

# Flavor Trivia and Tomato Aroma: Biochemistry and Possible Mechanisms for Control of Important Aroma Components

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Consumers are often dissatisfied with the flavor of fresh tomatoes (*Lycopersicon esculentum* Mill.) purchased in the supermarket. There are several reasons for this, ranging from poor genetic material to harvest and handling procedures. Research is ongoing to determine the important flavor components in tomato in order to give breeders and molecular biologists access to objective flavor criteria for use in selection of high-quality material. Furthermore, harvest and postharvest handling, and shipping and storage procedures can be analyzed for their effects on important flavor components.

## FLAVOR TRIVIA

To successfully conduct this research, one must first understand what comprises flavor and how it is perceived. The nose has olfactory nerve endings at the back with receptors that bind volatiles emanating from food. These reactions are somewhat analogous to enzyme/substrate stereochemical associations. Tastes such as sweet, sour, salty, or bitter are perceived because of reactions of sugars or polyalcohols, hydronium ions, sodium ions, glucosides, alkaloids, etc., with receptors located in certain regions of the tongue. The nerves in the tongue can also detect texture, temperature, metallic irritation, bite (carbonation), chemical heat [e.g., hot pepper (*Capsicum frutescens* L.)], etc., collectively known as trigeminal responses. The olfactory system is the more sensitive of the two organs, however, and the most sensitive of the five senses. It can detect odors in parts per trillion whereas receptors on the tongue can detect flavor compounds in parts per hundred. The adverse side of this extreme sensitivity is fatigue, which may be a protective mechanism against nerve damage (DeRovira, 1997). Nevertheless, the fatigue factor must be considered in sensory work with aroma compounds.

The extreme sensitivity of the olfactory organ has played an important role in the evolutionary development of mammals, allowing for the odor imprinting involved in olfactory recognition. This has helped in developing behavioral relationships, such as mother-offspring, consort interactions, and general kin recognition, not to mention detection of predator and prey (Margot and Salvadori, 1995;

Rouhi, 1996). Specific odors can evoke powerful thoughts and emotions in humans. The sense of smell, therefore, has played a key role in several areas important to species survival. In mammals at least 1000 receptor genes are devoted to encoding receptors that recognize odors; these comprise 1% to 2% of the mammalian genome (Rouhi, 1996).

Many factors can affect our perception of flavor, especially the components of flavor made up of aroma compounds. Smelling an aromatic food through the front of the nose may produce a different experience than when the aroma is perceived during chewing of food (Voirol and Daget, 1987). This difference is due to the temperature of the mouth, the disruption of food cells by chewing, and reduction of viscosity by mixing the food with saliva. Temperature, viscosity, and polarity of the food can effect relative vapor pressure and aroma release (Land, 1994; Taylor and Linforth, 1994; Voirol and Daget, 1987). This, in turn, alters the concentration in the headspace of the mouth of volatile compounds that rise through the back of the nose to bind olfactory receptors (Land, 1994; Taylor and Linforth, 1994). In addition, odor and taste can interact to give an integrated perception (Voirol and Daget, 1987). Texture can also play a role. A softer tomato may be perceived as more flavorful than a firm tomato, while a crisp, juicy apple will likely be perceived as more flavorful than a mealy one. Such observations may be related to texture and the state of the cell wall (in particular, the condition of the middle lamella). The mechanism of tissue disruption, i.e., whether fruit cells break across cell walls, releasing cellular components, or between cells (middle lamellae) as in mealy fruit, may affect juiciness and flavor impact (Vickers, 1977).

To make matters more complicated, not all odorants are alike. Primary odorants are like letters of the alphabet in that they define one odor individually and can then, in combination, define another aroma. They bind only one receptor in the olfactory bulb. Methyl salicylate, an important volatile in tomato, is an example; it alone is responsible for the aroma "wintergreen." Secondary odorants are like syllables and bind more than one olfactory receptor (Amoore, 1952). An example would be saffrole, which is thought to bind four receptors and is described as anise, with a wintergreen character, vanilla background, and camphoraceous overtone (= root beer flavor) (De Rovira, 1997). This food additive is carcinogenic and has been removed from the market. Manufacturers were able to duplicate its aroma by using a combination of primary odorants, including anise, methyl salicylate, vanillin and camphor, which bind the same four olfactory receptors as saffrole. And finally, to really confound the issue, flavors have top notes and background notes. Top notes are generally compounds of relatively low molecular weight and high volatility that are heat labile and polar (De Rovira, 1997). They are usually very noticeable in a food item. Background notes, on the other hand, are generally of low molecular weight, heat stable, and nonpolar, and have a more subtle impact on flavor than do top notes.

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## IMPORTANT AROMA COMPOUNDS FOR TOMATO FLAVOR

First, how do we measure odor compounds that are present in food? Earlier studies employed the classical flavor isolation procedures of steam distillation and/or solvent extraction (Teranishi and Kint, 1993), which can modify the flavor profile of a sample qualitatively and quantitatively (Schamp and Dirinck, 1982). This method is time-consuming and not easily applied to large numbers of fruit samples. Internal standards must be incorporated to determine recovery, but the resulting concentration of material allows identification of compounds by gas chromatography-mass spectrometry (GC/MS). More recently, investigators have employed purge and trap headspace sampling methods, which involve trapping and concentrating volatile components on a solid support. The trap is later heated to release volatiles into GC or GC/MS systems (Schamp and Dirinck, 1982; Teranishi and Kint, 1993). Gas chromatographs may be equipped with a sniff port to provide some sensory information as the compounds are eluted from the column. Static headspace methods are said to more closely reflect the true flavor profile, however, although the compounds are present at lower levels and some may not be detected. Cryofocusing (cold trapping) of static headspace volatiles (Teranishi and Kint, 1993) can help overcome this problem, since samples can be concentrated without heating and the associated possibility of adulteration. The newest method available is solid phase microextraction (SPME), a rapid sampling technique where volatiles interact with a fiber-coated probe that is inserted into the headspace of a sample and then transferred to a GC injection port where the volatiles are desorbed (Song et al., 1998). Aside from GC and GC/MS methods, there are new sensors available that have a broad range of selectivity. These sensor arrays (called "electronic noses") are useful for discriminating one sample from another based on the volatile profile, rather than for identification/quantification (Maul et al., 1998a).

