**The influence of lipid content and lipoxygenase on flavor
volatiles in the tomato peel and flesh**

THESIS

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Abstract

Ten different varieties of tomatoes were separated into peel and flesh and each portion was measured separately. Headspace volatiles were measured in real time using selected ion flow tube mass spectrometry (SIFT-MS). Lipoxygenase activity was measured using the adsorption of conjugated dienes formed by lipoxygenase. Lipid was extracted and fatty acids were quantified using a gas chromatograph. Volatiles were significantly greater in the peel than flesh when there was a significant difference. The lipoxygenase activity of the flesh and peel correlated with the volatiles produced by the lipoxygenase pathway. There was no correlation with the other volatiles, which are not dependent on lipid oxidation by lipoxygenase. The lipoxygenase activity, total fatty acid content, and linolenic acid of the peel was greater than the flesh, which is directly related to an increase in fresh, green volatiles. Addition of exogenous lipoxygenase had no effect on lipoxygenase derived volatiles formed in the peel and flesh. The addition of linoleic acid caused an increase in hexanal, 1-hexanol, and (*E*)-2-heptenal in the flesh and (*E*)-2-heptenal in the peel. Stored unrefrigerated peel had higher volatile concentrations whereas refrigerated peel had significantly lower concentration than Day 0. Storage decreased lipoxygenase activity in the unrefrigerated and refrigerated peel, but had no effect on the fatty acid content. Overall, linolenic acid was the most important to the

formation of headspace volatiles, but lipoxygenase activity and unknown factors are also important.

Practical Applications

The peel of a tomato is most beneficial to the production of volatiles associated with the fresh aroma of tomatoes; therefore, should be used in the processing of tomato products to produce a fresh, green aroma rather than being removed. Knowledge of the effects of lipoxygenase activity, total fatty acid content, and fatty acid profile on flavor volatiles will allow for better selection of a variety for raw consumption.

Dedicated to my family and friends

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Fields of Study

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Chapter 1: Introduction

Flavor is the critical quality characteristic in acceptance of fresh tomatoes and due to this fact consumers are willing to pay a premium for full-flavored fruit (Baldwin and others 2000). There have been over 400 flavor volatiles identified in tomato (Petro-Turza 1987), although the combination of 10 of these volatiles at varying concentrations produces the aroma of a fresh, ripe tomato (Buttery 1993). A majority of these volatiles are lipid derived and originate from the lipoxygenase pathway. This pathway is a cascade of enzymatic reactions that predominately form C-6 volatile compounds. The main enzymes involved are lipoxygenase (LOX), hydroperoxide lyase (HPL), alcohol dehydrogenase (ADH), isomerase, and lipase (Galliard and Matthew 1977). Stone and others (1975) were the first to propose the pathway that starts with tomato acyl lipids being broken down into free fatty acids, most importantly linoleic acid and linolenic acid. These fatty acids then react with LOX and HPL to form hexanal and (*Z*)-3-hexenal. (*Z*)-3-Hexenal is isomerized by (*Z*)-3/(*E*)-2 isomerase to form (*E*)-2-hexenal. ADH then converts these aldehydes into alcohols. There are other rearrangement reactions with

unknown enzymes that form (*E*)-2-octenal, (*E*)-2-heptenal, (*E*)-2-pentenal and 1-penten-3-one.

Different tissues of fruits and vegetables contain varying concentrations of flavor volatiles. Buttery and others (1988) analyzed a tomato broken down into four parts: pulp, skin, fluid, and seeds. The pulp contains similar values to the whole tomato except for higher levels of (*E*)-2-hexenal and C6 alcohols. The skin made up 10% of the whole tomato; however, it contained considerably higher levels of C6 aldehydes and several-fold higher concentrations of C6 alcohols than the whole tomato or the interior pulp. The concentrations in the fluid and seeds were generally lower. The reason for the difference in volatile concentrations in different tissues is not known, but lipoxygenase and fatty acid content are likely causes due to their role in the production of these volatiles.

The lipoxygenase pathway is the main creator of green, fresh volatiles in fruits and vegetables; therefore the level of lipoxygenase activity is important. The locular material has no lipoxygenase activity, while the skin with outer flesh has 71% of the activity, but when the flesh was removed from the skin the activity was drastically lower for the relatively pure skin (Hatanaka and others 1992). This suggests that lipoxygenase activity is greatest in the fleshy tissue adjacent to the skin. When an exogenous lipoxygenase, alcohol dehydrogenase or lipoxygenase/alcohol dehydrogenase was added, there was a steady decrease, not the expected increase, in the concentrations of hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal and 1-penten-3-one with the addition treatments (Yilmaz and others 2002). There are some inconsistencies in volatile results as researchers have

also found that decreasing the levels of lipoxygenase leads to a large reduction in fatty acid derived volatile aldehydes and alcohols in tomatoes (Chen and others 2004).

Fatty acids make up a very small portion of a tomato yet they are the precursors for the lipid derived volatiles formed in the lipoxygenase pathway (Petro-Turza 1987). To test the effect of fatty acid concentrations on lipid derived volatiles, there were tomato cultivars bred to contain different levels of linoleic and linolenic acids. When the ratio of linoleic acid to linolenic acid is higher there was a significant increase in the hexanal to hexenal ratio (Gray and others 1999). Similar findings were found when the expression of a yeast Δ -9 desaturase gene was altered to produce higher concentrations of oleic acid, linoleic acid, and total fatty acid concentration. Hexanal and 1-hexanol were more than two times greater in the tomato with higher levels of fatty acids than the control (Wang and others 1996). By altering the *Spr2* gene higher levels of linoleic acid were created and lower levels of linolenic acid formed, which caused an increase in hexanal and a decrease in (Z)-3-hexenal (Canoles and others 2006). Certain fatty acids have been found to correlate with the formation of some of their successor flavor volatiles.

Tomato volatiles have been previously studied using a gas chromatograph-mass spectrometer, atmospheric pressure ionization mass spectrometer, proton transfer reaction mass spectrometer and selected ion flow tube mass spectrometer (SIFT-MS) (Beltran and others 2006, Xu and Barringer 2009). The SIFT-MS is a relatively new technique for quantifying volatiles in tomatoes although it is able to do real time analysis and differentiate isomers without preconcentration, which the other methods cannot always do (Spanel and Smith 1999). This technique has been used in medical diagnosis (Smith

and others 2003b), monitoring environmental gas emission (Wilson and others 2003), as well as analysis of food, flavor and fragrance (Frank 2007). SIFT-MS has been useful in monitoring the real time release of food volatiles in oxidation of food products (Davis and others 2007), breathe analysis (Hansanugrum and Barringer 2009), enzyme activity (Azcarte and Barringer 2010), and roasting (Huang and Barringer 2011).

Tomato varieties differ in lipoxygenase activity, total fatty acid content, and fatty acid profile. These differences can have an impact on volatiles formed from raw tomatoes. The objective is to compare the peel and the flesh volatile concentrations and correlate these levels with lipoxygenase activity, total fatty acid content and individual fatty acids.

Chapter 2: Literature Review

2.1 Tomato

2.1.1 Tomato Fruit Background

Tomato (*Solanum lycopersicum*) belongs to the Solanaceae family where it is botanically classified as a fruit but is consumed as a vegetable. The Solanum genus has only eight species and two subgenerations with the tomatoes cultivated mainly belonging to the Eulycopersicon subgeneration (Petro-Turza 1987). There are around 7500 varieties grown for numerous reasons, including tomato paste, sauces and consumed raw. Plum tomatoes are a variety bred for higher solids content, which makes them useful for paste and sauce applications. On the other hand, beefsteak and grape tomatoes tend to be consumed raw. Tomatoes come in a variety of circular or oblong shapes and weigh anywhere from 1 oz to over 16 oz (Wolford and Banks 2011). Overall, United States is the second largest producer of tomatoes which accounts for 50.7% of the economic value of processed vegetables and 14.5% of the economic value of fresh market vegetables (Robertson and Labate 2007). This also makes tomato one of the most economically valuable crops in the United States (U.S. Department of Agriculture 2008).

2.1.2 Components of Tomato

Tomatoes vary greatly from fruit-to-fruit not only due to different cultivars but also between tomatoes from the same cultivar. The composition is quite variable depending on the cultivar but is also affected by the ripeness, year of growth and growing conditions. Due to tomato variation there are large fluctuations in published data (Petro-Turza 1987). Current data shows considerable differences in the nose-space volatile concentrations both among tomatoes of the same type as well as between different types (Brauss and others 1998).

The components of tomatoes are believed to play at least some role in the characteristic flavor. Table 2.1 contains composition data for yearly average, red, ripe tomato from the USDA Food Composition Database (USDA 2010). The effect of each component can be direct, as in flavor substances, or indirect. The indirect effect can include catalyzing reactions, being a precursor to flavor compounds, absorption of some volatiles or a medium for reactions to occur. (Petro-Turza 1987). There were no significant differences in the soluble solids content between the locular and pericarp tissue in a variety of cultivars. No significant difference was also found in the pH of the locular and pericarp tissue of most varieties except two varieties that had higher pH values in the locule than the pericarp. This can be significant due to the pH effects on lipoxygenase activity (Stevens and others 1977). The sweet and sour taste as well as the differences in flavor of different genotypes is attributed to the concentration and ratio of sugars and organic acids. The variation in sweetness is due to the fructose concentration

more than glucose. As for the organic acids, citric acid has more influence on tomato flavor than malic acid (Petro-Turza 1987).

Table 2.1 Tomato Composition (USDA 2010)

Component	Percent Composition
Water	94.52%
Protein	0.88%
Total Lipid	0.20%
Total Sugars	2.63%

2.2 Fatty Acids

The fatty acid content of tomatoes varies depending on the variety. Gray and others (1999) found the total content of fatty acids on a fresh weight basis for cherry and standard tomatoes to be 0.0006 and 0.0005 g g⁻¹, respectively. The USDA (2011) also reported that the fatty acid content on a fresh weight basis of an Roma tomato to be 0.0019 g g⁻¹, Cherry tomato to be 0.0018 g g⁻¹, and a plum tomato to be 0.0019 g g⁻¹.

Poly-unsaturated fatty acids prevail with linoleic acid present at 40% and linolenic acid present at 26% (Petro-Turza 1987), which are similar to the pericarp levels found by Bergevin and others (1993), (Table 2.2). These reported values vary greatly from the USDA values of 56.74% for linoleic acid and 2.13% for linolenic acid (USDA

2010) and the pericarp levels found by Cook and others (2002) to be 62.71% for linoleic acid and 3.99% for linolenic acid.

Table 2.2 Tomato Fatty Acid Profiles

Fatty Acid	Petro-Turza (1987)	Bergevin and others (1993)	USDA (2010)	Cook and others (2002)
Palmitic (16:0)	N/A	25.4%	14.18%	14.3%
Stearic (18:0)	N/A	N/A	5.67%	3.72%
Oleic (18:1)	N/A	2.2%	21.28%	15.21%
Linoleic (18:2)	40%	45.5%	56.74%	62.71%
Linolenic (18:3)	26%	21.6%	2.13%	3.99%

The difference in values can be accounted for by a different cultivar being used, class of lipid measured, storage conditions as well as the known variation among individual tomatoes. A difference in cultivars was found between cherry and standard tomatoes. Cherry has higher levels of linolenic and oleic acids whereas standard tomato has higher concentrations of linoleic and stearic acids (Gray and others 1999). A difference in type of lipid was also found. Neutral lipids are present in higher amounts than phospholipids and glycolipids, respectively. Linolenic acid was present in higher concentrations in the neutral lipid whereas oleic and linoleic acid are greater in the phospholipid portion (Gray and others 1999). Storage studies have found that there is a

decrease in linolenic acid concentration at 20°C. This is accompanied by an increase in palmitic, oleic and linoleic acids (Bergevin and others 1993). The levels of lipids, lipases and lipoxygenase varied during the stages of development as well as variety and storage treatment. Linolenic, linoleic, oleic, stearic, and palmitic acids increased during the period of greatest color development (Kazeniak and Hall 1970).

Even though fatty acids are present in such small amounts they seem to be much more important precursors to volatiles than carbohydrates which make up more than 50% of dry matter. Upon maceration of tomato tissues, the enzymatic breakdown of lipids occurs very rapidly. The fatty acids are the precursor molecules in the lipoxygenase pathway, which produces volatiles important to the fresh, green aroma of tomatoes. Researchers have found the same essential oils present in all varieties of tomatoes, although there are variations in the concentrations of each fatty acid depending on the variety. It has been hypothesized that the differences in aroma between varieties could be due to the dissimilarity in quantity as well as the relative ratios of other components in tomatoes (Petro-Turza 1987).

A relationship between the relative amounts of tomato fatty acid and C6 aldehydes has been identified when comparing cherry and standard tomatoes. A standard tomato has approximately twice the fatty acid ratio and three times the C6 aldehyde ratio as a cherry tomato. In directly comparing the concentrations, hexanal and hexenal produced from macerated tomato have similar ratios to the relative composition of their precursor fatty acids, linoleic and linolenic acids, respectively (Gray and others 1999). Although when comparing the volatiles with tomato size, (*Z*)-3-hexenal and (*E*)-2-

hexenal increase with decreasing size, whereas hexanal is less closely related with tomato size.

The fact that there is no overall increase in all of the lipid degradation products with size suggests that the lipid degradation pathway is specific, meaning there are differences in the metabolism of linoleic and linolenic fatty acids. Certain acyl hydrolase enzymes have been found to prefer to cleave more highly unsaturated fatty acid groups, monogalactosyldiacylglycerol and digalactosyldiacylglycerol, during chilling (Whitaker 1993). This correlates with the fact that more linolenic acid was lost upon maceration of tomato than linoleic acid (Gray and others 1999).

