Research Article

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Carotenoid content impacts flavor acceptability in tomato (*Solanum lycopersicum*)

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Abstract

BACKGROUND: Tomatoes contain high levels of several carotenoids including lycopene and β-carotene. Beyond their functions as colorants and nutrients, carotenoids are precursors for important volatile flavor compounds. In order to assess the importance of apocarotenoid volatiles in flavor perception and acceptability, we conducted sensory evaluations of near-isogenic carotenoid biosynthetic mutants and their parent, Ailsa Craig.

RESULTS: The carotenoid contents of these tomatoes were extremely low in the *r* mutant, increased in lycopene in *old gold*, and higher in tetra-*cis*-lycopene and ζ-carotene in *tangerine*. The volatiles derived from these carotenoids (β-ionone, geranylacetone and 6-methyl-5-hepten-2-one) were proportionally altered relative to their precursors. Fruits were also analyzed for soluble solids, sugars, acids and flavor volatiles. Consumer panels rated the *r* mutant lowest for all sensory attributes, while Ailsa Craig was generally rated highest. *Old gold* and *tangerine* were rated intermediate in two of the three harvests.

CONCLUSIONS: Several chemicals were negatively correlated with at least one of the hedonic scores while several others were positively correlated with tomato flavor acceptability. The results permitted identification of positive and negative interactions of volatiles with tomato flavor.

Keywords: tomato; flavor; carotenoids; apocarotenoid; volatiles

INTRODUCTION

The unique flavors of fresh fruits are the sum of a complex interaction among the taste and olfactory systems. Thus, the unique flavor of a fruit integrates chemical signals originating from distinct and spatially separated taste and olfactory receptors with visual and textural cues in the brain. Although these input signals clearly emanate from anatomically distinct sensory systems, it is clear that output from one can influence the perception of another sensory system. For example, the presence or absence of specific volatile chemicals perceived by the retronasal olfactory system can influence perceived sweetness, a signal that should be specific to sweet receptors in the mouth.[1,2]

The chemicals that contribute to the unique flavor of tomato fruit are largely known, although how they integrate to produce that flavor is not understood. Sugars and acids are perceived by taste receptors and contribute to good flavor. The principal sugar contributors are glucose and fructose while the principal acids are citrate, malate, ascorbate and glutamate. While a tomato fruit produces upward of 400 volatile chemicals, only 15–20 are generally accepted to be present in sufficient quantities to be detected by the olfactory system.[3,4] These volatiles are derived from a diverse set of precursors, including amino acids, fatty acids and carotenoids.[5] Their presence is absolutely essential to flavor. The poor flavor of modern commercial hybrids is at least in part associated with reduced production of these volatiles as well as reduced accumulation of sugars and acids.[5]

Among the most important volatile flavor chemicals are a set of apocarotenoid volatiles derived from carotenoids, including geranylacetone, 6-methyl-5-hepten-2-one (MHO), β-ionone and β-damascenone (Fig. 1). They are produced by non-enzymatic oxidative cleavage of various linear and cyclic carotenoids or by a family of carotenoid cleavage dioxygenases.[6,7] The apocarotenoid volatiles are generally described as having fruity and/or floral attributes. Although they are not abundant, they have very low odor thresholds and humans can be quite sensitive to them. Reconstitution experiments provide good evidence that these apocarotenoid volatiles broadly impact the perception of sweetness in tomato fruits.[2]

The distinctive red color of a tomato fruit is due to the accumulation of all-*trans*-lycopene. Tomato breeders have identified a

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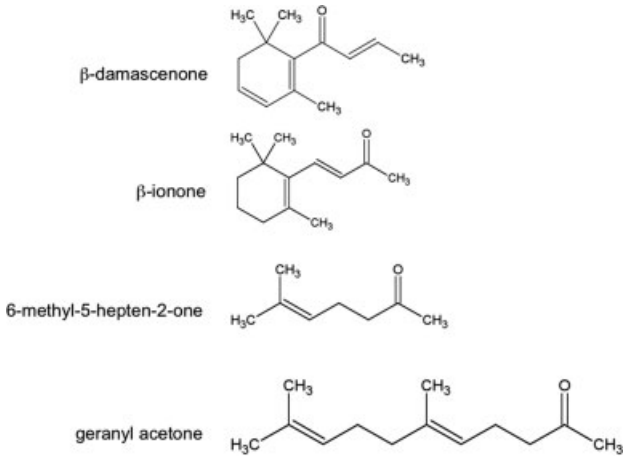

Figure 1. Structures of apocarotenoid volatiles contributing to tomato flavor.

number of fruit color mutants, ranging from yellow to dark red. The genes responsible for many of these color mutants have now been cloned, providing insight into carotenoid biosynthesis. For example, the *tangerine* mutant (*t*) of tomato has a deletion in the *CRTISO* gene. *CRTISO* encodes a carotene isomerase involved in the *cis*-to-*trans* isomerization of tetra-*cis*-lycopene (prolycopene) to all-*trans* lycopene.[8] The fruits of the *tangerine* mutant are orange, due to the accumulation of tetra-*cis*-lycopene (Fig. 2). The *old-gold* tomato is a recessive mutation in lycopene β-cyclase, the enzyme that converts lycopene to β-carotene.[9] This mutation results in decreased levels of carotenoids beyond lycopene in the biosynthetic pathway and a subsequent increase in lycopene. As such, the fruits of this mutant have a deeper red color (Fig. 2). The *yellow-flesh* (*r*) mutant is caused by loss of phytoene synthase 1 (PSY-1).[10] PSY-1 condenses two geranylgeranyl pyrophosphate molecules into phytoene. A mutation at this point in the biosynthetic pathway results in ripe fruits with a near complete loss of carotenoids (Fig. 2).

