EFFICIENCY OF COATING PROCESS AND REAL-TIME VOLATILE RELEASE IN TOMATILLO AND TOMATO

Dissertation

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ABSTRACT

Fifteen food powders were coated on aluminum targets at 0, + 25, and –25 kV using corona electrostatic coating at 20% to 80% relative humidity (RH). The effect of RH on 3 losses, that is, targeting loss, coating loss, and transportation loss, which contribute to coating efficiency, was studied. RH had no effect on targeting loss in either nonelectrostatic or electrostatic coating. In nonelectrostatic coating, increasing RH increased coating loss for powders with particle size ≥ 297 μm, but had no effect on powders ≤ 227 μm. Large powders were free-flowing and clumped with increasing RH, and then rolled off the targets, resulting in high coating loss. RH had no effect on nonelectrostatic transportation loss for all powders, except for very high absolute humidity when capillary forces dominated. Electrostatic charging efficiency and powder resistivity decreased with increasing RH. Electrostatic coating loss for salts increased with increasing RH. At high RH, powder resistivity decreases, increasing the charge decay rate, which decreases electrostatic adhesion. Electrostatic coating loss for powders other than salts, whose resistivities are much higher than salts, was not affected by RH until 80% RH. Electrostatic transportation loss for powders other than proteins was not affected by RH. There was no significant difference between positive and negative electrostatic transfer efficiency and adhesion, except for transfer efficiency of soy protein.
and pork gelatin, whose high positive tribocharging values cause higher positive electrostatic transfer efficiency. A 20% to 60% RH is recommended for both nonelectrostatic and electrostatic coating.

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

The release of volatiles from tomatillo and tomatoes in the mouthspace and nosespace was measured in real-time using selected ion flow tube mass spectrometry (SIFT-MS). cis-3-Hexenal, trans-2-hexenal, hexanal, and 1-penten-3-one increased, while isobutyl alcohol, nonanal and methylbutanal showed no significant change in the first 30 s of chewing. Cherry tomato released more trans-2-hexenal, cis-3-hexenal, and 1-penten-3-one than tomatillo, roma tomato and vine-ripened tomato during chewing. The ratio of
the average concentration of volatiles in the mouthspace after swallowing to before swallowing (MSas/MSbs) varied from 2.8 to 73.9%. Methylbutanal, hexanal, and nonanal were retained at a higher percentage in the mouth after swallowing than cis-3-hexenal, trans-2-hexenal, 1-penten-3-one and isobutyl alcohol. The mouthspace to headspace (MS/HS) ratio of 1-penten-3-one, hexanal, methylbutanal, and nonanal, and nosespace to headspace (NS/HS) ratio for 1-penten-3-one, hexanal, cis-3-hexenal, and nonanal was significant higher in tomatillo than in tomatoes. There was no difference between tomatoes of different varieties in NS/HS ratio.

The concentration of 31 volatiles were measured in the headspace of tomatillo using selected ion flow tube-mass spectrometry (SIFT-MS), and were compared with those in vine-ripened tomato, roma tomato, cherry tomato and grape tomato. None of volatiles was higher in the headspace of tomatilloes than of tomatoes. trans-2-Octenal and trans-2-pentenal, 2-isobutylthiazole, 6-Methyl-5-hepten-2-one and phenylacetaldehyde were significantly lower in tomatillo than in the tomato varieties in the headspace. After blending, volatiles in the headspace increased, and then decreased after reaching a maximum concentration, due to further degradation or depletion. trans-2-Pentenal and 1-Penten-3-one reached a maximum concentration later than trans-2-hexenal, cis-3-hexenal and hexanal for tomatillo and tomatoes. The slope of the ratio of trans-2-hexenal and cis-3-hexenal was not significantly different for any of the samples, implying that the activity of cis/trans isomerase was not different between tomatilloes and tomatoes.
Dedicated to my family
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>vi</td>
</tr>
<tr>
<td>VITA</td>
<td>vii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiv</td>
</tr>
<tr>
<td>CHAPTER 1</td>
<td>1</td>
</tr>
</tbody>
</table>

1.1 The influence of RH on corona electrostatic coating 1

1.1.1 The history of electrostatics 1

1.1.2 The introduction to electrostatics 2

1.1.3 Electrostatic coating 4

1.1.4 Adhesion 7

1.1.5 Transfer efficiency 13

1.1.6 The influence of relative humidity on powder coating 18

1.1.7 Powder properties 23
<table>
<thead>
<tr>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1.8 References</td>
</tr>
<tr>
<td>1. 2 Analysis of volatile release in tomato and tomatillo</td>
</tr>
<tr>
<td>1.2.1. Tomatillo</td>
</tr>
<tr>
<td>1.2.2. Tomato</td>
</tr>
<tr>
<td>1.2.3. Lipoxygenase pathway</td>
</tr>
<tr>
<td>1.2.4. Flavor release in the mouth</td>
</tr>
<tr>
<td>1.2.5. Analytical methods</td>
</tr>
<tr>
<td>1.2.6 Selected Ion Flow Tube Mass Spectrometry (SIFT-MS)</td>
</tr>
<tr>
<td>1.2.7 References</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Chapter 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Abstract</td>
</tr>
<tr>
<td>2.2 Introduction</td>
</tr>
<tr>
<td>2.3 Materials and methods</td>
</tr>
<tr>
<td>2.4 Results and Discussion</td>
</tr>
<tr>
<td>2.4.1 Effect of relative humidity on nonelectrostatic targeting loss</td>
</tr>
<tr>
<td>2.4.2 Effect of relative humidity on nonelectrostatic coating loss</td>
</tr>
<tr>
<td>2.4.3 Effect of relative humidity on nonelectrostatic transportation loss</td>
</tr>
<tr>
<td>2.4.4 Effect of relative humidity on electrostatic charging efficiency and targeting loss</td>
</tr>
<tr>
<td>2.4.5 Effect of relative humidity on electrostatic coating loss</td>
</tr>
<tr>
<td>2.4.6 Effect of relative humidity on electrostatic transportation loss</td>
</tr>
<tr>
<td>2.4.7 Positive vs. Negative electrostatic coating</td>
</tr>
<tr>
<td>2.5 Conclusions</td>
</tr>
</tbody>
</table>
2.6 References .............................................................................................................. 72

CHAPTER 3 ...................................................................................................................... 89
  3.1 Abstract .............................................................................................................. 89
  3.2 Introduction ....................................................................................................... 90
  3.3 Materials and Methods ....................................................................................... 92
    3.3.1 Analysis of volatiles with enzyme activity ............................................... 92
    3.3.2 Analysis of partition coefficient without enzyme activity ...................... 94
    3.3.3 Statistical analysis ....................................................................................... 95
  3.4 Results and Discussion ....................................................................................... 95
    3.4.1 Effect of temperature on linoleic acid related volatiles ....................... 95
    3.4.2 Effect of temperature on linolenic acid related volatiles .................... 100
    3.4.3 The effect of temperature on rate of production of lipid-related volatiles .......................................................................................... 103
  3.5 Conclusion ......................................................................................................... 104
  3.6 References ......................................................................................................... 104

CHAPTER 4 ...................................................................................................................... 120
  4.1 Abstract .............................................................................................................. 120
  4.2 Introduction ....................................................................................................... 121
  4.3 Materials and Methods ....................................................................................... 123
    4.3.1 Materials .................................................................................................... 123
    4.3.2 Instrument and sampling ........................................................................... 123
4.3.3 Headspace volatile analysis ............................................. 124
4.3.4 Mouthspace and nosespace volatile analysis .................... 125
4.3.5 Statistical analysis ......................................................... 126
4.4 Results and Discussion ..................................................... 126
  4.4.1 Real-time release of volatiles in the mouthspace ............... 126
  4.4.2 Comparison of mouthspace (MS) and headspace (HS) volatiles 130
  4.4.3 Comparison of nosespace (NS) and headspace (MS) volatiles 132
4.5 Conclusion ........................................................................ 133
4.6 References ....................................................................... 134

CHAPTER 5 ............................................................................ 147
  5.1 Abstract .......................................................................... 147
  5.2 Introduction ...................................................................... 148
  5.3 Materials and methods ..................................................... 150
    5.3.1 Materials .................................................................. 150
    5.3.2 Instrument and sampling ............................................ 150
    5.3.3 Headspace volatile analysis ......................................... 151
    5.3.4 Statistical analysis ..................................................... 152
  5.4 Results and discussion .................................................... 152
    5.4.1 Comparison of volatiles in the headspace between tomatillos and tomatoes ........................................ 152
    5.4.2 The real-time release of volatiles in the headspace .......... 155
  5.5 Conclusion ...................................................................... 157
# List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 3.1 Information for SIFT-MS analysis of selected volatile compounds.</td>
<td>108</td>
</tr>
<tr>
<td>Table 3.2 Ratio of volatiles in the headspace of grape tomato puree at different temperatures without enzyme activity.</td>
<td>109</td>
</tr>
<tr>
<td>Table 3.3 Time (min) to reach maximum level for lipid related volatiles from grape tomato after blending.</td>
<td>110</td>
</tr>
<tr>
<td>Table 4.1 Information for SIFT-MS analysis of selected volatile compounds.</td>
<td>138</td>
</tr>
<tr>
<td>Table 4.2 The ratios of volatiles in the mouth after swallowing (MSas) to before swallowing (MSbs) (%)</td>
<td>139</td>
</tr>
<tr>
<td>Table 4.3 The ratio of volatiles in the mouthspace (MS) to the headspace (HS) (%)</td>
<td>140</td>
</tr>
<tr>
<td>Table 4.4 The ratio of volatiles in the nosespace (NS) to the headspace (HS) %</td>
<td>141</td>
</tr>
<tr>
<td>Table 5.1 Information for SIFT-MS analysis of selected volatile compounds.</td>
<td>161</td>
</tr>
<tr>
<td>Table 5.2 Volatiles in the headspace of tomatillo and tomatoes.</td>
<td>164</td>
</tr>
<tr>
<td>Table 5.3 Time (min) to reach maximum level for lipid related volatiles in tomatoes and tomatillo after blending.</td>
<td>165</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1 Aldehyde and alcohol formation in tomato from linolenic and linoleic acid.</td>
<td>51</td>
</tr>
<tr>
<td>Figure 1.2 The principle of the Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS).</td>
<td>52</td>
</tr>
<tr>
<td>Figure 2.1 Schematic diagram of experiment setup.</td>
<td>75</td>
</tr>
<tr>
<td>Figure 2.2 RH had no effect on targeting loss of 434 μm and 19 μm NaCl with oil coated targets.</td>
<td>76</td>
</tr>
<tr>
<td>Figure 2.3 RH increased nonelectrostatic coating loss of powder with a particle size $\geq 297$ μm.</td>
<td>77</td>
</tr>
<tr>
<td>Figure 2.4 Cohesiveness of different food powders.</td>
<td>78</td>
</tr>
<tr>
<td>Figure 2.5 RH had no effect on nonelectrostatic coating for powders with a particle size $\leq 227$ μm.</td>
<td>79</td>
</tr>
<tr>
<td>Figure 2.6 RH had no effect on nonelectrostatic transportation loss from 20-80% RH.</td>
<td>80</td>
</tr>
<tr>
<td>Figure 2.7 Capillary forces decreased nonelectrostatic transportation loss at high absolute humidity (high water content of the air).</td>
<td>81</td>
</tr>
<tr>
<td>Figure 2.8 Corona charging efficiency decreased with increasing RH.</td>
<td>82</td>
</tr>
<tr>
<td>Figure 2.9 Improvement in electrostatic transfer efficiency (TE) and electrostatic adhesion of NaCl (434 μm) over nonelectrostatic coating for constant target voltage vs. constant applied voltage.</td>
<td>83</td>
</tr>
</tbody>
</table>
Figure 2.10 Decrease in coating loss with the use of electrostatics for NaCl and KCl
became lower with increasing RH................................................................. 84

Figure 2.11 Powder resistivity of proteins decreased largely with increasing RH......... 85

Figure 2.12 Effect of RH on the decrease in coating loss with the use of electrostatics for
powders with high resistivity. ................................................................. 86

Figure 2.13 Effect of RH on the improvement in transportation loss with the use of
electrostatics for proteins and sucrose, which have high resistivity............. 87

Figure 2.14 Effect of RH on the improvement in transportation loss with the use of
electrostatics for powders with low resistivity........................................ 88

Figure 3.1 The effect of temperature on hexanal generation................................. 111

Figure 3.2 Temperature dependent lipoxygenase pathway of linolenic and linoleic acids
in tomato. ................................................................................................. 112

Figure 3.3 Effect of temperature on trans-2-heptenal generation.......................... 113

Figure 3.4 Effect of temperature on trans-2-octenal generation............................ 114

Figure 3.5 The effect of temperature on hexanol generation............................... 115

Figure 3.6 The effect of temperature on cis-3-hexenal formation......................... 116

Figure 3.7 The effect of temperature on trans-2-pentenal generation.................... 117

Figure 3.8 The effect of temperature on 1-penten-3-one generation...................... 118

Figure 3.9 The effect of temperature on trans-2-hexenal formation....................... 119

Figure 4.1 Real-time release of volatiles from tomatillo in the mouthspace......... 142

Figure 4.2 Real-time release of volatiles from roma tomato in the mouthspace........ 143

Figure 4.3 Real-time release of volatiles from vine-ripened tomato in the mouthspace. 144

Figure 4.4 Real-time release of volatiles from grape tomato in the mouthspace........ 145

Figure 4.5 Real-time release of volatiles from cherry tomato in the mouthspace........ 146
Figure 5.1 The headspace concentration of cis-3-hexenal, trans-2-hexenal and hexanal after blending of tomatillos. ...................................................................................................................................... 166

Figure 5.2 The increase in the trans-2-hexenal/cis-3-hexenal ratio with time. ................. 167
CHAPTER 1

LITERATURE REVIEW

1.1 The influence of RH on corona electrostatic coating

1.1.1 The history of electrostatics

The electrostatic phenomenon has been known for several thousand years. First recorded history about the discovery of electrostatics is that the Greeks in early 600 BC found amber, which was rubbed with other materials, generated an unknown force to attract lightweight objects such as dried leaves and feathers. The knowledge of electrostatics did not progress a lot in the following 2000 years. In the early 1600s, William Gilbert found that there were some other materials can be charged and attract other objects (Herman 1995). Peoples’ knowledge on electrostatics expanded readily in later several hundred years. In 1700s, Charles DuFay found that charged glass not only attracts some objects but also repels some other charged ones. He then created two lists of materials, A and B. Any material in list A would attract any material in list B, but those materials in the same list would repel each other (McAteer 1990). Benjamin Franklin, whose famous kite experiment demonstrated that lightning is electricity, first developed terms of positive charge and negative charge for the two lists (McAteer 1990). Any object repelled by a piece rubbed by silk has a positive charge. Any object repelled by a
hard rubber rod rubbed by wool has a negative charge. Franklin later invented the first electrostatic motor, which is the precursor of today’s motor (McAteer 1990). Charles Augustin de Coulomb found that the force of attraction or repulsion between two charged objects has an inverse square relationship with distance between them, which is commonly known as Coulomb’s Law. As time goes into 1800s, Michael Faraday introduced the Faraday Cage concept, which showed a conductive enclosure attenuates an electrostatic field. In 1900s, most progress in electrostatic study focused on developing applications of electrostatics, such as electrostatic photography, electrostatic powder coating and ozone generation (Chang and others 1995). The development of novel electrostatic processing technology will still be an important research area in the future.

1.1.2 The introduction to electrostatics

The atom is the basic building block of the universe. The electron, neutron and proton are three principal parts of the atom. The proton has a positive charge, the electron has a negative charge and the neutron keeps no charge. The nucleus is consisted of protons and neutrons. The nucleus has a net positive charge due to the positive charge of the proton and the zero charge of the neutron. The number of protons, which is the same as the atomic number, determines what kind of element an atom is. Due to two basic laws of the physics, “opposite charges attract and like charges repel” and “a spinning object will pull away from its center point and that the faster it spins, the greater the centrifugal force becomes”, negative charged electrons are attracted to the positive charged nucleus, and the centrifugal force prevents the electron from falling into the nucleus. The combination of attraction and repulsion forces stabilizes the electrons’ spinning around the nucleus in
an atom. Each atom contains a number of electron orbits. The number of electrons can be accommodated in any orbit is determined by the formula of $2N^2$, in which $N$ stands for the number of orbits. The outer shell, also called the valence shell, can hold at most 8 valence electrons. Almost all electrostatic phenomena are related to the number of electrons in the valence shell. A conductor, which permits the electricity flow passing easily, contains one or two valence electrons. An insulator, which resists the flow of electricity, contains seven or eight valence electrons. A semiconductor, which is neither a good conductor nor good insulator, has four valence electrons. Any molecule or atom is normally electrically neutral. If one or more electrons are removed from any molecule or atom, the positive ion is formed. If extra electrons are added, a negative ion is produced. These charged ions can exist in liquid, solid and gas form (Herman 1995).

Electrostatics is defined as the class of phenomena which are recognized by the presence of electrical charges, either stationary or moving, and the interaction of these charges, this interaction being solely by reason of the charges themselves and their positions and not by the reason of their motions (Moore 1973). Electrostatics is based primarily on the facts that like charges repel and unlike charges attract. The force between two charges is described by Coulomb’s Law. Based on the Coulomb’s Law each charged body somehow affects the space around it. In order to explain the influence of one charged object on space, the idea of the electric field is developed. The electric field line is introduced, although it doesn’t exist in the real universe. Electric field lines originate from a positive charge to a negative charge and repel each other. Charged objects are free to move in the electrical field. The difference in electric field intensity $E$
between start and end determines the driving force (Moore 1973). Electrostatics has been widely applied into the daily use. The electrostatic motor is the application of electrostatic forces acting between electric charges. Also, the electrostatic force is the main concept of the electrostatic printing (Chang and others 1995).

