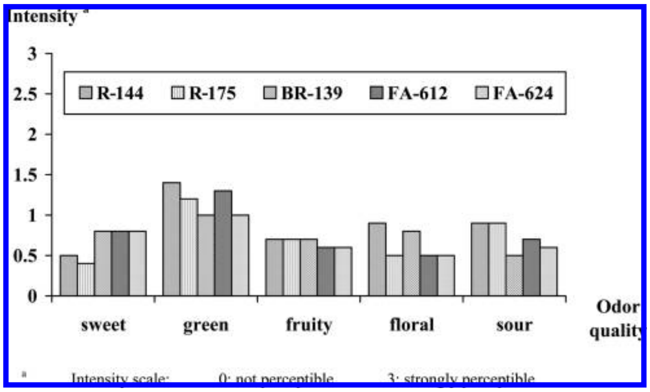

Figure 1. Flavor profiles of the five investigated tomato cultivars.

Table 2. Significant Differences in the Intensities of Certain Odor Qualities among the Five Investigated Tomato Cultivars (SAS GENMOD Procedure)

	pairs with a significant difference at			
odor quality	$p < 0.05$		$p < 0.1$	
sweet	144/139 175/139 175/612	144/624 175/624		
green	144/139 612/139	144/624 612/624	175/139	175/624
fruity				
floral	144/175			144/624
sour	144/139	175/139	144/624 612/139	175/624

The R-144 and R-175 tomato cultivars were rated significantly less sweet than the BR-139, FA-612, and FA-624 cultivars. The intensity scores for the green odor quality were significantly higher for R-144 and R-175 but also for FA-612 compared to the BR-139 and FA-624 cultivars. No significant differences were found for the fruity note. The R-144 and BR-139 cultivars were rated more floral than the other cultivars, but the only significant differences were between the R-144 and the R-175 and FA-624 tomatoes. The intensities of the sour odor quality of the R-144 and R-175 cultivars were significantly higher than those of the BR-139 and FA-624 cultivars; the FA-612 tomato was also rated significantly more sour than the BR-139 tomato. In summary, the less preferred tomato cultivars, R-144 and R-175, were rated less sweet, more green, and more sour than the other cultivars, with the R-144 tomato also having a more intense floral note. The more preferred cultivars, BR-139 and FA-624, in contrast, showed a higher intensity of the sweet and lower intensities of the green and sour odor qualities. The other highly accepted cultivar, FA-612, was somehow in between; its sweetness was rated similar to those of the BR-139 and FA-624 tomato, but the green and sour odor qualities were scored slightly higher, closer to the R-144 and R-175 cultivars.

AEDA. AEDA was performed on all five fresh tomato cultivars, and the results were compared with previously published data (*4, 21, 27–29*). We found the same odorants in the five investigated cultivars (**Table 3**) that others had previously found in fresh tomato, with three exceptions. Of the 19 flavor compounds we perceived in the samples here, 11 were already described by Buttery et al. (*21, 27*). Among those are (*Z*)-3-hexenal, β-ionone, hexanal, β-damascenone, 1-penten-3-one, 3-methylbutanal, (*E*)-2-hexenal, phenylacetaldehyde, 2-phenylethanol, 2-isobutylthiazole, and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol). Krumbein and Auerswald (*29*) used AEDA to find three other compounds, 1-octen-3-one, methional, and (*E*,*E*)-2,4-decadienal. Additionally, Guth and Grosch (*4*) used AEDA to characterize 3-methylbutyric acid and *trans*-4,5-epoxy-(*E*)-2-decenal as important fresh tomato odorants. The three compounds not previously mentioned as important contributors to fresh tomato aroma, but which we could perceive,

