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Effect of Temperature on Lipid-Related Volatile Production in Tomato Puree

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Lipid-related volatiles were measured in real time after the blending of grape tomatoes, using selected ion flow tube mass spectrometry (SIFT-MS). Measurements were made at 4, 23, or 37 °C. The volatiles in the headspace of the tomatoes, other than hexanal, increased with increasing temperature. The concentration of hexanal in the headspace increased from 4 to 23 °C, but decreased at 37 °C. The activity of hexanal-specific hydroperoxide lyase decreases at 37 °C. Moreover, precursors of hexanal may go through alternative pathways to form *trans*-2-heptenal and *trans*-2-octenal. The increase in concentration in the headspace for most volatiles can be explained by the increase in volatility, except for *trans*-2-heptenal, *trans*-2-octenal, and *trans*-2-pentenal. These three volatiles appear to be generated at a much higher rate at 37 °C due to the dominance of alternate pathways at this temperature. Temperature did not affect the time to peak level for most volatiles, except the time for hexanal was shorter with increasing temperature. A temperature-dependent lipoxygenase pathway was postulated.

KEYWORDS: Tomato; lipoxygenase pathway; temperature; volatile

INTRODUCTION

Aroma is one of the most important quality attributes of tomato (*1*). There are over 400 aroma volatiles identified in tomato (*2*), but only a dozen are critical to the characteristic tomato aroma, most of which are derived from the lipoxygenase pathway (*3*). The lipoxygenase pathway is ubiquitous in fruits and vegetables and refers to sequential reactions breaking down free fatty acids by an enzyme cascade involving lipoxygenase (LOX), hydroperoxide lyase (HPL), alcohol dehydrogenase (ADH), and possibly other enzymes (*4*, *5*). The widely accepted lipoxygenase pathway was first proposed by Stone and others (*4*) using $_{14}C$ labeling technology. Tomato acyl lipids (phospholipid, galactolipids, and triacylglycerols) are first broken down by acyl hydrolase enzymes to free fatty acids, such as linoleic acid and linolenic acid, which are quickly converted into hexanal and *cis*-3-hexenal, respectively, by LOX and HPL. *cis*-3-Hexenal is transformed to *trans*-2-hexenal by *cis*-3/*trans*-2 isomerase (Z3/E2-ISO). These aldehydes are further transformed into alcohols by the action of ADH.

A more complicated LOX pathway has been reported in other fruits and vegetables. There are other minor volatiles produced from the LOX pathway besides these major C6 aldehydes and alcohols. 10-Hydroperoxides and 12-hydroperoxides produced from rearrangement of 9- and 13-hydroperoxides degrade to *trans*-2-octenal and *trans*-2-heptenal, respectively, by unknown enzymes in bell peppers, and to 1-octen-3-ol in mushrooms (*5*). 9-Hydroperoxides of linoleic acid produce 2-pentylfuran and *trans*-2-nonenal in cucumbers and bell peppers (*5*). 13-Hydroperoxides of linolenic acid are also the precursors of 1-penten-3-one and *trans*-2-pentenal in soybeans and bell peppers (*5*).

Most studies on the enzymatic generation of tomato volatiles are carried out at room temperature; thus, there is limited information about the effect of temperature on volatile generation, although the activity of enzymes is known to be temperature-dependent. The activity of crude tomato LOX increases from 0 to 20 °C (*6*), and is at its peak at 20 −30 °C, after which the activity decreases gradually (*7*). In addition to its effect on enzyme activity, temperature also affects the partition coefficient of volatiles. The partition coefficient of lipid oxidation generated aldehydes increases from 20 to 40 °C (*8*).

A variety of analytical techniques have been developed for tomato volatile identification and quantification, such as gas chromatography−mass spectrometry (GC-MS), atmospheric pressure ionization mass spectrometry (API-MS), and proton transfer reaction mass spectrometry (PTR-MS) (*9*, *10*). However, these techniques either have difficulty monitoring real-time release of volatiles (GC-MS) or cannot differentiate isomers (API-MS and PTR-MS), such as *cis*-3-hexenal and *trans*-2-hexenal, in tomato (*11*). Selected ion flow tube mass spectrometry (SIFT-MS) allows real-time analysis of complex mixtures of volatile compounds without trapping or preconcentration and also provides differentiation of some isomers (*12*). SIFT-MS has been applied in the analysis of breath volatiles for medical diagnosis and therapeutic monitoring (*13*), in the detection of bacterial metabolites (*14*), and in the monitoring of air pollution (*15*), real-time release of volatiles in cut onion, crushed garlic, and ripe banana (*12*), and olive oil oxidation (*16*).