1.1.3 Electrostatic coating

Electrostatics has a wide range of applications, from printing to plant pollination. One of recent applications of electrostatics is in the coating industry. Coating refers to applying dry or wet materials over the surface of a product to produce a film, or coating. Electrostatic coating is similar to traditional coating, but electrostatic forces are brought in to deposit materials on the surface of the products. The origination of the conception of electrostatic coating was in USA in early 1940s, when Ransburg and Green (1941) got US patent on automatic electrostatic spray-coating system, which was used in painting or finishing industry. In 1960s, epoxy powders were electrostatically coated on pipe works to increase durability (Bailey 1998).

Electrostatic coating has been introduced into the food industry, especially the snack food industry, since 1950s. The traditional coating process is wasteful and inefficient. Traditional coating process, such as tumble drum and roll salter systems, only use gravity and the shaking movement of the machine, therefore resulting in uneven coating and poor powder adhesion (Pannell 1980). In order to reduce unevenness, more powder is applied in industry for traditional coating process, thus resulting in waste of food powder. The wastage is over 10% (Pannell 1980). Compared to traditional coating, electrostatic coating is more efficient and produces better coating quality. The advantage of
electrostatic coating includes more even distribution of food powder, less powder usage
and dust formation (Clark 1995).

Electrostatic coating has been used to coat potato chips and cheeses. Electrostatic
coating significantly increases transfer efficiency and decreases dust over nonelectrostatic
coating. Transfer efficiency can reach as high as 90%. Electrostatically coated potato
chips produce better coating uniformity by both sensory evaluation and colorimetry test
(Ratanatrimong and others 2003). When electrostatic coating is used to coat cheeses with
antimycotic and anticaking agents, electrostatic coating increases transfer efficiency of
natamycin on both shredded cheddar and mozzarella cheeses (Elayedath and Barringer
2002). Dust decreases significantly during electrostatic coating, compared to
nonelectrostatic coating (Elayedath and Barringer 2002).

1.1.3.1 Corona charging coating

Corona charging of particles is the most widely used method of particle charging in
powder coating (Hughes 1997). The fundamental theory is that corona charging generates
monopolar ions, which migrate rapidly outwards from a source, such as the region around
a sharp point, due to the Coulombic force of the intense electric field, and form an ion-
rich region. Any particle moving through such an ion-rich region will be charged (Bailey
1998).

Corona takes its name ("crown") from mariners’ observations of discharges from
their ships’ masts during electrical storms (Beuthe and Chang 1995). Corona is invariably
generated by strong electric fields using small diameter wires, needles or sharp edges
(Beuthe and Chang 1995). If the sufficient potential on a needle creates a local electric
field of about 3 MV/m, the electrical breakdown of air itself in the vicinity of the point will occur. The breakdown, also called the corona discharge, is a relatively low energy continuous process, and is ultimately responsible for particle charging in the electrostatic system (Hughes 1997). Air ions due to air breakdown form an ion-rich corona zone, where powder particles accept or lose electrons to be charged. There are several forms of the corona discharge, which depend on the polarity of the field and the geometrical configuration of electrode (Beuthe and Chang 1995). For negative corona charging, neutral oxygen molecules pick up electrons in corona zone to form negative oxygen ions, which then transfer the charge to powder particles by contact. Positive corona charging is similar except the charge carrier become the nitrogen ion, which takes away electrons from powder particle surface (Liberto 1994).

Charged powder particles repel each other due to the similar charge, causing the cloud formation. The charged powder cloud is then attracted to the ground target through the electric field and forms the uniform coating film (Hughes 1997). An electric field is created when a charged particle is positioned on the target surface, and a mirror image of opposite polarity forms on the target.

1.1.3.2 Tribocharging coating

The fundamental mechanism of tribocharging is the charge migration between two materials during the friction process. As two different solid materials come into the intimate contact, the imbalance in charge occurs between two molecular layers that are in contact. Reorganization of charge occurs across the interface in order to restore electrical balance (Hughes 1997). Thus, the charge migration occurs between two materials.
The work required to remove an electron from a surface during contact electrification is known as the electron work function (Mulligan 2003). Most materials are ranked in the Triboelectric Series based on its electron work function. The degree and direction of electron migration is dictated by the Triboelectric Series. Materials that give up electrons easily are considered to be electron donors and placed on the top of the series while those that readily accept electrons are treated as acceptors and placed at the bottom (Liberto 1994).

1.1.4 Adhesion

1.1.4.1 Nonelectrostatic adhesion

Adhesion is the physical phenomenon by which two materials stick together (Michalski and others 1997). Adhesion of powders to targets is important in numerous industries, especially in the food powder coating industry. Adhesion of particles plays a significant role in the performance of coating process. Poor adhesion results in product loss and poor product appearance in coating (Michalski and others 1997). Forces between solids in air are predominantly attractive and cause adhesion of particles to each other and to surface. Many interactions are involved in the adhesion phenomena between particles or particles and surface, such as molecular interactions, electrostatic interactions, mechanical interlocking, liquid bridges, double layer repulsion, and chemical bonds (Mullins and others 1992).

1.1.4.1.1 The influence of particle size on nonelectrostatic adhesion
Particle size, together with the chemical composition and moisture, is the most influential factor in the behavior of particles. In foods, it regulates a wide variety of physical phenomenas such as density, flowability, solubility, hygroscopicity, agglomerability, adhesion and more (Peleg 1987). Size of the particle with regular shapes, such as spherical and cubic, can be easily defined by a single length parameter (e.g. diameter).

Adhesion of dry seasoning particles onto tortilla chips increases with increasing particle size from 32-300 μm (Enggalhardjo and Narsimhan 2005). However, Mullins and others (1992) found the opposite results. Adhesion of glass beads on a glass substrate decreases with the increase of particle size from 5-62 μm (Mullins and others 1992). The powder particles may be bound to the surface by electrostatic image forces. The adhesion force between sugar powder and saltines crackers decreased with increasing particle size from 16-443 μm, because increasing particle size decreases the particle contact area which decreases the attraction force (Halim and Barringer 2006). For elastic powder particles, adhesion force increases in proportion to the diameter of the particles (Kendall and Stainton 2001). For other powder particles, adhesion force tends to decrease with increasing particle size, fine particles adhering best and large particles least (Kendall and Stainton 2001).

1.1.4.1.2 The influence of surface roughness on nonelectrostatic adhesion

Surface roughness plays an important role in determining adhesion of particles to substrates (Schaefer and others 1995). It is generally recognized that high surface
roughness acts to reduce adhesion between two solids. Schaefer and others (1995) measured lift-off forces of nominal 8 μm diameter spheres of glass, polystyrene, and tin from atomically flat substrates of mica and highly oriented pyrolitic graphite (HOPG), and found high surface roughness reduced adhesion between two solids. Smooth spherical polystyrene particles are applied on the silicon, aluminum polished, and aluminum PVD targets, whose roughness increases in order. Adhesion forces decreases with increasing roughness (Zhou and others 2003). Effective contact area is remarkably reduced between particles and rough targets, resulting in a significant reduction of the adhesion force.

1.1.4.1.3 The influence of geometry on nonelectrostatic adhesion

Geometry of a particle is an important factor in adhesion. Different geometries of powder, such as flat, cylindrical, and spherical shapes, have significant effects on powder adhesion on substrates (Mullins and others 1992). Aluminum flakes have better adhesion to flat aluminum substrates compared to aluminum spheres. Adhesion force decreases in order for plates, cylinders and spheres of a given mass (Mullins and others 1992).

1.1.4.2 Electrostatic adhesion

The electrostatic interaction is one of many important interactions for adhesion in electrostatic coating. The total force holding the layer of electrostatically charged powder onto the targets is due to the combination of effects: an interfacial electrochemical phenomenon at the surface, inter particle/particle cohesion and a bulk electrostatic attractive force (Stotzel and others 1997). The dominant force of adhesion derives from
the Coulombic electrostatic force and the corresponding image force that attracts the powder to the substrate (Singh and others 1978). Adhesion of electrostatically deposited powder layer depends on the following parameters: 1. conductivity of the substrate, 2. particle charge distribution, 3. electrical resistivity of the powder, 4. the thickness of the powder layer, and 5. particle size distribution (PSD) of the powder.

1.1.4.2.1 Adhesion forces for electrostatic coating

Electrostatic adhesion is affected by the combination of both the electrostatic force and the nonelectrostatic force (Stotzel and others 1997). Many forces are involved in electrostatic adhesion. Coulomb’s forces, the force between two unipolor charges, provide the fundamental attraction between charged powders and grounded targets. Charged particles are attracted to the substrate with opposite polarity according to the Coulomb’s Law (Hughes 1997). Coulomb’s Law can be expressed as:

\[ F = \frac{q_1 \times q_2}{4 \pi \varepsilon_o \varepsilon_i r^2} \]

Where:  
\( F \) = Coulomb’s force (F)  
\( q_1, q_2 \) = magnitude of charge (C)  
\( \varepsilon_o \) = permittivity of free space, which is \( 8.85 \times 10^{-12} \) (F/m)  
\( \varepsilon_i \) = relative permittivity of medium (F/m)  
\( r \) = distance between the two charges (m)

The strength of Coulomb’s forces depends on the charge on powder particles and the distance between powder particles and targets. Since the Coulomb’s forces between charges are an inverse square relationship, the electrostatic force is regarded as significant within the short range condition (Hughes 1997).
The Van der Waals force refers to intermolecular interactions that deal with forces due to the polarization of molecules. Food powder particles with the dipole moment attract each other due to the van der Waals force, so the van der Waals force is another influential force on adhesion. It can be calculated from:

\[ F_{\text{vdW}} = \frac{AR}{(6h^2)} \]

Where:  
\( A \) = Hamaker constant  
\( R = R_1R_2/(R_1+R_2) \); \( R_1, R_2 \) = radii of interacting spheres  
\( h \) = minimum gap between the two spheres

Since the distance between two particles has a large effect on the magnitude of the van der Waals force, the surface roughness greatly affects adhesion (Feng and Hays 2003). In electrostatic coating, charged powder deposited on the surface of the target results a layer of charged film, which brings in additional electrostatic forces. That is the electrical multilayer force. The more charged powder layers, the stronger the multilayer force thus the stronger the particle adhesion (Cross 1987). Saltine crackers, white bread, and aluminum foil were electrostatically coated with different food powders, and there was a significant improvement in adhesion (Halim and Barringer 2006). However, particle adhesion is self limiting when coating film reaches a certain thickness (Dastoori and others 2001). Some other forces, such as aerodynamic force and capillary force, have an effect on adhesion (Hughes 1997).

Electrostatic adhesion increases with decreasing particle size (Adhikari and others 2001). Both van der Waals and electrostatic forces play a significant role in particle adhesion for small particles (about 10 mm) (Feng and Hays 2003). As particle size
decreases, the van der Waals force increases. The charge to mass ratio decreases with increasing particle size. Although it is not clear whether van der Waals or electrostatic forces are greater, size of particles has effect on adhesion during electrostatic coating. Total adhesion between sugar particles and saltine crackers decreases with increasing size from 16 to 200 μm (Halim and Barringer 2006). Above 200 μm, there was no significant electrostatic adhesion (Halim and Barringer 2006). The charge to mass ratio decreased with increasing particle size, and larger particles have smaller electrostatic charge (Halim and Barringer 2006).

1.1.4.2.2 Measurements

Powder adhesion measurements have been obtained from three different techniques: (1) the centrifugal spinning of a powder coated cylinder, (2) measuring the mass of powder removed when a powder coated sample is subject to an impulse from a drop test, and (3) selectively removing powder under computer control with a series of micro air pulses (Stotzel and others 1997). The three powder adhesion measurement methods give good measurements. The centrifugal method is useful for understanding the structure through the layer. The centrifugal and micro air pulse methods are complex methods and provide a better understanding of adhesion through the powder layer. The drop test is a simple method, providing a quick, efficient method to analyze the coating efficiency and adhesion of a powder layer relevant to an industrial coating application (Stotzel and others 1997).
1.1.5 Transfer efficiency

1.1.5.1 Transfer efficiency of nonelectrostatic coating

1.1.5.1.1 The influence of particle size on transfer efficiency of nonelectrostatic coating

Transfer efficiency increases with increasing particle size in a nonelectrostatic coating system. Sixteen different food powder particles were tested, which include powdered sugar, corn starch, salt, cellulose, maltodextrin, cocoa powder, dry milk powder, all purpose wheat flour, soy flour and corn flour. The transfer efficiency of powder increased as particle size increased from 5.2 to 130 μm during nonelectrostatic coating. Large particles fall onto the target rather than remaining in the air that passes out of the coating system or drifting and settling beside the conveyor belt. Small particles tended to remain in the air as dust and did not fall as readily onto the target. Therefore transfer efficiency is lower for the small particles than for the large particles (Ricks and others 2002). Potato chips, which are nonelectrostatically coated with barbeque, sour cream and onion, salt and vinegar, nacho cheese, and salsa seasoning, have increasing transfer efficiency with increasing particle size from 25.7-331 μm (Ratanatriwong and others 2003).

However, the opposite results were found when testing the influence of powder particle size on transfer efficiency in a tumble drum system (Biehl and Barringer 2003). Popcorn was oiled and coated in a tumble drum nonelectrostatically with sugar, cornstarch, salt, cellulose, maltodextrin, all-purpose flour, whole wheat flour, and soy flour. Transfer efficiency increased as particle size decreased from 145-15 μm. Smaller particles are more easily carried by air currents induced in the tumble drum and disperse more evenly across the popcorn where large particles could not reach (Biehl and
More small particles could adhere per given surface area of the popcorn than large particles (Biehl and Barringer 2003). In automotive powder coating using spherical particles, a narrower size distribution and smaller particles produced higher transfer efficiency (Satoh and others 1998). Powders with 10 μm particles size and narrow particle size distribution produces higher transfer efficiency compared to particles with 30 μm.

**1.1.5.1.2 The influence of the powder charge on transfer efficiency of nonelectrostatic coating**

The powder charge is a significant factor for transfer efficiency of nonelectrostatic coating. The transfer efficiency decreased as the powder charge increased from 0-42%. From 42 to 100% charge there was no difference in coating improvement (Ricks and others 2002). The moderate charge on the powder decreased the powder landing on the target (Ricks and others 2002). Powder was tribocharged during being ground, transportation, and passing through coating system. Therefore, powder was charged.

**1.1.5.1.3 The influence of powder flowability on transfer efficiency of nonelectrostatic coating**

Flow characteristics measure how free flowing a powder is and how likely it is to clump (Ricks and others 2002). The transfer efficiency decreases with decreasing flowability for nonelectrostatic coating (Ricks and others 2002). Nonflowing powders tend to form clumps. Clumped powders get enough momentum to carry themselves beyond the target, resulting in the decrease of transfer efficiency. However, in tumble drum system, the more free-flowing the powder, the greater the nonelectrostatic coating
transfer efficiency (Biehl and Barringer 2003). Powder flowability has a significant effect on nonelectrostatically coating potato chips with seasonings. Free-flowing powders produce higher transfer efficiency (Ratanatriwong and others 2003).

1.1.5.2 Transfer efficiency of electrostatic coating

1.1.5.2.1 The influence of particle size on transfer efficiency of electrostatic coating

Powder particle size plays an important role in electrostatic powder coating. Size of the powder particle influences the behavior of the materials, in the delivery system, in the charging system, and of the final film characteristics, during the electrostatic coating process. The transfer efficiency of the powder coating process and the quality of the final coating depend critically on particle size (Bailey 1998). The typical value of mean particle diameter is approximately 30-40 μm (Bailey 1998). Smaller particle size and narrow width of the distribution function produce more consistent and uniform powder coating layer (Bailey 1998). Smaller particles carry more charge per unit weight and become self limiting at thinner films. Since back-ionization occurs at the self limiting point, a higher concentration of fine particles will contribute to surface disruptions at thinner films.

Charge-to-mass ratio increases as particle size decreases. A high charge-to-mass ratio causes the onset of back corona, which produces relatively thin coating film (Mazumder and others 1997). As the concentration of small particles increases, the coating powder will begin to clump together and resist steady fluidization, which is required to achieve a consistent flow. Smaller particles have more surface area per given weight than large
particle, and tend to pick up more moisture. The high moisture content causes clumping (Mazumder and others 1997).

Corona electrostatic coating provides average improvement of transfer efficiency up to 68% for all 16 tested food powders (Ricks and others 2002). Particle size of those 16 food powders ranges between 5.2 and 130.3 μm. Small particle size resulted in greater electrostatic coating improvement, which is 200% more than large particle size (Ricks and others 2002). In electrostatic coating, small particles can achieve a greater charge to mass than large particles. The increased charge to mass ratio improves coating efficiency because the particles are more attracted to the grounded surface (Ricks and others 2002). Powder particle size has a significant effect on transfer efficiency in coating potato chips with seasonings (Ratanatriwong and others 2003). Particle size of seasoning powders ranged between 25.7 and 331 μm. Smaller particles resulted in greater coating efficiency improvement than larger particles (Ratanatriwong and others 2003). In tumble drum system, transfer efficiency decreased as particle size increased from around 15 to 143 μm (Biehl and Barringer 2003).

However, transfer efficiency increased with increasing particle size in Mayr and Barringer’s (2006) study. Transfer efficiency reaches a maximum and leveled off near 80 μm in both coating system (Mayr and Barringer 2006). Mayr and Barringer’s study is more accurate because they used same powders with different particle size, while others compared different powders with different particle size, in which many other powder properties may be introduced.
1.1.5.2.2 The influence of flowability on transfer efficiency of electrostatic coating

The improvement in coating efficiency increases with decreasing powder flowability in the corona electrostatic coating system (Ricks and others 2002). The electrostatic forces improve the flowability of nonfree flowing powders. When nonfree flowing powders enter coating chamber, electrostatic forces promotes separation of powder particles from other particles due to the repulsion between same charged powders, thus the improvement is large (Ricks and others 2002). Powders with high flowability were less affected by electrostatic forces because they flowed and separated well even without the use of electrostatics (Ricks and others 2002). In electrostatically coating potato chips with seasonings, powers with lower flowability have a larger improvement in transfer efficiency compared to nonelectrostatic coating (Ratanatriwong and others 2003). The charge developed on the particles might cause the particles to repel each other and break up the clumps. This effectively decreased particle size, resulting in higher coating efficiency (Ratanatriwong and others 2003). Free-flowing powders have higher transfer efficiency in the conveyor system, but there is no significant difference in the tumble drum system (Biehl and Barringer 2004).