Table 3. Aroma Extract Dilution Analysis (AEDA) of Five Different Tomato Cultivars (Using the SAFE Method for Sample Preparation)

odorant	Kovats index on DB-Wax	flavor dilution factor				
		R-144	R-175	BR-139	FA-612	FA-624
3-methylbutanal	914	2	4	1	8	2
1-penten-3-one	1016	16	16	32	16	32
hexanal	1077	64	32	32	32	64
(*Z*)-3-hexenal	1135	1024	1024	2048	2048	2048
(*E*)-2-hexenal	1214	4	4	8	4	8
1-octen-3-one	1297	32	64	32	32	32
(*Z*)-1,5-octadien-3-one	1380	32	32	32	32	32
2-isobutylthiazole	1396	1	8	1	2	1
methional	1446	256	128	128	256	128
phenylacetaldehyde	1636	32	4	8	8	16
3-methylbutanoic acid	1680	8	16	8	8	8
(*E*,*Z*)-2,4-decadienal	1765	8	8	8	16	16
(*E*,*E*)-2,4-decadienal	1808	8	8	8	16	16
β-damascenone	1819	1024	1024	1024	1024	1024
2-phenylethanol	1910	32	16	16	16	16
β-ionone	1939	16	16	16	16	16
cis-4,5-epoxy-(*E*)-2-decenal	2000	16	16	16	16	16
trans-4,5-epoxy-(*E*)-2-decenal	2020	1024	1024	1024	1024	2048
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	2037	128	128	512	256	512

were (*E*,*Z*)-2,4-decadienal, *cis*-4,5-epoxy-(*E*)-2-decenal, and (*Z*)-1,5-octadien-3-one. The latter compound was recently identified as a fresh tomato odorant for the first time (*5*). In contrast, some other compounds previously reported as fresh tomato odorants could not be detected (by AEDA) in our samples, for example, 1-nitro-2-phenylethane, 6-methyl-5-hepten-2-one, or methyl salicylate (*21, 27*). Eugenol, reported by Guth and Grosch (*4*), could not be perceived in the present samples, but was detected in some supermarket hothouse tomatoes (results not shown). AEDA revealed no large differences among the five tomato cultivars. The same 19 compounds were perceived as the most important odorants in all five samples. Because the error range of the method is within one step up or down, a large difference in odorant concentration would be expected only if the dilution factors differ by a factor of 4 or more. With this consideration in mind, the FA-612 cultivar might have the highest concentration of 3-methylbutanal, whereas the BR-139 cultivar possibly has the lowest. 2-Isobutylthiazole seems to play a more important role in the flavor of the R-175 cultivar than for the other cultivars. The flavor dilution factors for phenylacetaldehyde and 2-phenylethanol were higher for the R-144 tomato than for the other tomatoes, so the R-144 cultivar probably contains higher amounts of those two compounds than the other cultivars. The BR-139 and FA-624 tomatoes had higher flavor dilution factors for 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol) than the R-144 and R-175 tomatoes, so the first two cultivars might contain higher concentrations of this compound than the latter two. For all of the other compounds perceived, the differences in flavor dilution factors were within the error range of the method, so it was not possible to draw further conclusions about differences in concentrations of these odorants in the five fresh tomato cultivars.

Quantification of Key Odorants. After comparing recovery values of the three sample preparation methods, we selected the SAFE method for the quantification studies. Quantitative results for the 19 odorants detected by AEDA are presented in **Table 4**. 1-Nitro-2-phenylethane was included (although it was not detected by AEDA) because its reported odor threshold was quite low (2 ppb) and its expected concentrations were higher than the reported odor threshold. (*Z*)-1,5-Octadien-3-one was not included (although it was perceived in all five cultivars by AEDA) because it could not be quantified with the usual amount of sample used for analysis (35 g) due to its low concentration. For its identification, about 900 g of tomatoes was needed along with preparative GC to get a clear mass spectrum of (*Z*)-1,5-octadien-3-one. The estimated concentration of (*Z*)-1,5-octadien-3-one in fresh tomato was <0.1 μg/kg (*5*). The values represent the mean of four samples. Each tomato cultivar was usually analyzed six times per season, but the results for the first and last sample of the season were not included. Early in the season the flavor was not yet fully developed, and very late in the season the tomatoes were already past their peak. The flavor of these samples did not match expectations and was different from the tomatoes picked during the rest of the season. Nevertheless, the standard deviation for some of the odorants was quite high as might be expected for a natural product. The 2 week interval in the time of harvest during the season influenced the amount of soluble solids and possibly flavor (precursor) production in the tomatoes.