So now that aroma compounds can be identified and quantified, how do we know which ones are contributing to flavor? One way is to do aroma extraction dilution analysis (AEDA) or "Charm" analysis, using a sniff port on a GC while diluting the sample (Acree, 1993). A simpler method is to establish odor thresholds (Teranishi and Buttery, 1987; Teranishi et al., 1991). This is done in the food or in some similar medium since odorants' volatility can change with polarity and viscosity (Buttery et al., 1973). In tomato, for example, >400 volatiles have been reported, but only 30 are present in concentrations >1 nL·L<sup>-1</sup>, as summarized in several reviews (Buttery, 1993; Buttery and Ling, 1993a, 1993b; Buttery et al., 1989). Buttery et al. (1971) determined odor thresholds (the level at which a compound can be detected by smell) for these 30 compounds. Log odor units can then be calculated from the ratio of the concentration of a component in a food to its odor threshold. Volatile compounds with positive odor units are assumed to contribute to the flavor of a food, while those with negative units may not (Buttery et al., 1989). Buttery (1993) determined concentrations, odor thresholds, and log odor units of volatiles present in tomato at levels of 1 nL·L<sup>-1</sup> or more (Table 1). Of these, 16 had log odor units >0, and therefore are likely to contribute to tomato flavor. Some fruits or vegetables have one or two odor-impact compounds that dominate the flavor of that particular commodity. Banana (*Musa acuminata* Colla) is a good example, with 3-methylbutyl acetate dominating its flavor (Berger, 1991). Not so for tomato, however, since no single compound has been found in this fruit that is reminiscent of a ripe tomato; a combination of at least 16 aroma compounds together give tomato its unique odor characteristics. Buttery (1993) has suggested that a combination of *cis*-3-hexenal, *cis*-3-hexenol, hexanal, 1-penten-3-one, 3-methylbutanal, *trans*-2-hexenal, 6-methyl-5-hepten-2-one, methyl salicylate, 2-isobutylthiazole, and  $\beta$ -ionone, at the appropriate concentrations, produces the aroma of a fresh, ripe tomato. However, compounds with negative odor units may still contribute to the overall flavor of tomato or other foods as background notes. For this reason synthetic flavors, vanillin, for example, do not have quite the same aroma as the natural vanilla extract, which contains many background notes not present in the synthetic product.

Tomato volatiles present at concentrations  $\geq 1$  nL·L<sup>-1</sup>, along with their concentrations in a typical ripe tomato, odor threshold, and log

odor units (Table 1) (Buttery, 1993), demonstrate that a volatile need not be present in high levels to have an impact on flavor. For example, *cis*-3-hexenal is the most abundant (12,000 nL·L<sup>-1</sup>) of the 30 volatiles present at  $>1$  nL·L<sup>-1</sup>, and has the highest log odor unit (3.7) (Table 1). However,  $\beta$ -ionone, a volatile present at one of the lowest concentrations (4 nL·L<sup>-1</sup>), is second highest in log odor units (2.8).

Odor thresholds in the above studies were determined in water, since tomato is considered an aqueous system. Tomato homogenate (which simulates masticated tomato), however, may bind or trap volatiles, and contains high levels of methanol and other alcohols (Baldwin et al., 1991a, 1991b) that could affect volatile solubility and, therefore, odor threshold. Threshold studies of tomato aroma compounds were carried out in an aqueous alcohol system (water with the amount of methanol and ethanol found in fresh tomato homogenate) and in bland homogenate (volatiles removed by distillation). Volatiles in homogenate generally had higher thresholds (i.e., suppressed sensory perception) and different odor descriptors than did those in water (Tandon, 1998).

Some investigators have attempted to determine the relationships of different flavor compounds (sugars, acids, and volatiles) to sensory descriptors in order to understand the contribution of individual components to overall flavor. Correlations were found between concentrations of aroma compounds and the intensity of aroma as well as taste descriptors (Baldwin et al., 1998). For example, geranylacetone was related to tomato-like flavor and sweetness whereas 6-methyl-5-hepten-2-one was associated with tomato-like flavor, overall acceptability, and spoiled aroma. In three seasonal studies, sweetness intensity was related to hexanal, with contributions from *cis*-3-hexenal, *trans*-2-hexenal, or *cis*-3-hexenol. Soluble solids were more closely related to sourness than to sweetness, which, in turn, correlated more closely with sucrose equivalents (combined sweetness value of glucose and fructose).

Table 1. Tomato volatiles present in fresh tomato at levels  $\geq 1$  nL·L<sup>-1</sup>, their odor threshold (in water) and their order of log odor units.<sup>z</sup>

Volatile	Concn (nL·L <sup>-1</sup> )	Odor threshold (nL·L <sup>-1</sup> )	Log odor units <sup>y</sup>
<i>cis</i> -3-Hexenal	12,000	0.25	3.7
$\beta$ -ionone	4	0.007	2.8
Hexanal	3,100	4.5	2.8
$\beta$ -Damascenone	1	0.002	2.7
1-Penten-3-one	520	1	2.7
2+3-Methylbutanal	27	0.2	2.1
<i>trans</i> -2-Hexenal	270	17	1.2
2-Isobutylthiazole	36	3.5	1.0
1-nitro-2-Phenylethane	17	2	0.9
<i>trans</i> -2-Heptenal	60	13	0.7
Phenylacetaldehyde	15	4	0.6
6-Methyl-5-hepten-2-one	130	50	0.4
<i>cis</i> -3-Hexenol	150	70	0.3
2-Phenylethanol <sup>x</sup>	1,900	1,000	0.3
3-Methylbutanol	380	250	0.2
Methyl salicylate	48	40	0.08
Geranylacetone	57	60	-0.02
$\beta$ -Cyclocitral	3	5	-0.2
1-Nitro-3-methyl-butane	59	150	-0.4
Geraniol	12	32	-0.4
Linalool	2	6	-0.5
1-Penten-3-ol	110	400	-0.6
<i>trans</i> -2-Pentenal	140	1,500	-1.0
Neral	2	30	-1.2
Pentanol	120	4,000	-1.5
Pseudoionone	10	800	-1.9
Isobutyl cyanide	13	1,000	-1.9
Hexanol	7	500	-1.9
Epoxy- $\beta$ -ionone	1	100	-2.0

<sup>z</sup>Adapted with permission from Buttery (1993), copyright 1993, American Chemical Society.

<sup>y</sup>Logarithm of odor unit value.

<sup>x</sup>The exact concentration and log odor unit values are uncertain.

## SYNTHESIS OF TOMATO VOLATILES

The biogenesis of aroma compounds in tomato has been the subject of several recent reviews (Buttery and Ling, 1993a, 1993b). Flavor volatiles are formed in the intact tomato fruit during ripening, or upon tissue disruption, which occurs when tomatoes are macerated, blended, or homogenized (Buttery, 1993). When cell disruption occurs, previously compartmentalized enzymes and substrates mix and new volatiles are formed. The ability to form flavor volatiles after cell disruption, however, can change during ripening, apparently because of changes in enzyme and substrate availability. To determine which volatiles are formed in intact tissue and which are formed after cell disruption, reactions were minimized by blending tomatoes in saturated calcium chloride (CaCl<sub>2</sub>) or rapidly heated in a microwave to deactivate enzymes (Buttery, 1993; Buttery and Ling, 1993a). This assumes that enzymes were, in fact, deactivated immediately and that nonenzymatic oxidative reactions did not result in production of some volatiles. Volatiles present at  $\geq 1 \text{ nL}\cdot\text{L}^{-1}$  that appeared when tissue was disrupted, those that increased upon tissue disruption, and those that showed little change are shown in Table 2 (Buttery and Ling, 1993a). Of those that appeared upon tissue disruption, only *trans*-2-pentenal was present in concentrations  $>1 \text{ nL}\cdot\text{L}^{-1}$ , although it has negative log odor units. Several of the volatiles that increased when tissue was disrupted, including the lipid oxidation compounds that are considered to be important to tomato flavor, had positive log odor units. All but two (1-penten-3-ol and geranylacetone) had positive odor units; these two, nevertheless, were present at  $>1 \text{ nL}\cdot\text{L}^{-1}$ . For volatiles that showed little change during tissue disruption, all but three (pentanol, hexanol, and geranial) had positive odor units and, thus, are likely to contribute to tomato flavor.