The objective of this study is to detect and quantify the level of volatiles generated from the LOX pathway in the grape tomato

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Table 1. Information for SIFT-MS Analysis of Selected Volatile Compounds

volatile compound	molecular formula	precursor ion	k (10^{-9}cm^3 s^{-1})	m/z	product ion	ref
1-penten-3-one	C_5H_8O	NO^+	2.5	114	$[C_5H_8O \cdot NO]^+$	*22*
cis-3-hexenal	$C_6H_{10}O$	H_3O^+	4.2	81	$[C_6H_9]^+$	*19*
cis-3-hexenol and *trans*-2-hexenol	$C_6H_{12}O$	NO^+	2.5	72	$[C_4H_8O]^+$	*22*
hexanal	$C_6H_{12}O$	NO^+	2.5	99	$[C_6H_{11}O]^+$	*19*
hexanol	$C_6H_{14}O$	NO^+	2.4	101	$[C_6H_{13}O]^+$	*18*
trans-2-heptenal	$C_7H_{12}O$	NO^+	3.9	111	$[C_7H_{11}O]^+$	*21*
trans-2-hexenal	$C_6H_{10}O$	H_3O^+	4.6	99 + 117 + 135	$[C_6H_{11}O]^+$, $[C_6H_{11}O \cdot H_2O]^+$, $[C_6H_{11}O \cdot 2H_2O]^+$	*19*
trans-2-octenal	$C_8H_{14}O$	NO^+	4.1	156	$[C_8H_{14}O \cdot NO]^+$	*21*
trans-2-pentenal	C_5H_8O	NO^+	4.0	83	$[C_5H_7O]^+$	*21*

and determine how temperature affects the volatile generation, using SIFT-MS.

MATERIALS AND METHODS

Grape tomatoes were used in this study due to their higher level of volatiles compared to regular tomatoes. Grape tomato samples were purchased from local food supermarkets in Columbus, OH. Grape tomato samples were washed and stored for 5 h at 4, 23, or 37 °C to equilibrate before testing. All testing was carried out at the same temperature as the sample's storage temperature.

Analysis of Volatiles with and without Enzyme Activity. Grape tomato (150 g) was blended for 30 s at the highest speed in a blender (Waring, Dynamics Corp.). Tomato puree (60 mL) was transferred into a 500 mL Pyrex bottle with minimal delay (typically 30 s), and the rest was discarded. Samples were held at 4, 23, or 37 °C during testing. The headspace volatile compounds were sampled directly by piercing the septum on a 500 mL Pyrex bottle with a 14-gauge passivated needle connected to the SIFT-MS. The needle point was 16 cm above the bottom of the bottle. A second long 14-gauge syringe needle pierced the septum to touch the bottom to maintain the pressure in the bottle at atmosphere pressure and also to provide oxygen for lipid oxidation. Analysis of volatile compounds in the headspace above the tomato puree was started immediately and continued for 60 min. The Pyrex bottles with tomato puree were held in a temperature-controlled water bath at 4, 23, or 37 °C during testing, and triplicate headspace analyses were carried out. The background for room air was subtracted from the data. The ratio of the concentration of volatiles between different temperatures was compared using the concentration at the peak level during the 60 min.

For samples without enzyme activity, the enzymes in the puree were allowed to be active for 5 min, and then 60 mL of saturated $CaCl_2$ solution was added to denature the enzymes and to inhibit further volatile generation. The puree was equilibrated and analyzed at 4, 23, or 37 °C in triplicate. To minimize the effect of test order on results, the first replicate was tested in the order of 4, 23, and 37 °C, the second replicate was tested in the order of 37, 23, and 4 °C, and the third replicate was tested in the order of 23, 4, and 37 °C.

Selected Ion Flow Mass Spectrometry. SIFT-MS (SYFT Voice100, Syft Ltd., Christchurch, New Zealand) was used to measure the volatile compounds released from grape tomatoes. The SIFT-MS instrument has been described elsewhere (*17*). Analysis was performed using selected ion mode (SIM) scans, and the concentration of volatile compounds was calculated using known kinetic parameters (**Table 1**) (*18*−*22*). Using the predetermined reaction rate constant for the volatile with that precursor ion, and accounting for dilution of the sample gas into the carrier gas, the concentration of the volatile was calculated (*15*). In SIFT-MS, analytes are identified from their reactions with the reagent ions specified. These reactions are instrument independent and have been studied in a number of laboratories; the data have been published for each analyte with the appropriate reagent ion (**Table 1**). Numerous publications have studied the quantification of SIFT-MS results, but in this study it is the relative changes, rather than absolute values, that are important. Standards showed a linear response between added and measured concentrations.