The quantitative results confirmed the concentration differences that were observed between the cultivars in AEDA. The FA-612 cultivar contained the highest amount of 3-methylbutanal, whereas the BR-139 cultivar had the lowest amount. The concentration of 2-isobutylthiazole was the highest in the R-175 cultivar. The R-144 cultivar had 3−8 times higher concentrations of phenylacetaldehyde and contained 4−6 times more 2-phenylethanol than the other cultivars. The concentration of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone in the BR-139, FA-612, and FA-624 cultivars was about 200 μg/kg, 2−7 times higher than in the R-144 and R-175 cultivars. The amounts of methional varied between 3 μg/kg (BR-139) and 30 μg/kg (R-144). Large concentration differences between the five cultivars were found for 3-methylbutanoic acid and the (*E*,*E*)- and (*E*,*Z*)-2,4-decadienal isomers that were not that obvious by AEDA. The content of 3-methylbutanoic acid was the highest in the R-175 cultivar, followed by the R-144 and FA-612 cultivars. Its concentration was 3−6 times lower in the BR-139 and FA-624 cultivars. The FA-624 tomato contained large amounts of the two decadienal isomers, 5−9 times more than the R-144 tomato. The FA-612 tomato also had twice as much of the decadienal isomers as the R-175 and BR-139 tomatoes. The differences in concentration of the other odorants in the five cultivars were rather small. The BR-139 and FA-624 cultivars had 1-penten-3-one concentrations 2−2.7 times higher than that of the R-144 and R-175 cultivars. The hexanal content in all five cultivars was between 1 and 1.9 mg/kg. The R-175 cultivar had the lowest concentration (4.9 mg/kg) of (*Z*)-3-hexenal, whereas the FA-624 cultivar (8.5 mg/kg) had the highest concentration. All cultivars contained between 80 μg/kg (R-175) and 180 μg/kg (BR-139) of (*E*)-2-hexenal and 4–6 μg/kg of 1-octen-3-one. The amount of β-damascenone in the five cultivars varied between 2 and 4 μg/kg, whereas the β-ionone content ranged from 7 μg/kg in the R-144 cultivar to 20 μg/kg in the FA-624 cultivar. The concentrations of the *cis*- and *trans*-4,5-epoxy-(*E*)-2-decenal in all five fresh tomato cultivars was between 110 and 160 μg/kg for the cis isomer and between 350 and 630 μg/kg for the trans isomer.

The quantitative results were in good accordance with the results of the flavor profile analyses. The higher intensity of the sweet odor quality in the more preferred tomato cultivars BR-139, FA-612, and FA-624 could be correlated to the higher amounts of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol). The more intense floral note in the R-144 tomato was caused

Table 4. Concentrations of Important Odorants in Five Different Fresh Tomato Cultivars