1.1.5.2.3 The influence of particle density on transfer efficiency of electrostatic coating

The transfer efficiency increases with increasing particle density (Ricks and others 2002). Most food powders have a density between 1.4 and 1.5 g/cm³ (Peleg 1983). Salt powders, which is 2.2 g/cm³, are much dense than common food powders, resulting high transfer efficiency in electrostatic coating (Ricks and others 2002). Less dense particles
showed higher transfer efficiency in conveyor and tumble drum electrostatic coating systems (Biehl and Barringer 2004). Electrostatic forces dominate over gravitational forces in powders with low particle densities. Columbic attractive forces exist between the charged powder particles and the substrate. Columbic forces dominate as the charged particles move closer to the substrate during the coating process (Biehl and Barringer 2004).

1.1.6 The influence of relative humidity on powder coating

Humidity is a state of moisture in the air. Relative humidity is the amount of moisture in a given volume of air as compared to the amount that it is capable of holding, and measured as a percentage at a given temperature. Relative humidity plays an important role in powder handling.

1.1.6.1 The influence of relative humidity on nonelectrostatic coating

RH either increases or has no effect on adhesion in nonelectrostatic coating. There is no influence of relative humidity on adhesion for CaF$_2$, but adhesion increases with increasing RH for KCl (Tang and others 2001). When dry powders are exposed to the surrounding atmosphere, adsorption of water molecules takes place on the particle surface. The extent of adsorption depends on the surrounding relative humidity, temperature and hygroscopic ability of powders. The absorbed water layers influence the interaction between powders and lead to unpredictable behaviors for processes, such as powder coating and deposition (Cleaver and Tyrrell 2004). Capillary meniscus increases with increasing RH. The increasing capillary meniscus increases adhesion of powder
particles. Adhesion depends on the thickness of the adsorbed layer of water, which in turn depends on the surface chemistry. High surface roughness prevents the formation of a complete capillary meniscus at the particle contact point until a critical humidity is reached (Cleaver and Tyrrell 2004).

The relative humidity of storage air strongly affects the median adhesion force at the interface of lactose monohydrate and salmeterol xinafoate (Podczeck and others 1997). Condensed water on the surface of particles and targets results in solid bridge bonds. Relative humidity of the air greater than 75% results in irreversible, strong forces acting at the interface of the particles. Below this humidity level, capillary forces can be removed by storing the mixtures at a very low relative humidity of about 5% for at least 72 h (Podczeck and others 1997).

RH of air influences the dustiness index (Hjemsted and Schneider 1996). In a rotating drum system, there is a decrease in the dustiness index when the relative humidity increases from 0 to 80% for some powders, but an increase in the dustiness index for some powders. However, specific powders are not mentioned. For powdered coal, the dustiness index increases when the RH increases from 0 to 50% RH and then decreases when the RH further increases (Hjemsted and Schneider 1996).

**1.1.6.2 The effect of relative humidity on tribocharging**

The surface charge density of polyamid-12 (PA) and polymethylmethacrylate (PMMA) strongly decreases with increasing RH from 20-80% RH (Nemeth and others 2003). The surface charge density of poly vinyl chloride (PVC), which is a polymer of high surface density, does not change with RH. The explanation of the charging behavior
change with RH is that PA and PMMA have a high ability to take up water. High RH provides more water to be absorbed into these polymers and form water containing swollen layer (Nemeth and others 2003). The formation of water containing layers decreases surface resistivity, resulting in low surface charge density (Nemeth and others 2003).

RH has a large effect on the tribocharging characteristic of ferrite powder (Nomura and others 2003). The absolute value of the saturated specific tribocharge of the powder decreases with increasing humidity within RH range of 8.5-85%. The charge of the dry powder was higher than that of the wet powder.

The moisture sorption isotherm can be used to predict charging of pharmaceutical excipient powders (Rowley and Mackin 2003). Relatively nonhygroscopic excipients, such as alpha-lactose monohydrate and dextrose monohydrate, show the negligible change in charge values in the RH range of 0-80%. In contrast, some hygroscopic excipients showed a continuous decrease in electrostatic charge in the RH range of 0-80%.

Triboelectric charging of pecan pollen by using Teflon charger has been studied by Banerjee and Law (1998). Pecan pollen charging is not affected by carrier-air humidity in the range of 18.1-47.6%. In those experiments, both the Teflon charger surface and the particulates remained in the humid air stream for a very short time (less than 5 min) for moisture to be absorbed by them, resulting in no change of particle charging (Banerjee and Law 1998).
The influence of the relative humidity of the fluidizing air on the maximum degree of electrification by fluidized bed is very complex and depends on the fluidization velocity, particle size as well as the type of fluidized bed (Guardiola and others 1996). Guardiola and others (1996) discovered the generalized characteristic curve of charge versus RH, which is valid for all types of the fluidized bed, fluidization velocity, and particle size. The whole RH range is divided to 5 segments from low to high: A, B, C, D, E.

Zone A: High level of bed electrification.

Zone B: Relative humidity has very little or no effect on the stationary charge. Humidities within this range produce the highest levels of bed electrification.

Zone C: Relative humidity has a strong effect on the degree of electrification and small increases in humidity produce sharp drops in the generated charge. Particle adhesion is not observed in this zone.

Zone D: For relative humidities greater than a certain value charge generation is not observed as all fluidization.

Zone E: At high humidities, high water content makes fluidization impossible, because glass hydrophilicity leads to the formation of an ultrathin liquid layer around the particles that strengthens their mutual cohesion.

The exact range depends on the specific powders (Guardiola and others 1996).

1.1.6.3 The influence of relative humidity on corona electrostatic charging

The types of ions generated by corona discharging are heavily dependant on the gas. In a example of air discharging, the ion generated by the positive corona are hydrated
ions with the formula \((\text{H}_2\text{O})_n\text{H}^+\), where \(n\) is an integer that increases with increasing relative humidity (Giacometti and Oliveira 1992). At low humidity, the dominate charged ions are \((\text{H}_2\text{O})_n\text{NO}^+\) and \((\text{H}_2\text{O})_n(\text{NO}_2)^+\). For negative corona discharging, the most dominate ions are \(\text{CO}_3^-\). At 50% RH, 10% of the ions are in the hydrated form \((\text{H}_2\text{O})_n\) \(\text{CO}_3^-\) (Giacometti and Oliveira 1992).

In the discharge development in a three electrode system (point-grid-plane), as the relative humidity increases to around 45%, less ionization takes place in the air gap and the corona inception voltage increases. However, with more increase in the relative humidity above 45%, more ionization takes place in the air gap and the corona inception voltage decreases. The breakdown streamer onset voltage decreases with increasing relative humidity. As the air relative humidity increases, the value of positive ion charge density around the point’s periphery becomes approximately constant and the glow mode appears at the onset (Fouad and Elhazek 1995).

Surface resistivity of LDPE film decreased with increasing RH (Sharma and others 2003). The surface resistivity of LDPE film increases from \(1.28\times10^{16}\) Ohms/Square at 18% RH to \(2.73\times10^{12}\) Ohms/Square at 90% RH. Charge decay time constant of acrylic polymer powders decreases significantly from 524 to 17 min when RH increases from 25 to 70%. The surface resistivity acrylic polymer powder also decreases with increasing RH. The increased surface conductivity of powders at high relative humidity is due to the formation of moisture layers on the particle surface (Sharma and others 2003).

RH not only has an effect on corona charging, but also has an effect on powder properties. The types of ions generated by corona charging depend on RH. More
ionization of air takes place with increasing RH. Surface resistivity and charge decay time decrease with increasing RH, which may have an effect on corona electrostatic coating.

1.1.7 Powder properties

Properties of powder, such as particle size distribution, chemical composition, tribo and corona charging characteristics, resistivity, hygroscopicity, and flow characteristics, play significant roles on the performance of powder coating such as transfer efficiency, adhesion, film thickness and appearance (Mazumder and others 1997).

1.1.7.1 Resistivity

Electrical resistivity is a measure indicating how strongly a material opposes the flow of electric current. A low resistivity indicates a material that readily allows the movement of electrons. Electrical resistivity of powder influences electrostatic coating process, and is a parameter to indicate how well the electrostatically deposited powder will adhere on the surface of a target. If the conductivity is too high, particles lose charge quickly upon contact with a grounded target and may detach from the surface (Bailey 1998). The powder with high resistivity produces high adhesion but the thin coating layer, whereas, a powder with low resistivity produces low adhesion but the thick coating layer (Sims and others 2000). The resistivity of a powder is a parameter that can indicate the quality of electrostatic coating.

Resistivity of a powder in excess of about $10^{15} \ \Omega m$ is generally considered as adequate for most electrostatic coating (Hughes 1997). However, Sims and others (2000)
considered that $10^{13} \Omega m$ is enough for good electrostatic coating. If the resistivity of powder is greater than $10^{13} \Omega m$, adhesion is good, but the accumulation of charge on the deposited powder layer often causes back corona, which limits further deposition of powder. However, if the powder has low resistivity, less than $10^{10} \Omega m$, the charge decay is rapid enough that back corona does not limit the deposition efficiency (Sims and others 2000). Bailey (1998) also identified three ranges of resistivity as of relevance to the powder coating process.

Range 1: $>10^{13} \Omega m$. Powders having a resistivity in this range are generally satisfactory with no problems occurring during coating. Adhesion is good and a self-limiting layer thickness builds up during the coating process.

Range 2: $>10^{10} \Omega m$ and $<10^{13} \Omega m$. It is very difficult to predict the powder coating performance for powders having a resistivity in this range. Charge decay is very short and adhesion is very poor. Some powders may offer good adhesion and are not due to electrostatic attraction but particle composition, surface chemistry and other factors.

Range 3: $<10^{10} \Omega m$. Powders act as conductors. Particles charge effectively in a corona discharge, but their charge is quickly lost before contacting with a metal workpiece. It is very possible to obtain satisfactory coatings for very fine particles below about 1 μm diameter. In this circumstance, there is no self-limiting process but particle adhesion is often poor.
1.1.7.2 Flow characteristics

The flowability of powder significantly affects the effectiveness of powder coating. All powder coating apparatus require a smooth delivery of fluidized powder at a powder flow rate of a few grams per second (Bailey 1998). A powder must be able to flow freely through the coating machine and deposit uniformly on the target (Mazumder and others 1997). Physical properties (surface properties, density, shape, size distributions etc.) and chemical composition (water content, fat, protein, sugar, etc.) of powders influence flowability (Peleg 1983).

Particle size and shape are related to adhesiveness and cohesiveness of powder (Adhikari and others 2001). Fine particles are more cohesive (Mazumder and others 1997). Smaller particles have larger surface area per unit mass, which increase cohesive forces and frictional forces to resist flow. The flowability of powder decreases with decreasing particle size (Fitzpatrick and others 2004). Particle shape could influence flowability by building mechanical interlock structures when particles with irregular or fibrous shape are vibrated (Peleg 1978; Adhikari and others 2001).

Environmental factors such as temperature and relative humidity influence the flowability of food powders. With increasing relative humidity and temperature, the flowability of tea and whey permeate powder decreases, while flour shows an increase in flowability with increasing temperature (Teunou and Fitzpatrick 1999). Relative humidity has a strong effect on the flowability of tea and whey permeate but less significant effect on flour. As relative humidity increases, water absorbed on the surface of flour tends to dissolve soluble components, forming liquid bridges between particles and making them
more cohesive, thus the flowability decreases (Teunou and Fitzpatrick 1999). The effects of relative humidity and temperature on powder flowability also depend on the time exposure to the condition.

Temperature causes structural changes in the amorphous parts of the structure (Bhandari and Howes 1999). Most food powders are amorphous due to the drying process from which they are produced. Food powders changed from solid glassy structure to a “rubbery” state when the surrounding temperature is above the glass transition temperature. Then, the increase of powder viscosity reduces the powder flowability (Bhandari and Howes 1999).

Angle of repose is an indicator of powder flowability. Powder with angle of 0-35° is free flowing; 35-45° is some cohesiveness, 45-55° is cohesiveness and above 55° indicate very high cohesiveness where powder has very limited flowability (Peleg 1983).

1.1.8 References


1. 2 Analysis of volatile release in tomato and tomatillo

1.2.1. Tomatillo

1.2.1.1 Tomatillo fruit

The tomatillo (Physalis ixocarpa Brot. Syn. Physalis philadelphica Lam) is a fruit of the solanaceae family, related to tomatoes, bearing small and spherical shape with a paper-like husk surrounding. Its skin varies from green, yellow, purple, or reddish, and flesh is pale-yellow, crisp, acid, sweet, and contains many tiny seeds (Morton 1987). This species is tropical, like the tomato, grows in summer with full sun, and can be grown in any soil suitable for tomatoes but not in wet situations (Morton 1987). The unhusked fresh fruits last for several months are stored properly in single layers in a cool and dry atmosphere (Morton 1987). Tomatillo is a major ingredient of green sources and salsas in various Mexican dishes, and it is also being utilized in a variety of recipes, which blends
Latin American flavor themes it contemporary North American tastes (McGorrin and Gimelfarb 2001). In Australia, tomatoillo is used to make jam commercially (Morton 1987). In Mexico, the decoction of the calyces of tomatillo is considered to help the cure of diabetes (Morton 1987)

1.2.1.2 Tomatillo flavor

Corresponding to extensive studies on volatile compounds of tomatoes, there was very little information on tomatillo volatile profile. A total of 52 volatile compounds were detected in tomatilloes, and were divided into 5 groups: lipid derived volatiles, esters, carotenoid related volatiles, lignin related volatiles, and amino acid related volatiles (McGorrin and Gimelfarb 2001). The most predominant volatiles in the tomatillo were lipid related saturated and unsaturated 6 carbon aldehydes and alcohols, in which cis-3-hexenal and trans-2-hexenal were present in significant concentrations, which provided tomatillo strong green leafy aroma. trans-2-Hexenol and cis-3-hexenol were detected, although at a lower concentration, and considered to be important in the tomatillo (McGorrin and Gimelfarb 2001). Other significant lipid related aldehydes detected in tomatilloes were 8- to 10-carbon enals, including 2-octenal, 2-nonenal, and 2,4-decadienal, which provides fatty green aroma (McGorrin and Gimelfarb 2001). Esters were the second largest group of volatiles detected in the tomatillo. Methyl hexanoate, ethyl octanoate, methyl and ethyl benzoate, methyl salicylate, and butyl, isobutyl, hexyl, cis-3- and trans-2-hexenyl acetates were identified in the tomatillo (McGorrin and Gimelfarb 2001). Carotenoid related volatiles were the third largest group of volatiles identified in the tomatillo. The oxidation of carotenoids leaded to the formation of some
unique terpenes in tomatillo, such as alpha-terpinolene, terpinen-4-ol, myrtenol, and cadinene (McGorrin and Gimelfarb 2001). Lignin related and amino acid related volatiles accounted for a small portion of volatiles identified in the tomatillo (McGorrin and Gimelfarb 2001).

The volatiles detected in the tomatillo were evaluated their importance to tomatillo flavor using the calculation of odor units. The odor unit was defined as the ratio of the concentration of volatiles in tomatillo to its odor threshold (McGorrin and Gimelfarb 2001). The logarithm of the ratio for each volatile detected in tomatillo was calculated. Once the logarithm of the ratio exceeded 1, the concentration of a volatile exceeded its threshold, and the volatile likely contributed significant flavor impact. Twelve volatile compounds identified in tomatillo were considered to be significant to tomatillo flavor, which included cis-3-hexenal, beta-ionone, trans, trans-2, 4-decadienal, nonanal, hexanal, beta-damascenone, decanal, trans, cis-2, 4-decadienal, hexyl acetate, trans-2-hexenal, hexanol, and cis-3-hexenol (McGorrin and Gimelfarb 2001).

The volatile compounds detected in the tomatillo were compared with those in the red plum tomato (McGorrin and Gimelfarb 1998). While most volatile compounds detected in tomatillo were similar to tomato, the tomatillo volatile profile missed some characteristic key tomato volatiles, such as iso-butylthiazole, nitrophenylethane, and phenylacetonitrile (McGorrin and Gimelfarb 1998). Volatile compounds unique to tomatillo aroma included hydroxy esters, aromatic esters, 8- to 12-carbon aldehydes, decanoic acids, and terpenes (McGorrin and Gimelfarb 1998).
1.2.2. Tomato

1.2.2.1 Tomato fruit

The tomato (*Lycopersicon esculentum*) is a fruit of solanaceae family with spherical shape. Botanically, tomato is a berry fruit, however, it is cultivated and consumed as a vegetable (Petro-Turza 1987). Tomatoes originated in South America, and are now cultivated and consumed all over the world. There are around 7500 tomato varieties grown for various purposes. The size of tomatoes ranges from 5 mm in diameter (tomberries) to 10 cm in diameter (beefsteak tomato) (Wikipedia 2009). A majority of tomato varieties have red color, but some rare varieties have yellow, orange, pink, black, brown, ivory, white, and purple color. The beefsteak tomato is large tomatoes often used for sandwiches and similar applications. Plum tomatoes are usually oblong and bred with a higher solid content, which is good for use in tomato sauce and paste (Wikipedia 2009). Cherry tomatoes are small, round, sweet, and generally eaten whole in salads, while grape tomatoes, which are a more recent introduction, are smaller and oblong, and are also used in salads (Wikipedia 2009).