2.3 Lipoxygenase Pathway

2.3.1 Lipoxygenase Pathway in the Formation of Tomato Volatiles

The lipoxygenase pathway is known as a cascade of enzymatic reactions that predominately form C-6 volatile compounds. The main enzymes involved in this pathway are lipoxygenase, hydroperoxide lyase, alcohol dehydrogenase, isomerase, and lipase (Galliard and Matthew 1977). These enzymes are active during ripening but the actual synthesis of C6 volatile compounds is more prevalent when the tomato tissue has been disrupted. This allows for the enzyme and substrate to be properly mixed (Prestage and others 1999). The two significant substrates of these volatiles are linoleic and linolenic acids. Upon disruption of the tomato tissue, the reactions will begin by initially breaking down unsaturated fatty acids that in turn form important volatile compounds. These volatiles are responsible for the key characteristic taste and aroma in tomatoes.

Lipoxygenase pathways have also been identified in many other fruits and vegetables although some of the pathways are more complicated.

The lipoxygenase pathway was first proposed by Stone and others (1975). The first lipid breakdown products that effect the odor and flavor are hexanal and (Z)-3-hexenal. Linoleic and linolenic acid are converted to hexanal and (Z)-3-hexenal, respectively, by reaction with lipoxygenase (LOX) and hydroperoxide lyase (HPL). (Z)-3-hexenal quickly isomerizes into (E)-2-hexenal by (Z)-3/(E)-2 isomerase (Z3/E2-ISO). Partly because of the limits of the experimental procedure, and partly because of the enzymatic activity occurring immediately upon maceration, it is difficult to establish which of the volatile components were present originally in the ripe fruit and which developed during processing (Petro-Turza 1987). But now with the addition of CaCl₂ solutions, researchers have been able to identify the original concentrations by deactivating the enzymes (Buttery and Ling 1993b). Then the aldehyde group of (Z)-3-hexenal and (E)-2-hexenal were reduced by alcohol dehydrogenase to form their alcohol counterparts. The lipoxygenase pathway produces the major C₆ volatiles as well as (E)-2-octenal, and (E)-2-heptenal from linoleic acid, as well as (E)-2-pentenal, and 1-penten-3-one from linolenic acid. They are formed by unknown enzymes from 10-hydroperoxides and 12-hydroperoxides, found in bell peppers (Luning and others 1995). Linolenic acid is also the precursor of (E)-2-pentenal and 1-penten-3-one in soybeans and bell peppers (Luning and others 1995). The formation of volatiles appears to go in a sequential fashion once the tomato tissue has been disrupted. There is a significant

difference in the rate of formation of the aldehydes and alcohols. Aldehydes form first which are then the precursor molecules of the alcohols (Linforth and others 1996).

Figure 2.1 Lipoxygenase pathway of linoleic and linolenic acids in tomato. Abbreviations: LOOH, hydroperoxide; [LOX], tomato lipoxygenase; [HPL], hydroperoxide lyase; [ADH], alcohol dehydrogenase; [Z3-E2 ISO], cis-3/trans-2 isomerase (Stone and others 1975)

2.3.2 Lipoxygenase

Lipoxygenase (LOX) plays a key role in the development of C6 volatile compounds in tomatoes. LOX is a nonheme iron-containing dioxygenase that catalyzes the reaction of the addition of oxygen into polyunsaturated fatty acids that contain a (Z,Z)-1,4-pentadiene and transform them into fatty acid hydroperoxides. The average molecular weight of LOX is 96 kDa. LOX has an optimum pH range of 4.2-8.0 and an optimum temperature range of 20-30°C (Yilmaz 2001b). Not only is LOX important to flavor volatile creation it has also been found to be involved in plant growth and development, biosynthesis of regulatory molecules, insect attraction and defense,

senescence, and response to wounding (Chen and others 2004). There are two classes of LOX enzymes: 9-LOX which forms 9-hydroperoxides, and 13-LOX which forms 13-hydroperoxides (Hatanaka 1993). Consideration must be made to the fact that the key volatiles are only derived from 13-hydroperoxides not the more plentiful 9-hydroperoxides. Of these two classes there are five LOX genes including Tomlox A, Tomlox B, Tomlox C, Tomlox D, and Tomlox E. The LOX isomers are different in their optimum pH, substrate and product specificity, location, and synthesis at certain developmental stages (Chen and others 2004). Tomlox A, Tomlox B, and Tomlox E generate 9-hydroperoxides. There are little or no hydroperoxide lyases to act on the 9-hydroperoxides therefore they accumulate and are not involved in the creation of C6 volatile compounds (Galliard and Matthew 1977). Tomlox D creates 13-hydroperoxides but does not play a role in creating C6 flavor compounds in tomatoes.

Studies by Chen and others (2004) found that decreasing the levels of Tomlox C led to a large reduction in fatty acid derived short chain volatile aldehydes and alcohols in tomatoes. Therefore, it is recognized that Tomlox C is the key isomer in the production of volatiles. Tomlox C is thought to be located in the chloroplast. The chloroplasts isolate LOX from other reactants in the lipoxygenase pathway so that may be why volatile production does not occur until the cells are disrupted. It has also been proposed that chloroplasts are the major site for fatty acid hydroperoxide metabolism. By disrupting the thylakoid membranes linoleic and linolenic acid may be produced. Consequently, Tomlox C is located in chloroplasts and can use both linoleic and linolenic

acids it is the prime candidate for being the main isomer in the lipoxygenase pathway (Chen and others 2004).

As for the particular location in the tomato, the flesh was measured by breaking it into two parts: pericarp and cytosol. LOX activity was found to be greatest in the pericarp with 65-80% occurring here (Riley and others 1996). Hatanaka and others (1992) tested the differences in LOX activity in three parts: skin with outer flesh, flesh without skin, and locular material. The locular material was found to have no activity. Whereas, 71% of the activity was in the skin with outer flesh, but when the flesh was removed from the skin the activity was drastically lower for the relatively pure skin. This suggests that LOX activity is greatest in the fleshy tissue adjacent to the skin.

2.3.3 *Hydroperoxide Lyase (HPL)*

Hydroperoxide Lyase (HPL) is a heme b protein with a molecular weight between 200-250 kDa that is thought to be a tetramer of 62 kDa (Zhuang and others 1998). It is most effective at a pH range of 5.5 to 8.0. Its location corresponds with that of lipoxygenase which is in chloroplasts and non-chloroplastic particles. It can be found in both soluble and membrane-bound forms (Yilmaz 2001a). HPL activity in tomato flesh is almost completely in the pericarp (Riley and others 1996). When Hatanaka and others (1992) measured HPL activity they looked at three parts: skin with outer flesh, flesh without skin and locular material. HPL activity was found to be distributed evenly through all three parts of the tomato. There are three kinds of HPLs: (1) 9-hydroperoxide specific which produces C9 aldehydes and oxoacids; (2) 13-hydroperoxide specific which creates C6 aldehydes and C12 oxoacid; (3) nonspecific that acts on both

substrates (Yilmaz 2001a). Hatanaka and others (1992) reported that tomato HPL utilized 85% of 13-hydroperoxide substrate over a 10 min period compared to only 7.1% of 9-hydroperoxide.

2.3.4 Alcohol Dehydrogenase (ADH)

Alcohol dehydrogenase (ADH) is a dimer with a molecular mass of 90-100 kDa and contains on average 15-48 zinc atoms per enzyme dimer (Yilmaz 2001a). ADH is a nonspecific oxidoreductase capable of using the primary formed volatile alcohols or aldehydes as substrates (Stone and others 1975). ADH is the enzyme that catalyzes the interconversion of (*E*)-2-hexenal and (*E*)-2-hexenol, as well as (*E*)-2-hexenal, hexanal and hexanal (Longhurst and others 1990). There are two ADH genes: Adh1 is common in developing seed and pollen whereas Adh2 is in a range of tissues in ripening tomatoes in response to stress. Longhurst and others (1990) found that ADH activity is induced by a change in cytoplasmic pH that is accompanied by changes in cytoplasmic ion concentrations. They also found that ADH activity is present before ripening then decreases until it increases again during the postclimacteric period.

2.4 Flavor

2.4.1 Tomato Volatiles

Flavor is the critical quality characteristic in acceptance of fresh tomatoes and due to this fact consumers are willing to pay a premium for full-flavored fruit (Baldwin and others 2000). The flavor of tomatoes has been extensively studied and there have been over 400 volatiles identified in tomato (Petro-Turza 1987). Of these 400 volatiles, only

29 have been found to be present in concentrations greater than 1 ppb. The log order units were quantified and 16 volatiles have a positive value which makes them likely to contribute to the characteristic tomato flavor. The 16 volatiles are (*Z*)-3-hexenal, β -ionone, hexanal, β -damascenone, 1-penten-3-one, 3-methylbutanal, (*E*)-2-hexenal, 2-isobutylthiazole, 1-nitro-2-phenylethane, (*E*)-2-heptanal, phenylacetaldehyde, 6-methyl-5-hepten-2-one, (*Z*)-3-hexanol, 2-phenylethanol, 3-methylbutanol, and methyl salicylate. However, the compounds with negative odor units may still play a role in the overall flavor (Buttery 1993). Buttery (1993) also found that by combining (*Z*)-3-hexenal, (*Z*)-3-hexenol, hexanal, 1-penten-3-one, 3-methylbutanal, (*E*)-2-hexenal, 6-methyl-5-hepten-2-one, methyl salicylate, 2-isobutylthiazole, and β -ionone at varying concentrations produces the aroma of a fresh, ripe tomato.

The volatile precursors in tomatoes include lipids, amino acids, carotenoids, terpenoids, lignin, and glycosides (Buttery and Ling 1993b, Williams 1993). The green, fresh volatiles characteristic of tomato come from the breakdown of lipids in the lipoxygenase pathway, more specifically linoleic and linolenic acids (Galliard and others 1977). Many of the volatiles found in tomatoes are developed during the blending of the product and result from the enzymatic and chemical reactions that occur. The blending allows for the enzymes and their substrates to be mixed as well as the addition of air. Lipid oxidizing enzymes react with fatty acids to create hydroperoxides that break down into carbonyl compounds. The latter can be further converted to the corresponding alcohols by alcohol dehydrogenase (Kazeniak and Hall 1970). A variety of compounds have been found in peas that originated from the enzymatic oxidation of lipids, including

acetaldehyde, 2-butenal, 2-pentenal, 2-hexenal, 2-heptenal, 2-octenal, 2-nonenal, 2,4-heptadienal and acetone (Kazeniak and Hall 1970). Alcohol dehydrogenase was found to be important to the aldehyde-alcohol ratio because it reduces the aldehydes to their alcohol counterparts, namely for acetaldehyde, 3-methylbutanal and hexenal (Kazeniak and Hall 1970). The formation of volatiles from the lipoxygenase pathway form in sequential reactions, therefore it takes different times for each volatile to reach their maximum concentration. Due to the enzymes generating new volatiles it took several minutes to reach their maximum concentrations, compared to within one minute for the volatiles not formed through enzymatic activity, that just equilibrate into the headspace (Xu and Barringer 2010a).

To find what volatiles are present prior to macerations, samples are blended with a saturated CaCl_2 solution or heated in a microwave to deactivate enzymes. This assumes that the enzymes were properly deactivated and that no enzymatic activity occurred prior to the CaCl_2 penetrating the entire sample (Buttery and Ling 1993b). The volatiles that showed no significant change due to tissue disruption are 3-methylbutanal, pentanol, (*Z*)-3-hexenol, hexanal, 6-methyl-5-hepten-2-one, phenylacetaldehyde and 2-phenylethanol. The volatiles that showed no change are not enzymatically formed through the lipoxygenase pathway. (*E*)-2-pentenal was the only one to appear after disruption, which means it was most likely formed solely through the lipoxygenase pathway. The volatiles that increase after tissue disruption include (*Z*)-3-hexenal, (*E*)-2-hexenal, hexanal, (*E*)-2-heptenal, 1-penten-3-one, 1-penten-3-ol, geranylacetone, 2-

isobutylthiazole and 1-nitro-2-phenylethane. These volatiles are from the lipoxygenase pathway (Buttery and Ling 1993b).

Two other volatiles in tomatoes are furfural and methanol. Higher amounts of each were found in over-ripe tomatoes which is consistent with high degrees of demethylation and furfural formation. Methanol is produced from pectins by the depolymerization and demethylation by pectic enzymes. The resulting uronic acids and ascorbic acid would be the most likely precursors for furfural (Kazeniak and Hall 1970).

The maturity stage of tomatoes has been found to be very important in the amounts of (*E*)-2-hexenal and (*Z*)-3-hexenal produced with the highest levels in the red mature stage. This may be due to the variation of lipids, lipases and lipoxygenase throughout maturation. They were found to reach the highest amounts at the climacteric stage in apples (Rhodes and Woollorton 1967). The total lipids were found to vary with fruit maturity at harvest as well as depending on the variety and storage conditions (Kazeniak and Hall 1970). The enzymes appeared to be at least one of the limiting factors in the concentration of (*Z*)-3-hexenal in green tomatoes (Kazeniak and Hall 1970).

Buttery and others (1988) analyzed a tomato broken down into four parts: pulp, fluid, seeds and skin. The fluid is the locular fluid which was obtained by filtering the fluid through a stainless steel screen to remove the seeds. None of the portions are able to be separated from one another without damaging the fresh condition, but volatile tests were conducted within 5 min to minimize changes in volatile release. The pulp make up about 70% of the tomato, yet contains similar volatile levels to the whole tomato with the exception of (*E*)-2-hexenal and C6 alcohols which it contained higher concentrations of.

