VOLATILE FLAVOR PROFILE OF TOMATO (*Lycopersicon esculentum* Mill.) AS AFFECTED BY TISSUE DISRUPTION

by

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(Under the Direction of Robert L. Shewfelt)

ABSTRACT

Consumer dissatisfaction with fresh tomato flavor has resulted in research that directly measures the volatile profile sensed by the consumer during consumption of the fruit. C$_6$ aldehydes (hexanal, cis-3-hexenal, and trans-2-hexenal) and alcohols (hexanol and cis-3-hexenol) are important contributors to the characteristic fresh tomato flavor. Effects of tissue disruption on the production of tomato flavor volatiles were determined. Differences among blended and masticated samples were identified. Hexanal, hexanol, 2-isobuty1thiazole, and 6-methyl-5-hepten-2-one were identified in all treatments. Additional C$_6$ volatile compounds were detected as the degree of tissue disruption increased. To be able to identify the compounds responsible for consumer perception of tomato aroma and the contribution of these compounds to fresh tomato flavor, it is important to identify those compounds that are actually generated during mastication.

INDEX WORDS: *Lycopersicon esculentum* Mill., tomato flavor, mastication, tissue disruption
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For my loving family.

And Tom.
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CHAPTER 1

INTRODUCTION

AND REVIEW OF LITERATURE
Introduction

The tomato (Lycopersicon esculentum Mill.) is an important vegetable crop of the potato (Solanceae) family. Botanically a berry fruit, it is cultivated and consumed as a vegetable. Native to South America, the tomato was domesticated in Mexico and later introduced into the United States, where Americans were reluctant to consume the tomato for fear of poisons (Petro-Turza, 1987). Today the tomato is the second largest vegetable crop in dollar value consumed in the United States (Thakur, et al., 1996).

Although a popular fresh produce item, consumers complain of a lack of characteristic flavor in supermarket tomatoes (Bruhn, et al., 1991). The lack of flavor experienced by consumers is a result of emphasized yield, fruit size, lack of defects, and disease resistance in the selection of tomatoes for the fresh market (Baldwin, et al., 1991). Consumer studies have shown that consumers are willing to pay premium prices for a more flavorful product (Bruhn, et al., 1991).

Tomato Flavor

Flavor is defined as the combination of taste and aroma. Taste is perceived on the tongue during the eating process, and aroma is perceived in the nose before or during the eating process (Acree, 1993). Orthonasal aroma occurs when the product is sniffed and the volatile compounds enter the nose through the nostrils before the food is consumed. Retronasal aroma is the odor sensation experienced during the consumption of a food product, when volatiles enter the nasal passages through the back of the throat (Roberts and Acree, 1995; Taylor and Linforth, 2000; Linforth, et al., 2002). Fresh tomato flavor
is a result of the complex interactions between non-volatile taste components and volatile aromatic compounds (Petro-Turza, 1987).

**Non-volatile Compounds**

The characteristic sweet-sour taste of tomatoes is due primarily to their sugar and organic acid content. Nearly 50% of the dry matter of tomatoes is made up of reducing sugars, largely glucose and fructose. Minute quantities of raffinose, arabinose, xylose, and galactose are occasionally present in the fruit. In the early stages of tomato development, only a small amount of sugar is present, although the sugar content increases significantly during growth and ripening. Organic acids, primarily citric and malic, constitute more than 10% of the dry content of tomato. The acid content of the fruit increases during growth and ripening, declining upon reaching breaker stage (Petro-Turza, 1987; Thakur, et al., 1996). Free amino acids comprise 2-2.5% of the total tomato dry matter. Glutamic acid is the most prevalent amino acid, while glutamine, gamma-amino-butyric acid, and aspartic acid are also found in the fruit. Minerals represent roughly 8% of the dry matter content of tomato. Potassium is the most abundant cation and phosphate the most abundant anion found in the fruit. It is the interaction of these non-volatile taste compounds with the volatile aroma compounds that is responsible for the characteristic tomato flavor (Petro-Turza, 1987).