The availability of these mutations in a near-isogenic background allows us to directly test an important question regarding tomato flavor: Do the apocarotenoid volatiles directly and positively contribute to tomato flavor? By using the near-isogenic mutants and the parent, we can alter the composition of tomato fruits in precise ways, altering apocarotenoid volatiles without impacting the other volatiles, sugars or acids. To address these questions, we used consumer sensory panels to determine flavor acceptability for *tangerine*, *r*, *og*, and the wild-type parent, Ailsa Craig.

EXPERIMENTAL

Plant growth and fruit harvest

The tomatoes used in this study were the *t*, *og*, and *r* mutants (LA3183, LA3179, LA3691, respectively), along with the control, Ailsa Craig (LA2838A). Seeds were kindly provided by the Tomato Genetics Resource Center (University of California, Davis). Plants were grown in Live Oak, Florida, USA (spring 2008, autumn 2008) using standard commercial practices in raised plastic mulch beds with drip irrigation. The spring 2009 crop was grown in a heated greenhouse on the University of Florida campus using standard commercial practices. Fruit were harvested the day before the taste panels, washed in water containing 10% commercial bleach, triple rinsed in distilled water, and air dried. Fruits were harvested from a minimum of 24 individual plants. In the field, fruits were staged by visual and tactile inspection. In the lab, staging was verified on representative fruit by cutting them open for visual inspection.

Volatile and carotenoid analysis

Volatile collection and analysis was performed as previously described.[11] Volatile determinations were made from at least four biological replicates, each consisting of six pooled fruits. Carotenoid isolation and HPLC analysis were performed as described[12] using three or four biological replicates, with each replicate consisting of six pooled fruits. Briefly, all HPLC grade solvents used were purchased from Fischer Scientific (Fair Lawn, NJ, USA) and degassed prior to use. Prior to HPLC, samples were dissolved in 0.5 mL of ethyl acetate and spun in a micro-centrifuge at 16 000 $\times$ *g* to clarify the samples. HPLC analysis was carried out on a Waters (Milford, MA, USA) system consisting of a 510 pump (pump C), two 515 pumps (pumps A and B), a 717 auto-sampler, and a 2996 photodiode array detector. A reverse-phase C_{30}, 5 µm column (250 $\times$ 4.6 mm) coupled to a 20 $\times$ 4.6 mm C_{30} guard (YMC Inc., Wilmington, NC, USA) was used. The mobile phases consisted of methanol (A), water/methanol (20/80 by volume) containing 0.2% ammonium acetate (B) and methyl-*tert* butyl ether (C). Peak areas were determined at the wavelength providing maximum absorbance. Astaxanthin was used as an internal standard to calculate % recovery and carotenoid concentration was calculated using a β-carotene standard curve (Sigma-Aldrich, St Louis, MO, USA). Data from each harvest were subjected to analysis of variance (significance at 0.05) and means were separated using least significant difference (LSD).

Sugar, acid and Brix analysis

Six tomato fruits were homogenized in a blender for 30 s and frozen at $-80\,^{\circ}$C until analysis. Samples were thawed and 1.5 mL was centrifuged at 16 000 $\times$ *g* for 5 min. The supernatant was analyzed using citric acid, malic acid, and glucose/fructose analysis kits (R-Biopharm, Marshall, MI, USA) according to the manufacturer's instructions. Average values were calculated from five biological replicates. Soluble solids ($^{\circ}$Brix) were measured using a handheld refractometer. The samples used to calculate $^{\circ}$Brix were the same as those used in the acids and sugars analysis. Data from each harvest were subjected to analysis of variance (significance at 0.05) and means were separated using least significant difference (LSD).

Sensory analysis

The four tomato varieties from each harvest were subjected to sensory evaluation for overall acceptability, tomato flavor acceptability, and sweetness acceptability using a nine-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely). Washed and dried tomatoes were cut into wedges (either four or eight wedges, depending on size). Wedges from several tomatoes were combined together to create a composite sample for testing. Two wedges of each variety were placed in black plastic cups labeled with three-digit random numbers. All orders of presentation were presented approximately an equal number of times. In order to mask color differences, the room housing the sensory evaluation booths was darkened and each booth was illuminated with red light (there was diffuse light from the LCD computer screens). Use of black cups further decreased color differences between tomato samples. Under these conditions, all