The fluid was about 20% of the tomato. (*Z*)-3-Hexenal, (*E*)-2-hexenal and 1-penten-3-one concentrations in the fluid were about half of that found in the entire tomato. The seeds made up a miniscule 0.3% of the tomato and had 100 times lower levels of most volatiles compared to the whole tomato except (*E*)-2-hexenal which was the same. The seeds make up such a small amount that it is unlikely that they contribute significantly to the flavor. The skin made up 10% of the whole tomato. However, it contained considerably higher levels of C6 aldehydes and several-fold higher concentrations of C6 alcohols than in the whole tomato or in the interior pulp. It must be noted that this could be partially due to the tissue damage during peeling. Buttery and others (1988) also studied 10 different lines of tomatoes and found no marked differences in concentrations of C5-C13 volatiles, although no thorough statistical analysis was done. Xu and Barringer (2010a) found that grape tomato had higher levels of volatiles than vine-ripened, cherry and roma tomatoes. It was also reported that the formation of a mixture of (*Z*)-3-hexenal and (*E*)-2-hexenal during chewing is greater in cherry tomato than plum and delice tomatoes (Brauss and others 1998). Nonanal levels were also found to be greater in tomatillo, cherry tomato and grape tomato than in roma and vine-ripened tomatoes (Xu and Barringer 2010b).

2.4.2 (*Z*)-3-Hexenal

(*Z*)-3-Hexenal is the key contributor to the characteristic green flavor of tomato. (*Z*)-3-Hexenal is formed through the lipoxygenase pathway by hydroperoxide lyase where its precursor lipid is linolenic acid. It is present in tomatoes at the highest concentration (12,000 ppb) and has the highest odor log unit (3.7) of the 30 volatiles

present at greater than 1 ppb (Buttery 1993). (Z)-3-Hexenal concentration in macerated tomato increases rapidly until it reaches its maximum around 3 min. It is gradually lost due to isomerization to (E)-2-hexenal, transformation to hexanal and reductase conversion to (Z)-3-hexenol (Buttery and Ling 1993b, Xu and Barringer 2009).

2.4.3 (Z)-3-Hexenol

(Z)-3-Hexenol is formed from the reduction of (Z)-3-hexenal by alcohol dehydrogenase. There is no significant change in concentration due to tissue disruption (Buttery and Ling 1993a). (Z)-3-hexenol has a green odor but due to its high odor threshold compared to (Z)-3-hexenal and (E)-2-hexenal it affects the flavor less (Petro-Turza 1987). The (Z)-2-hexenol concentrations in tomato are typically low (Yilmaz and others 2002).

2.4.4 Hexanal

Hexanal is produced via the lipoxygenase pathway by reaction of linoleic acid with hydroperoxide lyase. It can also be formed through the transformation of (Z)-3-hexenal, derived from linolenic acid. The conversion is only about 2% of the hexanal formation because hexanal formed from linoleic acid inhibits the formation of (Z)-3-hexenal from linolenic acid. Therefore linoleic acid is the controlling factor in the formation of hexanal (Xu and Barringer 2009). It has a green flavor at low concentrations but at higher concentration it has a rancid vegetable fat flavor. The green flavor is much less prevalent than (Z)-3-hexenal and (E)-2-hexenal (Petro-Turza 1987). Due to hexanal being formed through enzymatic activity it took approximately 6 min to reach its maximum concentration in tomatillo and then decreased due to degradation and

removal from sampling (Xu and Barringer 2010a). Hexanal had a greater concentration in cherry tomato and tomatillo than grape, roma and vine-ripened tomatoes (Xu and Barringer 2010b). An increase in temperature from 4 to 23°C correlated with an increase in hexanal concentration. The increase in hexanal concentration may be due to an increase in partition coefficient including increased volatility or an increase in the amount of volatiles generated by the enzymes (Xu and Barringer 2009).

2.4.5 1-Penten-3-one and (E)-2-Pentenal

1-Penten-3-one and (E)-2-pentenal reached their maximum concentrations later than (Z)-3-hexenal, hexenal and (E)-2-hexenal at 35 min for 1-penten-3-one and 28 min for (E)-2-pentenal in tomatillo. The maximum concentrations were earlier in tomatoes than tomatillos. They are formed through branch reactions of enzymatic oxidation of linolenic acid (Xu and Barringer 2010a). Cherry tomato and grape tomato had higher concentrations of 1-penten-3-one than roma and vine-ripened tomatoes during chewing (Xu and Barringer 2010b). The concentration of 1-penten-3-one increased with increasing temperature from 4 to 37°C with and without enzymatic activity. 1-Penten-3-one increases in the headspace after tissue disruption, and the addition of lipoxygenase and alcohol dehydrogenase in tomato homogenates decreases its concentration (Xu and Barringer 2009).

2.4.6 3-Methylbutanal and Phenylacetaldehyde

Tomato contains volatile nitro compounds which are not found in any other fruit or vegetable (Baldwin and others 2000). They appear in tomatoes as they ripen from breaker to mature red stages. The nitro compounds are formed by an amino acid being

converted by an enzyme to its corresponding oxime which is then converted by another enzyme system to a mixture of the nitro compound and nitrile. The enzymes in this reaction have not been identified. Both the nitro compound and nitrile compound can be found in fresh tomato. 1-Nitro-phenylethane was found to be converted to phenylacetaldehyde by making a water solution with the nitro compound at a pH of 10.0 and then making it mildly acidic (pH 4.5). This is referred to as the Nef reaction. This reaction occurs readily so it is believed to be mediated by an enzyme system and is most likely the cause of the formation of 3-methylbutanal and phenylacetaldehyde. The accepted pathway for these aldehydes is normally by Strecker degradation; however these samples were not heated (Buttery 1993). 3-Methylbutanal is from the enzymatic precursor amino acid leucine and has a caramel aroma (Xu and Barringer 2010a) whereas phenylacetaldehyde is enzymatically from phenylalanine and has a floral note (Buttery and others 1988).

2.4.7 (E)-2-Hexenal

(*E*)-2-Hexenal has a slightly less fresh, green aroma than (*Z*)-3-hexenal but its effect on the flavor is similar (Petro-Turza 1987). Isomerization of (*Z*)-3-hexenal creates (*E*)-2-hexenal, which can occur either enzymatically by *Z3/E2* isomerase or nonenzymatically (Galliard and Matthew 1977). The maximum concentration of (*E*)-2-hexenal is at 12 min in tomatillo, which is significantly later than (*Z*)-3-hexenal maximum formation. The extra enzyme step causes a delay in the maximum being reached (Luning and others 1995).

2.4.8 6-Methyl-5-hepten-2-one

The flavor of 6-methyl-5-hepten-2-one is debated. Kazeniac and Hall (1970) found it to have a fruity flavor but when added to tomato juice it did not enhance the tomatolike aroma. Others have found it to have a cooked or heat-treated flavor (Petro-Turza 1987). It is a terpene related compound found along with acetone. 6-Methyl-5-hepten-2-one and acetone have been found from lycopene degradation during the heating of tomato pulp. Lower concentrations of 6-methyl-5-hepten-2-one were found when tomatoes were blended under nitrogen rather than air (Kazeniac and Hall 1970). A high lycopene tomato was compared to other varieties of vine ripened tomatoes and found to contain similar concentrations of suspected lycopene degradation products 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol and geranylacetone.

2.4.9 Methyl Salicylate

Methyl salicylate is a lignin related compound that is incredibly variable in the concentrations present in tomato. The variability has yet to be linked with any difference in tomato lines, maturity or growing conditions (Buttery and Ling 1993b). Other lignin related compounds identified in tomato include eugenol, and guaiacol. These two were found in similar amounts to methyl salicylate and varied by variety as well (Kazeniac and Hall 1970).

2.4.10 2-Isobutylthiazole

Alkylthiazoles are commonly found in cooked products but tomato is one of the few raw foods to contain them. 2-Isobutylthiazole is the only alkylthiazole found in tomatoes. It is believed to develop from leucine. Leucine is first broken down into 3-

methylbutanal which then condenses with cystamine to give a thiazolidine. The thiazolidine oxidizes enzymatically to the thiazole (Buttery and Ling 1993b). 2-Isobutylthiazole has a spoiled vinelike and slightly horseradish flavor but when added to tomato improves the flavor by decreasing the harsh, unpleasant notes and intensifies the flavor (Petro-Turza 1987). The amounts of 2-isobutylthiazole are not dependent on the crushing procedure or presence of oxygen, therefore the majority of it should be present in the intact fruit. However, the concentrations vary from one variety of tomato to another (Kazeniak and Hall 1970).

2.4.11 β -Ionone

β -Ionone is present in tomato at the lowest concentration but due to its low odor threshold it has one of the highest log odor units (2.8) (Buttery 1993). β -Ionone is a carotenoid related volatile, along with 6-methyl-5-hepten-2-one. It is formed via an oxidative mechanism (Buttery and Ling 1993b). The decomposition of β -carotene is the proposed method of formation (Kazeniak and Hall 1970). A high β -carotene tomato was studied and it contained high levels of β -ionone and β -cyclocitral compared to regular vine-ripened tomato varieties (Buttery and others 1988).

2.4.12 Effect of Fatty Acid on Flavor

Lipids and fatty acids are precursors to the aldehydes (*Z*)-3-hexenal, (*E*)-2-hexenal, and hexanal, the alcohols (*Z*)-3-hexenol and 1-hexanol and the ketone 1-penten-3-one (Baldwin and others 2000). Due to the variation of fatty acid composition in tomatoes researchers have looked at the effects of adding linoleic and linolenic acids to tomato homogenates. The addition of linoleic acid was found to increase the levels of

hexanal and 1-hexanol whereas the addition of linolenic acid enhanced the production of (Z)-3-hexenal, (E)-2-hexenal and (Z)-3-hexenol (Boukobza and others 2001, Chen and others 2004, Riley and Thompson 1998). In green bell pepper, with the addition of linoleic acid there was a significant increase in the levels of hexanal, hexanal, 2-pentylfuran, (E)-2-heptenal, (E)-2-octenal, (E)-2-nonenal and (Z)-3-hexenal (Luning and others 1995). Instead of adding fatty acids, there are tomato cultivars that are bred to contain different levels of linoleic and linolenic acids. When the ratio of linoleic acid to linolenic acid is greater there was a significant increase in the hexanal to hexenal ratio (Gray and others 1999). Similar findings were found when the expression of a yeast Δ -9 desaturase gene was altered to produce higher concentrations of palmitoleic acid, oleic acid, linoleic acid, as well as total fatty acid concentration. Hexanal and 1-hexanol, believed to be derived from oleic and linoleic acids, were more than two times greater than the wild type (Wang and others 1996). By altering the *Spr2* gene higher levels of linoleic acid were created and lower levels of linolenic acid formed. This modification from the wild type caused an increase in hexanal and a decrease in (Z)-3-hexenal (Canoles and others 2006).

2.4.13 Effect of Lipoxygenase on Flavor

A mutant tomato line was bred to contain higher levels of linoleic levels and lower levels of linolenic acid. With this change in fatty acid content the linolenic derived (Z)-3-hexenal content increased while the linoleic acid derived hexanal did not. This change in volatile concentration is most likely not related to lipoxygenase since it has no specificity for linoleic or linolenic acids. Hydroperoxide lyase is most likely the enzyme

involved due to its higher affinity for linolenic acid (Canoles and others 2006). Tomatoes naturally containing varying concentrations of lipoxygenase, hydroperoxide lyase and alcohol dehydrogenase were found to have no significant correlation with hexanal, (Z)-3-hexenal, (E)-2-hexenal, (E)-2-heptenal, pentenone, and (Z)-3-hexenol (Yilmaz and others 2001). Similar results were found when the control group had consistently higher concentrations of all volatile compounds than any enzyme treatment. There was a steady decrease in the concentrations of hexanal, (Z)-3-hexenal, (E)-2-hexenal, and 1-penten-3-one with the lipoxygenase, alcohol dehydrogenase and lipoxygenase/alcohol dehydrogenase treatments in red tomatoes (Yilmaz and others 2002). Interestingly, when exogenous linoleic and linolenic acid is added to tomato homogenates made from tomatoes with low Tomlox C mRNA compared to wild type the increases in hexanal and hexenal levels were much lower than in the wild type controls (Chen and others 2004).

2.4.14 Effect of Storage on Flavor

Studies have shown that low storage temperatures (below 13°C) can cause poor tomato fruit quality, as well as chill damage (Hardenburg and others 1986). However, red-ripe fruit has been found to withstand low temperature storage for longer periods of time without chilling injury (Cook and others 1958). Ripe tomatoes stored at 5°C for 6 days had a significant decrease in volatiles and the main contributor being (Z)-3-hexenal (Stern and others 1994). After 2-12 days of storage at 5°C had markedly lower ripe aroma than those stored at 12.5 and 20°C. Hexanal, 2+3-methylbutanol, (E)-2-heptenal, and 2-isobutylthiazole were present in lower concentrations (Maul and others 2000).