	concentration[a] (μg/kg)				
odorant	R-144	R-175	BR-139	FA-612	FA-624
3-methylbutanal	78 ± 36	130 ± 80	19 ± 7	170 ± 80	21 ± 15
1-penten-3-one	250 ± 70	210 ± 50	500 ± 100	370 ± 160	560 ± 40
hexanal	1600 ± 600	1500 ± 700	1000 ± 200	1100 ± 600	1900 ± 300
(*Z*)-3-hexenal	5200 ± 2500	4900 ± 2800	6500 ± 800	6700 ± 3600	8500 ± 1100
(*E*)-2-hexenal	110 ± 50	79 ± 36	180 ± 40	130 ± 70	140 ± 10
1-octen-3-one	5 ± 2	6 ± 1	6 ± 1	4 ± 1	5 ± 1
2-isobutylthiazole	160 ± 30	350 ± 80	160 ± 80	250 ± 90	130 ± 40
methional	30 ± 30	14 ± 8	3 ± 2	16 ± 7	7 ± 3
phenylacetaldehyde	860 ± 360	190 ± 150	110 ± 60	230 ± 130	260 ± 180
3-methylbutanoic acid	550 ± 110	930 ± 720	180 ± 200	600 ± 400	150 ± 70
(*E*,*Z*)-2,4-decadienal	13 ± 5	40 ± 23	45 ± 25	98 ± 21	120 ± 30
(*E*,*E*)-2,4-decadienal	5 ± 2	8 ± 3	6 ± 3	14 ± 4	26 ± 11
β-damascenone	2 ± 1	4 ± 2	2 ± 1	3 ± 1	3 ± 1
2-phenylethanol	2300 ± 1000	500 ± 400	390 ± 200	550 ± 260	580 ± 330
β-ionone	7 ± 5	14 ± 9	12 ± 6	9 ± 3	20 ± 2
cis-4,5-epoxy-(*E*)-2-decenal	150 ± 90	150 ± 40	160 ± 50	110 ± 20	160 ± 30
trans-4,5-epoxy-(*E*)-2-decenal	570 ± 250	620 ± 150	630 ± 140	350 ± 40	650 ± 150
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	27 ± 11	84 ± 21	200 ± 20	190 ± 120	190 ± 70
1-nitro-2-phenylethane	190 ± 30	110 ± 40	22 ± 14	150 ± 60	100 ± 70

[a] Average of four samples including standard deviation.

by distinctly higher concentrations of phenylacetaldehyde and 2-phenylethanol. The R-144 and R-175 cultivars were perceived to have higher intensities of green odor compared to the BR-139 and FA-624 cultivars, although the latter two contained more (*Z*)-3-hexenal. This may be explained by lower concentrations of some other odorants in the first two mentioned cultivars, so that the green note was perceived much more intensely, whereas in the last two mentioned cultivars higher amounts of other odorants masked the green note, making it harder to perceive. The higher levels of 2-isobutylthiazole in the R-175 tomato might also contribute the higher intensity of the green odor quality.

Table 5. Comparison of Odor Units for Important Fresh Tomato Odorants in Five Different Cultivars

	odor units[a]				
odorant	R-144	R-175	BR-139	FA-612	FA-624
3-methylbutanal	390	650	95	850	105
1-penten-3-one	250	210	500	370	560
hexanal	356	333	222	244	422
(*Z*)-3-hexenal	20800	19600	26000	26800	34000
(*E*)-2-hexenal	6	5	11	8	8
1-octen-3-one	1000	1200	1200	800	1000
2-isobutylthiazole	35	76	35	54	28
methional	150	70	15	80	35
phenylacetaldehyde	215	48	28	58	65
3-methylbutanoic acid	2	4	<1	2	<1
(*E*,*Z*)-2,4-decadienal	118	364	409	891	1091
(*E*,*E*)-2,4-decadienal	45	73	55	127	236
β-damascenone	1000	2000	1000	1500	1500
2-phenylethanol	34	7	6	8	9
β-ionone	1000	2000	1714	1286	2857
cis-4,5-epoxy-(*E*)-2-decenal	nd[b]	nd	nd	nd	nd
trans-4,5-epoxy-(*E*)-2-decenal	28500	31000	31500	17500	32500
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	1	4	10	9	9
1-nitro-2-phenylethane	2	<1	<1	1	<1

[a] Odor units = average concentration of four samples/odor threshold in water. [b] Not determined; no odor threshold available.