1.2.2.2 Tomato volatiles

Desirable aroma and flavor is critical to the quality of tomatoes. The volatile compounds in tomato had been extensively studied, and over 400 volatiles were identified in tomato (Petro-Turza 1987). The volatile concentrations in tomatoes were usually low (Buttery and others 1988), and only 29 volatile compounds were quantified with the concentration exceeding 1 parts per billion (ppb) (Buttery 1993).
Volatile compounds in tomatoes can be divided into 5 groups based on their precursors: 1. Lipid related, 2. Carotenoid related, 3. Amino acid related, 4. Terpenoids, and 5. Lignin related and other miscellaneous (Buttery and Ling 1993). The odor unit was used to evaluate each volatile’s contribution to fresh tomato aroma (Buttery and others 1998). Eight volatile compounds, cis-3-hexenal, beta-ionone, hexanal, beta-damascenone, 1-penten-3-one, 3-methylbutanal, tran-2-hexenal, and 2-isobutylthiazole had a log odor unit higher than 1, which indicated a contribution to fresh tomatoes aroma.

Many possible flavor interactions of different volatile compounds in tomato made it difficult to evaluate the contribution of different volatiles to fresh tomato aromas. In order to study key “fresh” volatiles in tomatoes without sacrificing complicated interactions, some tomato volatile compounds were added into processed tomato juice and evaluated their “fresh” aroma ability by sensory panels (Kazeniac and Hall 1970, Guadagni and others 1972). cis-3-Hexenal, trans-2-hexenal, hexanal and 2-isobutylthiazole were found critical for fresh tomato aroma (Kazeniac and Hall 1970). Sensory panel showed addition of 2-isobutylthiazole gave processed tomato juice more intense and fresh tomato like flavor (Kazeniac and Hall 1970). Addition of cis-3-hexenal, trans-2-hexenal, or hexanal into processed tomato juice intensified “fresh” or “green” notes (Kazeniac and Hall 1970).

A mixture of cis-3-hexenal, 2-methylhept-2-en-6-one, eugenol, and beta-ionone significantly improved “fresh” aroma of processed tomato juice in sensory evaluation (Guadagni and others 1972). 2-methylhept-2-en-6-one, eugenol, or beta-ionone alone did not improve “fresh” aroma (Guadagni and others 1972). Buttery (1993) mimicked fresh tomato aroma using the mixture of 10 volatile compounds: cis-3-hexenal, cis-3-hexenol,
hexanal, 1-penten-3-one, 3-methylbutanal, trans-2-hexenal, 6-methyl-5-hepten-2-one, methyl salicylate, 2-isobutylthiazole, and beta-ionone. Sensory panel studies showed the synthetic mixture of these volatiles gave a very similar aroma to sliced fresh tomato (Buttery 1993). Maturity of tomatoes affected the concentration of 1-penten-3-one, cis-3-hexenal, hexanal, 6-methyl-5-hepten-2-one, 2-isobutylthiazole, 1-nitro-2-phenylethane. Ripe red tomatoes contained more these volatile compounds than green and pink tomatoes (Buttery 1993).

1.2.3. Lipoxygenase pathway

1.2.3.1 Lipoxygenase pathway in the biosynthesis of tomato volatiles

The lipoxygenase pathway refers to a series of sequential enzyme steps involving lipase, lipoxygenase, hydroperoxide lyase, hydroperoxide dehydrase, and alcohol dehydrogenase (Croft and others 1993, Prestage and others 1999). The activity of the lipoxygenase pathway gives rise to various products and intermediates, which contribute to characteristic aroma of tomato and tomatillo, by breaking down free fatty acids. The widely accepted lipoxygenase pathway was first proposed by Stone and others (1975) using $^{14}$C labeling technology (Figure 1.1). Tomato acyl lipids (phospholipid, galactolipids, and triacylglycerols) are first broken down by acyl hydrolase enzymes to free fatty acids, such as linoleic acid and linolenic acid, which are quickly converted into hexanal and cis-3-hexenal, respectively, by lipoxygenase (LOX) and hydroperoxide lyase (HPL). cis-3-Hexenal is transformed to trans-2-hexenal by cis-3/trans-2 isomerase (Z3/E2-ISO). These aldehydes are further transformed into alcohols by the action of alcohol dehydrogenase (ADH). A more complicated lipoxygenase pathway waw reported
in other fruits and vegetables. There are other minor volatiles produced from the lipoxygenase pathway besides these major C6 aldehydes and alcohols. 10-
Hydroperoxides and 12-hydroperoxides produced from rearrangement of 9- and 13-
hydroperoxides degrade to trans-2-octenal and trans-2-heptenal respectively by unknown enzymes in bell peppers, and to 1-octen-3-ol in mushrooms (Luning and others 1995). 9-
Hydroperoxides of linoleic acid produce 2-pentylfuran and trans-2-nonenal in cucumbers and bell peppers (Luning and others 1995). 13-Hydroperoxides of linolenic acid are also the precursors of 1-penten-3-one and trans-2-pentenal in soybeans and bell peppers (Luning and others 1995).

1.2.3.2 Lipoxygenase

Lipoxygenase is a family of iron-containing enzymes that catalyze dioxygenation of free fatty acids containing a cis, cis-1,4- pentadiene structure. Lipoxygenases are ubiquitous in plants, mammals, fungi, algae and other micro-organisms (Suurmeijer and others, 1998). Lipoxygenase plays an important role in the formation of C6 volatile compounds which is important to tomatoes and tomatillos. Although lipoxygenase is active during ripening, the actual synthesis of the C6 compounds only occur when tissue is damaged and enzyme and substrate are mixed (Prestage and others 1999). The production level of C6 volatiles was minimal when a tomato is macerated in nitrogen atmosphere (Kazeniac and Hall 1970; Prestage and others 1999). There are at least five lipoxygenase isomers present in tomatoes, including Tomlox A, Tomlox B, Tomlox C, Tomlox D, Tomlox E (Chen and others 2004). These lipoxygenase isomers have different substrate and product specificities. Tomlox A, Tomlox B, and Tomlox E produce 9-
hydroperoxides, which are not directly involved in the generation of the C6 aldehydes and alcohols (Chen and others 2004). Tomlox C uses both linoleic and linolenic acids as substrates and produce 13-hydroperoxy products, which are precursors of C6 volatile compounds, such as hexanal, hexenal, and hexenol (Chen and others 2004). Although Tomlox D generates 13-hydroperoxides, it does not play a key role in the generation of flavor volatiles in tomatoes (Chen and others 2004). The molecular weight of LOX ranges from 87 to 95 kDa. LOX has a wide optimum pH range of 4.2-8.0 (Yilmaz 2001b). The activity of LOX is optimum at room temperature range (20-30 °C), and decreases with further increases in temperature until it loses its activity at 80 °C (Yilmaz 2001b).

1.2.3.3 Hydroperoxide Lyase (HPL)

Hydroperoxide Lyase (HPL) is a heme protein with a molecular weight in the range of 200-250 kDa (Grechkin 1998). The enzyme is located in chloroplasts and non-chloroplastic particles, usually the same location of LOX found in the same tissue, and could be present in both membrane-bound and soluble forms (Hatanaka and others 1986; Grechkin 1998). HPL is divided into three classes based on substrate specificity: (1) 9-hydroperoxide specific HPL produces C9 aldehydes and C9 oxoacids, (2) 13-hydroperoxide specific HPL yields C6 aldehydes and C12 oxoacids, (3) nonspecific HPL acts on both substrates (Yilmaz 2001a). The effective pH range of tomato HPL is 5.5-8. The activity of HPL does not change during ripening of tomatoes (Riley and others 1996).
1.2.3.4 Alcohol dehydrogenase (ADH)

Alcohol dehydrogenase (ADH) in tomatoes is a relatively nonspecific oxidoreductase capable of using as substrates a range of primary alcohols or aldehydes (Longhurst and others 1990). The molecular weight of ADH is 90-100kDa (Yilmaz 2001). The ADH activity decreased during the early stages of ripening and then increased in the postclimacteric period (Longhurst and others 1990). In tomatoes, there are 2 ADH present: ADH 1 and ADH 2. ADH1 was expressed in developing seeds and pollen while ADH 2 accumulated in ripening fruit in response to a lowering of cytoplasmic pH (Prestage and others 1999).

1.2.4. Flavor release in the mouth

1.2.4.1 Volatile release in the mouth

Aroma being perceived by humans requires volatile compounds being transported to olfactory epithelium located high up in the nasal cavity. During eating, chewing movement breaks tissue in foods releasing volatile compounds into the mouthspace. The volatile compounds are transported through the posterior nares of the nasopharynx during respiratory exhalation or after swallowing to be perceived by olfactory epithelium (Diaz 2004). Not all volatile compounds released reach the olfactory epithelium at the same ratio as originally released, and may be lost through the upper airways (Dattatreya and others 2002). Linforth and others (2002) studied the retronasal transport of volatile compounds by evaluating the concentration of volatile compounds in the mouthspace relative to the headspace and the ratio of nosespace to the headspace. The ratio of mouthspace to headspace varied from 0.17% (pinene) to 70% (1-butanol), whereas, the
ratio of nosespace to headspace varied from 0.09% (pinene) to 11% (propan-2-ol). The release of volatiles in the mouthspace is affected by many factors, such as temperature, hydration, surface area, enzymes, dilution, interaction with salivary components, volatile loss during mouth movement, physicochemical properties of volatiles, and different food matrices (Taylor and Linforth 1996, Diaz 2004).

Linfoth and others (1994) measured eleven volatile compounds in the nosespace during eating tomatoes and compared these volatiles with the concentrations in the headspace. The ratio of the concentrations of volatiles in headspace to nosespace varied from 60 (dimethyl sulphide) to 2 (3-methyl nitrobutane) depending upon the volatiles and tester (Linfoth and others 1994). Brauss and others (1998) measured real-time volatile release in the nosespace during eating tomatoes. The concentration of isobutylthiazol, hexenal and hexenol reached the maximum concentration in the nosespace at different times during eating plum tomatoes. Isobutylthiazol, presented in the intact tomato in the active form, was released first (Brauss and others 1998). Hexenal reached the maximum concentration secondly due to the generation after chewing as a result of lipase, lyase, and lipoxygenase activity (Brauss and others 1998). Hexenol reached the maximum concentration later than hexenal due to the one more enzyme step (ADH) between aldehyde and the alcohol (Brauss and others 1998).

1.2.4.2 Long-term persistence of volatile in the breath

The aftertaste of food relies heavily on the continued perception of the volatile compounds after swallowing. Most studies on long-term persistence of volatiles in the breath were on single volatile compound solutions. Linfoth and Taylor (2000) evaluated
the ratios of the concentrations of 41 volatiles delivered to the nose during the second
breath after the swallow relative to the first breath before the swallow, and found the
values ranged from 0 (methyl furan) to 90% (3-ethyl-2-methylpyrazine). Four factors,
hydrophobicity, volatility, ether linkage, and carbonyl count, were found to affect
Persistence (Linforth and Taylor 2000). After swallowing, the surface of the throat is
coated with volatiles and there are also some volatile compounds in the gas phase above
it. During the subsequent exhalation, the gas phase component is expelled. Compounds
that are more water soluble or that have low volatility repartition more into the mucous
layers of the upper airways, whereas the more nonpolar compounds with higher volatility
partition into the mucous the least (Linforth and Taylor 2000).

Hodgson and others (2004) studied the long-term persistence of volatiles in the breath
by measuring the concentration of these volatiles continuously for 3 mins, and the decays
of the volatiles over time were evaluated. The decay rate of volatile compounds over time
was divided into two stages (Hodgson and others 2004). The first stage lasted up to 1 min
after the volatile solution was swallowed when the decay rate was very fast, whereas, the
later stage, usually 1 min later, showed a slower decay rate. The volatile with higher air-
water partition coefficient ($K_{aw}$) decayed at a much faster rate than compounds with
lower $K_{aw}$ (Hodgson and others 2004). The decay rate during the later stages was
constant for all volatiles, indicating that the individual properties of the volatiles had no
effect on the later stage of their persistence of volatiles (Hodgson and others 2004).
1.2.5. Analytical methods

Previous analysis of tomato and tomatillo volatile compounds was mainly based on gas chromatography-mass spectrometry (GC-MS) technology (Petro-Turza 1987, McGorrin and Gimelfarb, 1998). The volatile compounds in tomato and tomatillo are complex, and extraction of volatile compounds from tomatoes is usually required for quantitative analysis using GC-MS. Conventional sample extraction methods, such as static headspace, solvent extraction and purge-and-trap, have been used in GC-MS analysis of tomato volatiles (Buttery and others 1971, Beltran and others 2006). Solid-phase microextraction (SPME) is also used as an alternative extraction method for tomato volatile compounds analysis, providing shorter extraction time and lower cost per sample compared to conventional methods (Beltran and others 2006). Gas chromatography separates extracted volatile compounds mixture by different retention times of different compounds in a separation column. The eluates from gas chromatography were ionized for detection by mass spectrometry.

Sample preparation required by GC-MS makes it unsuitable for real-time analysis of volatiles. Conventional mass spectrometry generally utilizes low pressure electron ionization sources to determine molecular weights, elemental composition and structures of unknown substances. It is challenging to use electron ionization mass spectrometry when real-time detecting and quantifying the trace gasses in atmospheric air and exhaled breath, because the gas loading of the ion source by N₂, O₂, and water vapor can be excessive (Smith and Spanel 2005). The development of mass spectrometry introduced atmospheric pressure ionization mass spectrometry (API-MS), which by using chemical
ionization. API-MS allows real-time quantifying volatiles in the mixture in the headspace directly without sample preparation with detection level down to 10 ppb (Boukobza and others 2001). API-MS has been used to study the volatile release during eating of tomato (Brauss and others 1998), and to monitor lipid oxidation-derived volatiles in fresh tomato (Boukobza and others 2001). Proton transfer reaction mass spectrometry (PTR-MS), another chemical ionization based mass spectrometry, was also used to measure real-time volatile release in tomatoes (Masanobu and Hisakatsu 2005). Although these two techniques provide the ability of real-time analysis of volatile release, due to the lack of separation ability provided by GC, both techniques cannot distinguish the isomers. cis-3-Hexenal and trans-2-hexenal, these two key volatiles of tomato and tomatillo cannot be differentiated and are usually reported as hexenal.

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

1.2.6.1 Principle of the SIFT-MS

Selected Ion flow tube mass spectrometry (SIFT-MS) is a mass spectrometry technique for real-time trace gas analyses utilizing chemical ionization of sample trace gases by selected positive ions during a defined time period along a flow tube. The principle of the SIFT-MS can be explained in Figure 1.2. Judiciously chosen mass selected precursor ions generated by microwave gas discharge enter into a flow tube with an inert carrier gas at a known precisely controlled velocity via an upstream quadrupole mass filter. The sample gas mixture is introduced into the flow tube to react with precursor ions to produce characteristic product ions. The count rates of both the
precursors and products are determined by using downstream quadrupole mass spectrometer detection system (Spanel and Smith 1999).

Three precursor ions \( \text{H}_3\text{O}^+, \text{NO}^+, \) and \( \text{O}_2^+ \) are chosen because they do not react with the major components of air (\( \text{N}_2, \text{O}_2, \text{H}_2\text{O}, \text{Ar}\) and \( \text{CO}_2 \)) but react with volatile compounds rapidly (Spanel and Smith 1999). The concentration of a volatile compound \( M \) can be calculated using the following reaction:

\[
[M] = \frac{I_p}{I t k},
\]

in which

- \( I_p \): product count rate
- \( I \): precursor ions count rate
- \( k \): reaction rate coefficient
- \( t \): reaction time

The reaction rate coefficient, \( k \), was obtained from a previous study of the ion chemistry of tree precursor ions, \( \text{H}_3\text{O}^+, \text{NO}^+, \) and \( \text{O}_2^+ \) with a wide variety of volatile compounds, and was stored in the kinetic database of SIFT-MS. Reaction time \( t \) is accurately 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 volatile compounds in a wide range of sample formats, including whole air and headspace. It also has the ability to analyze high moisture samples, which makes it ideal for breath analysis. SIFT-MS uses multiple reagent ions (\( \text{H}_3\text{O}^+, \text{O}_2^+ \) and \( \text{NO}^+ \)) to chemically ionize volatile compounds.
Thus identification of some isomers without the need for chromatographic separation has become possible.

1.2.6.2 Ion chemistry

The essential requirement of the SIFT-MS is the reaction of each trace gas, M, with chosen precursor ions. The ion chemistry of tree precursor ions, H$_3$O$^+$, NO$^+$, and O$_2^+$ with a wide variety of volatile compounds had been detailed studied, which included alcohols, aldehydes, ketones, carboxylic acids, esters, ethers (Spanel and others 1997, Spanel and Smith 1997, Spanel and Smith 1998, Spanel and Smith 1999, Smith and Spanel 2005).

For the precursor ion H$_3$O$^+$, the reaction with volatile compounds M via rapid proton transfer producing MH$^+$ because their proton affinities are usually greater than that of H$_2$O (Spanel and Smith 1999). For OH-containing volatile compounds, such as alcohols, aldehydes, and carboxylic acids, the MH$^+$ ions partially or totally dissociate with the elimination of an H$_2$O molecule, leaving an [M-OH]$^+$ hydrocarbon ion (Spanel and Smith 1999). Aldehydes reactions produce either MH$^+$ or parallel MH$^+$ and [M-OH]$^+$. Ketones react with H$_3$O$^+$ to produce only MH$^+$ (Spanel and Smith 1999).