2.5 Analytical Methods for Headspace Analysis

Identification and quantification of tomato volatile compounds has been done by a variety of methods. Most methods require that the volatile compounds be extracted and/or concentrated prior to analysis. Common extraction methods include static headspace, steam distillation, solvent extraction, solid-phase microextraction (SPME), and purge-and-trap (Beltran and others 2006, Buttery and others 1971). SPME is commonly used because it is a rapid solvent free method and costs less compared to other extraction methods (Beltran and others 2006). A common method of tomato volatile quantification is using gas chromatography-mass spectrometry (GC-MS) which can be paired with most extraction methods (Petro-Turza 1987). Gas chromatography separates extracted volatiles based on each volatile's different retention times in a separation column. The gas chromatography eluates were then ionized for detection by mass spectrometry (Beltran and others 2006). A negative for GC-MS is that the sample preparation required makes it difficult to do real-time analysis of volatiles, as well as being able to follow the dynamics of the reaction. The time it takes to prepare the samples can be decreased by trapping volatiles on some form of trapping material, but this is an indirect measure of volatiles which is not favored (Boukobza and others 2001).

Atmospheric pressure ionization mass spectrometry (API-MS) is another method for identification and quantification of volatile compounds. API-MS can measure mixtures of volatiles with a millisecond delay and concentrations as low as 10 ppb (Boukobza and others 2001). API-MS methods require the volatiles emitted by foods to be collected prior to running the sample (Spanel and Smith 1999). Another method that

is able to measure volatile compounds is proton transfer reaction mass spectrometry (PTR-MS). PTR-MS is a chemical ionization based mass spectrometry that can measure real-time release of volatiles at concentrations as low as 1 ppb (Hansel and others 1995). API-MS and PTR-MS are both capable of doing real-time analysis but their major fault is in their ability to distinguish between isomers like (Z)-3-hexenal and (E)-2-hexenal, both of which are critical in tomato's flavor profile (Xu and Barringer 2009).

2.6 Selected Ion Flow Tube Mass Spectrometry (SIFT-MS)

2.6.1 Principles of SIFT-MS

Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) is a mass spectrometry technique used for real-time analysis that utilizes chemical ionization of sample trace gases by selected ions during a defined amount of time along a flow tube. It is used due to its ability to quantify volatile compounds in real time while distinguishing between key isomers in the headspace above tomato and does not require sample preparation (Xu and Barringer 2009).

The principle of SIFT-MS can be explained by Figure 2.2. Initially, precursor ions are generated by microwave gas discharge into an upstream chamber where a quadrupole mass filter selects only the reagent ions, H_3O^+ , NO^+ , and O_2^+ . These ions were selected based on their ability to not react with the major components of air (N_2 , O_2 , H_2O , Ar, and CO_2) but react with the sample volatile compounds and many inorganic molecules (Spanel and Smith 1999). Following the upstream chamber, the ions go through a Venturi inlet into an inert carrier gas, helium and argon, flowing along a flow tube at a known velocity. The sample gas enters at a controlled rate into the flow tube to

react with the precursor ions to produce product ions. The count rates of the precursor and product ions are determined using second quadrupole mass filter. Lastly, a particle multiplier detects the ions at the selected masses and the count rate which is used to calculate the concentration of each selected volatile.

Figure 2.2 Principles of Selected Ion Flow Tube Mass Spectrometry

SIFT-MS has been used for years to identify the kinetic data for a large number of ion-neutral reactions (Adams and Smith 1976). The kinetic data obtained includes the rate coefficient (k), the product ions and their relative amounts, which is all stored in the SIFT database library (Spanel and Smith 1999). Thus, the concentrations of individual compounds (M) in a sample can be instantly quantified from the ratio of the product to precursor ion count rates (I_p/I) using Equation 1:

$$[M] = \frac{I_p}{I k t} \quad (1)$$

The reaction time (t) can be defined by the known flow velocity of the helium carrier gas and the length of the flow tube (Spanel and Smith 1999).

SIFT-MS provides rapid identification and quantification of volatiles in a wide range of sampling methods. It does so by using soft chemical ionization which results in less compound fragmentation and a simpler mass spectrum when compared to electron impact ionization used in GC-MS. Another benefit to SIFT-MS is that it utilizes multiple reagent ions (H_3O^+ , O_2^+ and NO^+) to chemically ionize volatiles instead of only one ion used in PTR-MS, making the identification of isomers easier since there are one or two product ions from each volatile compound. The sensitivity of this method is dependent on the time allowed for collecting data. A compound can be identified in a few seconds if it is present at greater than 10 ppb but a level as low as 0.1 ppb takes closer to 10 seconds (Spanel and Smith 1999).

2.6.2 Full Scan vs. MIM Scan

SIFT-MS can be run in two different modes: Full Scan and Multiple Ion Monitoring mode (MIM). Full Scan is used when sample's volatile concentrations do not vary over time. The air mixture will flow at a known rate into the carrier gas and the spectrum of precursor and product ions are found through the downstream quadrupole ion sweeping over a selected mass-charge ratio (m/z) range. MIM is used to identify volatile compounds in a sample which the concentrations of volatiles vary over time. In this mode the downstream mass spectrometer rapidly switches between the masses of the precursor ions and the chosen product ions and dwelling on each mass for a predetermined short time interval. Quick sampling is possible because of the fast flow rate of the carrier gas along the flow tube and the sample gas along the inlet tube. The response time is approximately 20 ms (Smith and Spanel 2005).

2.6.3 Ion Chemistry

SIFT-MS works on the understanding that when each trace gas, M, reacts with chosen precursor ions, and the goal is that the product ion will be unambiguously identified. Past experiments have studied the reactions of the precursor ions with a wide array of classes of compounds including alcohols, aldehydes, ketones, carboxylic acids, esters and ethers (Spanel and Smith 1999). These reactions have been compiled to create a SYFT library containing more than 500 volatile compounds. Depending on the analyte and reagent ion, five types of reactions can occur (Harper and others 2010).

(1) Proton transfer (H^+):

The majority of reactions with H_3O^+ proceed by rapid proton transfer producing MH^+ ions because the sample volatile has a greater proton affinity than H_2O (Spanel and Smith 1999). Proton transfer is an exothermic reaction that normally results in one or two product ions. Sometimes the MH^+ ions will dissociate into $[\text{M-OH}]^+$ eliminating an H_2O . This most often happens after the protonation of some alcohols, aldehydes and carboxylic acids (Smith and Spanel 2005).

(2) Charge transfer:

For the charge transfer reaction to proceed the ionization energy (IE) of M must be less than the IE of NO (9.26 eV) and O_2 (12.06 eV) (Smith and Spanel 2005). The IE of O_2 is higher than most organic molecules, making it a valuable precursor for many

volatiles. NO has a lower IE than O₂, therefore the reactions with NO usually involve aromatic hydrocarbons, low molecular weight alkanes and organosulfur compounds (Spanel and Smith 1999).

(3) Dissociative charge transfer:

This reaction involves a charge transfer as well as the formation of fragments of the original compound. It frequently involves O₂⁺ instead of NO⁺ because of the greater IE of O₂⁺ (Smith and others 2003a).

(4) Association:

Association reactions are very common between NO⁺ and some types of polar organic compounds including carboxylic acids, esters and ketones. It becomes the most efficient when the IE(M) is not much different than IE(NO). The stabilizing agent for association reactions tends to be the helium carrier gas. Association reactions commonly occur in parallel with some form of a charge transfer (Smith and Spanel 2005).

(5) Hydride extraction:

Hydride extraction involves the transfer of a hydride atom to the analyte which most commonly results in a single product ion and an HNO molecule. This reaction is the main one between NO⁺ with aldehydes and ethers (Smith and Spanel 2005).

2.6.4 Validation of SIFT-MS

Unlike a GC-MS, SIFT-MS does not require a known concentration of a reference substance to validate itself. SIFT-MS is able to do an automatic, on-line validation completed by using a dilute gas mixture of benzene, ethylbenzene, toluene, m-xylene, o-xylene, and p-xylene. The validation process takes approximately 15 min. The validation process requires the SIFT-MS identify the rate constants and product ratios for the above gas samples (Smith and Spanel 2005).

2.6.5 Application of SIFT-MS in Food Science

SIFT-MS has been used on a multitude of applications in a variety of fields including environmental science, microbiological studies, clinical science, and food science. Non-food science studies include the analysis of breath volatile compounds for medical diagnosis and therapeutic monitoring (Senthilmohan and others 2000), detection of volatiles produced by microbial growth in urine (Storer and others 2011), and analysis of diesel engine exhaust (Smith and others 2004).

SIFT-MS is advantageous for studying food because it utilizes three precursor ions which allow it to distinguish between isomers (Smith and Spanel 2005). One of the major applications of SIFT-MS in the food industry is with fruits and vegetables. In other studies, the SIFT-MS has been used for the real-time analysis of tomato volatile release during chewing (Xu and Barringer 2010b), the effect of temperature on the lipid-related volatiles (Xu and Barringer 2009), as well as the comparison of tomato volatiles with those of tomatillo (Xu and Barringer 2010a). Other types of food that SIFT-MS has been used to analyze include swiss cheese (Harper and others 2010), cocoa (Huang and

Barringer 2010), and determining the olive oil oxidative status (Davis and McEwan 2007).

Chapter 3: Materials and Methods

Ten varieties of tomatoes were either purchased from supermarkets in Columbus, Ohio, USA (7 varieties) or grown in Fremont, OH (3 varieties). Red mature tomatoes of uniform size were used. Five replicates were run due to the large variation from tomato to tomato. Each replicate contained multiple tomatoes to reach sample weights.

Tomatoes were washed and stored at room temperature for a minimum of 5 h prior to testing. Tests were carried out at 20-23°C. Tomatoes were peeled whole using a paring knife and cutting as close to the peel as possible. Peel and flesh samples were analyzed separately.

3.1 Headspace Volatile Quantification

3.1.1 Sample Preparation

Each replicate contained multiple tomatoes to reach 37.50 g samples. In the chopper (Magic Bullet short cup, Homeland Housewares LLC, Sherman Oaks, CA, USA) 37.50 g of tomato flesh or peel was blended with 12.50 g water and 1.88 g NaCl for 30 sec. NaCl (Extra Fine 325 Salt, Morton Salt Inc., Chicago, IL, USA) was added to increase the amount of volatiles in the headspace. Nonvolatile solutes are added to

increase the partial pressures of the volatile compounds therefore increasing the concentration of volatile compounds in the vapor phase (Poll and Flink 1984).

3.1.2 SIFT-MS

The headspace volatile compounds were analyzed using a selected ion flow tube-mass spectrometer (SIFT-MS) (SYFT Voice100, Syft Ltd., Christchurch, New Zealand) using the method described by Xu and Barringer (2009). All samples were run using selected ion mode (SIM) scans. The concentration of each compound was found based on pre-determined reaction kinetics (Table 3.1). The absolute concentration of each volatile was calculated by taking into account the pre-determined reaction rate for the volatile with that precursor ion and the dilution of the sample gas into the carrier gas (Smith and Spangel 1996). Some volatiles are not reported due to low concentrations or conflicts.

Samples were measured by piercing a polytetrafluoroethylene faced silicone septum glued to the cap of a 300 mL chopper with a short 14-gauge passivated needle. The needle point was approximately 6 cm above the sample. A long 14-gauge syringe needle was 1 cm above the sample to maintain the pressure in the bottle at atmosphere pressure and to provide oxygen for lipid oxidation. Immediately following chopping, the cap with septum was placed on the small cup and samples were run for 180 sec on the SIFT-MS. Samples were run at 20-23°C. Inlet and outlet temperatures were set at 120°C. Validations were run prior to scanning the samples. The validations involve measuring the exact amount of the gas mixtures benzene, ethylbenzene, toluene, m-xylene, o-xylene and p-xylene and comparing the measured concentrations to the actual

concentrations to ensure the machine is calibrated correctly. Air samples were run between tests to remove any potential contaminants present during preparation.

Table 3.1 Reaction kinetics of the selected volatile compounds measured in the headspace of tomato

Compound	Precursor Ion	k (10^{-9} cm ³ /s)	m/z	Product Ion	Reference
(E)-2-heptenal	NO ⁺	3.9	111	C ₇ H ₁₁ O ⁺	Spanel and others 2002
(E)-2-hexenal	NO ⁺	3.8	71	C ₄ H ₇ O ⁺	Spanel and others 1997
(Z)-3-hexenal	H ₃ O ⁺	4.2	81	C ₆ H ₉ ⁺	Spanel and others 1997
1-butanol	NO ⁺	2.2	73	C ₄ H ₉ O ⁺	Spanel and Smith 1997
1-hexanol	NO ⁺	2.4	101	C ₆ H ₁₃ O ⁺	Spanel and others 1997
1-penten-3-one	NO ⁺	2.5	114	C ₅ H ₈ O ⁺ .NO ⁺	Syft Technologies 2009
1-propanol	NO ⁺	2.3	59	C ₃ H ₇ O ⁺	Spanel and Smith 1997
2,3-butanediol	NO ⁺	2.3	89+107	C ₄ H ₉ O ₂ ⁺ , C ₄ H ₉ O ₂ ⁺ .H ₂ O	Syft Technologies 2009
2,3-butanedione	NO ⁺	1.3	86	C ₄ H ₆ O ₂ ⁺	Spanel and others 1997
2-isobutylthiazole	H ₃ O ⁺	3	142	C ₇ H ₁₁ NS.H ⁺	Syft Technologies 2009
2-methylpropanal	O ₂ ⁺	3	72	C ₄ H ₈ O ⁺	Spanel and others 2002
2-pentanol	NO ⁺	2.5	87	C ₅ H ₁₁ O ⁺	Syft Technologies 2009
2-pentanone	NO ⁺	3.1	116	NO ⁺ . C ₅ H ₁₀ O	Spanel and others 1997
2-pentenal*	NO ⁺	4.0	83	C ₅ H ₇ O ⁺	Spanel and others 2002
3-methylbutanal	NO ⁺	3.0	85	C ₅ H ₉ O ⁺	Spanel and others 2002
6-methyl-5-hepten-2-one	H ₃ O ⁺	3.0	109	C ₈ H ₁₃ ⁺	Syft Technologies 2009
acetaldehyde	NO ⁺	6.0	43+61+79	CH ₃ CO ⁺ , CH ₃ CO ⁺ .H ₂ O, CH ₃ CO ⁺ .2H ₂ O	Spanel and others 1997