Odor Units. To gain additional insight into what compounds are the most important contributors to fresh tomato aroma and what odorants are responsible for the differences in flavor among the five cultivars, odor units (odor activity values) were calculated by dividing each odorant's concentration by its odor threshold in water (the water content of tomatoes is about 95%). The odor thresholds were previously reported by Mayer et al. (*7*) with the exception of 1-nitro-2-phenylethane, which was determined to be 120 μg/L water [in contrast to the 2 μg/L reported previously (*30*)]. The calculated odor units are shown in **Table 5**. In all investigated fresh tomato cultivars (*Z*)-3-hexenal and *trans*-4,5-epoxy-(*E*)-2-decenal were by far the most important odorants, with odor units between 17500 and 34000. It is noteworthy that *trans*-4,5-epoxy-(*E*)-2-decenal had higher odor units than that of (*Z*)-3-hexenal in three of the cultivars, whereas their values were similar in FA-624. *cis*-4,5-Epoxy-(*E*)-2-decenal is also thought to be a significant contributor to fresh tomato aroma, although its concentration was 3.1−4.2 times lower than that of *trans*-4,5-epoxy-(*E*)-2-decenal in the five tomato cultivars. Whereas the odor threshold of the trans isomer has been determined to be 0.02 μg/L in water (*7*) and 0.6–2.5 pg/L air (*8*), the threshold of the cis isomer has not yet been determined and, hence, odor units were not calculated. β-Ionone, β-damascenone, and 1-octen-3-one followed with odor units between 800 and 2857. In the more preferred FA-624 and FA-612 cultivars (*E*,*Z*)-2,4-decadienal had an odor unit value of 891−1091, whereas in the less preferred R-144 and R-175 cultivars its odor unit value varied between 118 and 364. (*E*,*E*)-2,4-Decadienal had higher odor units in the FA-624 and FA-612 cultivars than in the other three cultivars. The odor units for 1-penten-3-one were higher in the FA-624, FA-612, and BR-139 tomatoes than in the R-144 or R-175 tomatoes. 3-Methylbutanal had quite high odor units in the FA-612 and R-175 cultivars, with values of 850 and 650, respectively. These values were up to 8 times lower in the BR-139 and FA-624 cultivars. Hexanal had odor units of 222−422 in all five cultivars. In the R-144 cultivar phenylacetaldehyde had an odor unit value of 215, whereas its value in the other four cultivars was 3−7 times lower. The odor unit value of 2-phenylethanol was 4−6 times higher in the R-144 cultivar than in the other cultivars. The odor units of methional were the highest in the R-144 cultivar as well. In contrast, 2-isobutylthiazole had the highest odor units in the R-175 tomato compared to the other cultivars. 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone had odor units of only 1−10, but they were higher in the more preferred BR-139, FA-612, and FA-624 cultivars than in the less preferred R-144 and R-175 cultivars. The odor units of (*E*)-2-hexenal were

Table 6. Recipes for the Preparation of Fresh Tomato Aroma Models

odorant	*C* (stock solution) (μg/mL)	V^a (μL)				
		R-144	R-175	BR-139	FA-612	FA-624
3-methylbutanal	315	124	206	30	270	33
1-penten-3-one	3380	37^b	31^b	74^b	55^b	83^b
hexanal	3300	242	227	152	167	288
(*Z*)-3-hexenal	10000	260	245	325	335	425
(*E*)-2-hexenal	6500	8	6	14	10	11
1-octen-3-one	32	78	94	94	63	78
2-isobutylthiazole	700	114	250	114	179	93
methional	495	30	14	3	16	7
phenylacetaldehyde	1040	413	91	53	111	125
3-methylbutanoic acid						
(*E*,*Z*)-2,4-decadienal	1700	4	12	13	29	35
(*E*,*E*)-2,4-decadienal	186	13	22	16	38	70
β-damascenone	27	37^c	74^c	37^c	55^c	55^c
2-phenylethanol	10700	107	23	18	26	27
β-ionone	820	4	9	7	5	12
cis-4,5-epoxy-(*E*)-2-decenal	30	2500	2500	2660	1830	2660
trans-4,5-epoxy-(*E*)-2-decenal	180	1580	1720	1750	970	1800
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	2038	7	21	49	47	47

[a] Volume (μL) of stock solution added to 500 mL of H_2O, pH adjusted to 4.3 with citrate buffer. [b] Volume reduced to 50%. [c] Volume reduced to 50% (see explanation in text).