**Volatile Compounds**

Gas chromatography-mass spectrometry (GC-MS) has allowed for the identification of more than 400 tomato volatile compounds (Petro-Turza, 1987). Fewer
than thirty of these compounds are thought to contribute significantly to tomato aroma based on odor threshold values (Buttery, et al., 1987,1988; Buttery and Ling, 1993). The odor threshold is the concentration at which the compound can be detected. An odor unit ($U_o$) is the ratio of the concentration of the compound divided by the threshold concentration. Odor unit values give an indication of the importance of individual volatile compounds to the overall odor of the product (Buttery, et al., 1987). Based on odor unit values, the most important compounds contributing to tomato aroma include: hexanal, cis-3-hexenal, trans-2-hexenal, hexanol, cis-3-hexenol, 1-penten-3-one, 2-isobutylthiazole, 3-methylbutanal, 3-methylbutanol, 2-phenylethanol, 6-methyl-5-hepten-2-one, geranylacetone, and β-ionone (Buttery, et al., 1987; Buttery and Ling, 1993).

Free amino acids, carotenoids, terpenoids, and lipids serve as the primary precursors of tomato volatile compounds (Buttery and Ling, 1993). The degradation of free amino acids results in a large number of alcohols, carbonyls, acids, and esters.Alanine serves as the primary precursor for carbonyl compounds, and alcohols primarily originate from leucine and valine (Petro-Turza, 1987). Important amino acid related compounds include: 2-isobutylthiazole, 2-phenylethanol, 3-methylbutanal, and 2- and 3-methylbutanol (Buttery and Ling, 1993).

The characteristic color of tomatoes is due to the presence of carotenoids, namely lycopene and β-carotene. The carotenoids of a fully ripened tomato are 50-80% lycopene and 2-7% β-carotene. The degradation of these compounds occurs due to oxidation (Petro-Turza, 1987). The breakdown of lycopene yields a number of volatile tomato aroma compounds, including acetone, geranylacetone, farensylacetone, and 6-methyl-5-

Lipids serve as the source of the C₆ aldehydes and alcohols found in tomato fruit, primarily hexanal, cis-3-hexenal, trans-2-hexenal, hexanol, and, cis-3-hexenol (Kazeniac and Hall, 1970; Galliard, et al., 1977). During tissue disruption, lipid-degrading enzymes attack membrane and storage lipids (Galliard, et al., 1977). Linoleic and linolenic acids are converted to hexanal and cis-3-hexenal, respectively, by the enzymatic action of lipoxygenase and hydroperoxide lyase. Cis-3-hexenal can be isomerized to trans-2-hexenal by an isomerase enzyme (Figure 1). Aldehyde forms, hexanal and cis-3-hexenal, can be further reduced by alcohol dehydrogenase to their alcohol forms, hexanol and cis-3-hexenol, respectively (Kazeniac and Hall, 1970). An additional lipid derived volatile compound important to tomato aroma is 1-penten-3-one (Buttery and Ling, 1993).

The “fresh” flavors characteristic to tomatoes result from volatile carbonyl compounds, particularly cis-3-hexenal, trans-2-hexenal, hexanal, and 2-isobutylthiazole. As the flavor effects of these compounds reach levels no longer detected, alcohols and other compounds dominate, causing the flavor to appear “processed” or “enzymic” (Kazeniac and Hall, 1970).

cis-3-Hexenal is perhaps the most important flavor component of fresh tomato. Kazeniac and Hall (1970) confirmed the presence of cis-3-hexenal and found it to be unstable to heat and the acidic pulp and juice of the tomato. Upon standing and heating it is isomerized to the much more stable form, trans-2-hexenal. A solution of 1 ppm cis-3-hexenal in water resembled an odor similar to that of freshly cut green tomato. Buttery and co-workers (1971) determined the sensory threshold concentration of cis-3-hexenal,
finding it exceedingly low (0.25 ppb). At concentrations of 1 ppm and above, cis-3-hexenal is said to impart strong “green” rancid type flavors.

trans-2-Hexenal is said to impart a less intense and less fresh “green” character to tomato aroma than cis-3-hexenal (Kazeniac and Hall, 1970). Buttery and co-workers (1971) considered the odor of trans-2-hexenal different than that of cis-3-hexenal and much less characteristic of freshly cut tomato. The sensory threshold concentration of trans-2-hexenal in an aqueous solution was found to be 17 ppb (Buttery, et al., 1971).

Hexanal was found to impart a “green” flavor to tomato juice in the concentration range of 0.1-0.5 ppm. In comparison to cis-3-hexenal and trans-2-hexenal, the flavor effects of hexanal were much less prominent (Kazeniac and Hall, 1970). Hexanal was found to have a sensory threshold concentration of 4.5 ppb in aqueous solution (Buttery, et al., 1971). At concentrations of 0.5 ppm and above, the flavor of hexanal resembled that of rancid vegetable fat (Kazeniac and Hall, 1970).