Continued

Table 3.1 Continued

acetone	NO^+	1.2	88	$\text{C}_3\text{H}_6\text{O}.\text{NO}^+$	Spanel and others 1997
benzaldehyde	O_2^+	2.4	106	$\text{C}_7\text{H}_6\text{O}^+$	Spanel and Smith 1997
benzyl alcohol	NO^+	2.3	108	$\text{C}_7\text{H}_8\text{O}^+$	Wang and others 2004
cyclic terpenes**	NO^+	2.3	136	$\text{C}_{10}\text{H}_{16}^+$	Syft Technologies 2009
dimethyl disulfide	H_3O^+	2.6	95	$(\text{CH}_3)_2\text{S}_2.\text{H}^+$	Spanel and Smith 1998a
dimethyl sulfide	O_2^+	2.2	62	$(\text{CH}_3)_2\text{S}^+$	Spanel and Smith 1998a
ethanol	NO^+	1.2	45+63+ 81	$\text{C}_2\text{H}_5\text{O}^+, \text{C}_2\text{H}_5\text{O}^+.\text{H}_2\text{O},$ $\text{C}_2\text{H}_5\text{O}^+.\text{H}_2\text{O}$	Spanel and Smith 1997
ethyl acetate	NO^+	2.1	118	$\text{NO}^+.\text{CH}_3\text{COOC}_2\text{H}_5$	Spanel and Smith 1998b
hexanal	NO^+	2.5	99	$\text{C}_6\text{H}_{11}\text{O}^+$	Spanel and others 1997
hexen-1-ol***	NO^+	2.5	72	$\text{C}_4\text{H}_8\text{O}^+$	Syft Technologies 2009
isobutanol	O_2^+	3	72	$\text{C}_4\text{H}_8\text{O}^+$	Syft Technologies 2009
isobutyl alcohol	NO^+	2.4	73	$\text{C}_4\text{H}_9\text{O}^+$	Syft Technologies 2009
methanol	H_3O^+	2.7	33+51+ 65+69+ 83	$\text{CH}_3\text{O}^+, \text{CH}_3\text{OH}.\text{H}+\text{H}_2\text{O},$ $(\text{CH}_3\text{OH})_2.\text{H}+.\text{CH}_3\text{OH}.\text{H}$ $^+.(\text{H}_2\text{O})_2,$ $(\text{CH}_3\text{OH})_2.\text{H}+\text{H}_2\text{O}$	Spanel and Smith 1997
methional	O_2^+	2.5	104	$\text{C}_4\text{H}_8\text{OS}^+$	Syft Technologies 2009
methyl benzoate	H_3O^+	3.1	137	$\text{C}_6\text{H}_5\text{COOCH}_3.\text{H}^+$	Spanel and Smith 1998b
methylbutanoic acid****	NO^+	2.5	132	$\text{C}_5\text{H}_{10}\text{O}_2.\text{NO}^+$	Syft Technologies 2009
nonadienal*****	NO^+	2.5	168	$\text{C}_9\text{H}_{14}\text{O}.\text{NO}^+$	Syft Technologies 2009
nonanal	O_2^+	3.2	112	$\text{C}_8\text{H}_{16}^+$	Syft Technologies 2009
phenylacetaldehyde	NO^+	2.5	120	$\text{C}_8\text{H}_8\text{O}^+$	Syft Technologies 2009
propanal	NO^+	2.5	57	$\text{C}_3\text{H}_5\text{O}^+$	Spanel and others 1997
propanoic acid	O_2^+	2.2	74	$\text{C}_2\text{H}_5\text{COOH}^+$	Spanel and Smith 1998b

*2-Pentenal is a mixture of (*E*)-2-pentenal and (*Z*)-2-pentenal
**Cyclic terpenes are a mixture of 2-carene, (*R*)-limonene, and terpinen-4-ol.
***Hexen-1-ol is a mixture of (*E*)-2-hexenal, (*Z*)-2-hexenal, and (*Z*)-3-hexenal.
****Methylbutanoic acid is a mixture of 2-methylbutanoic acid and 3-methylbutanoic acid
*****Nonadienal is a mixture of (*E,Z*)-2,6-nonadienal and (*Z,Z*)-3,6-nonadienal

3.2 Moisture Content

Tomato peel was separated from the flesh using a paring knife. Approximately 2.5 g of peel and a cross section slice of the tomato flesh (≈ 5 g) were placed on separate aluminum weighing dishes. Samples were run in triplicate for both peel and flesh samples. Moisture was measured by placing the aluminum dishes in an Isotemp® Oven (Fisher Scientific, Pittsburgh, PA, USA) at 105°C for 16 h (AOAC 1990). Results are shown in Table 3.2.

Table 3.2 Weights of Tomato Samples for Lipid Extraction

Variety	Sample	Moisture (%)	Tomato Added (g)
Roma	Peel	93.52	42.77
	Flesh	95.39	41.93
Grape	Peel	92.01	43.47
	Flesh	93.01	43.01
Vine Ripened	Peel	93.43	42.81
	Flesh	95.16	42.03
Cherry	Peel	90.24	44.33
	Flesh	91.91	43.52
Heirloom	Peel	92.77	43.12
	Flesh	95.11	42.06
FG06-602	Peel	92.29	43.34
	Flesh	95.59	41.84
GEM 611	Peel	92.92	43.05
	Flesh	95.47	41.90
F006-609	Peel	93.52	42.77
	Flesh	95.86	41.73
Hydroponic	Peel	94.43	42.36
	Flesh	96.01	41.66
Campari	Peel	91.03	43.94
	Flesh	93.71	42.68

3.3 Lipoxygenase Activity

3.3.1 Extraction

Three tomatoes worth of peel/flesh were pureed in a chopper. For the enzyme extract, five replicates of 2 g were taken from this aliquot of pureed tomatoes and added to a mortar with 2 g of homogenization buffer (0.1 M Sodium Phosphate pH 7, 0.2% Triton X-100). The mixture was ground with a mortar and pestle. An aliquot of 1 mL was taken and centrifuged for 5 min at 16,000 xg in an Eppendorf® Centrifuge 5415R (Eppendorf N.A., Hauppauge, New York, USA) at 23°C. The least turbid portion of the

supernatant was removed and placed in a clean 1.5 mL centrifuge tube to form the enzyme extract. The substrate solution was made by adding 155 μ L linoleic acid (Sigma-Aldrich, St. Louis, MO, USA), 257 μ L Tween 20 and 5 mL distilled water to an amber container, which was mixed by drawing in and out of a pipette. Then 0.6 mL of 1.0 N NaOH was added to clarify the substrate and the overall solution was diluted to 25 mL by adding 0.1 M sodium phosphate buffer pH 7. Linoleic acid substrate solution was stored frozen in 1 ml aliquots.

3.3.2 Lipoxygenase Activity Quantification

The sample was assayed by adding 75 μ L of the enzyme extract, 60 μ L of substrate solution, and 3 mL 0.1 M sodium phosphate buffer pH 7 in a glass cuvette. Lipoxygenase activity was quantified by measuring the change in absorbance at 234 nm using a UV-2450 UV-Visible Spectrophotometer (Shimadzu Scientific Instruments Inc., Addison, IL, USA). Samples were measured for 4 min. Lipoxygenase activity was calculated as nmol of product formed per time on a fresh weight basis (nmols/g fwt/min). This was calculated by multiplying the average slope by 3 because the assay volume is 3 mL. The absorbance is then converted to nmoles by dividing by 0.023 because the molar extinction coefficient of the lipoxygenase product is 23,000 (Anthon 2011). Next, the value is converted from seconds to minutes by multiplying by 60. The value needs to be divided by the amount of tomato material in the assay. There were 75 μ L of homogenate added to the assay which is equal to 37.5 mg of tomato in the assay, which accounts for the dilution of the added sodium phosphate buffer.

3.4 Fatty Acid Composition

3.4.1 Lipid Extraction and Total Fatty Acid Determination

Tomato lipids were extracted using the procedure developed by Bligh and Dyer (1959). Each replicate contained multiple tomatoes to reach a specified amount of sample depending on the moisture content of each variety for a total of 40 mL of moisture from the tomato sample (See Appendix A for Table 3.2). The tomato sample was blended for 2 min with 50 mL chloroform and 100 mL methanol. Following blending, 50 mL chloroform was added. After 30 sec, 50 mL distilled water was added. The extract was blended for another 30 sec then transferred to centrifuge tubes to be centrifuged for 20 min at 9,000 RPM (Centrifric™ Centrifuge, Fisher Scientific, Pittsburgh, PA, USA). The supernatant was filtered through Whatman No. 1 filter paper in a funnel with suction. The filtrate was transferred to a separatory funnel and let stand for a few minutes. The alcoholic layer was removed by aspiration and then added to a round bottom flask. The sample was evaporated using a Büchi Rotavapor® R-210/R-215 (Büchi Co., New Castle, DE, USA). The total fatty acid was quantified by subtracting the round bottom flask weight from the weight of dried sample/round bottom flask weight.

3.4.2 Methylation

The extracted fatty acids were determined using a fatty acid methyl ester (FAME) method (Allendorf and others 2011). Lipids extracted from the tomato peel/flesh were esterified by adding 10 mL of 4% methanolic-sulfuric acid and 1 mL of benzene to 0.50 g of extracted lipid solubilized in chloroform into a glass test tube with a Teflon screw cap.

Samples were then boiled for 120 min at 80-90°C. After the samples cooled, 1 mL distilled water and 2 mL of hexane were added to help extract the methyl esters. One mL of the hexane layer was placed in a 1.5 mL GC vial and evaporated under nitrogen. Finally, 0.5 mL iso-octane was added to the dried sample to re-dilute it and the crimp top was crimped on.

3.4.3 Fatty Acid Composition

Methyl ester samples were analyzed using an HP-6890 GC equipped with a flame ionization detector (FID) (Hewlett Packard Co., Palo Alto, CA, USA). For automated injections an HP G 1513A autosampler and tray (Hewlett Packard Co., Palo Alto, CA, USA) were used. The components were then separated using an HP-FFAP 25 m x 0.32 mm x 0.5 µm column (Agilent part number 19019F-112) with helium as the carrier gas. One µL of sample was injected with a split ratio of 20:1. The oven was set at 110°C for 1 min then increased by 5°C/min to 220°C and held for 15 min. The injector temperature was 220°C and the detector temperature was 240°C. Run time was set at 30 min, during the first 20 min the sample was analyzed and the last 10 min the column was cleaned at a maximum temperature of 280°C. The retention times were compared with known fatty acid standards to identify the fatty acids present.

3.5 Storage Testing

Campani tomatoes were measured for volatile quantification, lipoxygenase activity, moisture content and total fatty acid determination on the day of purchase (Day 0) using the previous methods. Samples were then stored for 14 d in two separate conditions: room temperature at 20-23°C, and refrigerated at 2-5°C. Samples were stored

for 14 d and then reevaluated for volatiles, lipoxygenase activity, moisture and total fatty acid. Day 0 and the two day 14 samples were compared for any changes due to storage conditions.

3.6 Addition of Lipoxygenase and Linoleic Acid

Two mL of lipoxygenase homogenate (0.1 M sodium phosphate pH 7, 0.2% Triton X-100) was added prior to blending to 10.5 mL of water instead of 12.5 mL of water in the headspace volatile quantification method. For the linoleic acid addition, 5 mL of linoleic acid substrate solution (155 μ L linoleic acid, 257 μ L Tween 20, 5 mL distilled water, 0.6 mL of 1.0 N NaOH and 25 mL 0.1 M sodium phosphate buffer pH 7) was added prior to blending to 7.5 mL of water instead of 12.5 mL of water.

3.7 Statistical Analysis

Data was analyzed by using one-way analysis of variance (ANOVA) and the Tukey's family error rate with the Minitab program (Minitab 16, Minitab Inc., Pennsylvania USA). Significance was defined as $p \leq 0.05$. Headspace volatiles were also analyzed using Pirouette® (Version 4.0, Infometrix Inc., Woodville, WA, USA). Soft independent modeling of class analogy algorithm (SIMCA) with a probability threshold of 0.95 was used to identify variety differences.

Chapter 4: Results and Discussion

4.1 Comparison of Peel and Flesh of Tomato

4.1.1 Peel Volatiles vs. Flesh Volatiles

Volatile levels were significantly greater in peel than in flesh, when there was a significant difference (Tables 4.1 and 4.2). (Z)-3-Hexenal and methanol were significantly greater ($p \leq 0.05$) in peel than flesh for all 10 varieties, and 1-hexanol, 2-methyl propanal, dimethyl sulfide, hexanal, hexen-1-ol, methional, methyl benzoate, and propanoic acid were significantly greater in 6 of the 10 varieties. The volatiles in tomato peel and flesh can be broken down into five categories: lipid derived, amino acid derived, carotenoid derived, lignin derived and esters. The majority of volatiles measured were lipid or amino acid derived volatiles with 10 and 20, respectively.