Volatile precursors include lipids, amino acids, carotenoids, and terpenoids (Buttery and Ling, 1993b) (Table 3). Volatiles are formed from lipids via oxidation when cells are disrupted. The enzyme lipoxygenase (LOX), along with hydroperoxide lyase (HPL) and a

hydroperoxy cleavage enzyme, convert linoleic (18:2) and linolenic (18:3) acids to hexanal and *cis*-3-hexenal, respectively, via 9- and 13-hydroperoxy- C18:2 and -C18:3 intermediates (Fig. 1). Hexanal and *cis*-3-hexenal can be reduced to hexanol and *cis*-3-hexenol, respectively, by a reductase enzyme such as alcohol dehydrogenase (ADH). Further isomerization of *cis*-3-hexenal to *trans*-2-hexenal can occur, either enzymatically or nonenzymatically (Galliard et al., 1977; Jadhav et al., 1972; Riley et al., 1996; Stone et al., 1975). This process is similar for tomato leaves and fruits (Buttery and Ling, 1993b; Galliard et al., 1977). The ability to form volatiles upon tissue disruption, however, changes during ripening (Fig. 2). Levels of hexanal, *cis*-3- and *trans*-2-hexenal, formed after tissue homogenization, increase as the fruit ripens (Baldwin et al., 1991a).

Other volatiles arise from amino acid precursors, including alanine, valine, leucine, isoleucine, and phenylalanine (Table 3). This occurs mostly in the fruit during the ripening process rather than upon cell disruption. Tomato fruit contain some highly unusual, volatile, nitro compounds (Buttery, 1993), which are not found in any other fresh fruit or vegetable, but do occur in some flowers. They can, however, be formed by degradation of amino acids and sugars in cooked foods. They appear as tomatoes ripen from breaker to mature red stages. One proposed pathway is via an amino acid, such as phenylalanine, which can be converted to 1-nitro-phenylethane and thence to phenylacetaldehyde in vitro by reducing the pH from 10.0 to 4.5 (Nef reaction) (Buttery, 1993). The proposed pathway for phenylacetaldehyde and 3-methylbutanal, therefore, is from 1-nitro-2-phenylethane and 1-nitro-3-methylbutane, perhaps by some enzymatic system in the fruit.

Alternatively, volatile aldehydes such as phenylacetaldehyde and 3-methylbutanal could be formed from enzymatic oxidation of corresponding alcohols that are released by enzymatic hydrolysis of glycosides during ripening. Glycosides can also be precursors of volatile aroma compounds (Williams, 1993). Isolation of a fresh tomato glycoside fraction yielded 3-methylbutyric acid and  $\beta$ -damascenone

Table 2. Volatiles present in tomato at levels  $\geq 1 \text{ nL}\cdot\text{L}^{-1}$  that (A) appear after tissue disruption, (B) increase after tissue disruption, or (C) show no change after tissue disruption and are assumed to be present in the intact fruit.<sup>a</sup>

A) Appear after tissue disruption	B) Increase after tissue disruption	C) No significant change due to tissue disruption
<i>trans</i> -2-Pentenal	<i>cis</i> -3-Hexenal <i>trans</i> -2-Hexenal Hexanal <i>trans</i> -2-Heptenal 1-Penten-3-one 1-Penten-3-ol Geranylacetone	3-Methylbutanol Pentanol <i>cis</i> -3-Hexenol Hexanol 6-Methyl-5-hepten-2-one Phenylacetaldehyde 2-Phenylethanol
Geranial	2-Isobutylthiazole 1-Nitro-2-phenylethane	

<sup>a</sup>Buttery and Ling (1993a).

Table 3. Origins of tomato volatiles present in fresh tomato leaves (L), fruit (F) stems (S), and all plant parts<sup>a</sup>.

Lipid-L+F	Amino acid—mostly only in F		Carotenoid-F only
Hexanal	Alanine:	Acetaldehyde	Open chain:
Hexanol	Valine:	1-Nitro-2-methylpropane	Geranylacetone
<i>cis</i> -3-Hexenal	Leucine:	3-Methylbutanol	6-Methyl-5-hepten-2-one
<i>trans</i> -2-Hexenal		1-Nitro-2-methylpropane	6-Methyl-5-hepten-2-ol
<i>trans</i> -2-Heptenal		3-Methylbutanal	Pseudoionone
<i>cis</i> -3-Hexenol		3-Methylbutylnitrile	Cyclic:
Pentanol		3-Methylbutanol	$\beta$ -Ionone
1-Penten-3-one		1-Nitro-3-methylbutane	$\beta$ -Cyclocitral
1-Penten-3-ol		3-Methylbutyric acid	$\beta$ -Damascenone
2-Isobutylthiazole			
<u>Lignin and miscellaneous</u>			
—all parts	Isoleucine:	2-Methylbutanol	Terpenoid—mostly L+S
Methyl salicylate		2-Methylbutyric acid	$\alpha$ -Copaene (green fruit)
Eugenol	Phenylalanine:	Phenylacetaldehyde	Linalool <sup>b</sup>
Benzaldehyde		2-Phenylethanol	Neral <sup>b</sup>
Guaiacol		1-Nitro-2-phenylethane	Geranial <sup>b</sup>
		Phenylacetoneitrile	

<sup>a</sup>Buttery and Ling (1993b).

<sup>b</sup>Oxygenated.