2-Isobutylthiazole was described as having a spoiled vine-like, slightly horseradish type flavor in an aqueous solution. When added to tomato juice or paste, a more intense, fresh tomato-like flavor resulted. Buttery and co-workers (1971) determined a sensory threshold concentration of 2-isobutylthiazole of 3.5 ppb, which differs minimally from the 2 ppb value determined by Kazeniac and Hall (1970). At concentration levels above 50 ppb, the flavor of 2-isobutylthiazole was described as rancid, medicinal, or metallic.

cis-3-Hexenol has been described as important to tomato aroma due to its “green” notes (Kazeniac and Hall, 1970). The sensory threshold concentration of cis-3-hexenol was found to be 70 ppb in water (Buttery, et al., 1971).
Kazeniac and Hall (1970) described 6-methyl-5-hepten-2-one as having a fruit-like aroma. At high concentrations in tomato juice and paste, 6-methyl-5-hepten-2-one was described as decreasing the tomato-like notes, resulting in a flat, insipid flavor. The sensory threshold concentration of 6-methyl-5-hepten-2-one was established as 50 ppb in an aqueous solution. Geranylacetone and 1-penten-3-one, also imparting fruit-like aromas, were found to have sensory threshold concentrations of 60 ppb and 1 ppb, respectively (Buttery, et al., 1987).

**Tissue Disruption**

Tomato volatile compounds occur in the intact fruit or are formed during tissue disruption (Kazeniac and Hall, 1970). The rapid release of isobutylthiazole, methylbutanal, methylbutanol, and acetaldehyde immediately following tissue disruption suggests that these compounds are preformed in the ripe fruit (Boukobza, et al., 2001). The steady concentration increase of the lipid derived compounds, especially the C6 aldehydes and alcohols, following cutting, blending, and mastication indicates that these compounds are formed as a result of tissue disruption. According to Boukobza and co-workers (2001), hexanal was produced slowly upon tissue disruption, reaching a maximum intensity after 3 minutes, before slowly decreasing. Hexenal was released more rapidly in the first 2 minutes following tissue disruption, reaching a maximum intensity around 2-3 minutes, before decreasing.

To simulate the eating process and better account for the volatile compounds produced as a result of tissue disruption, tomatoes are often blended or homogenized prior to analysis. Blending results in the disintegration of the fruit tissue and the intimate
mixing of the natural tomato enzymes and substrates. These factors, in addition to air whipped into the mixture during blending, greatly favor the production of enzymatic oxidation products (Buttery, et al., 1971). Kazeniac and Hall (1970) determined cis-3-hexenal and hexanal the major compounds formed upon blending of tomato fruit and found the concentration of these aldehydes increased as did the severity of tissue disruption. According to a study conducted by Buttery and co-workers (1971), it was found that blending of tomato fruit increased the concentrations of trans-2-hexenal, hexanal, and geranylacetone. The greatest concentration of trans-2-hexenal was found in tomatoes blended in a high speed blender for 1 minute versus those tomatoes that were sliced, diced, or blended at lower speeds.

Quantitative analysis of tomato volatiles is difficult due to the dynamic nature of tomato fruit. Changes in volatile concentrations occur as enzymes and substrates are mixed as a result of tissue disruption and some of the enzyme-produced volatile flavor compounds are themselves degraded by other tomato enzymes before or during the isolation process (Buttery, et al., 1987). Kazeniac and Hall (1971) were first to discover that cis-3-hexenal was converted to trans-2-hexenal by the tomato medium in the amount of time needed to isolate the volatile compounds. Saturated calcium chloride (CaCl$_2$) and sodium chloride (NaCl) solutions have been found effective in deactivating tomato enzymes. Buttery and co-workers (1987) found that saturated CaCl$_2$ caused the concentration of cis-3-hexenal in macerated tomato tissue to remain unchanged for 3 hours.
Chemical Analysis

Qualitative studies of tomato flavor are abundant, but very few quantitative studies exist due to the variability between the results of different isolation methods, the low volatile concentrations, and the complex mixtures of tomato components (Buttery, et al., 1988). Numerous methods are used to separate tomato volatiles, including distillation, solvent extraction, solid phase microextraction (SPME), and headspace analysis; although, each method presents a different picture of the tomato flavor profile. Headspace sampling is thought by many as advantageous because it captures the same volatile compounds emitted from the tomato as are detected by the human nose prior to consumption (Sucan and Russell, 2001); however, it does not attempt to imitate the eating process and may not represent the volatile profile present at the receptors (Piggott and Schaschke, 2001).