The reaction of precursor ion NO$^+$ with volatile compounds variously by hydride ion, H$^-$, transfer producing [M-H]$^+$ions and HNO radicals, hydroxide ion, OH$^-$, transfer producing [M-OH]$^+$ ions and HNO$_2$ molecules, charge (electron) transfer producing M$^+$ ions and NO molecules, ion molecule association producing NO$^+$M ions, and OR radical abstraction producing [M-OR]$^+$ carboxy ions and RONO molecules (Spanel and Smith 1999). Alcohol reacts with NO$^+$ to form H$^-$ transfer (Spanel and Smith 1999). Hydride ion
transfer is the dominant process that occurs in the reactions of NO+ with aldehyde, resulting in a single product carboxy ion (Spanel and Smith 1999).

The reaction of the precursor ion \( \text{O}_2^+ \) with volatile compounds via a simple non-dissociative charge transfer producing the parent radical \( \text{M}^+ \), or via dissociative charge transfer reactions results in two or sometimes several fragment ions (Smith and Spanel 2005). \( \text{O}_2^+ \) precursor ions are most valuable for the detection and quantification of small molecules that do not react with either \( \text{H}_3\text{O}^+ \) or \( \text{NO}^+ \) (Smith and Spanel 2005).

1.2.6.3 Validation of the quantitative accuracy of SIFT-MS

The precision and accuracy for the analysis of the trace gases in air was carefully investigated. The validity of SIFT-MS was established by analyzing several organic volatile compounds in dry air over a wide range of partial pressures by preparing standard atmospheres using the syringe injection technique, which includes ethanol, benzene, toluene, xylene, acetone, 2-butanone, 1-methoxy-2-propanol, and trichloroethylene (Smith and Spanel 2005). The accuracy of measurement was better than 10% over a range of partial pressures (Smith and Spanel 2005).

The unique feature of SIFT-MS is the permission of the accurate quantification of volatiles in the humid air. The ion chemistry of precursors and volatile compounds shows the formation of hydrated ions in the humid air (Smith and Spanel 2005). The kinetics of hydrated ions of precursors and sample volatile compounds were detailed studied and included in the database. The quantification of volatile compounds in humid air accounts for the presence of water vapor and is independent of the humidity of the sample (Spanel and Smith 2000).
1.2.6.4 Application of SIFT-MS in Food Science

SIFT-MS has been successfully applied in different areas, such as the analysis of breath volatiles for medical diagnosis and therapeutic monitoring (Senthilmohan and others 2000; Smith and others 2003), detection of bacterial metabolites (Scotter and others 2005; Allardyce and others 2006), and monitoring air pollution (Smith and Spanel 1996). The applications of SIFT-MS have been reported on real-time quantification of volatile emissions from vegetables and fruits. The real-time release of diallyl disupphide and diallyl disulphide-s-oxide in crushed garlic cloves were measured and showed a later peak time for diallyl disulphide, which indicates the formation of diallyl disulphide is from the decomposition of diallyl disulphide-s-oxide (Smith and Spanel 1999). The volatile emission of volatiles from cut onions and ripe bananas were also measured using SIFT-MS (Smith and Spanel 1999). SIFT-MS was used for quality control of food products. Thirteen volatiles in the headspace of olive oil were monitored during accelerated thermal oxidation (Davis and McEwan 2007). A fast reliable method for prediction of olive oil peroxide values by SIFT-MS was developed (Davis and McEwan 2007). SIFT can also be used in brewing industry to monitor ethanol production in yeast fermentation (Smith and Spanel 2005).

1.2.7 References


Figure 1.1 Aldehyde and alcohol formation in tomato from linolenic and linoleic acid (Stone and others 1975).
Figure 1.2 The principle of the Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) (Syft technologies 2009).
CHAPTER 2

THE EFFECT OF RELATIVE HUMIDITY ON COATING EFFICIENCY IN NONELECTROSTATIC AND ELECTROSTATIC COATING

2.1 Abstract

Fifteen food powders were coated on aluminum targets at 0, + 25, and –25 kV using corona electrostatic coating at 20% to 80% relative humidity (RH). The effect of RH on 3 losses, that is, targeting loss, coating loss, and transportation loss, which contribute to coating efficiency, was studied. RH had no effect on targeting loss in either nonelectrostatic or electrostatic coating. In nonelectrostatic coating, increasing RH increased coating loss for powders with particle size ≥ 297 μm, but had no effect on powders ≤ 227 μm. Large powders were free-flowing and clumped with increasing RH, and then rolled off the targets, resulting in high coating loss. RH had no effect on nonelectrostatic transportation loss for all powders, except for very high absolute humidity when capillary forces dominated. Electrostatic charging efficiency and powder resistivity decreased with increasing RH. Electrostatic coating loss for salts increased with increasing RH. At high RH, powder resistivity decreases, increasing the charge decay rate, which decreases electrostatic adhesion. Electrostatic coating loss for powders
other than salts, whose resistivities are much higher than salts, was not affected by RH until 80% RH. Electrostatic transportation loss for powders other than proteins was not affected by RH. There was no significant difference between positive and negative electrostatic transfer efficiency and adhesion, except for transfer efficiency of soy protein and pork gelatin, whose high positive tribocharging values cause higher positive electrostatic transfer efficiency. A 20% to 60% RH is recommended for both nonelectrostatic and electrostatic coating.

2.2 Introduction

A flavored coating is applied to most snack foods and plays an important role in both the flavor and appearance of a snack. The snack food industry is constantly striving to obtain a competitive advantage by improving their flavor and appearance. Many powder coating systems, such as the roll salter and tumble drum, have been used to improve powder coating quality and efficiency. Electrostatic coating is one of the coating technologies used in the food industry. Electrostatic powder coating technology provides more uniform and efficient coating compared to traditional coating technologies in most circumstances (Mayr and Barringer 2006). Corona charging is one electrostatic charging method, which produces good coating quality and is popular in the USA (Mayr and Barringer 2006). In corona electrostatic powder coating systems, high voltage on the electrode breaks down the surrounding air and generates an ion rich region (Hughes 1997). When food powders are transported through the ion rich region, they pick up charge. Since charge on the powder directs the powder toward grounded targets in the
electric field, transfer efficiency is increased. Electrostatic force on the charged powder also increases adhesion.

Coating efficiency is important to the success of coating. It is affected by three losses during the coating process: targeting loss, coating loss and transportation loss. A change in the powder physical properties, such as powder particle size, resistivity, and flowability, affect coating efficiency (Ricks and others 2002, Ratanatriwong and others 2003, Biehl and Barringer 2004, Mayr and Barringer 2006). In addition, a change in charging efficiency should also affect coating efficiency.

Relative humidity affects corona discharge and charging efficiency. The corona initiation field strength, mobility of charge carriers, and plasma chemistry change due to the presence of water vapor in the air (Chen and Wang 2005). The decrease of mobility of charge carriers is critical in electrostatic powder coating, because it determines the charging efficiency. In positive corona discharge, increasing relative humidity reduces positive nitrogen and oxygen ions (Pavlik and Skalny 1997). Positive oxygen ions are quenched by H₂O in the air (Pavlik and Skalny 1997).

Relative humidity also affects many powder physical properties. Powder resistivity decreases with increasing RH. The resistivity of lactose, MMC and sorbitol decreased with increasing RH from 0-66% RH (Grosvenor and Staniforth 1996). Powder absorbs water and forms water bridges, which promotes conduction pathways, thus, powder resistivity decreases. Powder flowability also decreases with increasing RH. The flowability of feedstock powder (Stanford and DellaCorte 2002), tea powder and whey powder decreased with increasing RH (Teunou and Fitzpatrick 1999). Water absorbed
onto the surface of the particle tends to form water bridges between particles making them more cohesive (Teunou and Fitzpatrick 1999). The increased friction between powder particles lowers powder flowability. RH also affects powder particle size. Powder particles accumulate and form clumps when exposed to high RH, which will increase net particle size.

Environmental relative humidity varies across a wide range, 20 to 80%, from winter to summer and from North Europe to Southeast Asia. The relative humidity in the coating chamber may be differing from environmental relative humidity. Warm processed food products heat surrounding air in the coating chamber, which results in a decrease of relative humidity. Competent manufactures usually control the environment with the concern of moisture pickup by finished products. Since relative humidity influences electrostatic charging efficiency and powder properties, coating efficiency may be affected. In order to study how RH affects coating efficiency, how RH affects the three major losses during the coating process needs to be determined. The objective of this project is to determine how RH affects three losses, targeting loss, coating loss and transportation loss, during nonelectrostatic and electrostatic coating.

2.3 Materials and methods

Food powders tested in this project included sucrose-15 μm (confectioners powdered imperial sugar cane sugar, Imperial Savannah L. P., Sugar Land, TX), sucrose-439 μm (Meijer granulated sugar, Meijer distribution Inc., Grand Rapids MI), NaCl-297 μm (Premier fine flake, Cargill Inc., Minneapolis, MN), NaCl-227 μm (Alberger 50 fine flake, Cargill Inc., Minneapolis, MN), NaCl-434 μm (Culinox 999, Morton International Inc.,
Chicago Il), NaCl-19 µm (Extra Fine Salt 325, Morton International Inc., Chicago, Il), KCl-363 µm (A.C.S Reagent grade, Jenneile Enterprises, Cincinnati, OH), 87% soy protein isolate-73 µm (Protein Technologies International, Saint Louis, MO), 93.5% whey protein isolate-61 µm (WPI-90-regular, Industrial Food Ingredients, Minneapolis, MN), corn starch-15 µm (PURE-DENT B700, Grain Processing Corporation, Muscatine, IA), high amylose rice starch-192 µm (Remy B7, A&B Ingredients, Fairfield, NJ), potato starch-70 µm (unmodified food grade, AVEBE American Inc., Princeton, NJ), maltodextrin-75 µm (Maltrin M100, Grain Processing Corporation, Muscatine, IA), cellulose-80 µm (Solka-floc, Fiber Sales & Development Corp., Urbana, OH), and pork gelatin-110 µm (Flavex 95, Arnehem Inc., Cranford, NJ). All powders were dried in a vacuum oven with 30 mm Hg vacuum at 60 °C for 3 days, and stored in desiccators for future use. Mean diameters of powders were measured using the Malvern Mastersizer (Mastersizer X standard bench, Malvern Instruments Ltd., Worcestershire, UK). The volume mean diameter D[4,3] of each powder was measured 3 times and the average reported.

An electrostatic powder coating system (Terronics Development Corp., Elwood, IN) was used to coat targets at 0 kV for nonelectrostatic, -25 kV for negative corona electrostatic, and +25 kV for positive corona electrostatic coating. Negative polarity is reported unless otherwise noted. An aluminum sheet was cut into 9 targets of 10 cm × 15 cm. Nine targets were placed 3 × 3 on the grounded stationary conveyor belt in the coating chamber. Each target was left as is, except when determining targeting loss, six drops (0.065 gram per drop) of canola oil (Crisco, The J.M. Smucker Company, Orrville,
OH) were added and evenly spread across the targets by hand to produce 2mg/cm$^2$. An air compressor (5.0 hp, 15.0 gallon tank, Model WL650800AJ, Campbell Hausfeld, OH) was used to supply airflow, which drives food powder through the coating chamber. The airflow velocity was set at 3.3 m/s. The food powder was fluidized to pass two dc electrostatic wires, where food powders were corona charged with negative or positive charge. The schematic diagram of experiment setup is shown in Figure 2.1.

Dry food powders were coated in a relative humidity regulated coating room. Temperature was 21-25 C. Room RH was set to 20, 40, 60 or 80% during the whole coating process and test period by humidifiers (Environizer, Koz Inc., Southborough, MA; MoistAir, Emerson Electric, Hatfield, PA) or dehumidifiers (Maytag, Maytag Inc., Effingham, IL; Hampton bay, Fedders Corporation, Liberty Corner, NJ). Sucrose could not be tested at 80% because it is too hygroscopic and would not flow through the equipment. To reach higher water contents in the air, the temperature was raised to 27-29 C at 80% RH. Water content in the air was calculated from the temperature and RH using CYTSoft Psychrometric Chart software (version 2.1, CYTSoft Technology).

Transfer efficiency (TE) is a measurement of the percentage of applied powder deposited on the substrates. It was measured using the equation:

$$\text{TE} = \frac{M_d}{M_a} \times 100\%,$$

Where, $M_d$: mass of powders deposited on the substrates

$M_a$: Mass of powders applied

Improvement in transfer efficiency was calculated by subtracting nonelectrostatic transfer efficiency from electrostatic transfer efficiency.
Adhesion is a measurement of the strength of powders attaching to substrates. It was measured using the equation:

$$\text{Adhesion} = \frac{M_d}{M_s} \times 100\%,$$

Where, $M_d$: mass of powders deposited on the substrates

$M_s$: Mass of powders remained on the substrates after 5s shaking on a portable sieve shaker (Model RX-24, W.S. Tyler incorporated, Mentor OH).

Improvement in adhesion was calculated by subtracting nonelectrostatic adhesion from electrostatic adhesion.

In order to test corona charging efficiency, plastic foam (10 cm × 15 cm × 1cm) was placed on the conveyor belt at the center of the coating chamber to isolate the aluminum plate from ground. An aluminum plate (10 cm × 15 cm) was placed on the plastic foam. High voltage was applied to generate corona charges. A high voltage probe (Fluke 80K-40, Fluke corporation, Everett, WA) connected to a multimeter (Radio Shack, Tandy corporation, Fort worth, TX) was used to measure the target voltage on the center of the aluminum plate. Target voltage was measured at 10-35 kV between 20-80% RH. The 25 kV applied at 20% RH produced a target voltage of 3.7 kV. At 40-80% RH the applied voltage was increased until 3.7 kV target voltage was produced. Then the transfer efficiency and adhesion of NaCl-434 μm were tested.

Powder resistivities were tested using a powder test cell (Electrostatics Solutions, Bassett, U.K.), an electrometer (Model 614, Keithley Instruments, Inc., Cleveland, OH), a voltmeter (LCD Auto Range Digital Multimeter, Model 22-163, Radio Shack, Tandy.
Corporation, Fort Worth, TX) and a high voltage power supply (ABC Regulated DC Supply, Kepco, General Electric Co., Flushing, NY). The powder test cell was placed in a glove box, whose relative humidity was controlled using different saturated salt solutions. Resistivity tests were carried out in the glove box. Powders were first equilibrated in desiccators with RH in the range of 7.75-98%, which were set by saturated salt solutions: zinc bromide (RH 7.75%), lithium chloride (RH 11.3%), potassium acetate (RH 22.5%), magnesium chloride (RH 32.8%), potassium carbonate (RH 43.2%), magnesium nitrate (RH 52.9%), potassium iodide (RH 68.9%), sodium chloride (RH 75.3%), potassium chloride (RH 84.3%), and potassium nitrate (RH 93.6%). Powders were placed into a volumetric cylinder and firmly pressed to produce 5 cm$^3$ and then poured into the powder test cell. Air was dispelled by taping the cell for 5 s then pressed. Voltage applied was 171-172 V, which was read from the voltmeter. Current value was read from the electrometer when there was no change over a 15 s period. Resistivity was calculated by the following equation:

$$\rho = \frac{KV}{I}$$

Where, $\rho$ = resistivity ($\Omega \cdot m$),

$K$ = the cell constant = 0.014

$V$ = the voltage applied (V)

$I$ = current (A)

Cohesiveness of food powders was measured by angle of repose. Powders were first equilibrated in the salt controlled desiccators for 2 weeks. Equilibrated powders were slowly sifted by a powder sifter, through a funnel positioned 11 cm above a 3 cm radius
petri dish. The maximum peak height of the powder prior to collapse was measured with a caliper and recorded. Angle of repose is the angle between the powder and the disk. The angle of repose of the food powder pile was calculated using the arctangent of the radius and height of the pile, and converting to degrees.

\[
\alpha = \tan^{-1}\left(\frac{h}{r}\right) \times 57.3
\]

Where, \( h \) = peak height (mm)

\( r \) = dish radius (mm)

### 2.4 Results and Discussion

Coating efficiency is the powder remaining on the target after transportation divided by the powder applied during coating, and is an indicator of how successful a coating process is. The loss of powder during this process includes three kinds of loss. First is targeting loss, which is the loss that occurs after the powder enters the coating machine but before it lands on the target. Targeting loss is affected by engineering design and aerodynamic forces. Gravity, air viscosity, air velocity and powder cohesiveness affect powder aerodynamics. Second is coating loss, which occurs when some of the powder landing on the target does not adhere and is immediately lost. Aerodynamic forces, surface roughness, powder cohesiveness, and powder size affect the coating loss. Third is transportation loss, which is the loss of powder during transportation of coated targets. Transportation loss is affected by surface roughness and powder adhesiveness. In order to study coating efficiency, it is necessary to study each of these losses individually.
2.4.1 Effect of relative humidity on nonelectrostatic targeting loss

Relative humidity (RH) had no effect on targeting loss during nonelectrostatic coating (Figure 2.2). Transfer efficiency is the percentage of applied powder landing on the targets, and is the result of targeting loss and coating loss. When targets were coated with a layer of oil to hold all of the powder that landed and eliminated the effect of coating loss, transfer efficiency did not significantly change with increasing RH for nonelectrostatic coating (Figure 2.2). The targeting loss is mainly determined by the air velocity, air viscosity and the gravity. Air velocity was controlled to 3.3 m/s and was not subject to the change of RH. Gravity is not affected by relative humidity at all due to the nature. Although increasing relative humidity slightly increases the amount of water in the air, which increases the air viscosity, the influence is not significant. Thus, the nonelectrostatic targeting loss is not affected by relative humidity.

The smaller powder had more targeting loss than the larger powder (Figure 2.2). Small powders contain less weight and tend to float in the air. Some of those floating fine powders will remain in the air as dust, and some will fly out of the coating system, resulting in more targeting loss and low transfer efficiency (Ricks and others 2002). Large powders have more weight, thus, less dust loss and mistargeting loss, resulting in less targeting loss and high transfer efficiency.

2.4.2 Effect of relative humidity on nonelectrostatic coating loss

RH affected coating loss during nonelectrostatic coating of large powders only (Figure 2.3). Increasing RH decreased transfer efficiency of powders with a particle size
of 297 μm and larger. Since RH had no effect on targeting loss, the decrease in transfer efficiency was caused solely by the increase in coating loss with increasing RH.