## ENZYMATIC DEGRADATION OF LIPIDS

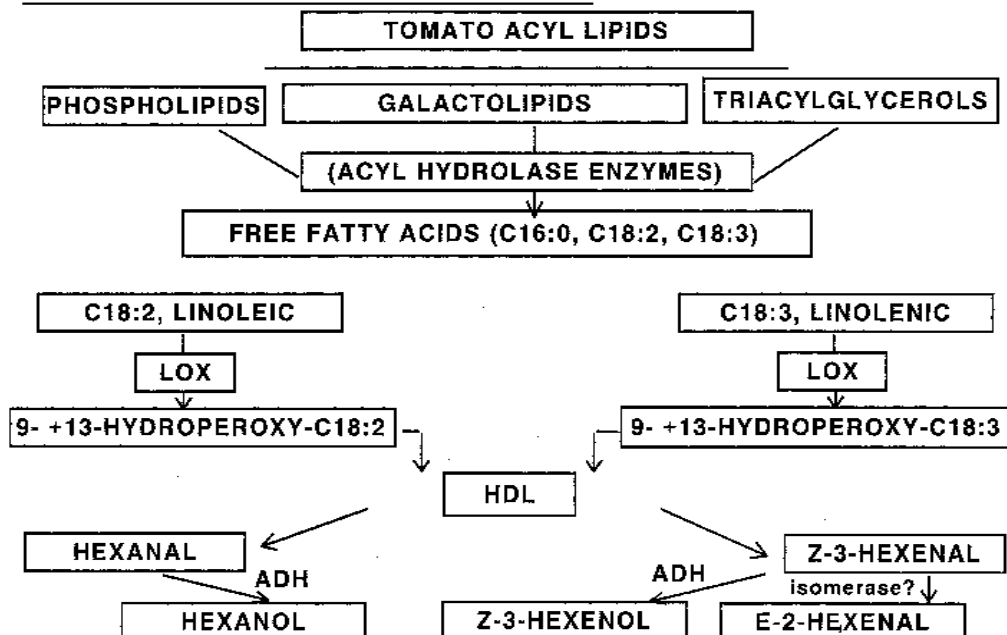


Fig. 1. Biosynthetic pathway for formation of  $C_6$  "green" aroma volatiles from degradation of acyl lipids in blended tomato fruit (Gallaird, et al., 1977; Riley et al., 1996). LOX = lipoxygenase, HDL = hydroperoxide lyase, and ADH = alcohol dehydrogenase.

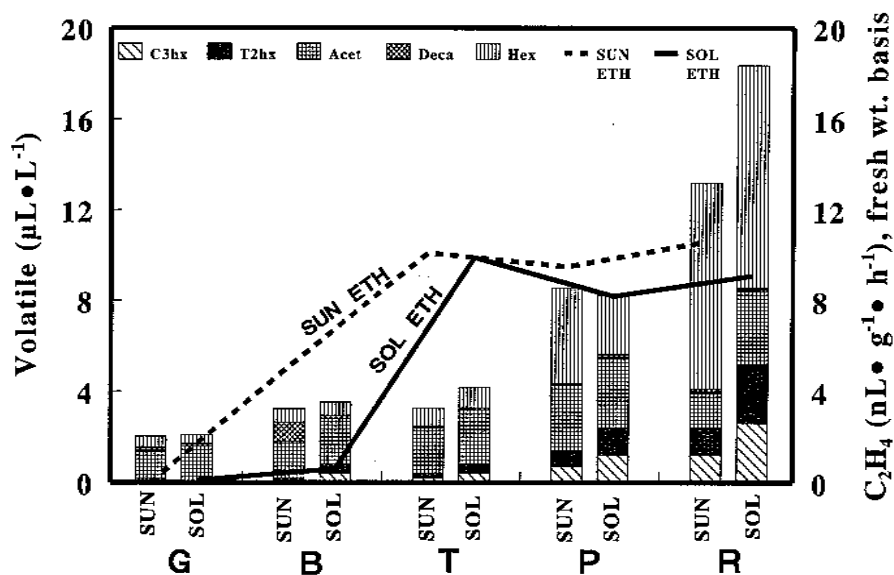


Fig. 2. Concentrations of aldehyde volatiles *cis*-3-hexenal (C3hx, HSD = 0.5), *trans*-2-hexenal (T2hx, HSD = 1.2), acetaldehyde (Acet, HSD = 1.0), *trans*-2-*trans*-4-decadienal (Deca, HSD = 0.07), hexanal (Hex, HSD = 3.1), and ethylene (ETH) production in homogenates of 'Sunny' (SUN ETH) and 'Solar Set' (SOL ETH) tomatoes sampled at the mature green (G), breaker (B), turning (T), pink (P), and red (R) ripening stages. Values are means for five replications. HSD (honestly significant difference) for ripening determined by Tukey's studentized range (after Baldwin et al., 1991a).

as major products upon hydrolysis. Other products included phenylacetaldehyde, 2-phenylethanol, linalool, linalool oxides, hotrienol,  $\alpha$ -terpineol, 4-vinylguaiacol, 4-vinylphenol (Buttery, 1993; Buttery et al., 1990). The volatile furanone [2,5-dimethyl-4-hydroxy-3-(2H)-furanone] may also be formed from enzymatic hydrolysis of furanone-glycoside during ripening, since its concentration is little affected by blending (Buttery et al., 1995). It has a pineapple-like odor and may contribute to flavor since it has relatively high odor units (1.4). Although hexanol, *cis*-3-hexenal, and 6-methyl-5-hepten-2-one are also found after hydrolysis of glycosides, they are probably not bound as glycosides, but formed after blending (Buttery et al., 1990).

Carotenoids may also be precursors of some important flavor

volatiles (Table 3) (Buttery and Ling, 1993a). They are formed from  $C_8$ ,  $C_{13}$ , and  $C_{18}$  linear and cyclic isoprene ( $C_5$ ) composites. This occurs only in the fruit and, in some cases, is dependent on cell disruption.

Finally, some volatiles are formed from terpenoids ( $C_{10}$  and  $C_{15}$ ), related to lignin or of unknown origin (Table 3). Only one terpenoid volatile ( $\alpha$ -copaene) has actually been identified in tomato fruit and occurred only in green fruit (Buttery and Ling, 1993b). However, several oxygenated terpenoid volatiles, which are important for flavor of other fruits, have been identified in ripe tomatoes, although their log odor units are below zero (linalool, nerol, and geranial). Lignin-related and miscellaneous volatiles include methyl salicylate, eugenol, benzaldehyde, and guaiacol. Of these, only methyl salicylate has log odor units  $>0$ .

## TARGETS FOR GENETIC CONTROL

Following tissue disruption, lipoxygenase is a key enzyme in the rapid formation of volatiles from lipids. The action of HPL ultimately forms hexanal and *cis*-3-hexenal. Reduction or isomerization of these compounds by ADH and perhaps an isomerase produces hexanol, *cis*-3-hexenol, and *trans*-2-hexenal (Galliard et al., 1977; Jadhav et al., 1972; Stone et al., 1975). All but hexanol in this group of volatiles have relatively high log odor units (Buttery, 1993). Conceivably, therefore, regulation of gene expression of LOX, HPL, and ADH might impact tomato fruit flavor. However, homogenation of tomato tissue may exaggerate the importance of lipoxygenase-derived volatiles because of the wounding effect, since wounding also induces formation of this enzyme (Hildebrand, 1989).

In a study of activities and subcellular location of LOX and HPL, Riley et al. (1996) reported that the majority of LOX activity was soluble, but the microsomal form of LOX changed most during ripening of tomato fruit. The activity of this isozyme increased between the green and breaker ripening stages and then decreased as the fruit turned red. Most of the HPL activity was found in the microsomal fraction and did not change during ripening. Riley et al. (1996) therefore suggested that volatile flavor formation takes place in the microsomal compartment. This was further confirmed by Riley and Thompson (1997), who also suggested that "blebbed" lipid particles (pinched off of membranes) may serve to move flavor volatiles out into the cytosol.