Many tomato volatiles are present in the fruit in considerably low concentrations (Buttery, et al., 1988), and quantitative data are necessary to fully understand the role of the individual volatile components in fresh tomato flavor (Buttery, et al., 1987). Tenax trapping is a concentration technique that can be used to enhance the detection of volatile compounds present in low concentrations, which may be important contributors to tomato aroma. Volatile components are trapped onto Tenax (2,6-diphenyl-1,4-phenylene oxide), a porous polymer adsorbent, and recovered for GC analysis using thermal or liquid desorption. This process is referred to as dynamic headspace sampling (DHS). (Sucan and Russell, 2001). Due to minimal interactions with the tomato volatile compounds (Buttery, et al., 1987), Tenax is the most widely used porous polymer adsorbent in flavor studies (Sucan, et al., 1998).
**Flavor Release**

Flavor is not perceived as a single event, but instead as a series of events during the consumption of a food product. Perception is dependent upon the nature and concentration of volatile aroma compounds and non-volatile taste components present in the food product (Overbosch, et al., 1991), as well as the release of these compounds from the food matrix and their transfer to receptor sites (Taylor, et al., 2001).

The volatilization of aroma compounds and the release of taste components from a food product in the mouth during consumption, termed flavor release are greatly influenced by mastication (Roberts and Taylor, 2000). Mastication causes deformation and breakdown of the food matrix, thereby eliminating concentration gradients and thus enhancing the release of flavor (Overbosch, et al., 1991) and retronasal perception (Van Ruth, et al., 1995). Different release rates of flavors result from variation in the deformation and breakdown of food products during consumption (Taylor and Linforth, 2000). In addition to mastication, the structure and composition of the food product, the temperature and pH of the mouth, saliva flow, breathing, and swallowing effect the rate of flavor release (Piggott and Schaschke, 2001).

Volatile aroma compounds and non-volatile taste components must be transferred to the appropriate receptor sites in order for perception to occur. Taste compounds are transferred to the receptors of the taste buds on the tongue, and aroma compounds are transferred to the receptor cells of the olfactory epithelium in the nose (Taylor and Linforth, 2000; Piggott and Schaschke, 2001). Aroma compounds can be transferred to the olfactory epithelium orthonasally through the nostrils prior to consumption, providing the first impressions of flavor, or retronasally through the back of the throat as the food is
consumed (Roberts and Acree, 1995; Taylor and Linforth, 2000; Linforth, et al., 2002). In contrast to orthonasal aroma, retronasal aroma is affected by salivation, mastication, and temperature changes in the food product as it enters the mouth (Roberts and Acree, 1995).

The respiratory tract drives the transport of volatile compounds through the upper airways to the olfactory epithelium in the nose. During the transport process, volatile compounds may be absorbed and desorbed in the mouth and airways. Due to absorption, the flavor released from the food matrix may not reach the olfactory epithelium in the same concentration as it was originally released from the food product into the mouth. As a result, the concentration of flavors released in the mouth is only a rough estimate of that which reaches the olfactory epithelium (Overbosch, et al., 1991).

**Nosespace and Mouthspace Analysis**

Traditional headspace analysis cannot account for the volatile compounds formed by enzymatic reactions or other reactions occurring during consumption, nor does it represent the volatile profile received by the olfactory epithelium in the nose. Therefore, it is difficult to correlate instrumental and sensory data (Taylor, et al., 2001). Attempts were made by Buttery and co-workers (1987) when volatiles were trapped and analyzed after blending of the fruit and enzyme deactivation using saturated CaCl₂. Although the high shear conditions of blending and homogenization are effective in tissue damage, localized heating, seed disintegration, air incorporation, and potential enzyme denaturation result (Linforth, et al., 1994a). These factors are not of concern during mastication, as shear in the mouth is much lower (Prestage, et al., 1999).
Recent developments, including nosespace and mouthspace techniques, attempt to provide information on the concentration of volatile compounds at or near the receptors (Piggott and Schaschke, 2001). Nosespace and mothspace analyses refer to the measurement of volatiles in expired air collected from the nose and mouth, respectively, during consumption of a food product. Linforth and Taylor (1993) found that volatile profiles in the mouth and nose can be measured during eating and that differences exist in the volatile profiles of mints obtained using headspace and nosespace techniques. Air from the nose was drawn across a Tenax trap as the mints were eaten and volatiles were desorbed and chromatographed. Mints proved an excellent sample because they contained large amounts of volatiles and a simple aroma profile (Linforth and Taylor, 1993).