The increase in coating loss was accompanied by increasing cohesiveness of the powders. As the relative humidity of the surrounding air is increased, powders tend to absorb water, and liquid bridges between powder particles increase, resulting in greater powder cohesiveness (Teunou and Fitzpatrick 1999). As the RH increased, the angle of repose of these powders increased (Figure 2.4). A large angle of repose indicates a highly cohesive food powder. Powders with an angle of repose lower than 45° are free-flowing (Carr 1976). The large powders have an angle of repose less than 45° at 20% RH, indicating that they are free-flowing. As RH increases, these large powders become more cohesive and tend to form larger clumps. These large clumps were observed bouncing off the target, resulting in more coating loss. A decrease in transfer efficiency due to clumps of powder rolling off the target during coating was also reported by Ricks and others (2002).

For the small powders, the cohesiveness also increased with RH. However, the coating loss did not significantly change (Figure 2.5), probably because these powders are cohesive and have an angle of repose larger than 45°, a level starting being regarded as high cohesiveness (Carr 1976), at all RH (Figure 2.4). Thus, increasing RH only made the cohesive powders more cohesive and had no effect on coating loss of powders with particles size 227 μm and smaller.
2.4.3 Effect of relative humidity on nonelectrostatic transportation loss

Relative humidity had no effect on transportation loss during nonelectrostatic coating of any of the powders tested at 20-80% (Figure 2.6). Adhesion is a measure of coating powder lost during transportation. Mechanisms involved in adhesion normally include molecular forces (such as van der Waals attractions, mechanical interlocking and chemical reactions), electrical forces (double layers), Coulombic forces (image attraction), and capillary forces (Michalski and others 1997; Johnson 1998; Zimon 1969; Podczeck and others 1997b). All of these forces combine to produce the total adhesion. Of these forces, capillary forces are the most RH-dependent. Capillary forces should increase with increasing RH, resulting in an increase in total adhesion. However, Zimon (1969) found capillary forces played no part in adhesion of glass particles on hydrophobic glass targets until 65% RH or > 90% RH, depending on the particle size. Podczeck and others (1997a) found RH had no effect on the adhesion between salmeterol xinafoate particles and salmeterol xinafoate targets from 5-95% RH, since there was no buildup of capillary forces on the hydrophobic targets. Xiao and Qian (2000) found that adhesion between Si₃N₄ and a hydrophobic OTE coated SiO₂ target did not change from 5-98% RH. Meniscuses of water are very difficult to form on a hydrophobic surface, which eliminates the effect of RH-dependent capillary forces and leads to domination by RH-independent adhesion forces especially from the van der Waals force (Xiao and Qian 2000). In order for RH to influence nonelectrostatic adhesion, enough meniscuses of water must form between the powders and targets. If the formation of water meniscuses is weak, even at RH above 90%, the adhesion force will not increase (Xiao and Qian...
Capillary forces have their greatest effect on a hydrophilic target, and their least effect on a hydrophobic target (Zimon 1969). Since hydrophobic aluminum sheets were used as targets in these experiments, water meniscuses are hard to form. Thus, RH had no effect on nonelectrostatic adhesion of these powders from 20-80%.

However, nonelectrostatic adhesion increased dramatically at higher absolute humidity (water contents of the air), resulting in low transportation loss (Figure 2.7). As the water content of the air increases, drops of vapor condense between powder particles and target surfaces. The presence of a liquid meniscus in the space between the particles and the target’s surface produces strong capillary forces. Once condensation is formed, the total adhesion is determined entirely by the capillary forces, which exceeds all other adhesive components (Zimon 1969). Zimon (1969) found adhesion of small glass beads and channel black powder on glass, a hydrophobic surface, increased when RH is above 65%, due to the presence of capillary force. The RH at which capillary forces start to significantly affect adhesion depends on the roughness and hydrophobicity of the target allowing water meniscuses to form. An increase in adhesion due to capillary forces can occur anywhere between 50 and 90% RH (Cleaver and Tyrrell 2004). In these experiments, hydrophobic aluminum targets needed a very high water content of the air to allow the formation of capillary forces.

2.4.4 Effect of relative humidity on electrostatic charging efficiency and targeting loss

Electrostatic charging transfers charge onto powders, which adhere to the grounded targets through electrostatic forces, resulting in higher transfer efficiency and adhesion
than in nonelectrostatic coating (Mayr and Barringer 2006). Charging efficiency determines the amount of charge being passed to powders during electrostatic coating. As RH increased, electrostatic charging efficiency decreased (Figure 2.8). The decrease in charging efficiency with increasing relative humidity can be measured by comparing the voltage produced on the target at different relative humidities (Figure 2.8). Under the same applied voltage, a higher target voltage indicates a higher charging efficiency. For applied voltage ranging from 10 to 35kV, the target voltage decreased when RH increased from 20 to 80% (Figure 2.8). As the RH increased, the target voltage and therefore charging efficiency decreased. Increasing RH increases the concentration of water in the air. Water molecules attach to nitrogen and oxygen ions in the air (Pavlik and Skalny 1997), which are the key charge carriers for corona electrostatic coating (Hughes 1997). Thus, the mobility of charge carriers decreases (Chen and Wang 2005), and charging efficiency also decreases.

The decrease in charging efficiency did not affect electrostatic transfer efficiency and adhesion at 20-60% RH, however, the transfer efficiency did decrease at 80% RH (Figure 2.9). The greatest decrease in charging efficiency occurred between 60 and 80% RH, which may account for the decrease in transfer efficiency (Figure 2.8). The change in charging efficiency did not significantly affect improvement in adhesion. Since the improvement in adhesion was low, below 5%, any decrease in adhesion at 80% RH would not be significant.

RH had no effect on electrostatic targeting loss (Figure 2.2). Electrostatic coating reduced targeting loss compared to nonelectrostatic coating for the small powder, as
would be expected. However, electrostatic coating did not reduce targeting loss compared to nonelectrostatic coating for the largest powder (Figure 2.2). In electrostatic coating, charge on the powders directs the powder toward the grounded targets, which causes more powder to land on the targets rather than be lost off the side of the targets (Biehl and Barringer 2004), resulting in less targeting loss compared to nonelectrostatic coating. For large powders, the charge to mass ratio is low (Mazumder and others 1997), thus the electrostatic force is low.

2.4.5 Effect of relative humidity on electrostatic coating loss

RH affected coating loss of only NaCl and KCl during electrostatic coating. Electrostatic coating improved transfer efficiency over nonelectrostatic coating, but the improvement became smaller with increasing RH for the salts (Figure 2.10). The resistivity of salts is low, in the range of $10^5$ - $10^7$ Ω m (Figure 2.11), which makes them conductors (Bailey 1998). If a powder is a conductor, the powder particles lose charge quickly upon contact with a grounded target and detach from the targets (Bailey 1998), resulting in high coating loss. As RH increases, powder absorbs water from the air, thereby decreasing its resistivity and increasing the charge decay rate (Sharma and others 2001; Sharma and others 2003; Ong and others 1975). Since the resistivity of salts is low, it can only hold a small amount of charge and the improvement with electrostatics is much lower than for the other powders. As the RH increases, the resistivity decreases and the amount of charge that can be held on the powder decreases, further increasing coating loss.
For all of the powders except the salts, RH had little effect on electrostatic coating loss except at 80% RH (Figure 2.12). Improvement in transfer efficiency from electrostatics was constant between 20 - 60% RH, but decreased at 80%. The resistivity of these powders is much higher than for salts, in the range of $10^8 - 10^{10} \, \Omega \, m$ (Figure 2.11). This improvement is constant from 20-60% RH because the resistivity remains high and charging efficiency did not change significantly. Therefore, the non-salt food powders can hold enough charge to show a large improvement with electrostatic coating (Figure 2.12). However, at 80% RH, the improvement with electrostatic coating significantly decreased. At this humidity, powder resistivity and charging efficiency both significantly decreased. Since the difference in transfer efficiency due to the change in charging efficiency was relatively small (Figure 2.9), the decrease in resistivity is likely to be the main cause for the decrease in improvement with electrostatic coating.

### 2.4.6 Effect of relative humidity on electrostatic transportation loss

Electrostatic coating improves adhesion due to the introduction of electrostatic forces. In electrostatic coating, powder particles are charged in the air by a high voltage field and deposit on the surface of targets. Charged powder particles induce image charges in the target. Coulombic forces between the charge and image charge are large due to the very short distance between charged powders and targets (Halim and Barringer 2006). Coulombic forces frequently exceed molecular forces and the electrical forces associated with contact potential differences, and dominate the adhesion of particles (Zimon 1969). Thus, transportation loss should be reduced with electrostatic coating.
Electrostatic coating improved adhesion over nonelectrostatic coating for powders with high resistivity (Figure 2.13). Resistivity determines the ability of a powder to hold charge and is critical to electrostatic adhesion. If powder resistivity is high, powder has a slow charge decay rate and retains more charge longer, producing high electrostatic adhesion (Bailey 1998). Sumawi and Barringer (2005) observed a correlation between powder resistivity and the adhesion improvement with electrostatic coating. In this study, three proteins produced significantly higher adhesion in electrostatic coating than in nonelectrostatic coating (Figure 2.13). These three powders have the highest resistivities, greater than $10^{10}$ ohm*m (Figure 2.11). Others have also found electrostatic coating significantly improved adhesion for proteins (Halim and Barringer 2006; Mayr and Barringer 2006; Sumawi and Barringer 2005).

The electrostatic transportation loss increased with increasing RH for powders with high resistivity (Figure 2.13). As RH increases, powder resistivity decreases, causing a decrease in the electrostatic adhesion. Resistivity of all of the powders decreased with increasing RH (Figure 2.11). Powders pick up water as relative humidity increases, which decreases powder resistivity and increases charge decay rate (Grosvenor and Staniforth 1996; Sharma and others 2001; Sharma and others 2003). Thus, the charge retained on the powders decreased with increasing relative humidity, resulting in a decrease in the electrostatic adhesion. The three proteins had the greatest decrease in resistivity with increasing RH (Figure 2.11) and thus, the greatest decrease in electrostatic adhesion. The electrostatic transportation loss of powders with low resistivity was small and not affected by relative humidity (Figure 2.14). If powder resistivity is low, powder has a fast
charge decay rate and retains less charge, producing low electrostatic adhesion (Bailey 1998). If the powder has low resistivity, less than $10^{10}$ ohm*m, the charge decay is rapid and the adhesion is so weak that the powder may not remain attached to the targets (Sims and others 2000). Since these powders have a resistivity lower than $10^{10}$ ohm*m, the improvement in adhesion was small, $\leq 5\%$. Corn starch has the highest resistivity of this set of powders and thus had the greatest improvement in adhesion with electrostatics.

Seasoning used in snack foods is usually a mixture of different flavoring powders, which can be as many as 20 different ingredients with particle sizes ranging from 20-500 $\mu$m. The different charging ability and particle size of powders provide different charge-to-mass ratio to each powder in the mixture. Powder mixture will separate during electrostatic coating due to the different charge-to-mass ratio of individual powder (Yousef and Barringer 2007). Separation should be aware of when applying electrostatic coating to a seasoning blend with wide range of charging abilities and particle sizes.

2.4.7 Positive vs. Negative electrostatic coating

There was no significant difference in electrostatic adhesion between positive and negative corona electrostatic coating for any of the tested powders. Sumawi and Barringer (2005) also found no significant difference in adhesion between positive and negative corona coating. There was also no significant difference between positive and negative electrostatic transfer efficiency for the powders, except soy protein and pork gelatin. Positive electrostatic coating produced higher transfer efficiency than negative electrostatic coating for soy protein and pork gelatin (Figure 2.12). Sumawi and Barringer
(2005) found that proteins tend to tribocharge positively and therefore produce higher transfer efficiency with positive than negative electrostatic coating.

2.5 Conclusions

Coating efficiency is critical to the success of the coating process, and is determined by three losses: targeting loss, coating loss, and transportation loss. Relative humidity had no effect on targeting loss in either nonelectrostatic or electrostatic coating. Increasing RH increased coating loss for powders with particle size ≥ 297 μm in nonelectrostatic coating. The large powders were free-flowing at 20% RH and became cohesive with increasing RH and formed clumps. Clumps rolled off the targets, resulting in high coating loss. Increasing RH had no effect on coating loss for powders with particle size ≤ 297 μm in nonelectrostatic coating. These powders were already cohesive at 20% RH, and the further increase in cohesiveness did not affect coating loss. For salts, electrostatic coating loss increased with increasing RH. The decrease in powder resistivity with increasing RH causes an increase in the charge decay rate, resulting in higher coating loss. For powders other than salts, whose resistivity is high, RH had no effect on electrostatic coating loss until 80% RH when resistivity and charge efficiency significantly decreased.

RH had no effect on transportation loss in nonelectrostatic coating, except when capillary forces dominated at high water contents in the air. Electrostatic transportation loss for proteins increased with increasing RH. Proteins have the highest resistivity among the powders. The decrease in resistivity with humidity increased transportation
loss. RH had no effect on electrostatic transportation loss for powders other than proteins and powdered sucrose.

There was no significant difference between positive and negative electrostatic transfer efficiency and adhesion, except for transfer efficiency of soy protein and pork gelatin. High positive tribocharging values of these two powders cause higher positive electrostatic transfer efficiency.

Among the three losses, transportation loss was frequently the biggest loss during the coating process. Electrostatic coating reduces targeting loss for small powders, coating loss at 20-60% RH, and transportation loss for powders with high resistivity. 20-60% RH is recommended for both nonelectrostatic and electrostatic coating.

2.6 References


Figure 2.1 Schematic diagram of experimental setup.
Figure 2.2 RH had no effect on targeting loss of 434 μm and 19 μm NaCl with oil coated targets.
Figure 2.3 RH increased nonelectrostatic coating loss of powder with a particle size \( \geq 297 \, \mu\text{m} \).
Figure 2.4 Cohesiveness of different food powders.
Figure 2.5 RH had no effect on nonelectrostatic coating for powders with a particle size ≤ 227 μm.
Figure 2.6 RH had no effect on nonelectrostatic transportation loss from 20-80% RH.
Figure 2.7 Capillary forces decreased nonelectrostatic transportation loss at high absolute humidity (high water content of the air).
Figure 2.8 Corona charging efficiency decreased with increasing RH.
Figure 2.9 Improvement in electrostatic transfer efficiency (TE) and electrostatic adhesion of NaCl (434 μm) over nonelectrostatic coating for constant target voltage vs. constant applied voltage.
Figure 2.10 Decrease in coating loss with the use of electrostatics for NaCl and KCl became lower with increasing RH.
Figure 2.11 Powder resistivity of proteins decreased largely with increasing RH.
Figure 2.12 Effect of RH on the decrease in coating loss with the use of electrostatics for powders with high resistivity.
Figure 2.13 Effect of RH on the improvement in transportation loss with the use of electrostatics for proteins and sucrose, which have high resistivity.
Figure 2.14 Effect of RH on the improvement in transportation loss with the use of electrostatics for powders with low resistivity.
CHAPTER 3

THE EFFECT OF TEMPERATURE ON LIPID RELATED VOLATILE PRODUCTION IN TOMATO PUREE

3.1 Abstract

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

Aroma is one of the most important quality attributes of tomato (Dirinck and others 1977). There are over 400 aroma volatiles identified in tomato (Petro-Turza 1987), but only a dozen are critical to the characteristic tomato aroma, most of which are derived from the lipoxygenase pathway (Buttery and others 1987). The lipoxygenase pathway is ubiquitous in fruits and vegetables, and refers to sequential reactions breaking down free fatty acids by an enzyme cascade involving lipoxygenase (LOX), hydroperoxide lyase (HPL), alcohol dehydrogenase (ADH) and possibly other enzymes (Stone and others 1975, Luning and others 1995). The widely accepted lipoxygenase pathway was first proposed by Stone and others (Stone and others 1975) using \(^{14}\text{C}\) labeling technology.

Tomato acyl lipids (phospholipid, galactolipids, and triacylglycerols) are first broken down by acyl hydrolase enzymes to free fatty acids, such as linoleic acid and linolenic acid, which are quickly converted into hexanal and \(\text{cis}-3\)-hexenal, respectively, by lipoxygenase (LOX) and hydroperoxide lyase (HPL). \(\text{cis}-3\)-Hexenal is transformed to \(\text{trans}-2\)-hexenal by \(\text{cis}/\text{trans}-2\) isomerase (Z3/E2-ISO). These aldehydes are further transformed into alcohols by the action of alcohol dehydrogenase (ADH).

A more complicated lipoxygenase pathway has been reported in other fruits and vegetables. There are other minor volatiles produced from the lipoxygenase pathway besides these major C6 aldehydes and alcohols. 10-Hydroperoxides and 12-hydroperoxides produced from rearrangement of 9- and 13-hydroperoxides degrade to \(\text{trans}-2\)-octenal and \(\text{trans}-2\)-heptenal respectively by unknown enzymes in bell peppers, and to 1-octen-3-ol in mushrooms (Luning and others 1995). 9-Hydroperoxides of
linoleic acid produce 2-pentylfuran and \textit{trans}-2-nonenal in cucumbers and bell peppers (Luning and others 1995). 13-Hydroperoxides of linolenic acid are also the precursors of 1-penten-3-one and \textit{trans}-2-pentenal in soybeans and bell peppers (Luning and others 1995).

Most studies on the enzymatic generation of tomato volatiles are carried out at room temperature, thus there is limited information about the effect of temperature on volatile generation, though the activity of enzymes is known to be temperature-dependent. The activity of crude tomato LOX increases from 0 to 20°C (Jadhav and others 1972), and is at its peak at 20-30°C, after which the activity decreases gradually (Yilmaz 2001). In addition to its effect on enzyme activity, temperature also affects the partition coefficient of volatiles. The partition coefficient of lipid oxidation generated aldehydes increases from 20 to 40°C (Hall and Andersson 1983).