The lipoxygenase pathway is very important to the production of C-6 volatiles in tomatoes (Galliard and Matthew 1977). The lipid derived volatiles are formed from the breakdown of linoleic and linolenic acids which react with lipoxygenase to form 13-hydroperoxy intermediates. The 13C compounds further react with other enzymes in the lipoxygenase pathway to form the lipid related volatile compounds. Of the 10 lipid derived volatiles, 8 had significantly higher levels in the peel than the flesh in at least one

variety (Tables 4.1 and 4.2). (*Z*)-3-Hexenal was significantly different for all 10 varieties. 1-Hexanol, hexanal, hexen-1-ol, nonadienal, and (*E*)-2-hexenal were significantly different in a majority of the varieties. Similar findings were reported by Buttery and others (1988), who found that the skin contained considerably higher levels of C6 aldehydes and several-fold higher concentrations of C6 alcohols than the whole tomato or the interior pulp.

There were six amino acid derived volatiles that were significantly greater in the peel than the flesh in six or more varieties: 2-methyl propanal, acetone, dimethyl sulfide, isobutanal, methional, and propanoic acid (Tables 4.1 and 4.2). 2-Methyl-propanal, isobutanal, and propanoic acid are products of the precursor amino acid valine (Bernalte and others 1998, Gibson and others 1998, Urbach 1995). Dimethyl sulfide and methional are produced from methionine (Wong and Carson 1966, Mayer and others 2008).

The other reported volatiles that were greater in the peel than flesh fall into the categories of carotenoid, lignin, ester and pectin derived volatiles. Methyl benzoate was the only volatile of these that was significantly different in a majority of the varieties (Tables 4.1 and 4.2). The lignin derived volatile benzyl alcohol was the only volatile to be significantly greater in the flesh than peel and this was only for one variety. Overall there was no pattern among the volatiles that are not related to amino acids or lipids.

Thirteen volatiles were not significantly different in all 10 varieties: 1-butanol, 1-propanol, 2,3-butanedione, 2-isobutylthiazole, 2-pentanol, 3-methylbutanal, ethanol,

ethyl acetate, isobutyl alcohol, methyl butanoic acid, nonanal, phenylacetaldehyde, and propanal. The majority of the non-significant volatiles are amino acid derived.

Combining (*Z*)-3-hexenal, (*Z*)-3-hexenol, hexanal, 1-penten-3-one, 3-methylbutanal, (*E*)-2-hexenal, 6-methyl-5-hepten-2-one, methyl salicylate, 2-isobutylthiazole, and β -ionone at varying concentrations produces the aroma of a fresh, ripe tomato (Buttery 1993). Of these volatiles only (*Z*)-3-hexenal, (*Z*)-3-hexenol, hexanal, and 1-hexanol were significantly greater in the peel than the flesh in more than half the varieties. The others were either present in too low of amounts or were significantly different in only a few varieties.

Table 4.1 Peel volatiles (ppm) of 10 tomato varieties. (Each volatile with different letters in Tables 4.1 and 4.2 are significantly different)

Volatile	Campari	FG06-602	Hydroponic	Vine Ripened	Heirloom	Roma	Grape	Cherry	GEM 611	F006-609
(E)-2-heptenal	6 ^{cd}	5 ^{cd}	5 ^{cd}	5 ^{cd}	5 ^{cd}	7 ^{cd}	19 ^a	7 ^{cd}	3 ^{cd}	4 ^{cd}
(E)-2-hexenal	394 ^a	273 ^{abc}	295 ^{abc}	273 ^{abc}	290 ^{abc}	322 ^{ab}	258 ^{bcd}	181 ^{bcd}	139 ^{cde}	163 ^{cde}
(Z)-3-hexenal	4048 ^a	3482 ^{ab}	3423 ^{ab}	3418 ^{bc}	2942 ^{ab}	2690 ^{bc}	2470 ^{cd}	2134 ^e	2023 ^{cde}	1995 ^{cde}
1-butanol	14 ^{ef}	18 ^{def}	24 ^{cdef}	27 ^{bcd}	40 ^{ab}	21 ^{cdef}	19 ^{ef}	12 ^f	8 ^f	15 ^{ef}
1-hexanol	30 ^a	22 ^{abc}	17 ^{bcd}	23 ^{abc}	19 ^{bcd}	23 ^{ab}	22 ^{cdef}	16 ^{cde}	10 ^{defg}	9 ^{efg}
1-penten-3-one	47 ^{cde}	48 ^{de}	51 ^{cde}	45 ^{de}	48 ^{de}	48 ^{cde}	213 ^a	15 ^e	39 ^{de}	39 ^{de}
1-propanol	22 ^{abc}	55 ^a	22 ^{abc}	28 ^{abc}	28 ^{abc}	24 ^{abc}	14 ^{bc}	54 ^a	15 ^{bc}	43 ^{ab}
2,3-butanediol	11 ^{ab}	11 ^a	9 ^{abcd}	9 ^{abc}	8 ^{abcde}	9 ^{abc}	8 ^{abcdef}	7 ^{abcdef}	10 ^{abc}	7 ^{abcdef}
2,3-butanedione	16 ^{abc}	11 ^{bc}	17 ^{bc}	12 ^{bc}	13 ^{abc}	13 ^{abc}	26 ^{ab}	5 ^c	9 ^{bc}	9 ^{bc}
2-isobutylthiazole	18 ^{abc}	1 ^d	3 ^{cd}	16 ^{abc}	5 ^{bcd}	2 ^{cd}	11 ^{bcd}	11 ^{abcd}	2 ^{cd}	10 ^{abcd}
2-methylpropanal	293 ^a	180 ^{cd}	151 ^{cde}	202 ^{bc}	186 ^{bcd}	233 ^{ab}	160 ^{def}	168 ^{cd}	90 ^{efg}	93 ^{efg}
2-pentanol	21 ^{abcd}	13 ^{cdefg}	20 ^{abcde}	19 ^{abcde}	27 ^a	23 ^{abc}	18 ^{abcdefg}	8 ^{efg}	9 ^{defg}	9 ^{defg}
2-pentanone	16 ^{ab}	16 ^a	14 ^{abcd}	15 ^{abc}	12 ^{abcdef}	16 ^{abc}	16 ^{abcde}	9 ^{cdefg}	11 ^{abcdefg}	9 ^{bcdefg}
2-pentenal	42 ^{abcd}	45 ^{abc}	40 ^{abcdef}	37 ^{bcd}	35 ^{bcd}	36 ^{abcdef}	68 ^a	20 ^{efgh}	28 ^{bcdefgh}	25 ^{cdefgh}
3-methylbutanal	42 ^{abcd}	21 ^{bcd}	48 ^{abcd}	30 ^{bcd}	29 ^{bcd}	36 ^{bcd}	68 ^{ab}	8 ^d	15 ^{bcd}	12 ^{cd}
6-methyl-5-hepten-2-one	24 ^{cd}	68 ^a	13 ^d	18 ^d	16 ^d	15 ^d	29 ^d	16 ^d	49 ^{abc}	45 ^{bc}
acetaldehyde	264 ^{abcdef}	442 ^a	287 ^{abcdef}	237 ^{abcdef}	217 ^{abcdef}	212 ^{abcdef}	313 ^{abc}	345 ^{abcd}	229 ^{abcdef}	325 ^{abcde}
acetone	477 ^a	337 ^{abcdef}	350 ^{abcdef}	395 ^{abcd}	348 ^{abcde}	335 ^{abcdef}	375 ^{cdefg}	254 ^{efgh}	454 ^{abc}	254 ^{defgh}

Continued

Table 4.1 Continued

Volatile	Campari	FG06-602	Hydroponic	Vine Ripened	Heirloom	Roma	Grape	Cherry	GEM 611	F006-609
benzaldehyde	16 ^{abcd}	17 ^{abc}	15 ^{abcde}	21 ^{ab}	14 ^{bcde}	20 ^a	18 ^{bcdef}	8 ^{defg}	11 ^{cdefg}	7 ^{defg}
benzyl alcohol	10 ^{de}	43 ^{ab}	5 ^e	6 ^e	12 ^{de}	7 ^e	9 ^e	2 ^e	30 ^{bc}	23 ^{cd}
cyclic terpenes	35 ^a	10 ^{bc}	6 ^c	18 ^b	5 ^c	6 ^c	6 ^c	4 ^c	4 ^c	5 ^c
dimethyl disulfide	6 ^{bcde}	15 ^{abcd}	8 ^{abcde}	16 ^{abc}	6 ^{cde}	7 ^{bcde}	23 ^a	18 ^{ab}	9 ^{abcde}	13 ^{abcde}
dimethyl sulfide	591 ^a	332 ^{cd}	326 ^{cd}	500 ^{ab}	403 ^{bc}	534 ^a	415 ^{cde}	336 ^{cd}	158 ^{efg}	161 ^{efg}
ethanol	123 ^a	409 ^a	122 ^a	108 ^a	99 ^a	92 ^a	148 ^a	193 ^a	137 ^a	274 ^a
ethyl acetate	12 ^{ab}	59 ^a	9 ^{ab}	13 ^{ab}	10 ^{ab}	11 ^{ab}	11 ^{ab}	7 ^{ab}	15 ^{ab}	20 ^{ab}
hexanal	4270 ^a	2492 ^{bc}	2185 ^c	2994 ^{bc}	2550 ^{bc}	3550 ^{ab}	2528 ^{cd}	2242 ^{bc}	1051 ^{de}	1076 ^{de}
hexen-1-ol	28 ^b	42 ^a	20 ^{bcd}	28 ^b	21 ^{bcd}	25 ^{def}	24 ^{bcd}	16 ^{cde}	29 ^b	25 ^{bc}
isobutanal	293 ^a	180 ^{bcd}	151 ^{cde}	202 ^{bc}	186 ^{bcd}	233 ^{ab}	160 ^{def}	168 ^{cd}	90 ^{efg}	93 ^{efg}
isobutyl alcohol	13 ^{ef}	16 ^{def}	22 ^{cdef}	25 ^{bcde}	36 ^{ab}	19 ^{cdef}	17 ^{ef}	11 ^f	7 ^f	14 ^{ef}
methanol	31400 ^{de}	48300 ^{ab}	31275 ^{de}	55360 ^a	24080 ^e	29640 ^{de}	40150 ^{de}	39640 ^{bcd}	44467 ^{bc}	36425 ^{cd}
methional	46 ^{ab}	38 ^{bcd}	24 ^{def}	55 ^a	22 ^{defg}	40 ^{abc}	44 ^{cdef}	27 ^{cde}	18 ^{efgh}	17 ^{efgh}
methyl benzoate	133 ^a	50 ^d	40 ^{de}	95 ^b	53 ^{cd}	68 ^{bc}	64 ^{de}	55 ^{cd}	21 ^{ef}	20 ^{ef}
methylbutanoic acid	2 ^{de}	14 ^{bc}	4 ^{de}	5 ^d	4 ^{de}	3 ^{de}	2 ^e	1 ^e	3 ^{de}	11 ^c
nonadienal	31 ^a	21 ^{abc}	24 ^{ab}	25 ^{ab}	22 ^{abc}	17 ^{bc}	16 ^{cd}	8 ^{de}	4 ^{de}	6 ^{de}
nonanal	11 ^{abc}	15 ^{ab}	4 ^c	11 ^{abc}	8 ^{abc}	10 ^{abc}	25 ^a	9 ^{abc}	9 ^{abc}	7 ^{abc}
phenylacetaldehyde	16 ^{ab}	14 ^{abc}	11 ^{bcdef}	18 ^a	10 ^{cdef}	12 ^{abcd}	17 ^{bcde}	8 ^{cdefg}	7 ^{defgh}	8 ^{cdefgh}
propanal	17 ^{abc}	15 ^{abc}	15 ^{abc}	12 ^{abc}	13 ^{abc}	14 ^{abc}	27 ^{abc}	9 ^{abc}	8 ^{abc}	9 ^{abc}
propanoic acid	14 ^{ab}	17 ^a	15 ^{ab}	15 ^{ab}	13 ^{abc}	14 ^{ab}	12 ^{bcde}	8 ^{cde}	19 ^a	16 ^{ab}

Table 4.2 Flesh volatiles (ppm) of 10 tomato varieties. (Each volatile with different letters in Tables 4.1 and 4.2 are significantly different)