Unlike mints, tomatoes proved a challenge for nosespace analysis in terms of sensitivity and the complexity of the volatile profile. Tomatoes are natural products and variation between fruit is considerably greater than that of mints, a fabricated food. Linforth and co-workers (1994a) compared the headspace profiles of diced and pureed tomatoes to nosespace profiles. The headspace profiles of diced and pureed tomatoes were similar to one another but distinctly different from the nosespace profile. Additional work performed by Linforth and co-workers (1994b) showed that 2- and 3-methylbutanals and alcohols, 2-isobutylthiazole, 3-methylnitrobutane, and cis-3-hexenal, initially present in the breath of operators, are present at higher levels in the nosespace during consumption. trans-2-Hexanal, hexanal, geranylacetone, dimethyl disulphide, and 1-penten-3-one were not present in higher levels in the nosespace as compared to blank breath samples. cis-3-Hexenal and hexanal were found in much higher levels in the
headspace samples than in nosespace samples, and the inverse was found for 2-isobutylthiazole and the 2-methyl aldehydes. The nosespace analyses suggest that compounds such as 3-methylbutanal are more important to fresh tomato flavor than the \textit{C}_6 alcohols and aldehydes, which are often regarded as the major flavor components of tomato (Linforth, et al., 1994b).

**Objective**

The objectives of this thesis are to determine the effects of tissue disruption on the production of tomato flavor volatiles and to identify differences among intact, sliced, masticated, and blended tomato samples.
LIPIDS

Lipases

FREE FATTY ACIDS

Linolenic Acid (18:3) + Linoleic Acid (18:2)

Lipoxygenases

HYDROPEROXIDES


Hydroperoxide lyases

cis-3-HEXENAL

HEXANAL

trans-2-HEXENAL

Figure 1- Schematic of the C₆ aldehydes formed during disruption of the tomato fruit (adapted from Gray, et al., 1999; Griffiths, et al., 1999).
References


CHAPTER 2

VOLATILE FLAVOR PROFILE OF TOMATO (*Lycopersicon esculentum* Mill.) AS
AFFECTED BY TISSUE DISRUPTION

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Abstract

Consumer dissatisfaction with fresh tomato flavor has resulted in research that directly measures the volatile profile sensed by the consumer during consumption of the fruit. The effects of tissue disruption on the production of tomato flavor volatiles were identified using GC and GC-MS. Hexanal, hexanol, 2-isobutylthiazole, and 6-methyl-5-hepten-2-one, key flavor compounds, were identified in all treatments. Additional C₆ volatile compounds were detected as the degree of tissue disruption increased. Mastication was found to elicit a different volatile profile than did blending. Therefore, to be able to identify the compounds responsible for consumer perception of tomato aroma and the contribution of these compounds to fresh tomato flavor, it is important to identify those volatiles that are actually generated during mastication.
Introduction

Fresh tomato flavor is a result of the complex interactions between non-volatile taste components and volatile aromatic compounds. Sugars, organic acids, free amino acids, and minerals comprise the non-volatile substances affecting taste. Gas chromatography-mass spectrometry (GC-MS) has allowed for the identification of more than 400 tomato volatile compounds (Petro-Turza, 1987). Of these compounds identified, fewer than 30 are thought to be important contributors to tomato aroma based upon their odor threshold values. The most important contributors to tomato aroma include: hexanal, cis-3-hexenal, trans-2-hexenal, hexanol, cis-3-hexenol, 1-penten-3-one, 2-isobutylthiazole, 3-methylbutanal, 3-methylbutanol, 2-phenylethanol, 6-methyl-5-hepten-2-one, geranylacetone, and β-ionone (Buttery and others, 1987, 1988; Buttery and Ling, 1993).

Tomato volatile compounds are present in the intact fruit while others are formed upon tissue disruption (Kazeniac and Hall, 1970). Substrates for these compounds include lipids, carotenoids, amino acids, lignin, terpenoids, and other miscellaneous compounds (Buttery and Ling, 1993). The steady concentration increase of lipid-derived compounds, especially hexanal, cis-3-hexenal, trans-2-hexenal, hexanol, and cis-3-hexenol, following cutting, blending, and mastication indicates that these compounds are formed as a result of tissue disruption (Boukobza and others, 2001).

During tissue disruption, lipid-degrading enzymes attack membranes and storage lipids (Galliard and others, 1977). Linoleic and linolenic acids are converted to hexanal and cis-3-hexenal, respectively, by the enzymatic action of lipoxygenase and hydroperoxide lyase. cis-3-Hexenal can be isomerized to trans-2-hexenal by an
isomerase enzyme. Aldehyde forms, hexanal and cis-3-hexenal, can be furthered reduced to their alcohol forms, hexanol and cis-3-hexenol, by alcohol dehydrogenase (Kazeniac and Hall, 1970).