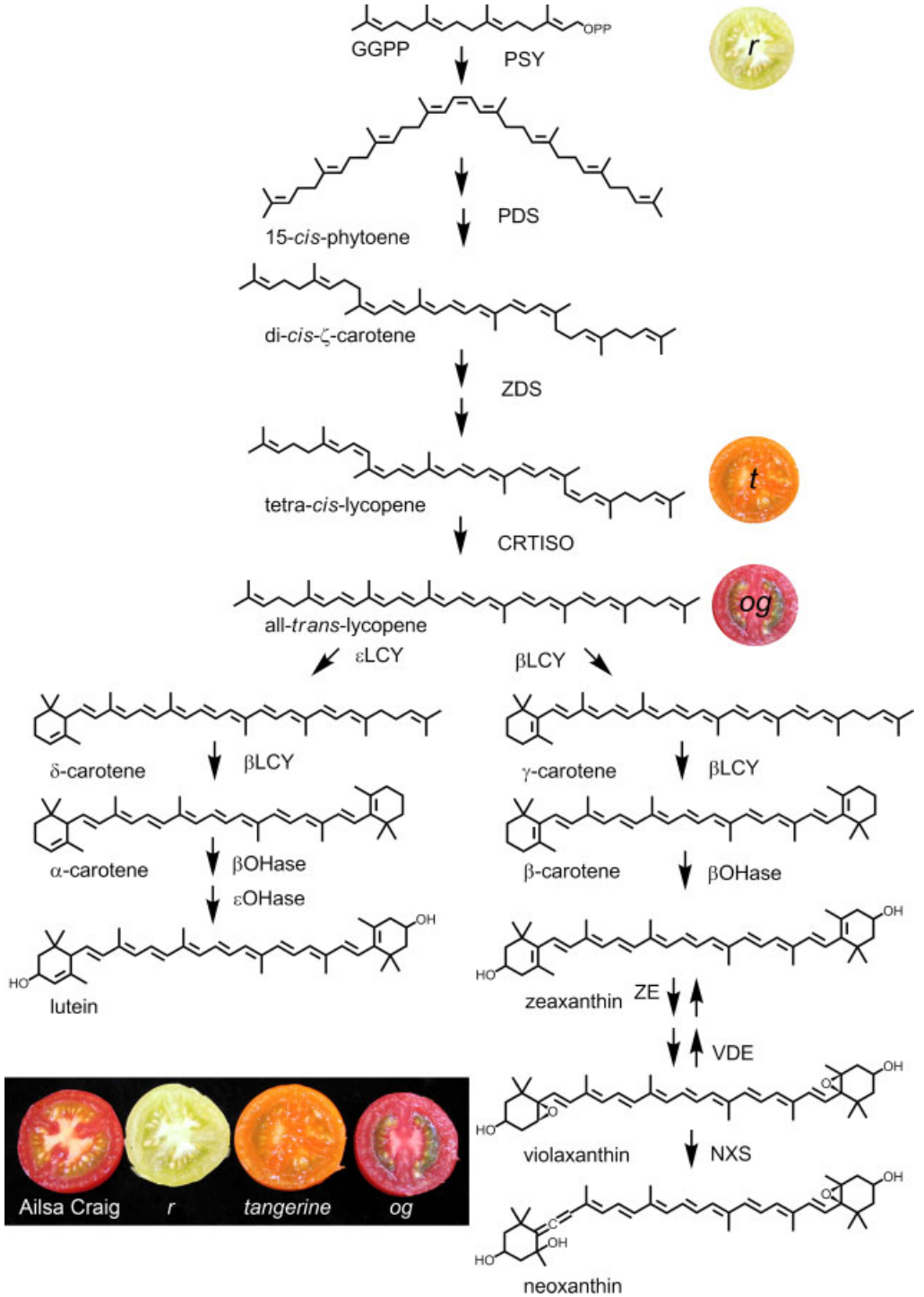

Figure 2. The carotenoid biosynthesis pathway and tomato mutants used in this study. The tomato near-isogenic lines used in this study are shown: *r* (*yellow flesh*), *og* (*old gold*), and *t* (*tangerine*). GGPP, geranylgeranyl diphosphate; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, zeta-carotene desaturase; CRTISO, carotenoid isomerase; βLYC, lycopene beta-cyclase; βOHase, beta ring hydrolase; εOHase, epsilon ring hydrolase; ZE, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; NXS, neoxanthin synthase.

four tomato samples appeared red in color and this eliminated or greatly decreased any color bias.

One hundred panelists (different panelists for each harvest) evaluated the samples in private booths equipped with a computer data entry system (Compusense, Ontario, CA). The panelists were recruited from the University of Florida campus. Panelists were approximately 50% male and 50% female, and 60–85% of the panelists were between the ages of 18–29 years. A randomized complete block design was used for each harvest. All four samples were presented simultaneously to panelists, who then rated the samples for overall acceptability, tomato flavor acceptability and sweetness acceptability as described above. Water and unsalted crackers were provided to panelists, and panelists were instructed to take a bite of a cracker and sip of water before

Table 1. Fruit carotenoid composition (mg kg^{-1} dry weight) – June 2008 harvest

Component	Ailsa Craig	*r*	*t*	*og*
Lutein	53.5 ± 7.6	16.2 ± 3.6	31.8 ± 6.4	64.5 ± 7.8
Phytoene	71.6 ± 6.6	–	498 ± 114.7	98.3 ± 9.5
Phytofluene	28.8 ± 3.2	–	245.1 ± 55.1	43.3 ± 6.8
ζ-Carotene	–	–	1715.6 ± 395.8	–
β-Carotene	210.4 ± 17.2	19.1 ± 9.4	–	136.7 ± 31.3
Poly *cis*-lycopene	–	–	716 ± 173.5	–
δ-Carotene	16.5 ± 2.7	–	–	37 ± 9.2
Neurosporene	–	–	213.9 ± 49.9	–
cis-Lycopene	119.1 ± 24.1	–	28 ± 9.6	326.5 ± 107.8
All *trans*-lycopene	2153.2 ± 340.2	2 ± 0.6	10.8 ± 1.4	2823.8 ± 297.3

Each mean represents four biological replicates, ± SD. All values except for lutein in *og* are significantly different to Ailsa Craig (*t*-test, $P < 0.05$).