A variety of analytical techniques have been developed for tomato volatile identification and quantification, such as gas chromatography-mass spectrometry (GC/MS), atmospheric pressure ionization mass spectrometry (API-MS), and proton transfer reaction mass spectrometry (PTR-MS) (Brauss and others 1998, Beltran and others 2006). However, these techniques either have difficulty monitoring real-time release of volatiles (GC/MS) or cannot differentiate isomers (API-MS and PTR-MS), such as \textit{cis}-3-hexenal and \textit{trans}-2-hexenal in tomato (Boukobza and others 2001). Selected ion flow tube mass spectrometry (SIFT-MS) allows real-time analysis of complex mixtures of volatile compounds without trapping or pre-concentration, and also provides differentiation of some isomers (Spanel and Smith 1999). SIFT-MS has been
applied in the analysis of breath volatiles for medical diagnosis and therapeutic monitoring (Smith and others 2003), detection of bacterial metabolites (Scotter and others 2005), monitoring air pollution (Smith and Spanel 1996), monitoring real-time release of volatiles in cut onion, crushed garlic and ripe banana (Spanel and Smith 1999) and monitoring olive oil oxidation (Davis and others 2007).

The objective of this study is to detect and quantify the level of volatiles generated from the lipoxygenase pathway in the grape tomato, and determine how temperature affects the volatile generation, using selected ion flow tube mass spectrometry (SIFT-MS).

3.3 Materials and Methods

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

3.3.1 Analysis of volatiles with enzyme activity

Grape tomato (150 g) was blended for 30 s at the highest speed in a blender (Waring, Dynamics Corp, USA). Tomato puree (60 ml) was transferred into a 500 ml pyrex bottle with minimal delay (typically 30 sec), and the rest was discarded. Selected ion flow tube mass spectrometry (SIFT-MS) (SYFT Voice100, Syft Ltd, Christchurch, New Zealand) was used to detect and quantify volatile compounds released from grape tomatoes.
The SIFT-MS instrument used for real-time analysis of tomato volatiles has been elaborated previously (Smith and Spanel 2005). The headspace volatile compounds were sampled directly by piercing the septa on a 500 ml Pyrex bottle with a 14-gauge passivated needle connected to the SIFT-MS. The needle point was 16 cm above the bottom of the bottle. A second long 14-gauge syringe needle pierced the septa to touch the bottom to maintain the pressure in the bottle at atmosphere pressure and also to provide oxygen for lipid oxidation. Analysis of volatile compounds in the headspace above the tomato puree was started immediately and continued for 60 min. The Pyrex bottles with tomato puree were held in a temperature controlled water bath at 4, 23 or 37°C during testing and triplicate headspace analyses were carried out. The background for room air was subtracted from the data. The ratio of the concentration of volatiles between different temperatures was compared using the concentration at the peak level during the 60 min.

Analysis was performed using selected ion mode (SIM) scans, and the concentration of volatile compounds was calculated using known kinetic parameters (Table 3.1) (Spanel and Smith 1997, Spanel and others 1997, Spanel and Smith 1998, Spanel and others 2002). Using the pre-determined reaction rate constant for the volatile with that precursor ion, and accounting for dilution of the sample gas into the carrier gas, the absolute concentration of the volatile was calculated (Smith and Spanel 1996). The soft chemical ionization used in SIFT-MS yields a smaller range of product ions than is common in electron impact mass spectrometry (as used by gas chromatography mass spectrometry (GC-MS), for example). Hence the need for gas chromatographic
separation of the sample is circumvented. However, the measured product mass produced by reaction with one of the three reagent ions, must be carefully chosen. Many of the masses produced are produced by several different volatiles, which creates an interference that must be removed or the results must be reported as a mixture. *trans*-2-Hexenal has an interference with *cis*-3-hexenal at m/z 99, 117, and 135. At these m/z, 35% of *cis*-3-hexenal and 100% of *trans*-2-hexenal are measured (Spanel and others 1997). However, since *cis*-3-hexenal has no interference at the m/z at which it was measured, 35% of *cis*-3-hexenal was subtracted from the concentration of *trans*-2-hexenal. Also *cis*-3-hexenol and *trans*-2-hexenol had irresolvable interferences and were reported as a mixture. Concentrations were reported in ppb.

### 3.3.2 Analysis of partition coefficient without enzyme activity

In order to compare the partition coefficient of different volatiles, the grape tomatoes were held at 4, 23 or 37°C, and prepared as in the analysis of volatiles of different aroma compounds with enzyme activity. The enzymes in the puree were allowed to be active for 5 min, and then 60 ml of saturated CaCl₂ solution was added to denature the enzymes and to inhibit further volatile generation. The puree was equilibrated and analyzed at 4, 23 or 37°C in triplicates. In order to minimize the effect of test order on results, the first replicate was tested in the order of 4, 23 and 37°C, the second replicate was 37, 23, and 4°C, and the third replicate was 23, 4, and 37°C.
3.3.3 Statistical analysis

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

3.4 Results and Discussion

3.4.1 Effect of temperature on linoleic acid related volatiles

3.4.1.1 Hexanal

Hexanal is associated with the “green” aroma of tomato (Kazeniac and Hall 1970). Due to its low threshold value, 4.5 ppb in aqueous solution, hexanal is a strong component of fresh tomato aroma (Petro-Turza 1987). After blending, the headspace concentration of hexanal increased and reached a peak level, then decreased (Figure 3.1). This release pattern was also found in previous studies (Brauss and others 1998, Boukobza and others 2001). In the first few minutes after tomato tissue disruption, volatiles are generated and accumulate due to enzyme activity. The rate of release of the volatiles from the tomato puree into the headspace, which is affected by the volatiles’ generation rate and volatility, is higher than the rate of loss caused by degradation and removal during sampling. After reaching a peak, the loss of volatiles is faster than the generation rate and the net concentration in the headspace decreases (Brauss and others 1998, Boukobza and others 2001).

The concentration of hexanal in the headspace increased from 4°C to 23°C (Figure 3.1). Two factors may be responsible for the increase: increase in partition coefficient and increase in the amount of volatiles generated by the enzymes. Partition coefficient
refers to the ratio of a certain volatile in gas phase and liquid phase, and is greatly
affected by matrix composition and temperature. For the same matrix, the partition
coefficient is highly dependent on temperature (Ruth and Roozen 2000). As temperature
increases, volatile molecules have more energy, thus, molecules escape more easily from
the medium, resulting in higher concentration in the gas phase. The partition coefficient
of pure lipid-oxidation-related aldehydes increases with increasing temperature in a
gas/water system (Hall and Andersson 1983). However, a determination made from one
matrix cannot be directly applied to another matrix (Hall and Andersson 1983), because
volatiles have different affinity to different matrices. The temperature dependence is
different for different volatiles due to the nature of the compounds’ molecular structure
(Hall and Andersson 1983).

The relative partition coefficient of compounds in the tomato puree was compared at
different temperatures. A saturated CaCl₂ solution was added to the blended tomato puree
to inactivate enzymes, and then tested at different temperatures. Since no additional
volatiles could be generated by the enzymes, the concentration of a volatile in the
headspace is determined by the partition coefficient. The partition coefficient of hexanal
in the tomato puree increased with increasing temperature (Table 3.2). The concentration
of hexanal with enzyme activity increased from 4 to 23°C in a ratio similar to the increase
in partition coefficient, so that the increased levels of hexanal can be explained by the
increased partition coefficient (Figure 3.1, Table 3.2).

The concentration of hexanal in the headspace decreased from 23°C to 37°C (Figure
3.1). The increase in partition coefficient of hexanal should increase the concentration
from 23°C to 37°C (Table 3.2). Instead, the increased temperature reduced the total amount of hexanal generated. Hexanal is derived from the oxidation of the free unsaturated fatty acids, linoleic and linolenic acid, which account for over 50% of the total free fatty acids in tomatoes (Gray and others 1999). Linoleic acid, 43.5% of the total free fatty acid, is the major precursor of hexanal (Gray and others 1999), and is converted by LOX to form 13-hydroperoxides, which are then cleaved to hexanal by HPL (Figure 3.2) (Gray and others 1999). Hexanal is also formed from the transformation of cis-3-hexenal, derived from linolenic acid, which is about 11% of the total free fatty acid (Stone and others 1975, Gray and others 1999). However, the conversion of cis-3-hexenal to hexanal is minimal and only accounts for 2% of the total hexanal, because hexanal formed from linoleic acid inhibits the formation of hexanal from linolenic acid (Stone and others 1975). Therefore, the linoleic acid pathway is the controlling factor in the formation of hexanal.

There are two possible causes for the reduction of hexanal production at 37°C: reduced enzyme activity and reduction of the availability of the 13-hydroperoxide precursor due to the presence of other pathways. The activity of tomato LOX on linoleic acid is optimum at 20-30°C (Jadhav and others 1972, Yilmaz 2001). At 37°C, the activity of tomato LOX on linoleic acid decreases to 40% of its optimum activity (Yilmaz 2001), which reduces the rate of hexanal generated at this temperature. If the value measured at 23°C is adjusted upward based on the partition coefficient differences shown in Table 2, and multiplied by a factor of 0.4 to account for the decrease in enzyme activity, the calculated line is close to the measured values at 37°C (Figure 3.1), thus, the reduced
enzyme activity is a major factor in the decreased concentration of hexanal in the headspace.

Reduction of the amount of 13-hydroperoxide precursor available to be converted into hexanal, due to the presence of other pathways, may be another reason for the reduced hexanal in the headspace at 37 °C (Figure 3.1). In addition to the linoleic acid to hexanal pathway confirmed in tomatoes (Stone and others 1975), there are other possible pathways for linoleic acid. Linoleic acid is first broken down by tomato LOX to produce 9-hydroperoxides (96%) and 13-hydroperoxides (1%) (Suurmeijer and others 1998). 18O labelled linoleate hydroperoxides were shown to rearrange to form 9-, 10-, 12-, and 13-hydroperoxides in equal amounts when held at 40 °C (Chan and others 1979, Tressl and others 1981). Thus thermal rearrangement will create the 12- and 10-hydroperoxides necessary for other pathways.

3.4.1.2 trans-2-Heptenal

The ratio of trans-2-heptenal in the headspace at 4:23:37°C was 1:3:336 (Figure 3.3). The ratio of trans-2-heptenal in the headspace at 4:23:37°C without enzyme activity was 1:4:6 (Table 3.2). Thus, the increase in trans-2-heptenal between 4°C and 23°C can be explained by the increase in partition coefficient. However, the increase at 37°C was much higher than can be explained by the partition coefficient (Figure 3.3). When the enzyme activity was stopped by adding a saturated CaCl₂ solution, the concentration of trans-2-heptenal in the headspace increased only 6-fold at 37°C, whether through increased partition coefficient or thermal generation of the compounds (Table 3.2), however, when enzymes were present, the increase was 336 times.
trans-2-Heptenal is known to increase after tomato tissue disruption due to the activity of unknown enzymes (Buttery and Ling 1993b). The addition of LOX and ADH lower the trans-2-heptenal in the headspace above tomato homogenates (Yilmaz and other 2002), likely because they encourage the 13-hydroperoxides pathway, which depletes the precursors for trans-2-heptenal. In green bell pepper homogenates, the addition of linoleic acid doubled the concentration of trans-2-heptenal in the headspace due to cleaving of 12-hydroperoxides by an unidentified enzyme (Luning and others 1995). It is likely that an enzyme with a similar function also exists in the tomato lipoxygenase system, possibly an isomer of HPL. If at 37˚C, rearrangement of the hydroperoxides of linoleic acid occurred and the activity of 12-hydroperoxide-specific HPL significantly increased, the total amount of trans-2-heptenal would greatly increase. This would also contribute to the total amount of enzymatically generated hexanal decreasing at 37˚C, because more of the precursor hydroperoxides would be converted to trans-2-heptenal, instead of hexanal.

3.4.1.3 trans-2-Octenal

The concentration of trans-2-octenal also greatly increased in the headspace at 37˚C, and may represent another pathway important at higher temperatures (Figure 3.4). The ratio of trans-2-octenal at 4:23:37˚C without enzyme activity was 1:3:7 (Table 3.2), and was 1:3:21 with enzyme activity (Figure 3.4). The increase of trans-2-octenal in the headspace from 4˚C to 23˚C can again be explained by the increased partition coefficient, but not the increase at 37˚C. At 37˚C, the increase of trans-2-octenal in the headspace was also likely due to an increase in volatile generation. trans-2-Octenal is a lipid related volatile (Yilmaz 2001), and produces a sweet and phenolic aroma with a low threshold of
3 ppb (Stevens and others 1977). The level of trans-2-octenal increases when the fatty acids are increased by altering the gene expression in tomato (Wang and others 1996). The enzymatic generation of trans-2-octenal has also been reported in soybean and bell pepper, from decomposition of 10-hydroperoxides produced by the rearrangement of hydroperoxides of linoleic acid (Luning and others 1995). The pathway may also exist in tomato, and is another alternate pathway for the precursors to hexanal.

3.4.1.4 Hexanol

The concentration of hexanol in the headspace increased with increasing temperature, proportional to what was expected based on the increased partition coefficient (Figure 3.5, Table 3.2). Hexanol is formed from hexanal by the action of alcohol dehydrogenase (ADH) (Prestage and others 1999). The optimum pH for ADH is near neutrality (Longhurst and others 1990), and the pH of tomato puree is acidic (typically pH 4.0-4.5) (Prestage and others 1999), thus, the ADH activity in tomato puree is low.

3.4.2 Effect of temperature on linolenic acid related volatiles

3.4.2.1 cis-3-Hexenal

cis-3-Hexenal is characterized as a pleasant “green” aroma, and has an extremely low threshold of 0.25 ppb (Petro-Turza1987). It is a characteristic volatile for the fresh aroma of tomato (Kazeniac and Hall 1970). The concentration of cis-3-hexenal in the headspace increased with increasing temperature (Figure 3.6). The ratio of cis-3-hexenal in the headspace at 4:23:37˚C was 1:19:62 without enzyme activity (Table 3.2), but only 1:6:10 with enzyme activity (Figure 3.6). When enzymes were active, the concentration was
lower than when they were not present, thus, the generation of cis-3-hexenal must decrease at higher temperatures. cis-3-Hexenal is the decomposition product of 13-hydroperoxides of linolenic acid (Figure 3.2). There are alternative pathways for 13-hydroperoxides to generate both trans-2-pentenal and 1-penten-3-one, which would reduce the 13-hydroperoxides available for cis-3-hexenal generation (Figure 3.2).

3.4.2.2 trans-2-Pentenal

The ratio of trans-2-pentenal in the headspace without enzyme activity at 4:23:37°C was 1:6:18, and with enzyme activity was 1:4:105 (Table 3.2, Figure 3.7). At 37°C, the total amount of trans-2-pentenal was much higher than can be explained by the increase in partition coefficient. trans-2-Pentenal appears after tomato tissue disruption, when enzyme activity is the highest (Buttery and Ling 1993b). In soybean and bell pepper, 13-hydroperoxides of linolenic acid, the precursors of cis-3-hexenal, are broken down to form trans-2-pentenal (Luning and others 1995, Grosch and Laskawy 1975). This branch pathway may also exist in tomatoes, and may be greatly affected by temperature. At room temperature and lower, 13-hydroperoxides may be mainly metabolized through the major pathway to form cis-3-hexenal. But as temperature increases to 37°C, the activity of trans-2-pentenal specific HPL may reach its optimum, producing a large amount of trans-2-pentenal (>1000 ppb) (Figure 3.7), while also significantly reducing the amount of cis-3-hexenal formed.
3.4.2.3 1-Penten-3-one

The concentration of 1-penten-3-one in the headspace also increased with increasing temperature (Figure 3.8). The ratio of 1-penten-3-one in the headspace without enzyme activity at 4:23:37°C was 1:4:5, and with enzyme activity was 1:3:5 (Table 3.2, Figure 3.8), which means the increase in concentration can be explained by the increased partition coefficient. The concentration of 1-penten-3-one in the headspace is known to increase after tomato tissue disruption (Buttery and Ling 1993b), and the addition of LOX and ADH into tomato homogenates decreases the concentration of 1-penten-3-one (Yilmaz and others 2002), but even though the 1-penten-3-one pathway will consume a certain amount of 13-hydroperoxides, the generation of 1-penten-3-one would not noticeably affect cis-3-hexenal because the amount formed is small.

3.4.2.4 trans-2-Hexenal

Cis-3-Hexenal is isomerized to the more stable trans-2-hexenal by Z3/E2-ISO in the tomato puree, and then is further degraded to alcohols (Figure 3.2) (Stone and others 1975). Similar to cis-3-hexenal, the ratio of the trans-2-hexenal without enzyme activity at 4:23:37°C was 1:16:56, but with enzyme activity was 1:13:21 (Table 2, Figure 9). There was less trans-2-hexenal when enzymes were present than when they weren’t. Less cis-3-hexenal available to be converted to the trans form may be responsible the less amount of trans-2-hexenal.
3.4.2.5 *cis*-3-Hexenol and *trans*-2-Hexenol

* cis*-3-Hexenol and *trans*-2-hexenol are derived from *cis*-3-hexenal and *trans*-2-hexenal respectively by ADH. The concentration of the mixture of *cis*-3-hexenol and *trans*-2-hexenol is low, below 25 ppb, and the increase with temperature can be explained by the increased partition coefficient. The concentration of *cis*-3-hexenol and *trans*-2-hexenol is typically low in tomato (Yilmaz and others 2002).

3.4.3 The effect of temperature on rate of production of lipid-related volatiles

At 4°C, most lipid related volatiles reached their maximum level within 5 min, and there was no significant difference in time to reach the maximum level, except for hexanal, which peaked significantly later at about 12 min (Table 3.3). At this temperature, the activity of hexanal specific LOX is below its optimum activity (Jadhav and others 1972, Yilmaz 2001), so the generation rate of hexanal is low, and it takes a longer time to reach its peak level.