The tomato volatiles produced as a result of tissue disruption, primarily the C₆ alcohols and aldehydes, are thought to be important contributors to the characteristic fresh tomato flavor. In many studies, samples are blended to achieve similar profiles as those sensed during consumption of the fruit (Buttery and others, 1971). However, shear in the mouth is much lower, and blending may not accurately represent the profile sensed by the consumer (Prestage and others, 1999).

This study was conducted to determine the effects of tissue disruption on the production of tomato flavor volatiles and to identify differences among intact, sliced, masticated, and blended tomato samples.

**Materials and Methods**

**Volatile Compounds**

Volatile compounds previously identified as important contributors to fresh tomato aroma (Buttery and others, 1987; Buttery and Ling, 1993) were evaluated. Reference chemicals of these compounds were obtained from commercial sources. These compounds and their sources include: hexanal (Sigma Chemical Company, St. Louis, MO), cis-3-hexenal (50% solution in triacetin; Aldrich Chemical Company, Inc., Milwaukee, WI), trans-2-hexenal (98%; Aldrich Chemical Company, Inc., Milwaukee, WI), hexanol (98%; Aldrich Chemical Company, Inc., Milwaukee, WI), and cis-3-hexenol (Sigma Chemical Company, St. Louis, MO).
Tomatoes

Vine-ripened tomatoes of the cultivar ‘Sunguard’ (Seminis Seed Co.) were grown in Palmetto, Florida and obtained from a local farmers market (Atlanta, Ga). Tomatoes were stored at 20°C prior to analysis and used within 3 days of purchase.

Volatile Collection

Whole Tomatoes

The volatile collection apparatus and procedures used were adapted from Buttery et al (1987). Whole tomatoes (ca. 500 g) were sealed in a glass sample container fitted with a Tenax trapping system. Purified air was passed through an activated charcoal filter and drawn across the sample by a laboratory vacuum line at the rate of 300 mL/min. Volatiles were collected on a Tenax trap (8 cm length x 1.0 mm internal diameter, Tenax-TA, 60/80 mesh; Alltech Associates, Inc., Deerfield, IL) for 4 hrs. The volatile compounds were desorbed from the Tenax by rinsing with 250 µL hexane (J.T. Baker, Phillipsburg, NJ) containing (-)-trans-caryophyllene (ca. 300 µg/mL; Fluka Chemika, Switzerland) as an internal standard. A total of two analyses were performed on each sample.

Sliced Tomatoes

Three tomatoes were cut into quarters and cut again to give 8 sections per tomato. The sections (ca. 500 g) were sealed in a glass sample container fitted with a Tenax trapping system and sampled as previously described.
Masticated Tomatoes

Three tomatoes were cut into quarters and cut again to give 8 sections per tomato. The sections (ca. 500 g) were masticated by the primary investigator (one half section chewed 10 times) and expectorated. The masticated tomato mixture was sealed in a glass sample container fitted with a Tenax trapping system and sampled as previously described.

Blended Tomatoes

Three tomatoes were cut into quarters and cut again to give 8 sections per tomato (adapted from Buttery and others, 1987). The sections (ca. 500 g) were blended for 30 sec in a Waring Commercial Blender (Waring, Torrington, CT), and the mixture was held for 180 sec. Saturated CaCl₂ (125 mL) was added and to the mixture to inactivate the tomato enzyme system, and the mixture was blended for 10 sec. The blended tomato mixture was sealed in a glass sample container fitted with a Tenax trapping system and sampled as previously described.

Gas Chromatography (GC) Analysis

Analyses were performed using a HP 5890 series II gas chromatograph (GC) (Hewlett Packard, San Fernando, CA) with an injection port temperature of 225°C and a flame ionization detector (FID) temperature of 250°C. Separations were made on a 30 m x 0.25 mm i.d. and 0.25 μm film thickness EC-Wax capillary column (Alltech Associates, Inc., Deerfield, IL). A 2 μL sample was injected in the splitless mode with a purge time of 0.5 min. The GC oven was held for 10 min at 40°C, after which the temperature was increased to 120°C at 2°C/min and held at that temperature for 1 min.
The temperature was then increased to 250°C at 20°C/min and held at the final temperature for 5 min. For quantification of peak areas, signal output was integrated using an HP 3396 Series III integrator (Hewlett Packard, San Fernando, CA).