Table 2. Fruit carotenoid composition (mg kg^{-1} dry weight) – November 2008 harvest*

Component	Ailsa Craig	*r*	*t*	*og*
Lutein	70.2 ± 11.6	17 ± 6*	41.2 ± 0.8*	81.7 ± 4
Phytoene	221.2 ± 18.3	–	658.1 ± 148.9*	276 ± 43.8*
Phytofluene	95.3 ± 9.9	–	318 ± 78.2*	122.8 ± 15.1
ζ-Carotene	3.6 ± 0.7	–	708.3 ± 115.8*	4.3 ± 0.5
β-Carotene	861.1 ± 180.8	18.7 ± 1.1*	88.4 ± 14.9*	376.5 ± 72.4*
Poly *cis*-lycopene	–	–	2799.3 ± 765.3*	–
δ-Carotene	6.8 ± 2.4	–	–	7.7 ± 2
Neurosporene	–	–	134.9 ± 30.5*	–
cis-Lycopene	165.7 ± 60.4	–	38.3 ± 4.1*	181.5 ± 42.3
All *trans*-lycopene	3038.5 ± 207.2	–	28.4 ± 8.8*	4886.6 ± 383*

* Each mean represents three biological replicates, ± SD. Values indicated by an asterisk are significantly different to Ailsa Craig (*t*-test, $P < 0.05$).

starting and between each sample. Data from each harvest were subjected to analysis of variance (significance at 0.05) and means were separated using least significant difference (LSD). Principal component analysis (PCA) was performed on the analytical data and the mean hedonic ratings (based on the correlation matrix) using SAS software (v. 9.1 SAS Institute, Cary, NC).

RESULTS

Carotenoid and apocarotenoid volatiles

In order to directly address the contribution of apocarotenoid volatiles to perceived flavor, a set of near-isogenic carotenoid mutants, all in the Ailsa Craig background, were characterized. The study included *tangerine*, *r* and *og*. All lines were grown over three different seasons, either in the field or a greenhouse. The carotenoid contents of fruits from each line were determined in the first and second seasons (Tables 1 and 2). Although the absolute amounts of carotenoids in ripe fruits varied between seasons, the relative amounts were reproducible and consistent with prior characterizations of these mutants in multiple genetic backgrounds.[8–10] As expected, *r* was nearly deficient in all carotenoids, with most below the limits of detection. The *tangerine* mutant exhibited significant reductions in all carotenoids downstream of tetra-*cis*-lycopene (Fig. 2). This mutant also accumulated significantly higher levels of carotenoids preceding *CRTISO* in the biosynthetic pathway, most notable, phytoene, phytofluene and ζ-carotene. The *og* mutant, deficient in lycopene β-cyclase, contained somewhat reduced levels of β-carotene and higher levels of all *trans*-lycopene.

The apocarotenoid volatile emissions from fruits were generally consistent with the carotenoid composition of those fruits (Tables 3–5). Although not always significant ($P < 0.05$), trends were apparent. The *r* fruits always exhibited large reductions in β-ionone, MHO, geranylacetone and β-cyclocitral. The *tangerine* fruits emitted reduced levels of the cyclic apocarotenoids, β-ionone and β-cyclocitral while emitting significantly higher levels of the linear apocarotenoids, geranyl acetone and MHO. Similarly, *og* produced significantly lower levels of β-ionone and β-cyclocitral while emitting higher levels of the linear apocarotenoids, pseudoionone, geranyl acetone and MHO. Although the levels of these latter linear apocarotenoids were always higher, they were generally below the 0.05 level of significance. Taken together, the three mutants over the three seasons exhibited substantial differences from the control Ailsa Craig sufficient to test the importance of the apocarotenoid volatiles to flavor preferences. There were a few significant differences in non-apocarotenoid volatile emissions observed in each season. However, these differences were not consistent within lines or seasons. Such differences were expected due to the large number of volatiles assayed and the environmental variations between seasons and locations. These differences permitted subsequent analyses on their relative contributions to consumer acceptability (described below).

Sweetness should be related to the sugar content of the fruit, but could also be influenced by acids. The major sugars in a ripe