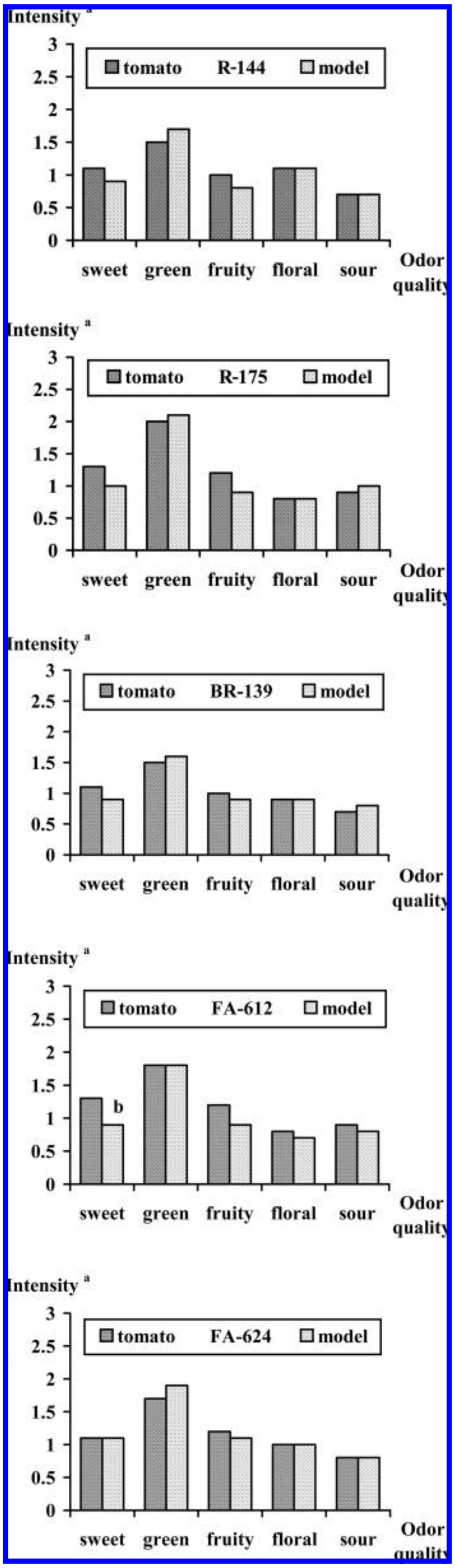

Figure 2. Comparison of the flavor profiles of five different fresh tomato cultivars and their corresponding aroma models. [a]Intensity scale: 0, not perceptible; 3, strongly perceptible. "b" indicates a significant difference in intensity ($p < 0.05$).

also low, although there were no large differences between the cultivars. 3-Methylbutanoic acid had odor units that were <1 in the BR-139 and FA-624 tomatoes, whereas the other three cultivars had odor units of only 2−4.

Comparison of Fresh Tomato Cultivars to their Corresponding Aroma Model. To confirm that all important odorants of fresh tomato aroma were identified, that their concentrations were determined correctly, and that the differences among the five cultivars were caused by concentration differences, aroma model solutions for the five cultivars were prepared by combining the odorants in the determined concentrations. Each aroma model was compared to its corresponding fresh tomato. The aroma models were prepared in citrate buffer at pH 4.3 according to the recipes listed in **Table 6** (using the concentrations shown in **Table 4**). (*Z*)-1,5-Octadien-3-one was not included in the models because it was found in a separate experiment that our panel could not distinguish between a model mixture containing (*Z*)-1,5-octadien-3-one and another model in which this compound was omitted (data not shown). The aroma model mixture and corresponding tomato juice (prepared immediately prior to sensory evaluation by squeezing fresh tomato pieces) were presented in red tubes. The sensory evaluation results of the aroma models of the tomato cultivars compared to the corresponding fresh tomato samples are shown in **Figure 2**. It is evident that the flavor profiles of all aroma models were very similar to the flavor profiles of the fresh tomato samples. Statistical evaluation of the results revealed no significant differences between the models and the fresh tomatoes in any of the odor qualities except one. The intensity of the sweet odor quality of the FA-612 tomato was significantly different ($p < 0.05$) from the model. The problems of comparing a liquid aroma model to a real tomato for similarity leaves some room for further improvement, especially in finding ways to simulate the tomato matrix to create realistic conditions for the distribution of odorants between the matrix phase and the vapor phase. We tried to compensate for differences in vapor pressure between the aroma models and the fresh tomatoes by adjusting the concentrations of the two most dominantly perceived odorants in the models, 1-penten-3-one and β-damascenone. Recent investigations (*31*) have shown that there is a difference in odorant amounts released into the headspace from a buffer solution compared to tomato matrix. The release of odorants from a buffer solution is generally higher than from tomato matrix. Therefore, when an aroma model is prepared in a buffer