**Gas Chromatography-Mass Spectrometry (GC-MS) Analysis**

Volatile compounds were identified using a HP 5970 mass selective detector (MSD) coupled with a HP 5890 GC (Hewlett Packard, San Fernando, CA). A scanning range of 50-400 atomic mass units and a scanning speed of 2 scans/sec were used. The solvent delay was set at 6 min.

**Results and Discussion**

The procedure previously developed by Buttery and others (1987) for the quantitative analysis of tomato volatiles was adapted in the current study to identify compounds present in intact, sliced, masticated, and blended tomato samples. Seven compounds, previously identified as important contributors to tomato aroma (Buttery, et al., 1987; Buttery and Ling, 1993), were identified and quantified using GC and GC-MS.

Flavor profiles differed among the four treatments. Hexanal, hexanol, 2-isobutylthiazole, and 6-methyl-5-hepten-2-one were identified in all treatments; however, cis-3-hexenal, trans-2-hexenal, and cis-3-hexenol were formed with tissue disruption. Their concentrations increased as the degree of tissue disruption increased (Figures 1 – 4).

The results indicate an increase in the production of the lipid-derived C₆ volatile compounds with increasing degrees of tissue disruption, with the exception of hexanol
and cis-3-hexenol which decreased in the blended sample (Table 1). Concentrations of the volatiles followed the same general trend as previously reported by Buttery and others (1988). Hexanal had the highest concentration among all other volatiles in all treatments. These findings agree with those of Kazeniac and Hall (1970) and Buttery and others (1971) who determined hexanal as a major compound formed upon disruption of the tomato fruit.

Trans-2-Hexenal and trace amounts of cis-3-hexenal were found in the masticated and blended samples, while neither compound was identified in the intact or sliced samples. cis-3-Hexenal is a major compound formed as a result of tissue disruption, although it is easily isomerized to the more stable form, trans-2-hexenal. The absence of cis-3-hexenal is likely attributed to the conversion of cis-3-hexenal to trans-2-hexenal by the tomato medium in the time interval necessary to isolate the volatile compounds (Kazeniac and Hall, 1970). As a result, it is possible that the current method of volatile collection reveals smaller amounts of cis-3-hexenal than may be present in the tomato samples.

Hexanol was identified in all treatments, while cis-3-hexenol was identified in the sliced, masticated, and blended samples. Both compounds increased with increasing degrees of tissue disruption; however, they were significantly lower in the blended samples. Saturated CaCl₂ was added to the blended samples to deactivate the tomato enzyme system (Buttery and others, 1987). The lower concentrations of hexanol and cis-3-hexenol in the blended samples may be a result of chemical interactions caused by the addition of saturated CaCl₂.
2-Isobutylthiazole was identified in all treatments. Comparison of concentrations of 2-isobutylthiazole among the different treatments suggests that tissue disruption has little effect. Sliced and blended sample concentrations were higher than that of the intact fruit but similar to one another. These results coincide with those of Boukobza and others (2001), who reported 2-isobutylthiazole as a compound present in the ripened fruit. The greatest concentration of 2-isobutylthiazole was present in the masticated sample. These results agree with Linforth and others (1994b), who found 2-isobutylthiazole present at higher levels during consumption.

6-Methyl-5-hepten-2-one was identified in all treatments with the concentration increasing with increasing degrees of tissue disruption. These results agree with Boukobza and Taylor (2003), who identified 6-methyl-5-hepten-2-one as a carotenoid-derived compound formed upon tissue disruption.

The largest number of volatile compounds was identified in the blended sample. Blending serves to simulate the eating process and better account for the volatile compounds produced as a result of tissue disruption (Buttery and others, 1971). However, the high shear conditions of blending often result in localized heating, seed disintegration, incorporation of air into the mixture, and potential enzyme denaturation (Linthforth and others, 1994a). These factors are not of concern during mastication, as the shear in the mouth is much lower (Prestage and others, 1999). Therefore, the flavor profile of the masticated sample, which differed from that of the blended sample, may be more representative of the profile sensed during the consumption of the fruit.

To be able to identify the compounds responsible for consumer perception of tomato aroma and the contribution of these compounds to fresh tomato flavor, it is
important to identify those volatiles that are actually generated during mastication. Linforth and others (1994a) found the headspace profiles of diced and pureed tomatoes similar to one another but distinctly different from nosespace profiles. Additional studies found hexanal and cis-3-hexenal in much higher levels in headspace samples, while 2-isobutylthiazole and the 2-methyl aldehydes were found in much higher levels in the nosespace samples. These results suggest that compounds such as 3-methylbutanal are more important to fresh tomato flavor, rather than the C₆ alcohols and aldehydes which are most often named as the major contributors to tomato flavor (Linforth and others, 1994b).