Table 3. Tomato aroma volatiles (ng g fw^{-1} h^{-1}) – June 2008 harvest*

	Ailsa Craig	Old Gold	*r*	*t*
3-Methylbutanal	12.3^{a}	10.1^{a}	14.4^{a}	10.0^{a}
2-Methylbutanal	5.9^{b}	7.5^{a}	5.2^{b}	4.9^{b}
Ethyl vinyl ketone	1.4^{a}	1.3^{a}	1.2^{a}	1.0^{a}
3-Methyl-1-butanol	57.5^{a}	39.8^{ab}	44.0^{ab}	28.4^{b}
Z-3-Hexanal	110.3^{a}	49.2^{ab}	92.1^{ab}	41.1^{b}
Hexanal	146.6^{a}	64.3^{b}	62.6^{b}	41.5^{b}
E-2-Hexenal	8.3^{a}	4.2^{a}	6.7^{a}	3.4^{a}
Z-3-Hexen-1-ol	89.0^{a}	53.5^{ab}	49.5^{b}	32.9^{b}
E-2-Heptenal	0.9^{a}	0.7^{ab}	0.3^{b}	0.3^{b}
6-Methyl-5-hepten-2-one	2.9^{b}	3.4^{b}	0.3^{c}	8.4^{a}
2-Isobutylthiazole	3.2^{a}	1.2^{b}	2.5^{a}	2.7^{a}
Phenylacetaldehyde	1.1^{a}	0.7^{b}	1.1^{a}	1.1^{a}
2-Phenylethanol	0.14^{b}	0.27^{ab}	0.19^{ab}	0.28^{a}
Methylsalicylate	0.2^{a}	0.2^{a}	0.4^{a}	0.1^{a}
β-Cyclocitral	0.17^{a}	0.05^{c}	0.02^{d}	0.08^{b}
Neral	0.4^{a}	0.3^{a}	0.3^{a}	0.4^{a}
Geranial	0.14^{b}	0.15^{ab}	0.02^{c}	0.21^{a}
Geranylacetone	0.9^{b}	1.4^{b}	0.0^{b}	11.8^{a}
β-Ionone	0.10^{a}	0.02^{bc}	0.01^{c}	0.05^{b}
Z-Pseudoionone	0.02^{bc}	0.04^{a}	0.01^{c}	0.03^{ab}
E-Pseudoionone	0.006^{b}	0.009^{ab}	0.014^{ab}	0.017^{a}
Farnesyl acetone	0.09^{ab}	0.09^{b}	0.00^{c}	0.16^{a}
Guaiacol	0.78^{a}	0.77^{a}	0.76^{a}	0.68^{a}

* Within a row, means followed by the same letter are not significantly different (LSD, 0.05).

Table 4. Tomato aroma volatiles (ng g fw^{-1} h^{-1}) – November 2008 harvest*

	Ailsa Craig	Old Gold	*r*	*t*
3-Methylbutanal	34.9^{ab}	33.4^{ab}	43.5^{a}	17.6^{b}
2-Methylbutanal	7.2^{a}	7.0^{a}	5.0^{ab}	4.5^{b}
Ethyl vinyl ketone	2.5^{a}	1.8^{a}	2.0^{a}	1.7^{a}
3-Methyl-1-butanol	140.5^{b}	143.5^{b}	242.6^{a}	87.0^{b}
Z-3-Hexanal	177.0^{a}	129.3^{a}	220.0^{a}	149.7^{a}
Hexanal	230.4^{a}	210.2^{a}	144.0^{a}	163.6^{a}
E-2-Hexenal	18.0^{a}	16.7^{a}	13.3^{a}	18.4^{a}
Z-3-Hexen-1-ol	126.5^{a}	88.9^{a}	89.5^{a}	108.0^{a}
E-2-Heptenal	0.5^{a}	0.6^{a}	0.5^{a}	0.5^{a}
6-Methyl-5-hepten-2-one	4.4^{b}	6.7^{b}	0.3^{c}	9.9^{a}
2-Isobutylthiazole	6.5^{a}	6.2^{a}	9.1^{a}	7.8^{a}
Phenylacetaldehyde	1.2^{b}	1.1^{b}	1.8^{ab}	2.2^{a}
2-Phenylethanol	0.05^{a}	0.02^{ab}	0.00^{b}	0.01^{b}
Methylsalicylate	0.07^{b}	0.07^{b}	0.56^{a}	0.35^{ab}
β-Cyclocitral	0.20^{a}	0.10^{b}	0.02^{c}	0.06^{bc}
Neral	0.2^{a}	0.3^{a}	0.2^{a}	0.3^{a}
Geranial	0.2a	0.3a	0.0^{b}	0.2a
Geranylacetone	0.8^{c}	1.6^{b}	0.0^{d}	5.3^{a}
β-Ionone	0.06^{a}	0.03^{b}	0.00^{c}	0.02^{bc}
Z-Pseudoionone	0.006^{bc}	0.011^{a}	0.004^{c}	0.012^{ab}
E-Pseudoionone	0.01^{a}	0.01^{a}	0.01^{a}	0.02^{a}
Farnesyl acetone	0.04^{b}	0.06^{a}	0.00^{c}	0.07^{a}
Guaiacol	1.20^{b}	1.42^{b}	3.88^{a}	2.57^{ab}

* Within a row, means followed by the same letter are not significantly different (LSD, 0.05).