The soft chemical ionization used in SIFT-MS yields a smaller range of product ions than is common in electron impact mass spectrometry (as used by GC-MS, for example). Hence, the need for GC separation of the sample is circumvented. However, the measured product mass/charge produced by reaction with one of the three reagent ions must be carefully chosen. Many of the mass/charge values are produced by several different volatiles, which creates an interference that must be removed or the results must be reported as a mixture. The m/z values in **Table 1** were chosen because they were not produced by the other compounds in the sample and, thus, uniquely measured the stated compound, with the following exceptions. *trans*-2-Hexenal has an interference with *cis*-3-hexenal at m/z 99, 117, and 135. At these m/z values, 35% of *cis*-3-hexenal and 100% of *trans*-2-hexenal are measured (*19*). However, because *cis*-3-hexenal has no interference at the m/z at which it was measured, 35% of *cis*-3-hexenal was subtracted from the concentration of *trans*-2-hexenal. At m/z 99, the product forms clusters with water, and so the water clusters at 117 and 135 had to be added to the results at 99, to capture all of the reaction that occurred (*19*). Also, *cis*-3-hexenol and *trans*-2-hexenol had irresolvable interferences and were reported as a mixture. Concentrations were reported in micrograms per liter.

The parameters of the SIFT-MS were set as follows: scan time, 2 min; calculation delay time, 5 s; product sample period, 100 ms; precursor sample period, 20 ms; heated inlet temperature, 120 °C; carrier gas argon pressure, 200 kPa; helium pressure, 30 psi. The flow tube vacuum pressure was 0.038 ± 0.003 Torr.

Statistical Analysis. Data were analyzed by one-way analysis of variance (ANOVA) using the least significant differences for means (LSD) technique with the SAS program (SAS 9.1, SAS Institute Inc.). Significance was defined as $p \leq 0.05$.

RESULTS AND DISCUSSION

Effect of Temperature on Linoleic Acid Related Volatiles. *Hexanal.* Hexanal is associated with the "green" aroma of tomato (*23*). Due to its low threshold value, 4.5 μg/L in aqueous solution, hexanal is a strong component of fresh tomato aroma (*2*). After blending, the headspace concentration of hexanal increased, reached a peak level, and then decreased (**Figure 1**). This release pattern was also found in previous studies (*9*, *11*). In the first few minutes after tomato tissue disruption, volatiles are generated and accumulate due to enzyme activity. The rate of release of the volatiles from the tomato puree into the headspace, which is affected by the volatiles' generation rate and partition coefficient, is higher than the rate of loss caused by degradation and removal during sampling. After reaching a peak, the loss of volatiles is faster than the generation rate, and the net concentration in the headspace decreases (*9*, *11*).

The concentration of hexanal in the headspace increased from 4 to 23 °C (**Figure 1**). Several factors may be responsible for the increase: increase in partition coefficient including increased volatility, changes in water clustering that change how the SIFT-MS reports the concentrations, and increase in the amount of volatiles generated by the enzymes. The partition coefficient and volatility are greatly affected by matrix composition and temperature (*8*, *24*). As temperature increases, molecules have more energy, escape more readily from the medium, and produce a higher concentration in the gas phase. The temperature dependence is different for different volatiles due to the nature of the compounds' molecular structure (*8*). As temperature increases, more water is present in the air. Humidity affects the amount and

ratio of water clusters formed during SIFT-MS (*12*) and thus could change the measured concentrations.

To eliminate all but the enzyme effects, the relative headspace concentrations of compounds in the tomato puree were compared at different temperatures. A saturated $CaCl_2$ solution was added to the blended tomato puree to inactivate enzymes and then tested at different temperatures. Because no additional volatiles could be generated by the enzymes, the concentration of a volatile in the headspace is determined by the partition coefficient, and any humidity effects on the measurement are taken into account. The partition coefficient of hexanal in the tomato puree increased with increasing temperature (**Table 2**). The concentration of hexanal with enzyme activity increased from 4 to 23 °C in a ratio similar to the increase in partition coefficient, so that the increased levels of hexanal can be explained by the increased partition coefficient (**Figure 1**; **Table 2**).