Table 7. Concentrations of Important Odorants in Tomato with Labeled $[^{13}C]$Linoleic Acid Added

	concentration (μg/kg)		
odorant	unlabeled (U)	labeled (L)	ratio (U/L)
(*Z*)-3-hexenal	4000		
(*E*)-2-hexenal	225		
hexanal	925	173	5.3:1
(*E*,*Z*)-2,4-decadienal	17	341	1:20
(*E*,*E*)-2,4-decadienal	2	15	1:7.5
cis-4,5-epoxy-(*E*)-2-decenal	35	54	1:1.5
trans-4,5-epoxy-(*E*)-2-decenal	77	113	1:1.5

solution, the concentration of many odorants should be lower compared to the determined concentrations in tomatoes. For example, the amounts of (*E*,*E*)-2,4-decadienal, β-damascenone, β-ionone, and 1-penten-3-one released into the headspace from tomato matrix were 6.5-, 2.3-, 3.3-, and 1.7-fold lower, respectively, compared to that released from buffer solution (*31*). Therefore, reducing the concentrations of many odorants by certain factors would probably further improve the aroma models by compensating for the differences in vapor pressure of these odorants in the buffer solution compared to real tomato matrix.

Nevertheless, by reducing the concentrations of the two most dominantly perceived odorants, β-damascenone and 1-penten-3-one, the flavor profiles of the models and their corresponding fresh samples were very similar. The sensory evaluations confirmed the results of instrumental analysis; all important odorants for fresh tomato aroma were identified, and odorant concentrations were determined correctly. We showed that the differences among the five cultivars that are responsible for differences in preference are due to variations in the concentrations of certain flavor compounds. Higher amounts of the (*E*,*E*)- and (*E*,*Z*)-2,4-decadienal isomers and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol) had a positive influence on preference, whereas high concentrations of methional, phenylacetaldehyde, 2-phenylethanol, or 2-isobutylthiazole had a negative influence.