Despite numerous studies, no universally accepted model has been developed to describe fresh tomato flavor (Petro-Turza, 1987). Some studies emphasize cis-3-hexenal (Kazeniac and Hall, 1970), others the C₆ aldehydes and alcohols (Buttery and others, 1987; Buttery and Ling, 1993), and still others compounds such as 3-methylbutanal (Linforth and others, 1994b). The importance of the degree and type of tissue disruption to the volatiles generated and perceived by the consumer has only been recently studied (Linforth and Taylor, 1993; Linforth and others, 1994a; Linforth and others, 1994b).

Flavor improvement by breeders will require a better understanding of the descriptive flavor notes affecting consumer acceptability, the chemical compounds affecting these critical notes, as well as the genetic and environmental factors affecting the chemical composition of fresh tomatoes.
Figure 1 – Chromatogram of volatile flavor compounds found in intact ‘Sunguard’ tomatoes.
Figure 2 - Chromatogram of volatile flavor compounds found in sliced ‘Sunguard’ tomatoes.
Figure 3 - Chromatogram of volatile flavor compounds found in masticated ‘Sunguard’ tomatoes.
Figure 4 - Chromatogram of volatile flavor compounds found in blended ‘Sunguard’ tomatoes.
Table 1 - Concentrations (µg/kg) of volatile flavor compounds found in intact, sliced, masticated and blended ‘Sunguard’ tomatoes\(^1\).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Intact</th>
<th>Sliced</th>
<th>Masticated</th>
<th>Blended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexanal</td>
<td>6.34 ± 0.85</td>
<td>15.01 ± 1.25</td>
<td>51.74 ± 2.82</td>
<td>81.95 ± 9.66</td>
</tr>
<tr>
<td>cis-3-Hexenal</td>
<td>-</td>
<td>-</td>
<td>TR*</td>
<td>TR*</td>
</tr>
<tr>
<td>trans-2-Hexenal</td>
<td>-</td>
<td>-</td>
<td>3.77 ± 0.75</td>
<td>10.10 ± 0.52</td>
</tr>
<tr>
<td>Hexanol</td>
<td>1.07 ± 0.14</td>
<td>1.44 ± 0.16</td>
<td>6.76 ± 0.48</td>
<td>0.72 ± 0.02</td>
</tr>
<tr>
<td>cis-3-Hexenol</td>
<td>-</td>
<td>2.42 ± 0.37</td>
<td>9.19 ± 0.66</td>
<td>0.88 ± 0.00</td>
</tr>
<tr>
<td>2-Isobutylthiazole</td>
<td>1.57 ± 0.16</td>
<td>4.37 ± 0.31</td>
<td>6.69 ± 0.25</td>
<td>4.61 ± 0.66</td>
</tr>
<tr>
<td>6-Methyl-5-hepten-2-one</td>
<td>1.46 ± 0.13</td>
<td>4.05 ± 0.44</td>
<td>9.57 ± 0.13</td>
<td>14.96 ± 0.98</td>
</tr>
</tbody>
</table>

\(^1\) Mean ± S.D., \(n = 2\).

*Trace amounts of cis-3-hexenal were identified within the sample.
References


CHAPTER 3

SUMMARY AND CONCLUSIONS
This thesis described the effects of tissue disruption on tomato flavor volatile production. Results indicate an increase in the production of the lipid-derived C$_6$ volatile compounds, with the exception of hexanol and cis-3-hexenol in the blended sample, and the carotenoid-derived compound, 6-methyl-5-hepten-2-one, with increasing degrees of tissue disruption. Tissue disruption was shown to have little effect on 2-isobutylthizole, a compound thought to be present in the intact fruit. In addition, it was found that blending and mastication result in different volatile profiles. While the largest number of volatile compounds was identified in the blended sample, the flavor profile of the masticated sample is thought to be more representative of that sensed at the receptors during consumption of the fruit.

Although tomato flavor has been the subject of many studies, much is still unknown regarding the compounds most important to consumer perception of tomato flavor. To be able to identify the compounds responsible for consumer perception of tomato aroma and the contribution of these compounds to fresh tomato flavor, it is important to identify those volatiles that are actually generated during mastication. With the introduction of nosespace and mouthspace analytical techniques, attempts have been made to collect such data, although further research is necessary to integrate chemical, sensory, and consumer studies of tomato flavor.