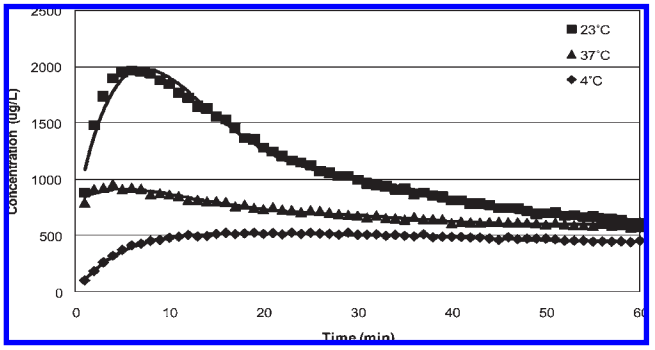

Figure 1. Effect of temperature on hexanal generation.

Table 2. Ratio of Volatiles in the Headspace of Grape Tomato Puree at Different Temperatures, without Enzyme Activity[a]

volatile	4 °C	23 °C	37 °C
1-penten-3-one	1 a	4 b	5 c
cis-3-hexenal	1 a	19 b	62 c
cis-3-hexenol and *trans*-2-hexenol	1 a	2 a	5 b
hexanal	1 a	15 b	19 c
hexanol	1 a	14 b	33 c
trans-2-heptenal	1 a	4 a	6 b
trans-2-hexenal	1 a	16 b	56 c
trans-2-octenal	1 a	3 b	7 c
trans-2-pentenal	1 a	6 b	18 c

[a] Values with different letters at the different temperatures are significantly different.

The concentration of hexanal in the headspace decreased from 23 to 37 °C (**Figure 1**). The increase in partition coefficient of hexanal should increase the concentration from 23 to 37 °C (**Table 2**). Instead, the increased temperature reduced the total amount of hexanal generated. Hexanal is derived from the oxidation of the free unsaturated fatty acids, linoleic and linolenic acid, which account for >50% of the total free fatty acids in tomatoes (*25*). Linoleic acid, 43.5% of the total free fatty acid, is the major precursor of hexanal (*25*) and is converted by LOX to form 13-hydroperoxides, which are then cleaved to hexanal by HPL (**Figure 2**) (*25*). Hexanal is also formed from the transformation of *cis*-3-hexenal, derived from linolenic acid, which is about 11% of the total free fatty acid (*4*, *25*). However, the conversion of *cis*-3-hexenal to hexanal is minimal and accounts for only 2% of the total hexanal, because hexanal formed from linoleic acid inhibits the formation of hexanal from linolenic acid (*4*). Therefore, the linoleic acid pathway is the controlling factor in the formation of hexanal.

There are two possible causes for the reduction of hexanal production at 37 °C: reduced enzyme activity and reduction of the availability of the 13-hydroperoxide precursor due to the presence of other pathways. The activity of tomato LOX on linoleic acid is optimum at 20−30 °C (*6*, *7*). At 37 °C, the production of hexanal by tomato LOX decreases to 40% of its level at its optimum temperature (*7*). If the value measured at 23 °C is adjusted upward on the basis of the partition coefficient differences shown in **Table 2** and multiplied by a factor of 0.4 to account for the decrease in enzyme activity, the calculated line is close to the measured values at 37 °C; thus, the reduced enzyme activity is a major factor in the decreased concentration of hexanal in the headspace.

Reduction of the amount of 13-hydroperoxide precursor available to be converted into hexanal, due to the presence of other pathways, may be another reason for the reduced hexanal in the headspace at 37 °C (**Figure 1**). In addition to the linoleic acid to hexanal pathway confirmed in tomatoes (*4*), there are other possible pathways for linoleic acid. Linoleic acid is first broken down by tomato LOX to produce 9-hydroperoxides (96%) and