Formation of 4,5-Epoxy-(*E*)-2-decenal and Methional in Tomato. Labeled $[^{13}C]$linoleic acid (4.5 mg) was added to fresh tomatoes (30 g) and internal standards, and the resulting mixture was blended in a Waring blender for 30 s. In our usual tomato flavor studies the blended mixture is allowed to stand for 3 min to permit the enzymatic generation of flavor constituents such as (*Z*)-3-hexenal. At the end of 3 min, enzyme action is stopped by the addition of saturated $CaCl_2$ solution. In these studies we did not stop enzymatic activity, and no saturated $CaCl_2$ solution was added. Flavor extracts were prepared using the SAFE technique. Quantitative results for some important odorants are presented in **Table 7**. Labeled (*Z*)-3-hexenal was not found because it is derived from the enzymatic degradation of linolenic acid in blended tomatoes (*32, 33*). Similarly, labeled (*E*)-2-hexenal was not present because it is formed from the isomerization of (*Z*)-3-hexenal (*31*). Gaillard and Matthew (*33*) showed that the reaction of linoleic acid with tomato homogenates produced fatty acid hydroperoxides in 60% yield with the ratio of 9- to 13-hydroperoxides at least 95:5 in favor of the 9-hydroperoxide isomer. In tomato, the 9-hydroperoxide of linoleic acid is not subject to the cleavage reaction, whereas the minor 13-hydroperoxide of linoleic acid is readily cleaved to hexanal (*34*). Small amounts of hexanal are also produced in tomato from linolenic acid as shown by experiments using ^{14}C-labeled linolenic acid (*33, 35*). Much less labeled hexanal (5.3 times less) was formed compared to unlabeled hexanal, which is in contrast to the results with the C10 aldehydes. The concentrations of labeled (*E*,*Z*)-2,4-decadienal and (*E*,*E*)-2,4-decadienal were 20 and 7.5 times higher, respectively, than the concentrations of the unlabeled isomers. The levels of the labeled *cis*-4,5-epoxy-(*E*)-2-decenal and *trans*-4,5-epoxy-(*E*)-2-decenal were 1.5 times higher than the levels of the unlabeled isomers. The formation of *trans*-4,5-epoxy-(*E*)-2-decenal by thermal reactions has been discussed by Gardner and Selke (*36*) and Gassenmeier and Schieberle (*37*). Model studies revealed that thermal degradation of methyl 12,13-epoxy-9-hydroperoxy-10-octadecenoate led to formation of the target compound (*36*). It was postulated that the starting compound underwent homolysis to an oxy radical. β-Scission of the oxy radical at C8,9 would form the target compound. Thermal degradation of 13-hydroperoxy-9,11-octadecadienoic acid (13-HPOD) and 9-hydroperoxy-10,12-octadecadienoic acid (9-HPOD) led to significant yields of *trans*-4,5-epoxy-(*E*)-2-decenal (*37*). 9-HPOD was demonstrated to give higher amounts of the epoxyaldehyde than 13-HPOD. The role of 2,4-decadienal as a key intermediate of epoxydecenal was established by heating (*E*,*E*)-2,4-decadienal in the presence and absence of 9-HPOD. It was found that heating of 2,4-decadienal in the presence of 9-HPOD yielded 4.8 times more of the epoxydecenal than from 9-HPOD itself. In their studies on black tea, Kumazawa and co-workers (*16*) found 2,4-alkadienals ranging from 6 to 10 carbons. They reasoned that if the 2,4-alkadienals were key intermediates, the corresponding 4,5-epoxy-2-alkenals should be present. However, only the 4,5-epoxy-2-heptenals and 4,5-epoxy-(*E*)-2-decenals were found in black tea, whereas the other 4,5-epoxy-2-alkenals could not be detected. The researchers postulated that the 4,5-epoxy-(*E*)-decenals in black tea were generated from linoleic acid during the manufacturing process. Lipoxygenase converts linoleic acid to its 13(*S*)-hydroperoxide, which leads to the epoxydecenals via the *cis*- and *trans*-epoxyallylic radicals. The formation mechanism of the epoxydecenals in tomato is still unknown. To clarify the possible role of 2,4-decadienal as an intermediate in the formation *cis*- and *trans*-4,5-epoxy-(*E*)-2-decenal, we need to perform additional experiments with labeled 2,4-decadienal. It would also be informative to add labeled 9-HPOD and 13-HPOD to tomatoes followed by blending and sample workup.

Reaction of labeled methionine with blended tomato yielded 2 μg/kg of the unlabeled methional and 17 μg/kg of the labeled methional. Although methional can be generated thermally, some studies have also shown that methional can be produced under mild conditions (*38, 39*). Amárita and co-workers (*40*) have shown that *Lactococcus lactis* can enzymatically convert methionine to methional in a process mediated by aminotransferase and α-keotacid decarboxylase activities. They proposed that the primary step is the transamination of methionine to 4-methylthio-2-ketobutyrate. Decarboxylation of the latter compound to methional is mediated by the α-ketoacid decarboxylase activity present in this strain. Due to the mild conditions and short sample preparation time, it seems likely that the formation of methional from methionine is an enzymatic process also in tomatoes, in which up to 30 μg/kg was found.

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