Volatile	Campari	FG06-602	Hydroponic	Vine Ripened	Heirloom	Roma	Grape	Cherry	GEM 611	F006-609
(E)-2-heptenal	4 ^{bc}	3 ^d	4 ^{bc}	4 ^{cd}	3 ^d	4 ^{bc}	11 ^{ab}	8 ^{bc}	3 ^d	3 ^d
(E)-2-hexenal	255 ^{abc}	43 ^e	88 ^{de}	85 ^{de}	214 ^{abcd}	151 ^{cde}	151 ^{cde}	75 ^e	37 ^e	38 ^e
(Z)-3-hexenal	1230 ^{def}	434 ^{fg}	520 ^{fg}	566 ^{efg}	961 ^{fg}	687 ^{fg}	915 ^{fg}	771 ^{fg}	308 ^{fg}	233 ^g
1-butanol	17 ^{ef}	17 ^{def}	32 ^{bcd}	37 ^{abc}	46 ^a	19 ^{def}	22 ^{def}	19 ^{def}	9 ^f	18 ^{def}
1-hexanol	6 ^{fg}	3 ^g	3 ^g	5 ^g	5 ^g	5 ^g	7 ^{fg}	6 ^g	2 ^g	2 ^g
1-penten-3-one	85 ^{bc}	40 ^{de}	32 ^{de}	49 ^{cde}	46 ^{de}	57 ^{cd}	111 ^b	20 ^{de}	28 ^{de}	18 ^{de}
1-propanol	12 ^{bc}	45 ^{ab}	13 ^{bc}	16 ^{bc}	22 ^{abc}	11 ^{bc}	7 ^c	33 ^{abc}	8 ^{bc}	57 ^a
2,3-butanediol	8 ^{abcde}	3 ^{ef}	4 ^{ef}	4 ^{def}	6 ^{abcdef}	5 ^{cdef}	6 ^{bcdef}	5 ^{cdef}	9 ^{abcde}	2 ^f
2,3-butanedione	28 ^a	8 ^{bc}	9 ^{abc}	15 ^{abc}	18 ^{abc}	13 ^{abc}	21 ^{ab}	4 ^c	4 ^c	4 ^c
2-isobutylthiazole	22 ^a	4 ^{cd}	2 ^d	22 ^a	7 ^{bcd}	4 ^{bcd}	9 ^{bcd}	9 ^{bcd}	2 ^{cd}	3 ^{cd}
2-methylpropanal	78 ^{fg}	23 ^g	46 ^g	53 ^g	73 ^{fg}	67 ^{fg}	80 ^{fg}	61 ^{fg}	26 ^g	23 ^g
2-pentanol	18 ^{abcdef}	6 ^{efg}	9 ^{defg}	14 ^{abcdefg}	26 ^{ab}	16 ^{abcdef}	14 ^{bcdefg}	5 ^{fg}	6 ^{efg}	2 ^g
2-pentanone	7 ^{defgh}	3 ^{gh}	3 ^{gh}	4 ^{gh}	7 ^{efgh}	14 ^{abcd}	9 ^{cdefg}	4 ^{gh}	5 ^{fgh}	1 ^h
2-pentenal	41 ^{abcde}	20 ^{defgh}	20 ^{fgh}	28 ^{cdefgh}	50 ^{ab}	36 ^{bcdefg}	45 ^{abc}	15 ^{gh}	10 ^h	10 ^h
3-methylbutanal	105 ^a	15 ^{cd}	39 ^{bcd}	61 ^{abcd}	75 ^{abc}	58 ^{abcd}	81 ^{abcd}	14 ^d	11 ^{cd}	8 ^d
6-methyl-5-hepten-2-one	19 ^d	49 ^{ab}	7 ^d	10 ^d	14 ^d	13 ^d	14 ^d	16 ^d	72 ^a	21 ^d
acetaldehyde	122 ^{cdef}	79 ^{ef}	102 ^{def}	74 ^{ef}	127 ^{cdef}	173 ^{bcdef}	381 ^{ab}	217 ^{abcdef}	87 ^{cdef}	46 ^f
acetone	328 ^{bcdef}	166 ^{ghi}	164 ^{hi}	185 ^{ghi}	210 ^{fghi}	213 ^{efghi}	297 ^{defgh}	181 ^{ghi}	483 ^{ab}	97 ⁱ

Continued

Table 4.2 Continued

Volatile	Campari	FG06-602	Hydroponic	Vine Ripened	Heirloom	Roma	Grape	Cherry	GEM 611	F006-609
benzaldehyde	10 ^{cdefg}	7 ^{efg}	4 ^{fg}	8 ^{efg}	11 ^{cdefg}	10 ^{cdefg}	8 ^{defg}	5 ^{fg}	3 ^{fg}	2 ^g
benzyl alcohol	9 ^{de}	40 ^{ab}	2 ^e	6 ^e	10 ^{de}	8 ^{de}	6 ^e	2 ^e	53 ^a	15 ^{cde}
cyclic terpenes	5 ^c	5 ^c	4 ^c	10 ^{bc}	1 ^c	1 ^c	2 ^c	2 ^c	4 ^c	3 ^c
dimethyl disulfide	4 ^{de}	3 ^{de}	3 ^e	2 ^e	4 ^e	5 ^{cde}	9 ^{abcde}	4 ^e	4 ^{cde}	2 ^e
dimethyl sulfide	154 ^{fg}	39 ^g	90 ^{fg}	126 ^{fg}	149 ^{fg}	146 ^{fg}	205 ^{def}	137 ^{fg}	36 ^g	35 ^g
ethanol	43 ^a	38 ^a	67 ^a	39 ^a	79 ^a	56 ^a	196 ^a	296 ^a	44 ^a	30 ^a
ethyl acetate	4 ^b	5 ^{ab}	4 ^b	5 ^{ab}	8 ^{ab}	5 ^{ab}	5 ^{ab}	3 ^b	6 ^{ab}	6 ^{ab}
hexanal	772 ^e	218 ^e	506 ^e	566 ^{de}	724 ^e	732 ^e	1039 ^{de}	835 ^e	182 ^e	177 ^e
hexen-1-ol	14 ^{cdef}	11 ^{def}	5 ^f	6 ^{ef}	12 ^{def}	8 ^f	11 ^{def}	5 ^{ef}	13 ^{cdef}	5 ^{ef}
isobutanal	78 ^{fg}	23 ^g	46 ^g	53 ^g	73 ^{fg}	67 ^{fg}	80 ^{fg}	61 ^{bc}	26 ^g	23 ^g
isobutyl alcohol	15 ^{ef}	16 ^{def}	29 ^{bcd}	34 ^{abc}	42 ^a	17 ^{def}	20 ^{def}	18 ^{def}	8 ^f	16 ^{def}
methanol	4140 ^f	9253 ^f	6604 ^f	7366 ^f	7080 ^f	8370 ^f	8418 ^f	6254 ^f	10533 ^f	8905 ^f
methional	6 ^{gh}	3 ^h	3 ^h	5 ^h	6 ^h	9 ^{fgh}	11 ^{fgh}	4 ^h	3 ^h	2 ^h
methyl benzoate	25 ^{ef}	9 ^f	13 ^f	24 ^{ef}	17 ^{ef}	17 ^{ef}	23 ^{ef}	16 ^{ef}	7 ^f	8 ^f
methylbutanoic acid	2 ^{de}	21 ^a	4 ^{de}	4 ^{de}	4 ^{de}	2 ^{de}	1 ^e	1 ^e	1 ^{de}	18 ^{ab}
nonadienal	4 ^{de}	0 ^e	0 ^e	1 ^e	3 ^{de}	1 ^e	3 ^{de}	3 ^{de}	0 ^e	0 ^e
nonanal	8 ^{abc}	10 ^{abc}	7 ^{abc}	7 ^{abc}	6 ^{bc}	9 ^{abc}	15 ^{ab}	7 ^{abc}	13 ^{abc}	8 ^{abc}
phenylacetaldehyde	5 ^{fgh}	3 ^{gh}	3 ^h	4 ^{gh}	3 ^{gh}	4 ^{gh}	7 ^{defgh}	4 ^{efgh}	1 ^h	3 ^{gh}
propanal	21 ^a	7 ^{abc}	8 ^{abc}	10 ^{abc}	19 ^{abc}	22 ^{ab}	21 ^{ab}	7 ^{abc}	4 ^{bc}	3 ^c
propanoic acid	6 ^{de}	7 ^{cde}	6 ^{de}	6 ^{de}	6 ^{de}	6 ^{cde}	7 ^{cde}	4 ^e	12 ^{abcd}	4 ^e

4.1.2 Effect of Lipoxygenase Activity on Volatile Concentration

Lipoxygenase (LOX) is the first enzyme in the pathway which creates lipid volatiles in tomatoes therefore it may play a role in the differences that occur in the peel and flesh. LOX activity was significantly higher in peel than flesh for all 10 varieties (Table 4.3). Others have found similar results, where the locular material has no LOX activity, while the skin with outer flesh has 71% of the activity, but when the flesh was removed from the skin the activity was drastically lower for the relatively pure skin (Hatanaka and others 1992). This suggests that LOX activity is greatest in the fleshy tissue adjacent to the skin. The peel portion in this study contained small amounts of flesh next to the peel which may be why the peel portion was significantly greater than the flesh. These results for LOX are different from those for hydroperoxide lyase (HPL) activity, which was evenly distributed throughout the tomato (Hatanaka and others 1992). LOX produces 9 and 13-hydroperoxides which then react with HPL to form C9 and C6 aldehydes, respectively. HPL activity is critical because it helps in the creation of the fresh, green C6 aldehydes, such as hexanal and (*Z*)-3-hexenal in the lipoxygenase pathway.

Table 4.3 Lipoxygenase activity (nmol/g fwt/min) in different varieties of tomato

	Lipoxygenase Activity	
	Peel	Flesh
Campari	144 ^{ab}	63 ^{de}
FG06-602	130 ^{ab}	69 ^{de}
Hydroponic	151 ^a	56 ^{de}
Vine Ripened	139 ^{ab}	71 ^{de}
Heirloom	143 ^{ab}	53 ^e
Roma	166 ^a	96 ^{cd}
Grape	138 ^{ab}	93 ^{cd}
Cherry	163 ^a	77 ^{de}
GEM 611	110 ^{bc}	65 ^{de}
F006-609	136 ^{ab}	84 ^{de}

^a Samples with different letters in the table are significantly different

Lipoxygenase activity may have a significant effect on the formation of volatiles in the lipoxygenase pathway, such as (Z)-3-hexenal which originates from linolenic acid (Figure 2.1). There is a distinct separation between the peel and flesh with peel being greater than flesh in both lipoxygenase activity and the concentration of (Z)-3-hexenal (Figure 4.1). However, the correlation between (Z)-3-hexenal and lipoxygenase activity is not significant within the flesh or peel groups. Similar results were found with hexanal, 1-hexanol, hexen-1-ol, (E)-2-hexenal, and nonadienal.

Figure 4.1 Effect of lipoxygenase activity on (Z)-3-hexenal formation in flesh (diamond) and peel (square)

To test the effect of lipoxygenase on lipoxygenase generated volatiles, an exogenous lipoxygenase homogenate was added (Table 4.4). Lipoxygenase addition had no significant effect on the lipoxygenase generated volatiles for either peel or flesh. This is somewhat consistent with a study where there was a steady decrease in the concentrations of hexanal, (Z)-3-hexenal, (E)-2-hexenal, and 1-penten-3-one with the addition of lipoxygenase, alcohol dehydrogenase or lipoxygenase/alcohol dehydrogenase in red tomatoes, as these volatiles also tended to decrease in this study (Yilmaz and others 2002). Tomatoes naturally containing varying concentrations of lipoxygenase,

hydroperoxide lyase and alcohol dehydrogenase were found to have no significant correlation with hexanal, (Z)-3-hexenal, (E)-2-hexenal, (E)-2-heptenal, pentenone, or (Z)-3-hexenol (Yilmaz and others 2001). However, when linoleic and linolenic acid was added, levels of hexanal and hexenal were much lower in tomatoes with low levels of lipoxygenase activity than tomatoes with high levels of lipoxygenase, thus there is some effect (Chen and others 2004).

Table 4.4 Effect of lipoxygenase addition on the lipoxygenase generated volatiles (ppm). There were no significant differences between the control and lipoxygenase addition.

Volatile	Control Peel	Peel with Addition	Control Flesh	Flesh with Addition
hexanal	4398	3980	748	931
1-hexanol	32	37	6	7
(E)-2-heptenal	8	6	4	4
(Z)-3-hexenal	3650	3480	1172	1122
(E)-2-hexenal	391	340	200	190
(Z)-3-hexen-1-ol	30	36	11	13
(E)-2-pentenal	42	40	40	39
1-penten-3-one	64	31	89	87

4.1.3 Effect of Total Fatty Acid Content and Fatty Acid Profile on Volatile Concentration

Peel total fatty acid content was significantly greater than flesh in six of the varieties (Table 4.5). No other researchers have looked at the variations in total fatty acid content between the flesh and peel in tomato. Others have reported fatty acid contents of 0.5 to 1.9 g/kg in whole tomato (Gray and others 1999, USDA 2011). Differences in

values can be accounted for by the different cultivars being used, storage conditions, as well as the known variation among individual tomatoes.

Table 4.5 Total fatty acid content (g per fresh weight kg) of different tomato varieties

	Total Fatty Acid	
	Peel	Flesh
Campari	2.11 ^{abc}	1.50 ^{efg}
FG06-602	1.90 ^{bcde}	1.11 ^{gh}
Hydroponic	2.41 ^a	2.00 ^{abcd}
Vine Ripened	2.09 ^{abc}	0.90 ^h
Heirloom	2.08 ^{abc}	1.68 ^{cdef}
Roma	1.84 ^{bcde}	0.83 ^h
Grape	1.95 ^{bcde}	1.61 ^{def}
Cherry	1.73 ^{cde}	1.23 ^{fgh}
GEM 611	2.12 ^{abc}	1.57 ^{def}
F006-609	2.21 ^{ab}	2.12 ^{abc}

^a Samples with different letters in the table are significantly different

The total fatty acid content includes linoleic, palmitic, linolenic, oleic, and stearic acids (Table 4.6). Linoleic acid (39-51%) is present in the highest concentrations followed by palmitic acid (26-33%). Linolenic (5-19%) and oleic (4-19%) acid concentrations vary by variety. Stearic acid (2-4%) is always present at the lowest concentration. Others have reported that linoleic acid was present in the highest concentration followed by oleic acid, palmitic acid, then stearic acid or linolenic acid for the whole tomato or the pericarp (USDA 2010, Gray and others 2002). Linoleic acid is always reported to be present in the highest concentrations but the other fatty acids vary.

The differences in order and percentages can be accounted for by a different cultivar being used, different tissue measured, as well as the known variation among tomatoes.

The peel contained significantly more linolenic acid than flesh in all varieties except two. For oleic acid, the flesh was significantly greater than peel for half of the varieties. Palmitic, stearic, and linoleic acids had minimal differences throughout the varieties. There have been no previous comparisons made for the fatty acids in the peel versus the flesh portions of a tomato.