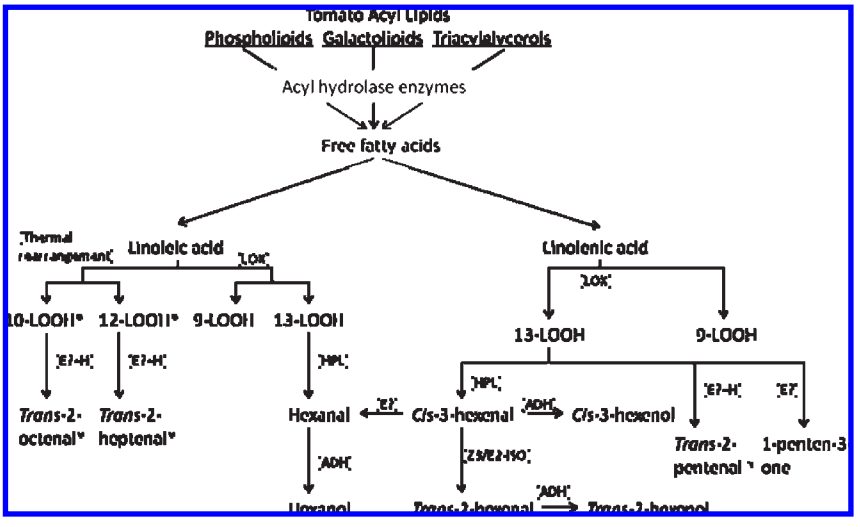

Figure 2. Temperature-dependent lipoxygenase pathway of linolenic and linoleic acids in tomato. Abbreviations: LOOH, hydroperoxide; [LOX], tomato lipoxygenase; [HPL], hydroperoxide lyase; [ADH], alcohol dehydrogenase; [Z3/E2-iso], *cis*-3/*trans*-2 isomerase; [E?], unknown enzyme; [E?+H], unknown enzyme plus heat. Asterisked compounds: significant formation at 37 °C.

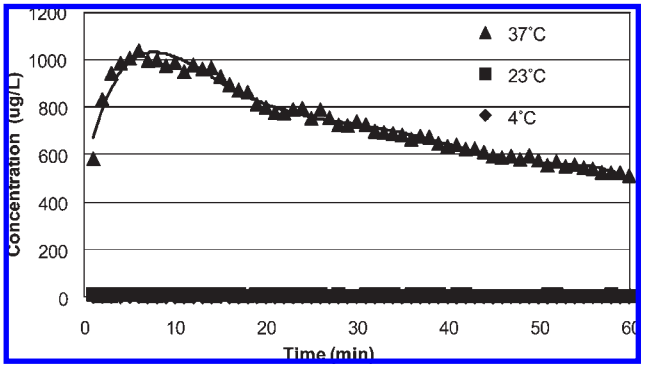

Figure 3. Effect of temperature on *trans*-2-heptenal generation.

13-hydroperoxides (1%) (*26*). $_{18}$O-labeled linoleate hydroperoxides were shown to rearrange to form 9-, 10-, 12-, and 13-hydroperoxides in equal amounts when held at 40 °C (*27*, *28*). Thus, thermal rearrangement will create the 12- and 10-hydroperoxides necessary for other pathways.

trans-2-Heptenal. The ratio of *trans*-2-heptenal in the headspace at 4:23:37 °C with enzyme activity was 1:3:336 (**Figure 3**) but without enzyme activity was 1:4:6 (**Table 2**). Thus, the increase in *trans*-2-heptenal between 4 and 23 °C can be explained by the increase in partition coefficient. However, the increase at 37 °C was much higher than can be explained by the partition coefficient. When the enzyme activity was stopped by adding a saturated $CaCl_2$ solution, the concentration of *trans*-2-heptenal in the headspace increased only 6-fold at 37 °C (**Table 2**), whether through increased partition coefficient or thermal generation of the compounds; however, when enzymes were present, the increase was 336 times (**Figure 3**).

trans-2-Heptenal is known to increase after tomato tissue disruption due to the activity of unknown enzymes (*29*). The addition of LOX and ADH lower the *trans*-2-heptenal in the headspace above tomato homogenates (*30*), likely because they encourage the 13-hydroperoxides pathway, which depletes the precursors for *trans*-2-heptenal. In green bell pepper homogenates, the addition of linoleic acid doubled the concentration of *trans*-2-heptenal in the headspace due to cleaving of 12-hydroperoxides by an unidentified enzyme (*5*). It is likely that an enzyme with a similar function also exists in the tomato lipoxygenase system, possibly an isomer of HPL. If at 37 °C, rearrangement of the hydroperoxides of linoleic acid occurred and the activity of 12-hydroperoxide-specific HPL significantly increased, the total amount of *trans*-2-heptenal would greatly increase. This would also contribute to the total amount of enzymatically generated hexanal decreasing at 37 °C, because more of the precursor hydroperoxides would be converted to *trans*-2-heptenal, instead of hexanal.