Table 5. Tomato aroma volatiles (ng g fw^{-1} h^{-1}) – February 2009 harvest*

	Ailsa Craig	Old Gold	*r*	*t*
3-Methylbutanal	14.8^{a}	10.4^{a}	14.0^{a}	12.1^{a}
2-Methylbutanal	5.4^{a}	4.7^{a}	3.8^{a}	4.2^{a}
Ethyl vinyl ketone	2.7^{a}	3.0^{a}	3.4^{a}	2.2^{a}
3-Methyl-1-butanol	48.6^{a}	20.2^{b}	30.4^{ab}	22.7^{b}
Z-3-Hexanal	98.8^{a}	55.5^{a}	83.9^{a}	25.7^{a}
Hexanal	60.1^{a}	41.8^{ab}	44.5^{ab}	19.8^{b}
E-2-Hexenal	3.8^{a}	2.5^{a}	2.9^{a}	1.4^{a}
Z-3-Hexen-1-ol	21.0^{a}	17.4^{a}	23.9^{a}	10.5^{a}
E-2-Heptenal	0.10^{a}	0.14^{a}	0.18^{a}	0.04^{a}
6-Methyl-5-hepten-2-one	0.15^{bc}	0.33^{ab}	0.04^{c}	0.39^{a}
2-Isobutylthiazole	0.47^{a}	0.47^{a}	1.42^{a}	0.43^{a}
Phenylacetaldehyde	0.06^{a}	0.07^{a}	0.09^{a}	0.04^{a}
2-Phenylethanol	0.009^{a}	0.007^{a}	0.006^{a}	0.008^{a}
Methylsalicylate	0.03^{a}	0.06^{a}	0.08^{a}	0.02^{a}
β-Cyclocitral	0.02^{a}	0.01^{b}	0.01^{b}	0.01^{b}
Neral	0.04^{ab}	0.05^{a}	0.05^{a}	0.02^{b}
Geranial	0.01^{a}	0.00^{a}	0.00^{a}	0.01^{a}
Geranylacetone	0.06^{bc}	0.12^{b}	0.01^{c}	0.29^{a}
β-Ionone	0.02^{a}	0.01^{b}	0.01^{b}	0.01^{b}
Z-Pseudoionone	0.02^{bc}	0.04^{a}	0.01^{c}	0.03^{ab}
E-Pseudoionone	0.006^{b}	0.009^{ab}	0.014^{ab}	0.017^{a}
Farnesyl acetone	0.01^{a}	0.01^{a}	0.01^{a}	0.01^{a}
Guaiacol	0.10^{b}	0.10^{b}	0.18^{a}	0.07^{b}

* Within a row, means followed by the same letter are not significantly different (LSD, 0.05).

Table 6. Sugars and acids – June 2008 harvest*

	Ailsa Craig	*og*	*r*	*t*
Citric acid (g L^{-1})	3.7^{b}	4.7^{a}	3.1^{b}	3.5^{b}
Malic acid (g L^{-1})	1.2^{a}	0.4^{a}	1.1^{a}	1.1^{a}
Fructose (g L^{-1})	17.2^{a}	16.2^{a}	17.3^{a}	17.0^{a}
Glucose (g L^{-1})	14.4^{ab}	13.7^{b}	15.8^{a}	15.5^{ab}
Brix (%)	4.7^{a}	4.5^{ab}	4.2^{b}	4.5^{ab}
Total sugars (g L^{-1})	31.6^{a}	29.8^{a}	33.2^{a}	32.4^{a}
Total acids (g L^{-1})	4.9^{a}	5.1^{a}	4.2^{a}	4.6^{a}

* Within a row, means followed by the same letter are not significantly different (LSD, 0.05).

Table 7. Sugars and acids – November 2008 harvest*

	Ailsa Craig	*og*	*r*	*t*
Citric acid (g L^{-1})	4.8^{b}	5.3^{a}	3.3^{d}	4.0^{c}
Malic acid (g L^{-1})	1.1^{a}	0.5^{b}	1.2^{a}	1.2^{a}
Fructose (g L^{-1})	15.6^{a}	13.5^{b}	12.7^{b}	14.0^{ab}
Glucose (g L^{-1})	10.9^{a}	8.5^{b}	7.5^{b}	8.6^{b}
Brix (%)	4.6^{a}	4.1^{b}	3.6^{c}	4.0^{b}
Total sugars (g L^{-1})	26.4^{a}	22.0^{b}	20.1^{b}	22.6^{b}
Total acids (g L^{-1})	5.9^{a}	5.7^{a}	4.5^{c}	5.2^{b}

* Within a row, means followed by the same letter are not significantly different (LSD, 0.05).

Table 8. Sugars and acids – February 2009 harvest*

	Ailsa Craig	*og*	*r*	*t*
Citric acid (g L^{-1})	4.2^{b}	5.7^{a}	3.3^{c}	3.8^{b}
Malic acid (g L^{-1})	0.8^{a}	0.3^{c}	0.8^{a}	0.6^{b}
Fructose (g L^{-1})	23.5^{a}	19.7^{b}	20.6^{b}	23.4^{a}
Glucose (g L^{-1})	19.7^{a}	16.5^{b}	17.0^{b}	20.6^{a}
Brix (%)	5.5^{a}	5.0^{b}	4.8^{b}	5.5^{a}
Total sugars (g L^{-1})	43.2^{a}	36.1^{b}	37.6^{b}	44.0^{a}
Total acids (g L^{-1})	5.0^{b}	6.0^{a}	4.2^{c}	4.4^{c}

* Within a row, means followed by the same letter are not significantly different (LSD, 0.05).

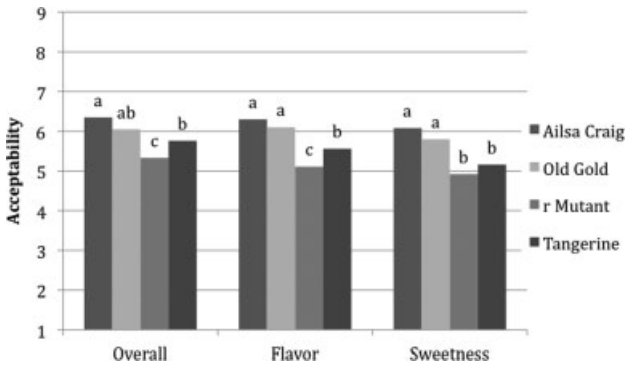

Figure 3. Overall acceptability, flavor acceptability, and sweetness acceptability of tomato varieties from June 2008 harvest. Means followed by the same letter are not significantly different (LSD, 0.05).

tomato fruit are glucose and fructose while the major contributors to acidity are citric and malic acids. Typically a measurement of total soluble solids, Brix, is used as an indirect measure of sugar content. We measured Brix for each line, as well as glucose, fructose, citric acid and malic acid. We further determined the total sugars and total acids. The data are presented in Tables 6–8. The *og* mutant was significantly lower in total sugars in two out of the three seasons. The *r* mutant had lower citric acid, glucose, fructose, total sugars and total acid levels in two of the three seasons.