Two genes for LOX, *tomloxA* and *tomloxB*, have been cloned in tomato, using degenerate oligonucleotides corresponding to a purified, partially sequenced (Ferrie et al., 1994), membrane-associated LOX of  $\approx$ 97-kD (Bowsher et al., 1992) by probing breaker fruit cDNA library. *TomloxA* is expressed in seeds and fruit, reaches its highest level in fruit during the breaker stage and corresponds to the membrane-associated LOX. *TomloxB* is fruit-specific and reaches its highest level in ripe fruit. Another report describes two LOX activities in tomato fruit pericarp that increase in early stages of ripening and subsequently decrease, roughly paralleling ethylene production. The activities were associated with proteins of 95- and 97-kD (Ealing, 1994).

A 94-kD LOX was purified and cloned from a red-ripe fruit cDNA library (Kausch and Handa, 1997); it corresponded with a 94-kD protein that accumulated in the fruit during ripening, and reached a maximum concentration at the red-ripe stage. Expression of this gene was highest in the radial pericarp, but the highest LOX activity was in the locular gel, suggesting that LOX might have been synthesized in the pericarp and transported to the locular tissue where it accumulated during ripening. Buttery et al. (1988) reported that levels of hexanal, *cis*-3-hexenal, and *cis*-3-hexenol in the locular fluid of dissected fruit was 50% to >100% that of levels in intact fruit. The pulp contained >100% of the levels found in the intact fruit for these volatiles. This increase in volatile concentrations during or following dissection was attributed to wounding (Buttery et al., 1988). Interestingly, the 94-kD LOX gene was not expressed in the nonripening (*nor*) mutant, whereas the never-ripe (*Nr*) mutant accumulated the LOX mRNA, but not the protein. Nonexpression in *nor* fruit indicates that expression of the 94-kD LOX is linked to the ripening process. Since the *Nr* mutation blocks ethylene perception (Lanahan et al., 1994), accumulation of the LOX mRNA, but not the protein, indicates that ethylene may play a role in posttranscriptional regulation of the 94-kD LOX, perhaps through translation, or that ethylene affects the stability of the protein. Apparently ethylene perception is not required for gene expression and subsequent mRNA accumulation. The ability to synthesize hexanal, *cis*-3-hexenal, *cis*-3-hexenol, and *trans*-2-hexenal increases during ripening, peaking at the pink to red-ripe stage, and is closely associated with ethylene production (Fig. 2) (Baldwin et al., 1991a).

Another important enzyme is ADH, which reduces hexanal and *cis*-3-hexenal to hexanol and *cis*-3-hexenol, respectively. Two genes for ADH have been identified in tomato. One (*adh1*) is expressed in developing seed and pollen and responds to anaerobic stress (Longhurst et al., 1990). Hypoxic conditions are well known to increase ADH gene transcription in many plant parts (DeLisle and Ferl, 1990). The other (*adh2*) is expressed in a range of tissues, including the fruit, and

responds to anaerobic stress but also increases during ripening in the presence of normal oxygen levels. The *adh2* gene product is present in tomato pericarp 15 d prior to the initiation of ripening. The activity decreases and then increases late in ripening. Changes in cytoplasmic pH may induce ADH activity, since anaerobic stress is not a factor during tomato fruit ripening (Longhurst et al., 1990). A recent study demonstrated the effect of genetic manipulation of ADH levels in ripening tomato fruit. Tomato plants were transformed with constructs containing a tomato *adh2* cDNA. The resulting fruit, with higher or lower levels of ADH activity, exhibited corresponding higher and lower levels of hexanol and *cis*-3-hexenol. Fruit with increased levels of these alcohols were rated higher in "ripe fruit" flavor by a sensory panel (Speirs et al., 1998).

In one study, alteration of the fatty acid composition of tomato fruit led to changes in the flavor profile (Wang et al., 1996). Over-expression of the yeast  $\Delta$ -9 desaturase gene increased concentrations of unsaturated and saturated fatty acids in transformed tomato fruit, some of which are precursors of important flavor volatiles. The most dramatic increases were in levels of palmitoleic acid (16:1), 9,12-hexadienoic acid (16:2), oleic acid (18:1), and linoleic acid (18:2), the latter being a precursor of hexanal. Because of the increased LOX substrates, transformed plants produced fruit with higher levels of hexanal and hexanol. This was expected since higher levels of hexanal precursor (linoleic acid) were present. Surprisingly, fruit from transformed plants also had higher levels of linolenic acid peroxidation products, such as *cis*-3-hexenal, *trans*-2-hexenal, and, subsequently, *cis*-3-hexenol via ADH.

## CONSEQUENCES OF GENETIC CONTROL

Down-regulation of cell wall-digesting enzymes, such as polygalacturonase (PG) and pectinmethylesterase (PME), can alter the cell wall structure of tomato fruit. The enzyme PME demethylates pectin and PG hydrolyzes mainly demethylated pectin (Huber, 1983; Pressey and Avants, 1982). Transformation of fruit with the antisense genes for these enzymes inhibited PG to <1% of the normal level, extended shelf life, and increased disease resistance and solids content (Hobson and Grierson, 1993; Kramer et al., 1992; Schuch et al., 1991). Because cell wall structure can impact aroma binding and release, we assessed, in a preliminary study, the release of flavor volatiles into the headspace of homogenates prepared from transgenic, ripe tomato fruit with down-regulated PG, PME, and PG + PME activities (Table 4 A and B). The flavor volatile data from transgenic fruit, obtained by the method of Baldwin et al. (1992a), are shown as percentages of the values for nontransformed fruit. In the first study (Table 4A), red-ripe tomatoes with down-regulated PG (Kramer et al., 1992; Sheehy et al., 1988) produced lower levels of some volatiles, including methanol, ethanol, 1-penten-3-one, hexanal, 2+3-methylbutanol, *trans*-2-hexenal, *trans*-2-heptenal, 6-methyl-5-hepten-2-one, *cis*-3-hexenol, 2-isobutylthiazole, and geranylacetone, than did nontransformed fruit. In the second study (Table 4B), transformation of 'Ailsa Craig' fruit down-regulated PG (pTOM 6) (Grierson and Schuch, 1993; Smith et al., 1988) and reduced PME (PE1) (Hall et al., 1993) and PG+PME activities. In this case, the levels of flavor volatiles were similar to those of nontransformed controls except for a reduction in methanol in antisense PME and PG+PME fruit (Table 4B). Thus, at least some of the methanol observed in the headspace of homogenized tomato fruit (Baldwin et al., 1991a, 1991b) comes from demethylation of cell walls by PME once enzyme and substrate are mixed upon cell disruption. Nisperos-Carriedo and Shaw (1990) also suggested that this was the source of the methanol observed in the headspace of orange [*Citrus sinensis* (L.) Osbeck] juice, which also has active PME. Fruit with down-regulated PG+PME also exhibited low levels of 2+3-methylbutanol, 2-isobutylthiazole, and 6-methyl-5-hepten-2-one, the first two of which are derived from amino acids (Table 3). The third is thought to arise from carotenoid breakdown (Buttery and Ling, 1993b) and is similar in structure to geranylacetone (one isoprene unit difference between the two compounds), which was at normal levels in the transformed fruit. Despite their apparently similar origin, these two volatiles often behave differently, indicating a possible alternate mechanism for biosynthesis of 6-methyl-5-hepten-2-one. For ex-

Table 4. Tomato flavor volatiles in ripe homozygous and heterozygous transgenic fruit expressed as a percentage of concentrations in non-transformed controls (NT) including antisense PG (AS-PG), antisense ACC (AS-ACC) and its hybrid (AS-ACC x NT), antisense PME (AS-PME), antisense PG+PME (AS-PG+PME), antisense ACO (AS-ACO), pTOM99 (AS-99), and antisense phytoene synthase (AS-PSY).