Table 4.6 Fatty acid profiles (%) of different tomato varieties

		Linoleic Acid	Palmitic Acid	Linolenic Acid	Oleic Acid	Stearic Acid
FG06-602	Flesh	47.89 ^{ab}	30.96 ^{abcd}	12.11 ^{cde}	6.32 ^{gh}	2.71 ^b
	Peel	39.62 ^e	33.96 ^a	19.15 ^a	4.08 ^h	3.19 ^{ab}
Vine Ripened	Flesh	40.41 ^e	32.60 ^{ab}	13.64 ^{bcd}	11.13 ^{cdef}	2.81 ^b
	Peel	39.19 ^e	30.83 ^{bcd}	14.66 ^{bc}	12.29 ^{bcd}	2.85 ^b
Heirloom	Flesh	50.36 ^a	26.36 ^{ef}	7.03 ^{gh}	14.00 ^{bcd}	2.25 ^b
	Peel	42.93 ^{de}	31.23 ^{abcd}	14.67 ^{bcd}	8.25 ^{efg}	2.91 ^b
Roma	Flesh	46.41 ^{bc}	31.31 ^{abc}	8.99 ^{efg}	8.83 ^{fgh}	4.46 ^a
	Peel	44.71 ^{bcd}	28.67 ^{def}	16.42 ^{ab}	7.04 ^{gh}	3.16 ^{ab}
Grape	Flesh	46.12 ^{bcd}	26.18 ^f	5.18 ^h	19.47 ^a	3.05 ^{ab}
	Peel	45.11 ^{bcd}	26.61 ^{ef}	9.41 ^{fg}	15.37 ^b	3.50 ^{ab}
Cherry	Flesh	50.45 ^a	29.19 ^{bcd}	8.63 ^{fgh}	8.38 ^{defg}	3.35 ^{ab}
	Peel	51.72 ^a	30.27 ^{cde}	10.55 ^{defg}	4.47 ^h	2.99 ^b
GEM 611	Flesh	43.07 ^{cde}	31.29 ^{abcd}	9.45 ^{fg}	14.13 ^{bc}	2.05 ^b
	Peel	41.16 ^e	33.92 ^a	17.03 ^{ab}	4.38 ^h	3.51 ^{ab}

^a Fatty acids with different letters in the same column are significantly different

Even though fatty acids are present in small amounts they are the precursors for the lipid derived volatiles in the lipoxygenase pathway, which produces volatiles important to the green, fresh aroma of tomatoes (Petro-Turza 1987). Linolenic acid is converted into (Z)-3-hexenal as the first volatile produced from the pathway. Fatty acid content and amount of linolenic acid may affect the production of lipoxygenase generated volatiles such as (Z)-3-hexenal (Figure 4.2). There is a noticeable separation between the peel and flesh samples for both total fatty acid and linolenic acid content. Peel has higher concentrations of total fatty acids, linolenic acid, and (Z)-3-hexenal than flesh. Within the peel or flesh groups there is no significant correlation with lipid derived volatiles and either total fatty acid content or linolenic acid content.

There is some correlation between volatile levels and total fatty acid content for (Z)-3-hexenal, hexen-1-ol, (E)-2-hexenal, hexanal, and 1-hexanol. However, linolenic acid has a stronger correlation than total fatty acid content with (Z)-3-hexenal, (E)-2-hexenal, and hexen-1-ol. Linoleic acid content had no correlation to hexanal and 1-hexanol likely because there was no difference between peel and flesh linoleic acid content. For most of the lipid derived volatiles the correlation to total fatty acid content was the weakest with the correlations to lipoxygenase activity and linolenic content being similar (Figures 4.1 and 4.2).

Figure 4.2 Effect of total fatty acid content (left) and linolenic acid (right) on (Z)-3-hexenal formation in flesh (diamond) and peel (square)

Exogenous linoleic acid was added to flesh and peel to test the effect on linoleic acid derived volatiles (Table 4.7). In the flesh, hexanal, 1-hexanol, and (*E*)-2-heptenal were significantly greater after linoleic acid addition. In the peel, only (*E*)-2-heptenal was greater. The difference in effect on peel versus flesh may mean that linoleic acid is the limiting factor in the flesh but not in the peel, since linoleic acid content is the same in flesh and peel but the lipoxygenase activity is significantly greater in the peel (Tables 4.3, 4.5 and 4.6). This is somewhat consistent with results of other studies that found an increase in the levels of hexanal and 1-hexanol in a whole tomato with the addition of linoleic acid (Boukobza and others 2001, Chen and others 2004, Riley and Thompson 1998). In green bell pepper, with the addition of linoleic acid there was a significant increase in the levels of hexanal, 1-hexanol, 2-pentylfuran, (*E*)-2-heptenal, (*E*)-2-octenal,

(*E*)-2-nonenal and (*Z*)-3-hexenal (Luning and others 1995). With tomato cultivars that are bred to contain different levels of linoleic and linolenic acids, when the ratio of linoleic acid to linolenic acid is greater there is a significant increase in the hexanal to hexenal ratio (Gray and others 1999). Similar findings were found when the expression of a yeast Δ -9 desaturase gene was altered to produce higher concentrations of oleic acid and linoleic acid, which caused hexanal and 1-hexanol to be more than two times greater than the control (Wang and others 1996). Breeding tomatoes with higher levels of linoleic acid and lower levels of linolenic acid caused an increase in hexanal and a decrease in (*Z*)-3-hexenal formation (Canoles and others 2006).

Table 4.7 Effect of linoleic acid addition on the formation of linoleic acid derived volatiles (ppm)

	Volatile	Control	Sample with Addition
Flesh	hexanal	748 ^b	1393 ^a
	1-hexanol	6 ^b	9 ^a
	(<i>E</i>)-2-heptenal	5 ^b	28 ^a
Peel	hexanal	3597 ^a	3243 ^a
	1-hexanol	28 ^a	19 ^a
	(<i>E</i>)-2-heptenal	5 ^b	26 ^a

^a Samples with different letters in the same row are significantly different

4.2 Variety Differences

The variety differences in concentrations of volatiles are great. When samples were analyzed by chemometrics, the flesh and peel samples of any given variety were classified as closer together than peel or flesh samples between different varieties.

Campari, FG06-602, and Grape had higher concentrations of volatiles than most varieties

(Tables 4.1 and 4.2). Grape had significantly higher levels of (*E*)-2-heptenal and 1-penten-3-one than any other variety, as well as noticeably higher levels of (*E*)-2-pentenal and acetaldehyde. Campari had concentrations significantly higher in cyclic terpenes and methyl benzoate with noticeably higher levels of (*Z*)-3-hexenal and 2-methylpropanal. There was no real pattern with the type of compounds, although 3 of the compounds having higher concentrations in Grape are produced in the lipoxygenase pathway. Interestingly, Grape and Campari are two of the smallest varieties of those measured. Results by Xu and Barringer (2010a) were similar in that grape tomato had higher levels of volatiles than vine-ripened, cherry and roma tomatoes. It was also reported that the formation of a mixture of (*Z*)-3-hexenal and (*E*)-2-hexenal during chewing is greater in cherry tomato than plum and delice tomatoes (Brauss and others 1998).

Each variety had major differences in which volatiles were significantly higher in peel than flesh (Tables 4.1 and 4.2). FG06-602, Vine Ripened, and Hydroponic had the greatest number of significantly greater peel volatiles than flesh volatiles with 19, 18, and 17 different volatiles respectively. The three varieties were significantly greater in 16 of the same volatiles. The different volatiles were fairly split between amino acid and lipid derived volatiles with slightly more being amino acid derived. Grape and GEM 611 had the least amount of volatile differences with only 3 and 4 volatiles, respectively. Neither Grape nor GEM 611 was significantly different in an amino acid derived volatile but were significantly different in lipid and pectin derived volatiles.

4.3 Effect of Storage Temperature on Volatiles

4.3.1 Day 0 vs. Day 14 Unrefrigerated and Refrigerated Storage

Campani tomatoes were stored in unrefrigerated and refrigerated conditions for 14 days. Concentrations of volatiles, lipoxygenase activity and fatty acid content were measured at day 0 and day 14. Stored unrefrigerated peel was significantly greater and refrigerated peel was significantly lower than before storage in hexanal, 1-hexanol, (*E*)-2-hexenal, and dimethyl sulfide contents (Table 4.8). Refrigerated peel was also significant lower than day 0 in (*Z*)-3-hexenal. Overall, the flesh and peel showed the same trends but the flesh samples showed few significant differences which is possibly due to the overall levels being much lower. The increase in unrefrigerated peel volatiles is most likely due to ethylene production which allows the tomato to further ripen and continue producing flavor volatiles up until precursors are limiting. The exact mechanism by which the volatile concentrations decrease during refrigeration is unknown but could be related to reduced ethylene synthesis at low temperatures (Baldwin and others 2000). Other studies found similar results of those to refrigerated peel in that whole tomatoes stored at 5°C for 6 days had a significant decrease in volatiles with the main contributor being (*Z*)-3-hexenal (Stern and others 1994). After 2-12 days of storage at 5°C, tomatoes had significantly lower ripe aroma and hexanal, 2+3-methylbutanol, (*E*)-2-heptenal, and 2-isobutylthiazole were at significantly lower concentrations than the tomatoes stored at 12.5 and 20°C (Maul and others 2000).

Table 4.8 Effect of storage temperature on flesh and peel volatile production (ppm) after 14 days of storage

	Peel			Flesh		
	Day 0 ^b	Unrefrigerated	Refrigerated	Day 0 ^b	Unrefrigerated	Refrigerated
hexanal	3743 ^b	5793 ^a	1705 ^c	809 ^d	1140 ^{cd}	489 ^d
1-hexanol	26 ^b	35 ^a	17 ^c	5 ^d	7 ^d	4 ^d
(<i>E</i>)-2-heptenal	7 ^a	5 ^{ab}	5 ^{ab}	5 ^{ab}	4 ^b	3 ^b
(<i>Z</i>)-3-hexenal	3155 ^a	3538 ^a	2230 ^b	1103 ^c	1113 ^c	1017 ^c
(<i>E</i>)-2-hexenal	319 ^b	430 ^a	215 ^c	151 ^{cd}	156 ^{cd}	144 ^d
(<i>Z</i>)-3-hexen-1-ol	26 ^{ab}	34 ^a	21 ^b	9 ^c	9 ^c	9 ^c
(<i>E</i>)-2-pentenal	39 ^{ab}	36 ^{ab}	40 ^a	41 ^a	26 ^b	41 ^a
1-penten-3-one	55 ^b	26 ^b	55 ^b	104 ^a	57 ^b	55 ^b
dimethyl sulfide	544 ^b	896 ^a	280 ^c	145 ^d	206 ^{cd}	111 ^d

^a Samples with different letters in the same row are significantly different

^b Initial value before storage

4.3.2 Storage Effects on Lipxygenase Activity and Fatty Acid Content

Storage decreased lipxygenase activity in the unrefrigerated and refrigerated peel (Table 4.9). However, storage had no effect on the fatty acid content or flesh lipxygenase activity. Due to the fact that fatty acid and flesh lipxygenase activity did not change during storage they could not have played a role in the changing volatiles. Peel lipxygenase activity was highest on before storage followed by the refrigerated and unrefrigerated samples, respectively. However, unrefrigerated peel had the highest levels of the lipxygenase generated volatiles (*E*)-2-hexenal, (*Z*)-3-hexenal, hexanal, and 1-hexanol followed by day 0 and refrigerated. This correlates with the fact that lipxygenase was lower in refrigerated peel than in day 0, but not with the fact that

lipoxygenase activity was highest in unrefrigerated peel. Thus clearly other factors than lipoxygenase activity and fatty acid content also affect volatile production including temperature and rate of ethylene production.

Table 4.9 Effect of storage temperature on flesh and peel lipoxygenase activity (nmol/g fwt/min) and total fatty acid content (g/kg fwt) after 14 days of storage

		Lipoxygenase Activity	Fatty Acid Content
Flesh	Day 0 ^b	67 ^d	1.49 ^b
	Unrefrigerated	61 ^d	1.43 ^b
	Refrigerated	68 ^d	1.46 ^b
Peel	Day 0 ^b	169 ^a	2.14 ^a
	Unrefrigerated	123 ^c	2.11 ^a
	Refrigerated	149 ^b	2.11 ^a

^a Samples with different letters in the same column are significantly different

^bDay 0 is the initial value before storage

Chapter 5: Conclusions

Tomato peel had significantly higher concentrations of volatiles than flesh, when there was a significant difference. Lipoxygenase activity, total fatty acid content and linolenic acid content were also significantly greater in peel than in flesh for all varieties. Due to the high linolenic acid content of the peel there were higher levels of substrates to react with lipoxygenase producing an increase in successor molecules with an emphasis on (*Z*)-3-hexenal and other lipoxygenase generated volatiles. A correlation was identified between both lipoxygenase activity and linolenic acid content, and the formation of lipid derived volatiles. However, within peel or flesh groups, lipid derived compounds did not correlate well with total fatty acid content, lipoxygenase activity or linolenic acid. The addition of exogenous lipoxygenase had no effect on lipoxygenase derived volatiles formed in the peel and flesh, while the addition of linoleic acid caused an increase in hexanal, 1-hexanol, and (*E*)-2-heptenal in the flesh and (*E*)-2-heptenal in the peel. Stored unrefrigerated peel had the highest volatile concentrations followed by initial values at Day 0 then refrigerated peel, but this only partially matched changes in lipoxygenase activity while there was no change in the fatty acid content. Overall, linolenic acid was the most important to the formation of fresh, green volatiles, but lipoxygenase activity and unknown factors are clearly also important.

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