trans-2-Octenal. The concentration of *trans*-2-octenal also greatly increased in the headspace at 37 °C and may represent another pathway important at higher temperatures (**Figure 4**). The ratio of *trans*-2-octenal at 4:23:37 °C without enzyme activity was 1:3:7 (**Table 2**) and was 1:3:21 with enzyme activity (**Figure 4**). The increase of *trans*-2-octenal in the headspace from 4 to 23 °C can again be explained by the increased partition coefficient, but not the increase at 37 °C. At 37 °C, the increase of *trans*-2-octenal in the headspace was also likely due to an increase in volatile generation. *trans*-2-Octenal is a lipid-related volatile (*7*) and produces a sweet and phenolic aroma with a low threshold of 3 μg/L (*31*). The level of *trans*-2-octenal increases when the fatty acids are increased by altering the gene expression in tomato (*32*). The enzymatic generation of *trans*-2-octenal has also been reported in soybean and bell pepper, from decomposition of

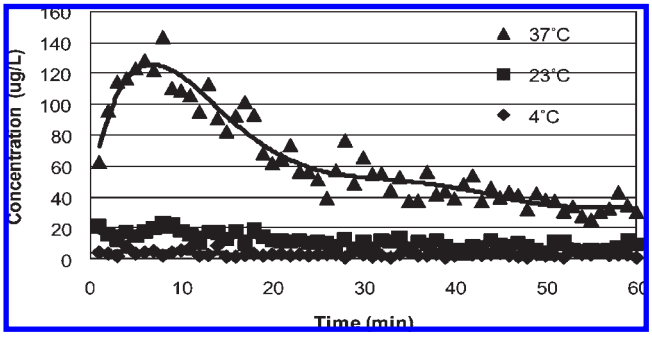

Figure 4. Effect of temperature on *trans*-2-octenal generation.

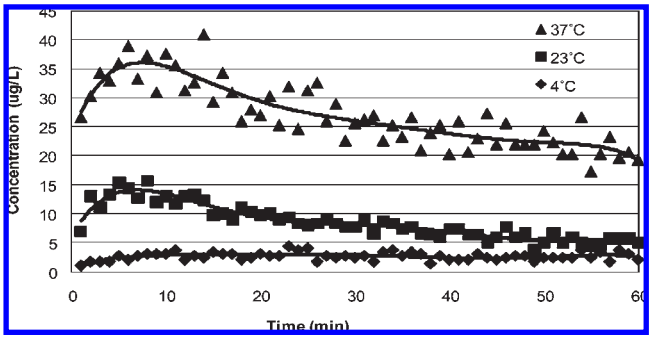

Figure 5. Effect of temperature on hexanol generation.

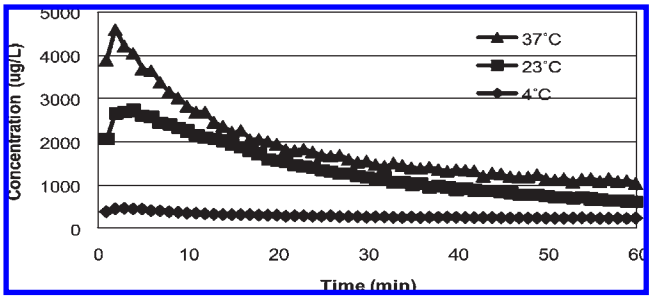

Figure 6. Effect of temperature on *cis*-3-hexenal formation.

10-hydroperoxides produced by the rearrangement of hydroperoxides of linoleic acid (*5*). The pathway may also exist in tomato and is another alternate pathway for the precursors to hexanal.

Hexanol. The concentration of hexanol in the headspace increased with increasing temperature, proportional to what was expected on the basis of the increased partition coefficient (**Figure 5**; **Table 2**). Hexanol is formed from hexanal by the action of ADH (*33*). The optimum pH for ADH is near neutrality (*34*), and the pH of tomato puree is acidic (typically pH 4.0−4.5) (*33*); thus, the ADH activity in tomato puree is low.