Fruit type	Volatile (% of nontransformed controls) <sup>2</sup>														
	acet	meoh	etoh	pent	hex	c3hx	mbut	t2hx	t2hp	mhep	c3hol	iso	nphn	ger	bio
<i>Study A<sup>3</sup></i>															
AS-PG	79	59	56	67	39	123	49	68	54	46	53	50	77	61	218
AS-ACC	69	67	51	48	48	82	44	64	54	37	59	40	85	46	173
AS-ACC x NT	67	87	74	60	80	105	49	69	69	48	65	60	77	55	173
<i>Study B<sup>4</sup></i>															
AS-PG	85	79	---	100	108	104	102	125	---	85	167	100	---	118	102
AS-PME	116	46	---	98	102	102	114	110	---	80	117	100	---	130	102
AS-PG+PME	69	42	---	102	84	107	57	88	---	63	175	0	---	98	102
AS-ACO	78	82	---	78	121	117	31	74	---	52	108	100	---	73	150
AS-99	113	104	---	82	108	77	83	138	---	107	175	200	---	148	271
AS-PSY	54	92	53	80	140	172	58	104	66	56	50	35	69	14	16

<sup>2</sup>Volatiles analyzed: acetone (acet), methanol (meoh), ethanol (etoh), 1-penten-3-one (pent), hexanal (hex), *cis*-3-hexenal, (c3hx), 2+3-methylbutanol (mbut), *trans*-2-hexenal (t2hx), *trans*-2-heptenal (t2hp), 6-methyl-5-hepten-2-one (mhep), *cis*-3-hexenol (c3hol), 2-isobutylthiazole (iso), 1-nitro-2-phenylethane (nphn), geranylacetone (ger), and  $\beta$ -ionone (bio).

<sup>3</sup>Data are means of two samples, each a composite of three ripe fruit, harvested field ripe.

<sup>4</sup>Data are means of three to five samples, each a composite of two to three ripe fruit, harvested 6 d past breaker stage; "—" indicates not measured.

ample, this compound did not increase following cell disruption, as did geranylacetone (Buttery and Ling, 1993a), and was a product of glycoside hydrolysis, whereas geranylacetone was not (Buttery et al., 1990).

One way to extend shelf life is to delay or reduce ethylene production. Transformed fruit with down-regulated key enzymes in the ethylene biosynthesis pathway exhibited extended shelf life (Murray et al., 1993; Oller et al., 1991). Ethylene is synthesized from *S*-adenosyl methionine via the intermediate, 1-aminocyclopropane-1-carboxylic acid (ACC), by action of ACC synthase; ACC in turn, is oxidized to ethylene via ACC oxidase (ACO, also known as ethylene-forming enzyme) (Adams and Yang, 1979; John, 1997). Fruit in which expression of ACC synthase is inhibited (Oeller et al., 1991) produce only 1% to 5% of normal ethylene levels and do not ripen completely without treatment with exogenous ethylene. Fruit in which ACO expression is inhibited (Hamilton et al., 1990) produce only 3% of normal ethylene levels, yet ripen slowly, especially if left on the plant. Ripening can be accelerated by treatment with exogenous ethylene (Murray et al., 1993). Gene pTOM99, which is identical to E8 (Slater et al., 1985), is reportedly regulated by both ethylene and other fruit-ripening signals. Meanwhile, the gene appears to negatively regulate ethylene biosynthesis (Deikman, 1997; Penarrubia et al., 1992). Since ethylene production often correlated with synthesis of pigments and flavor volatiles (Baldwin et al., 1991a; Buttery, 1993), we investigated the effect of altered ethylene production on levels of flavor volatiles.

In one study, red-ripe fruit with down-regulated ACC synthase (Oeller et al., 1991) produced lower levels of all volatiles measured except *cis*-3-hexenal, 1-nitro-2-phenylethane, and  $\beta$ -ionone (Table 4A). Hybrid fruit, resulting from a cross between plants with antisense ACC synthase and nontransformed plants, generally showed levels of volatiles intermediate between those of the two parents. The differences between ACC nontransformed control, hybrid, and antisense ACC fruit were significant ( $P \leq 0.05$ ) for nine volatiles (Baldwin et al., unpublished). Transgenic fruit had less red color development, as evidenced by lower "a\*" values when measured by a chromameter by the method of Baldwin et al. (1991a) than did nontransformed fruit. The hybrid fruit again showed "a\*" values intermediate between those of the two parents ( $a^* = 3.1, 4.0$ , and  $6.8$  for the transformed, hybrid, and nontransformed fruit, respectively). Levels of the major sugars, glucose and fructose, as analyzed by HPLC (Baldwin et al., 1991a, 1991b), were similar in transformed, hybrid, and nontransformed fruit (Baldwin et al., unpublished).

Fruit from the transgenic 'Ailsa Craig' line (same line as the antisense PG, PME, and PG+PME in Table 4B) exhibited less activity of ACO (pTOM13) (Hamilton et al., 1990; Murray et al., 1993), a key enzyme for the formation of ethylene, and had longer shelf life (Murray et al., 1993; Pictou et al., 1993). Volatile production was little affected except for reduction in 2+3-methylbutanol and 6-methyl-5-hepten-2-one (Table 4B). Fruit with the antisense gene pTOM99

produced normal to high levels of flavor volatiles. The antisense ACC synthase fruit from the first study and the antisense ACO and pTOM99 fruit from the second study all had abnormally high levels of  $\beta$ -ionone.

Phytoene synthase (PSY) is a key enzyme in the synthesis of phytoene, a precursor of carotenoids (Gross, 1991). Down-regulation of this enzyme in pTOM5 tomatoes resulted in fruit with no red color (Bird et al., 1991). This resulted in negative "a\*" values when measured with a chromameter ( $a^* = -4.0$  and  $+6.4$  for antisense PSY and nontransformed control fruit, respectively). Analysis of flavor volatiles from antisense PSY fruit revealed lowered levels of all but methanol and some lipid-derived volatiles (1-penten-3-one, hexanal, *cis*-3-hexenal, and *trans*-2-hexenal). Levels of most of the amino acid and carotenoid-derived volatiles were reduced, and in the case of geranylacetone and  $\beta$ -ionone, to nearly trace levels. Surprisingly, however, 6-methyl-5-hepten-2-one was reduced to only 56% of the levels in nontransformed controls. This again suggests an alternate route for synthesis of this compound. The levels of eight volatiles, including the amino acid and carotenoid-derived compounds, were significantly higher in nontransformed fruit.