Sensory acceptability

The *r* mutant was consistently rated the lowest for overall, flavor and sweetness acceptability (Figs 3–5) in all three harvests. Ailsa Craig tended to be rated the highest for overall, flavor and sweetness acceptability, although the differences were not always significant. Ailsa Craig was rated higher than the *tangerine* mutant in two of the harvests (June and November), but was only significantly higher than the *og* mutant in the November harvest. The only significant differences in acceptability in the February harvest were between the *r* mutant and the other three varieties, which were rated the same.

Correlations of chemical composition and sensory acceptability

Pearson correlation coefficients revealed that samples high in soluble solids and citric acid tended to have higher scores for overall, flavor and sweetness acceptability (Table 9). Fructose, glucose and total sugars content were positively correlated

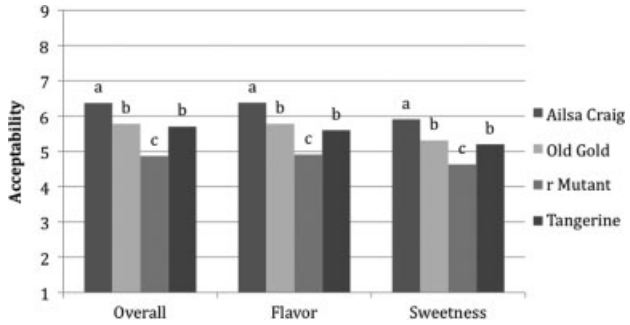

Figure 4. Overall acceptability, flavor acceptability, and sweetness acceptability of tomato varieties from November harvest. Means followed by the same letter are not significantly different (LSD, 0.05).

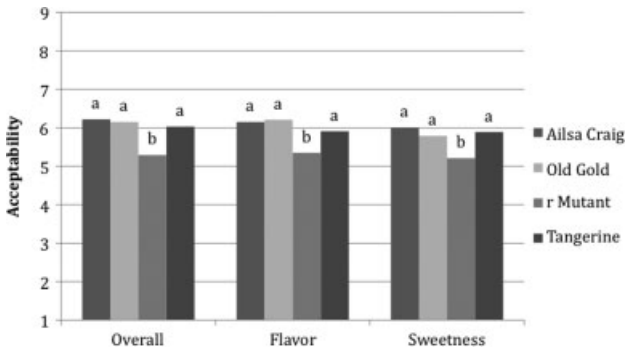

Figure 5. Overall acceptability, flavor acceptability, and sweetness acceptability of tomato varieties from February harvest. Means followed by the same letter are not significantly different (LSD, 0.05).

with sweetness acceptability, only. Total acids were positively correlated with overall and tomato flavor acceptability but not with sweetness acceptability. Samples high in the volatiles β-ionone and β-cyclocitral tended to receive higher hedonic ratings of tomato flavor acceptability and overall acceptability. Methylsalicylate and guaiacol, on the other hand, were negatively correlated with all of the hedonic scores. Sweetness acceptability tended to be rated lower for those samples high in 2-isobutylthiazole, phenylacetaldehyde and pseudoionone#2.

Principal component analysis performed on the mean volatile, sugar and acid content and mean hedonic scores showed that the first two principal components (PCs) explained 43 and 22 (65% total) of the variability in the data. Figure 6 shows the biplot of sample scores. PC1 separated the February harvest (greenhouse fruit) from June and November harvests (field grown fruit). The *r* fruits with lower acceptability scores and reduced levels of apocarotenoid volatiles such as β-ionone, MHO and β-cyclocitral were separated along PC2 from the other fruit within a given harvest.

DISCUSSION

The flavor of fresh commercial tomatoes is generally accepted as poor by consumers. We have sought to better understand the components of flavor with a goal of ultimately improving flavor quality. Determining the contributions of individual volatiles, both positive and negative, is an important aspect of flavor enhancement. What is perceived as tomato 'flavor' is highly complex, being the sum of a large set of volatile and non-

Table 9. Pearson correlation coefficients between hedonic scores and sugar, acid and volatiles content*

	Overall acceptability	Tomato flavor acceptability	Sweetness acceptability
Soluble solids	0.63	0.59	0.75
Total sugars	N.S.	N.S.	0.54
Total acids	0.62	0.69	N.S.
Citric acid	0.58	0.67	0.50
Fructose	N.S.	N.S.	0.56
Glucose	N.S.	N.S.	0.51
2-Isobutylthiazole	N.S.	N.S.	−0.52
Phenylacetaldehyde	N.S.	N.S.	−0.53
Methylsalicylate	−0.68	−0.68	−0.70
β-Cyclocitral	0.53	0.53	N.S.
β-Ionone	0.54	0.52	N.S.
E-Pseudoionone	N.S.	N.S.	−0.61
Guaiacol	−0.54	−0.50	−0.61

* Volatiles that were not significantly correlated (N.S., $\alpha = 0.10$) with any of the hedonic scores are omitted from the table.