Effect of Temperature on Linolenic Acid Related Volatiles. *cis-3-Hexenal*. *cis*-3-Hexenal is characterized as a pleasant "green" aroma (*2*) and has an extremely low threshold of 0.25 μg/L in aqueous solution (*2*). It is a characteristic volatile for the fresh aroma of tomato (*23*). The concentration of *cis*-3-hexenal in the headspace increased with increasing temperature (**Figure 6**). The ratio of *cis*-3-hexenal in the headspace at 4:23:37 °C was 1:19:62 without enzyme activity (**Table 2**), but only 1:6:10 with enzyme activity (**Figure 6**). When enzymes were active, the concentration was lower than when they were not present; thus, the generation of *cis*-3-hexenal must decrease at higher temperatures. *cis*-3-Hexenal is the decomposition product of 13-hydroperoxides of linolenic acid (**Figure 2**). There are alternate pathways for

13-hydroperoxides to generate both *trans*-2-pentenal and 1-penten-3-one, which would reduce the 13-hydroperoxides available for *cis*-3-hexenal generation (**Figure 2**).

trans-2-Pentenal. The ratio of *trans*-2-pentenal in the headspace without enzyme activity at 4:23:37 °C was 1:6:18 and with enzyme activity was 1:4:105 (**Table 2**; **Figure 7**). At 37 °C, the total amount of *trans*-2-pentenal was much higher than can be explained by the increase in partition coefficient. *trans*-2-Pentenal appears after tomato tissue disruption, when enzyme activity is highest (*29*). In soybean and bell pepper, 13-hydroperoxides of linolenic acid, the precursors of *cis*-3-hexenal, are broken down to form *trans*-2-pentenal (*5*, *35*). This branch pathway may also exist in tomatoes and may be greatly affected by temperature. At room temperature and lower, 13-hydroperoxides may be mainly metabolized through the major pathway to form *cis*-3-hexenal. However, as the temperature increases to 37 °C, the activity of *trans*-2-pentenal-specific HPL may reach its optimum, producing a large amount of *trans*-2-pentenal (> 1000 μg/L) (**Figure 7**) while also significantly reducing the amount of *cis*-3-hexenal formed.

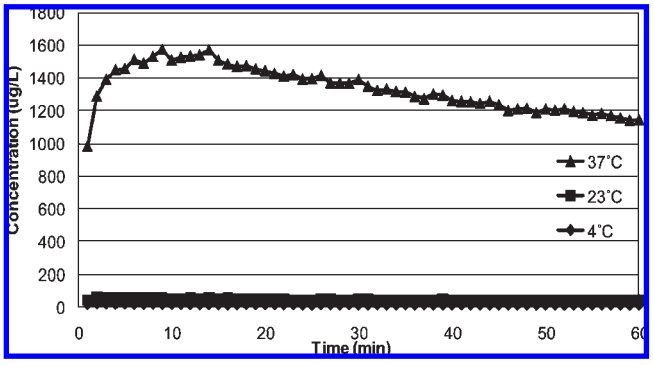

Figure 7. Effect of temperature on *trans*-2-pentenal generation.

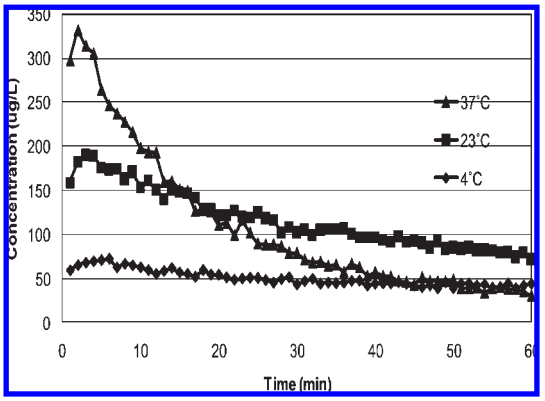

Figure 8. Effect of temperature on 1-penten-3-one generation.

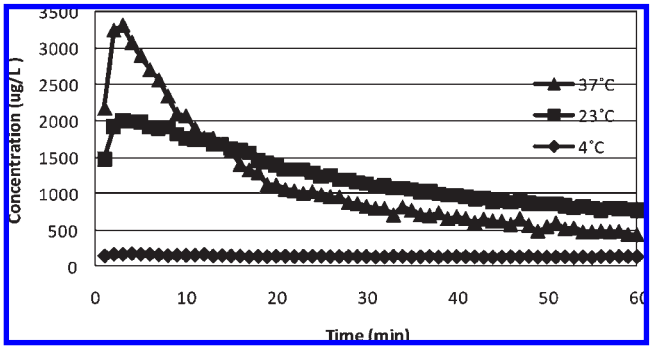

Figure 9. Effect of temperature on *trans*-2-hexenal formation.