The effects of the various antisense transformations may have been due to repression of a specific gene product, but could also have been related to the site at which the antisense gene was inserted. In the latter case, different transgenic lines could exhibit different volatile levels.

## CONVENTIONAL BREEDING AND POSTHARVEST TREATMENTS

Selecting tomatoes with higher levels of carotenoids may result in higher levels of carotenoid-derived volatiles, such as 6-methyl-5-hepten-2-one,  $\beta$ -ionone,  $\beta$ -damascenone, and geranylacetone, that have positive or borderline log odor units. Stevens (1970) reported high correlations between certain carotenoid-derived volatiles and specific carotenoids in tomato. Buttery et al. (1988) found that tomato cultivars containing higher levels of carotenoids also contained higher levels of 6-methyl-5-hepten-2-one, geranylacetone, and  $\beta$ -ionone than did those with lower concentrations of carotenoids. The volatile 6-methyl-5-hepten-2-one was higher in the high lycopene cultivar, geranylacetone was higher in the high lycopene and high  $\beta$ -carotene cultivars, and  $\beta$ -ionone was higher in the high  $\beta$ -carotene cultivar. Tomatoes cultured *in vitro* were induced to produce more lycopene pigment by addition of 2-(4-chlorophenylthio)triethylamine (CPTA), which resulted in an increase of some carotenoid-derived volatiles (Ishida et al., 1998).

Reducing ethylene production and thereby slowing down the ripening and softening process can also be accomplished using the ripening mutants, including nonripening (*nor*), ripening inhibitor (*rin*), never ripe (*Nr*), and alcobaca (*alc*). The mutants *rin* and *nor* do not ripen, do not display the climacteric rise in carbon dioxide or ethylene, and contain little PG activity. The *rin* and *nor* fruit fail to

develop normal red color. *Nr* fruit will ripen to a deeper red than either *rin* or *nor* and may contain low PG activity. *Alc* fruit have prolonged keeping qualities and can develop a light orange-red color on the plant, but do not ripen off the plant if harvested when mature green (Mutschler and Guttieri, 1987). All of these fruit have been used by breeders in crosses with normal-ripening lines to produce hybrids with extended shelf life characteristics, although *rin* hybrids have been the most successful commercially. It was generally assumed, as with the antisense PG, ACC, or EFE fruit, that quality of the ripened product would be improved because of the possibility of advanced harvest maturities (past color-break). This was based on work by Kader et al. (1977), where tomatoes ripened past breaker stage had better flavor.

Over the past several years, our laboratories have been analyzing *rin* hybrids from different sources and with different genetic backgrounds. Hybrids with the *rin* gene often have a pale red color (Baldwin et al., 1995) and both sensory and volatile analyses indicated that *rin* hybrids had lower levels of many important volatiles at the red-ripe stage (Baldwin et al., 1992a, 1992b, 1995) (Fig. 3A–D). This was true even in fruit harvested past the breaker stage when compared with normal tomatoes harvested mature green (Fig. 3A–D). Yet there was no consistent pattern of difference in soluble solids or titratable acidity (Baldwin et al., 1995) (Fig. 3 E and F). Firmness and shelf life, however, were often greater in the hybrids. Sensory work indicated

that *rin* hybrids were ranked lower than other normal fruit by consumer and experienced panels (Baldwin et al., 1995) (Fig. 3E) and were ranked lower in flavor intensity by trained panels (Baldwin et al., 1998, unpublished data). Nevertheless, incorporation of the *rin* gene into high flavor and color backgrounds may compensate for the *rin* effect on flavor and color while improving shelf life. Another study showed that both *rin* and *nor* hybrid fruit (backcross 6 generation with 'Rutgers') were deficient in volatiles found in 'Rutgers' tomatoes (McGlasson et al., 1987). The author is not aware of published information on flavor or volatile levels in *Nr* fruit.

In addition to using antisense technology and ripening-impaired mutants to indirectly improve flavor through advanced harvest maturity, breeders try to select for better-flavored tomatoes. The problem is that identifying objective measurements that signify good flavor is difficult (Baldwin et al., 1998). Which flavor components are important and what are the appropriate levels and balance for good flavor is still not understood. Analysis of flavor compounds in the aromatic component requires expensive equipment and training. Breeders can use sensory analysis, but this is often difficult to perform and requires access to a panel and considerable expertise. Even sensory descriptive data are limited in applicability as they provide information on specific flavor characteristics, but do not indicate consumer acceptability (Shewfelt, 1993). Nevertheless, significant differences exist between