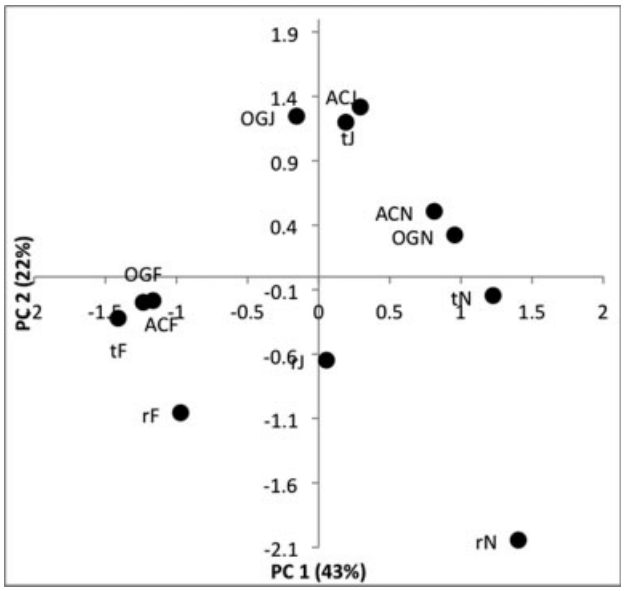

Figure 6. PCA biplot of tomato samples from different harvests (scores). Letters to follow the sample name (AC, OG, t, r) in the scores plot refer to the harvest (F: February 2009, J: June 2008, N: November 2008).

volatile chemicals. The interaction between individual volatiles and integration with sugars and acids is not well understood. However, it is clear that volatiles influence the perception of sweetness, despite being recognized by independent sensory systems.[1,2] We have taken a systematic approach to dissect out the interactions between sets of chemicals that contribute to overall flavor. Using mutants altered in specific pathways together with precisely engineered transgenic plants, we can alter individual chemicals or sets of related chemicals and assess the effects of these changes on consumer perception. Here, we used a set of mutants altered in carotenoid metabolism. The carotenoid contents of tomato fruits are predictive of the apocarotenoid volatiles produced by those fruits.[11,13] Thus, we have been able

to evaluate the effects of changing one related set of volatiles on flavor. By further incorporating natural environmental variations over multiple seasons we identified chemicals that both positively and negatively affect flavor, as assessed by consumer panels.

The apocarotenoid volatiles can be divided into two separate structural and perceptual classes. The linear apocarotenoids such as geranylacetone and pseudoionone have relatively high odor thresholds. For example, the odor thresholds for MHO and geranylacetone are 50 nL L^{-1} and 60 nL L^{-1}, respectively.[3,14] The cyclic apocarotenoids such as β-ionone and β-cyclocitral have odor thresholds of 0.007 nL L^{-1} and 5 nL L^{-1}, respectively.[3,14] Overall, the three carotenoid mutants affected the apocarotenoid pools in three distinct patterns. The *r* mutant, which is blocked in the first step of carotenoid synthesis, produced substantially lower levels of both the linear and cyclic carotenoids. The *tangerine* mutant, blocked in the isomerase that produces all-*trans*-lycopene, was reduced in the cyclic carotenoids but had elevated levels of linear carotenoids such as MHO, pseudoionone and geranylacetone. The *og* mutant, with reduced levels of the enzyme responsible for β-cyclization of lycopene, also produced lower levels of the cyclic apocarotenoids and higher levels of the linear carotenoids. However, the absolute levels of the linear apocarotenoids were generally intermediate between those of *tangerine* and Ailsa Craig. Thus, the three mutants provided distinct patterns of apocarotenoid production. The consumer panels consistently rated overall acceptability, tomato flavor and sweetness similarly. They rated the most severely affected *r* mutant as having the poorest overall flavor, acceptability and sweetness in all three seasons. The line with the second most affected apocarotenoid volatile profile, *tangerine*, was rated significantly worse in all three attributes in two out of three seasons.

The sweetness of the *r* mutant was liked less than the control in every season. Although the glucose and fructose levels were significantly lower than Ailsa Craig in two of the three seasons, these levels were comparable to those of *og*. In all three seasons, the sweetness of *og* was liked significantly better than *r* suggesting that the reduced levels of apocarotenoid volatiles in *r* negatively affect the acceptability of sweetness, as well as the acceptability of tomato flavor and the product overall.

There was a strong negative correlation between flavor and acceptability with guaiacol, methylsalicylate and the branched chain amino acid-derived volatiles. Methylsalicylate and guaiacol are structural similar compounds whose synthesis may be coordinately regulated. The aroma profiles of both have been described as 'medicinal-like' and 'pharmaceutical'.[15] The branched-chain amino acid derived volatile 2-isobutylthiazole is described as 'pungent' in tomato homogenate[14] Although methylsalicylate and 2-isobutylthiazole are major contributors to overall tomato flavor,[14] it is possible that lower levels of these volatiles could be lead to better flavor.

CONCLUSIONS

Taken together, these results support a major role for the apocarotenoid volatiles in tomato flavor. Reduction of the major apocarotenoids negatively impacts flavor acceptability as well as the sweetness acceptability of the fruits with the most significant effect in the most severely altered *r* mutant. The cyclic apocarotenoids β-cyclocitral and β-ionone had the strongest positive correlations with acceptability. Targeting fruits with higher concentrations of these volatile compounds while reducing levels of guaiacol and methylsalicylate should be major goals of breeding for improved tomato flavor.

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