1-Penten-3-one. The concentration of 1-penten-3-one in the headspace also increased with increasing temperature (**Figure 8**). The ratio of 1-penten-3-one in the headspace without enzyme activity at 4:23:37 °C was 1:4:5 and with enzyme activity was 1:3:5 (**Table 2**; **Figure 8**), which means the increase in concentration can be explained by the increased partition coefficient. The concentration of 1-penten-3-one in the headspace is known to increase after tomato tissue disruption (*29*), and the addition of LOX and ADH into tomato homogenates decreases the concentration of 1-penten-3-one (*30*); even though the 1-penten-3-one pathway will consume a certain amount of 13-hydroperoxides, the generation of 1-penten-3-one would not noticeably affect *cis*-3-hexenal because the amount formed is small.

trans-2-Hexenal. *cis*-3-Hexenal is isomerized to the more stable *trans*-2-hexenal by Z3/E2-ISO in the tomato puree and then is further degraded to alcohols (**Figure 2**) (*4*). Similar to *cis*-3-hexenal, the ratio of the *trans*-2-hexenal without enzyme activity at 4:23:37 °C was 1:16:56, but with enzyme activity was 1:13:21 (**Table 2**; **Figure 9**). There was less *trans*-2-hexenal when enzymes were present than when they were not. Less *cis*-3-hexenal available to be converted to the trans form may be responsible for the lower amount of *trans*-2-hexenal.

cis-3-Hexenol and trans-2-Hexenol. *cis*-3-Hexenol and *trans*-2-hexenol are derived from *cis*-3-hexenal and *trans*-2-hexenal, respectively, by ADH. The concentration of the mixture of *cis*-3-hexenol and *trans*-2-hexenol is low, below 25 ppb, and the increase with temperature can be explained by the increased partition coefficient. The concentrations of *cis*-3-hexenol and *trans*-2-hexenol are typically low in tomato (*30*).

Effect of Temperature on Rate of Production of Lipid-Related Volatiles. At 4 °C, most lipid-related volatiles reached their maximum level within 5 min, and there was no significant difference in time to reach the maximum level, except for hexanal, which peaked significantly later at about 12 min (**Table 3**). At this temperature, the activity of hexanal-specific LOX is below its optimum activity (*6*, *7*), so the generation rate of hexanal is low, and it takes a longer time to reach its peak level.

At 23 °C, the increase in temperature did not significantly change the time to reach the maximum level for most volatiles, except for hexanal (**Table 3**). The time for hexanal to reach its maximum level was significantly shortened, so that it was not significantly different from the other volatiles (**Table 3**). The increase in enzyme activity at 23 °C significantly increases the generation rate. The concentration of *cis*-3-hexenal in tomato was also found by others to reach its maximum level at about 3 min

Table 3. Time (Minutes) To Reach Maximum Level for Lipid-Related Volatiles from Grape Tomato after Blending[a]

temperature (°C)	*cis*-3-hexenal	*trans*-2-hexenal	1-penten-3-one	hexanal	hexanol	*trans*-2-octenal	*trans*-2-heptenal	*trans*-2-pentenal	*cis*-3-hexenol and *trans*-2-hexenol
4	2.7 a A	2.7 a A	4.3 a A	12.7b B	N/A	N/A	N/A	N/A	N/A
23	3.3 a A	3.7 a A	3.0 a A	4.7 a A	3.7 aA	N/A	N/A	N/A	N/A
37	2.0 a A	2.7 a A	2.0 a A	2.7 a A	5.3 abA	5.3 ab	7.3 b	7.0 b	N/A

[a] Values with different lower case letters at the same temperature are significantly different. Values with different capital letters for the same compound are significantly different. N/A, no detectable peak at this temperature.

after blending and then to decrease gradually due to further degradation at room temperature (*11*, *36*).

At 37 °C, the increase in temperature did not significantly change the time to reach the maximum level for most volatiles (**Table 3**). However, the volatiles with significant formation from branch reactions at this temperature, such as *trans*-2-heptenal and *trans*-2-pentenal, peaked significantly later than the other volatiles (**Table 3**). *trans*-2-Pentenal and *trans*-2-heptenal took longer to reach the peak level either because the activity of the enzyme controlling the reaction is slow or because the thermal arrangement of 13-hydroperoxides to the 10- and 12-hydroperoxide precursors is slow.

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