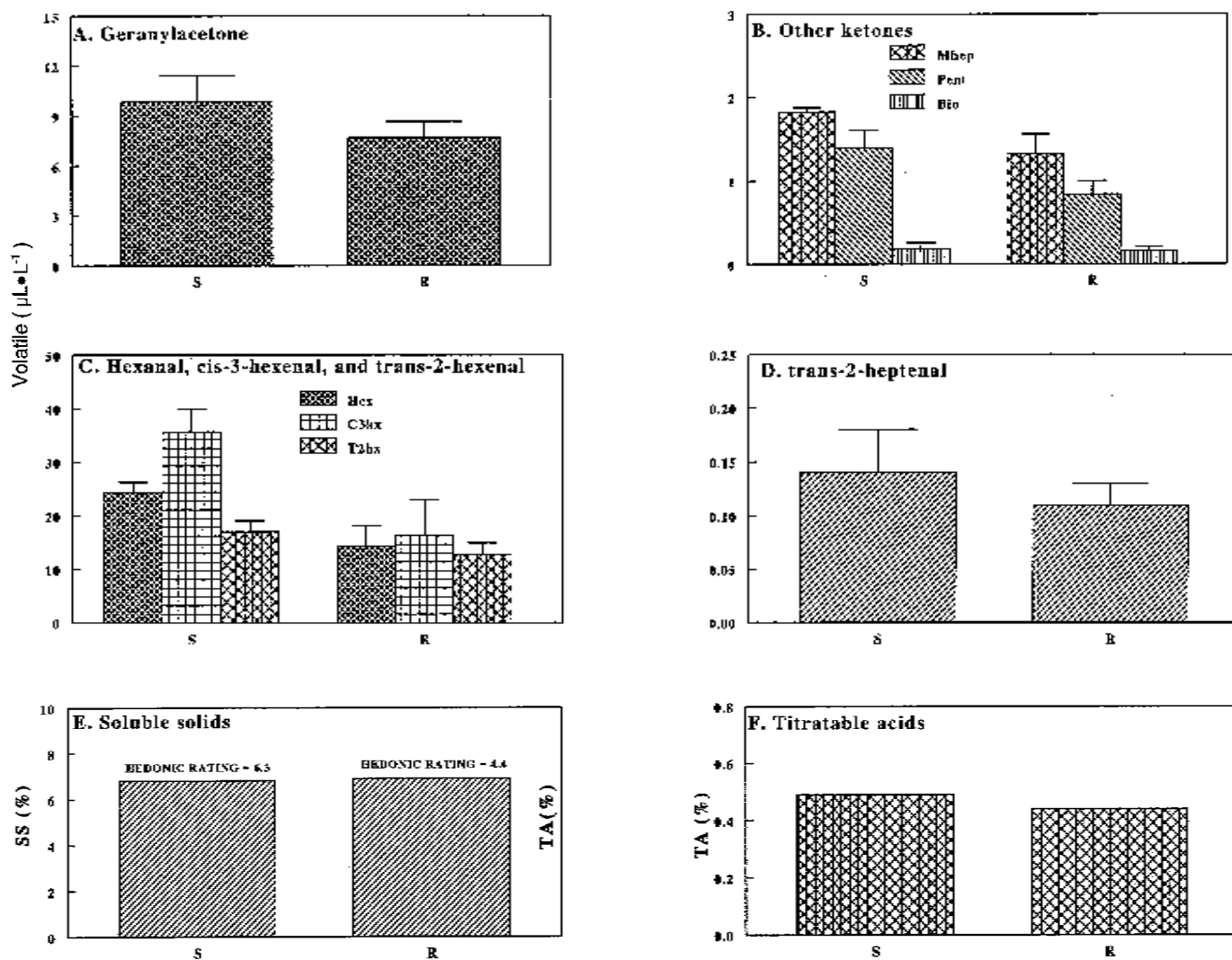


Fig. 3. Headspace ketone volatiles (A) geranylacetone (Ger), (B) 6-methyl-5-hepten-2-one (Mhep), 1-penten-3-one (Pent), and  $\beta$ -ionone (Bio); headspace aldehyde volatiles (C) hexanal (Hex), *cis*-3-hexenal (C3hx), *trans*-2-hexenal (T2hx), and (D) *trans*-2-heptenal (T2hp) in homogenates of 'Solar Set' (S) tomato harvested mature green and in a *rin* hybrid (R) harvested at breaker stage; and (E) soluble solids (SS) and (F) titratable acidity (TA) in homogenized fruit tasted by 28 experienced panelists and rated on a hedonic scale of 1 to 9 for overall flavor (E). Data for (A–D) are means of three replicate samples  $\pm$ SD, while (E) and (F) represent one composite sample for SS and TA, all from the same fruit (10 fruit/cultivar) sampled by the 28 panelists (E).

cultivars in levels of important aroma compounds (Baldwin et al., 1991b, 1995).

Climatic and cultural conditions can also affect tomato flavor. For example, heavy rains prior to harvest appear to dilute the concentrations of flavor compounds (Baldwin et al., 1995). Heavy fertilization with nitrogen and potassium reduced sensory analysis scores, and increased levels of titratable acidity, soluble solids, and several volatiles (including hexenal, phenylacetaldehyde,  $\beta$ -ionone, and 6-methyl-5-hepten-2-one among others) in tomato fruits (Wright and Harris, 1985). Levels of 12 out of 15 volatiles were determined to be higher in field-grown tomatoes than in greenhouse-grown fruit (Dalal et al., 1967).

Finally, harvesting and handling techniques impact the flavor of the ripened tomato fruit. The immature green fruit (determined non-destructively by number of days to break color under ethylene treatment) that can make up a significant percentage of the harvest in a gas green operation, do not ripen with acceptable flavor, as evidenced in sensory and volatile data (Maul, 1999; Maul et al., 1998ab). Bruising of fruit during harvest and handling also alter aroma volatile profiles (Maul et al., 1997; Moretti et al., 1997). Certain volatiles that increase in controlled atmosphere storage (5% O<sub>2</sub> and 4.4% CO<sub>2</sub>) do not do so in air storage (Crouzet et al., 1986). Temperature abuse is also a problem that could be controlled. Temperatures below 16 °C may impair tomato flavor by lowering the volatile content and reducing "tomato-like" flavor (Kader et al., 1978). Storage at 2, 5, 10, 12.5 and 13 °C reduced levels of important volatiles (Baldwin et al., 1992b; Buttery et al., 1987; Maul, 1999). The mechanism by which volatile levels are reduced is not known, but could be related to reduced ethylene synthesis at low temperatures. Tomatoes stored at 2, 5, 10 or 12.5 °C also had less ripe aroma and tomato flavor, as well as more off-flavor, when analyzed by a trained descriptive panel (Maul, 1999).

More studies are needed to really understand the effects of climate, and of cultural and postharvest handling practices on tomato flavor. Nevertheless, poor flavor quality in tomato appears to be a result of breeding practices that do not select for flavor (because of lack of information), harvesting of green fruit (because of prevalence of immature green fruit in commercial harvests), and temperature abuse (because of storage of fruit below 16 °C, which results in impaired volatile levels). Harvest and handling practices could conceivably be altered, but information on flavor for use by breeders and molecular biologists is lacking. Considerable progress has been made in the identification of important flavor components in tomato and the determination of their concentrations in fresh fruit. Additional information is needed, however, on the optimal ranges and ratios for sugars, acids and aromatics required for good flavor. Future work needs to address what instrumental and sensory methods are most effective for evaluating important tomato flavor components. Likewise, establishing the relationship between instrumental measurements and sensory analysis is essential and will allow a more reliable assessment of the effects of breeding, genetic transformation, harvest maturity and postharvest handling on product quality.

The biosynthetic pathway for lipid-derived volatiles has been largely determined, but specific information is lacking on enzyme systems and other possible mechanisms of formation of amino acid and carotenoid groups. This information is necessary for the pragmatic manipulation of levels of flavor volatiles in the fruit. The relationship between volatile formation and ethylene-induced ripening events is not clearly understood, although lipoxygenase appears to be linked to such events. This enzyme may require posttranscription regulation by ethylene as well (Kausch and Handa, 1997). Ethylene is necessary for normal carotenoid development and, thus, may at least indirectly control synthesis of carotenoid-derived volatiles. Control mechanisms for amino acid-derived and other miscellaneous volatiles are little understood.

Thus far, flavor quality for tomato (or any other horticultural product) has been an elusive trait. We still lack quantifiable definition for tomato flavor. Increased performance of analytical instruments, availability of new sensor technology, advances in biotechnology, and development of powerful computer programs make identification and quantification of important chemical components possible. Furthermore, these components can then be traced back to sensory descrip-

tors, key enzymes, and gene products. Ultimately, identification and isolation of genes that influence eating quality would be useful. Integrating the fields of plant breeding, molecular biology, postharvest physiology and food science should provide superb opportunities for major breakthroughs in this area.

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