At 23°C, the increase in temperature did not significantly change the time to reach the maximum level for most volatiles, except for hexanal (Table 3.3). The time for hexanal to reach its maximum level was significantly shortened, so that it was not significantly different from the other volatiles (Table 3.3). The increase in enzyme activity at 23°C significantly increases the generation rate. The concentration of *cis*-3-hexenal in tomato was also found by others to reach its maximum level at about 3 min after blending, and then decrease gradually due to further degradation at room temperature (Boukobza and others 2001, Buttery and Ling 1993a).
At 37˚C, the increase in temperature did not significantly change the time to reach the maximum level for most volatiles (Table 3.3). However, the volatiles with significant formation from branch reactions at this temperature, such as trans-2-heptenal and trans-2-pentenal, peaked significantly later than the other volatiles (Table 3.3). trans-2-Pentenal and trans-2-heptenal took longer to reach the peak level either because the activity of the enzyme controlling the reaction is slow, or the thermal arrangement of 13-hydroperoxides to the 10- and 12-hydroperoxide precursors is slow.

3.5 Conclusion

Lipid volatiles in the headspace increase after tomato tissue disruption, and reach a maximum level within 10 min, then decrease due to air dilution and degradation. Although volatiles in the headspace decreased after reaching a maximum level, the remaining concentration was still significantly above the threshold for many of the characteristic tomato volatiles 60 min after blending, thus long processing times do not destroy tomato aroma. However, a short processing time, within 10 min, to retain maximum volatiles is recommended for tomato products to produce the freshest aroma.

3.6 References


104


Table 3.1 Information for SIFT-MS analysis of selected volatile compounds.

<table>
<thead>
<tr>
<th>Volatile Compound</th>
<th>Molecular formula</th>
<th>Precursor Ion</th>
<th>k (10⁻⁹ cm³ s⁻¹)</th>
<th>m/z</th>
<th>Ion Responsible</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Penten-3-one</td>
<td>C₅H₈O</td>
<td>NO⁺</td>
<td>2.5</td>
<td>144</td>
<td>[C₅H₆O-NO]⁺</td>
<td>Syft Technologies 2009</td>
</tr>
<tr>
<td>cis-3-Hexenal</td>
<td>C₆H₁₀O</td>
<td>H₃O⁺</td>
<td>4.2</td>
<td>81</td>
<td>[C₆H₆]⁺</td>
<td>Spanel and others 1997</td>
</tr>
<tr>
<td>Hexenol(cis-3-Hexenol and trans-2-Hexenol)</td>
<td>C₆H₁₂O</td>
<td>NO⁺</td>
<td>2.5</td>
<td>72</td>
<td>[C₆H₆]⁺</td>
<td>Syft Technologies 2009</td>
</tr>
<tr>
<td>Hexanal</td>
<td>C₆H₁₂O</td>
<td>NO⁺</td>
<td>3.7</td>
<td>99</td>
<td>[C₆H₁₁O]⁺</td>
<td>Spanel and others 1997</td>
</tr>
<tr>
<td>Hexanol</td>
<td>C₆H₁₄O</td>
<td>NO⁺</td>
<td>2.4</td>
<td>101</td>
<td>[C₆H₁₃O]⁺</td>
<td>Spanel and Smith 1997</td>
</tr>
<tr>
<td>trans-2-Heptenal</td>
<td>C₇H₁₂O</td>
<td>NO⁺</td>
<td>3.9</td>
<td>111</td>
<td>[C₇H₁₁O]⁺</td>
<td>Spanel and others 2002</td>
</tr>
<tr>
<td>trans-2-Hexenal</td>
<td>C₆H₁₀O</td>
<td>H₃O⁺</td>
<td>4.6</td>
<td>99</td>
<td>[C₆H₁₁O]⁺</td>
<td>Spanel and others 1997</td>
</tr>
<tr>
<td>trans-2-Hexenal</td>
<td>C₆H₁₀O</td>
<td>H₃O⁺</td>
<td>4.6</td>
<td>117</td>
<td>[C₆H₁₁O+H₂O]⁺</td>
<td>Spanel and others 1997</td>
</tr>
<tr>
<td>trans-2-Hexenal</td>
<td>C₆H₁₀O</td>
<td>H₃O⁺</td>
<td>4.6</td>
<td>135</td>
<td>[C₆H₁₁O+2H₂O]⁺</td>
<td>Spanel and others 1997</td>
</tr>
<tr>
<td>trans-2-Octenal</td>
<td>C₈H₁₄O</td>
<td>NO⁺</td>
<td>3.9</td>
<td>156</td>
<td>[C₈H₁₄O-NO]⁺</td>
<td>Spanel and others 2002</td>
</tr>
<tr>
<td>trans-2-Pentenal</td>
<td>C₅H₈O</td>
<td>NO⁺</td>
<td>3.9</td>
<td>83</td>
<td>[C₅H₆O]⁺</td>
<td>Spanel and others 2002</td>
</tr>
</tbody>
</table>
Table 3.2 Ratio of volatiles in the headspace of grape tomato puree at different temperatures without enzyme activity.

<table>
<thead>
<tr>
<th>Volatiles</th>
<th>4 °C</th>
<th>23 °C</th>
<th>37 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Penten-3-one</td>
<td>1 ( ^a )</td>
<td>4 ( ^b )</td>
<td>5 ( ^c )</td>
</tr>
<tr>
<td>( cis-3 )-Hexenal</td>
<td>1 ( ^a )</td>
<td>19 ( ^b )</td>
<td>62 ( ^c )</td>
</tr>
<tr>
<td>( cis-3 )-Hexenol and ( trans-2 )-Hexenol</td>
<td>1 ( ^a )</td>
<td>2 ( ^a )</td>
<td>5 ( ^b )</td>
</tr>
<tr>
<td>Hexanal</td>
<td>1 ( ^a )</td>
<td>15 ( ^b )</td>
<td>19 ( ^c )</td>
</tr>
<tr>
<td>Hexanol</td>
<td>1 ( ^a )</td>
<td>14 ( ^b )</td>
<td>33 ( ^c )</td>
</tr>
<tr>
<td>( trans-2 )-Heptenal</td>
<td>1 ( ^a )</td>
<td>4 ( ^a )</td>
<td>6 ( ^b )</td>
</tr>
<tr>
<td>( trans-2 )-Hexenal</td>
<td>1 ( ^a )</td>
<td>16 ( ^b )</td>
<td>56 ( ^c )</td>
</tr>
<tr>
<td>( trans-2 )-Octenal</td>
<td>1 ( ^a )</td>
<td>3 ( ^b )</td>
<td>7 ( ^c )</td>
</tr>
<tr>
<td>( trans-2 )-Pentenal</td>
<td>1 ( ^a )</td>
<td>6 ( ^b )</td>
<td>18 ( ^c )</td>
</tr>
</tbody>
</table>

Values with different superscripts at the different temperature are significantly different.
Table 3.3 Time (min) to reach maximum level for lipid related volatiles from grape tomato after blending.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>cis-3-Hexenal</th>
<th>trans-2-Hexenal</th>
<th>1-Penten-3-one</th>
<th>Hexanal</th>
<th>Hexanol</th>
<th>trans-2-Octenal</th>
<th>trans-2-Heptenal</th>
<th>trans-2-Pentenal</th>
<th>cis-3-Hexenol and trans-2-Hexenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>4°C</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt; A</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt; A</td>
<td>4.3&lt;sup&gt;a&lt;/sup&gt; A</td>
<td>12.7&lt;sup&gt;b&lt;/sup&gt; B</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>23°C</td>
<td>3.3&lt;sup&gt;a&lt;/sup&gt; A</td>
<td>3.7&lt;sup&gt;a&lt;/sup&gt; A</td>
<td>3.0&lt;sup&gt;a&lt;/sup&gt; A</td>
<td>4.7&lt;sup&gt;a&lt;/sup&gt; A</td>
<td>3.7&lt;sup&gt;a&lt;/sup&gt; A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>37°C</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt; A</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt; A</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt; A</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt; A</td>
<td>5.3&lt;sup&gt;ab&lt;/sup&gt; A</td>
<td>5.3&lt;sup&gt;b&lt;/sup&gt; A</td>
<td>7.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Values with different superscripts at the same temperature are significantly different.
Values with different subscripts for the same compound are significantly different.
N/A: No detectable peak at this temperature
Figure 3.1 The effect of temperature on hexanal generation.
Figure 3.2 Temperature dependent lipoxygenase pathway of linolenic and linoleic acids in tomato.
Abbreviations: LOOH: hydroperoxide; [LOX]: Tomato lipoxygenase; [HPL]: Hydroperoxide lyase; [ADH]: Alcohol dehydrogenase; [Z3/E2-iso]: cis-3/trans-2 isomerase; [E?]: Unknown enzyme; [E?+H]: Unknown enzyme plus heat. Asterisked compounds: Significant formation at 37˚C.
Figure 3.3 Effect of temperature on *trans*-2-heptenal generation.
Figure 3.4 Effect of temperature on *trans*-2-octenal generation.
Figure 3.5 The effect of temperature on hexanol generation.
Figure 3.6 The effect of temperature on cis-3-hexenal formation.
Figure 3.7 The effect of temperature on \textit{trans}-2-pentenal generation.
Figure 3.8 The effect of temperature on 1-penten-3-one generation.
Figure 3.9 The effect of temperature on trans-2-hexenal formation.
CHAPTER 4

COMPARISON OF REAL-TIME VOLATILE RELEASE IN TOMATILLO AND TOMATO DURING CHEWING BY SELECTED ION FLOW TUBE MASS SPECTROMETRY (SIFT-MS)

4.1 Abstract

The release of volatiles from tomatillo and tomatoes in the mouthspace and nosespace was measured in real-time using selected ion flow tube mass spectrometry (SIFT-MS). cis-3-Hexenal, trans-2-hexenal, hexanal, and 1-penten-3-one increased, while isobutyl alcohol, nonanal and methylbutanal showed no significant change in the first 30 s of chewing. Cherry tomato released more trans-2-hexenal, cis-3-hexenal, and 1-penten-3-one than tomatillo, roma tomato and vine-ripened tomato during chewing. The ratio of the average concentration of volatiles in the mouthspace after swallowing to before swallowing (MSas/MSbs) varied from 2.8 to 73.9%. Methylbutanal, hexanal, and nonanal were retained at a higher percentage in the mouth after swallowing than cis-3-hexenal, trans-2-hexenal, 1-penten-3-one and isobutyl alcohol. The mouthspace to headspace (MS/HS) ratio of 1-penten-3-one, hexanal, methylbutanal, and nonanal, and nosespace to headspace (NS/HS) ratio for 1-penten-3-one, hexanal, cis-3-hexenal, and
nonanal was significant higher in tomatillo than in tomatoes. There was no difference between tomatoes of different varieties in NS/HS ratio.

### 4.2 Introduction

The tomatillo (*Physalis ixocarpa Brot*) is a solanaceous fruit originating in Mexico. It is very similar to the green tomato in appearance, except for featuring a paper-like husk formed from the calyx. The tomatillo is a major ingredient in green sauces and salsas in Mexican cuisine, and also is used to make jam in Australia (Morton 1987). It has been adopted in a variety of recipes to create Latin American flavor themes (McGorrin and Gimelfarb 1998).

Tomatillo produces a strong green flavor note, which is similar to tomato, but lacks the typical tomato viney and sulfur notes when evaluated by sensory evaluation (McGorrin and Gimelfarb 1998). There are over 400 volatile compounds identified in tomato, of which 30 have been shown to contribute to the characteristic aroma of tomatoes (Petro-Turza 1987). In contrast to extensive studies that have been done on tomato volatiles, there is limited information about the flavor profile of tomatillo. Only a total of 52 volatile compounds have been identified in tomatillo, and of these, 6 volatiles, *cis*-3-hexenal, *trans*,*trans*-2,4-decadienal, nonanal, hexanal, hexanol, and *cis*-3-hexenol, are reported to be important to tomatillo aroma (McGorrin and Gimelfarb 2001). A comparison of the volatile profile between tomatillo and red plum tomato using GC/MS revealed that some important tomato volatiles, such as 2-isobutylthiazole, nitrophenelethane, and phenylacetonitrile, are not detected in tomatilloes (McGorrin and Gimelfarb 1998). Compounds unique to tomatillo flavor, as compared to tomatoes, are
hydroxy esters, aromatic esters, 8- to 12-carbon aldehydes, decanoic acid and terpenes (McGorrin and Gimelfarb 1998).

Most aroma studies focus on headspace analysis. However, volatile release is more complicated in the mouth. The structure and composition of the food, the temperature and pH of the mouth, saliva flow, the rate at which the food is broken down and mixed during chewing, breathing, swallowing, and losses due to adsorption through the airways all affect the volatile release in the mouth (Piggott and Schaschke 2001). Moreover, some volatiles are generated when the food is eaten, which adds complexity to volatile analysis (Linforth and others 1994). Direct mouth and nose tests better represent the volatiles reaching the olfactory epithelium (Piggott and Schaschke 2001). Different tomato types contained significantly different concentrations of each compound in nosespace tests (Brauss and others 1998).

Real-time release of volatiles during eating was previously analyzed by using atmospheric pressure ionization mass spectrometry (API-MS) and proton transfer reaction mass spectrometry (PTR-MS) (Hodgson and others 2003). However, they cannot differentiate isomers, such as cis-3-hexenal and trans-2-hexenal (Boukobza and others 2001). Selected ion flow tube mass spectrometry (SIFT-MS) allows real-time analysis of complex mixtures of volatile compounds without trapping or pre-concentration, and also provides differentiation of some isomers (Spanel and Smith 1999). It has been successfully used to monitor real-time release of volatiles in cut onion, crushed garlic and ripe banana (Spanel and Smith 1999) and olive oil oxidation (Davis and McEwan 2007).
The objective of this study is to detect and compare volatile compounds released from tomatillos and tomatoes in the headspace, mouthspace, and nosespace using SIFT-MS. The retention of volatiles in the mouthspace after swallowing is also compared between tomatillos and tomatoes.

4.3 Materials and Methods
4.3.1 Materials

Tomatillos, vine-ripened tomatoes, roma tomatoes, cherry tomatoes, and grape tomatoes were purchased from local food supermarkets in Columbus OH, USA. Samples were washed and stored for 5 h at room temperature to equilibrate before testing. All testing was carried out at 23 °C.

4.3.2 Instrument and sampling

The selected ion flow tube-spectrometry (SIFT-MS) instrument (SYFT Voice100, Syft Ltd, Christchurch, New Zealand) was used for real-time analysis of tomato and tomatillo volatiles, using the method described by Xu and Barringer (2009). Analysis was performed using selected ion mode (SIM) scans, and the concentration of volatile compounds was calculated using known kinetic parameters (Table 4.1) (Spanel and others 1997; Spanel and others 2002; Spanel and Smith 1997; Syft Technologies 2009). Using the pre-determined reaction rate constant for the volatile with that precursor ion, and accounting for dilution of the sample gas into the carrier gas, the absolute concentration of the volatile was calculated (Smith and Spanel 1996). Numerous studies have quantified concentrations, but in this study it is the comparison of values over time.
and between samples that is most important. The soft chemical ionization used in SIFT-MS yields a smaller range of product ions than is common in electron impact mass spectrometry (as used by gas chromatography mass spectrometry (GC-MS), for example). Hence the need for gas chromatographic separation of the sample is circumvented. However, the measured product mass produced by reaction with one of the three reagent ions must be carefully chosen. Many of the masses produced are produced by several different volatiles, which creates an interference that must be removed or the results must be reported as a mixture. *trans*-2-Hexenal has an interference with *cis*-3-hexenal at \( m/z \) 99, 117, and 135. At these \( m/z \), 35% of *cis*-3-hexenal and 100% of *trans*-2-hexenal are measured (Spanel and others 1997). However, since *cis*-3-hexenal has no interference at the \( m/z \) at which it was measured, 35% of *cis*-3-hexenal was subtracted from the concentration of *trans*-2-hexenal. Also 2-methylbutanal and 3-methylbutanal have irresolvable interferences and are reported as a mixture (methylbutanal). Concentrations are reported in ppb.

### 4.3.3 Headspace volatile analysis

The headspace volatile compounds were sampled directly by piercing the septa on a 500 ml Pyrex bottle with a 14-gauge passivated needle connected to the SIFT-MS. The needle point was 16 cm above the bottom of the bottle. A second long 14-gauge syringe needle pierced the septa to touch the bottom to maintain the pressure in the bottle at atmosphere pressure and also to provide oxygen for lipid oxidation. Tomatoes or tomatillos were blended for 30 sec at the highest speed using a blender (Waring, Dynamics corp, USA). Puree (60 ml) was transferred into a 500 ml pyrex bottle with
minimal delay (typically 30 sec). NaCl (3 g) (Alberger flake salt, Cargill Incorporated, Minneapolis, MN USA) was added into the pyrex bottle to increase the amount of volatile compounds in the headspace. A magnetic stirrer (PC-351, Corning Incorporated, Corning, NY, USA), was used at the highest speed to stir the puree during sampling. The average concentration of volatile compounds above the headspace of puree in the first minute was measured in triplicate.

### 4.3.4 Mouthspace and nosespace volatile analysis

The volatile compounds in the mouthspace and the nosespace were sampled by piercing the middle of an 8 cm Teflon coated plastic tube with the passivated sampling needle, and exhaling into the tube. A portion of tomatillo or tomato (20±1 g) was cut from an intact fruit. The sample was placed into the mouth with minimal delay. The sample was chewed for the first 15 sec, during which period no exhale or swallow were allowed. The first exhale was made for 5 sec after the chewing period ended. No chewing or swallowing was allowed during the exhale. The exhale occurred 3 times every other 5 sec. Volatile compounds in the mouth were tested in this period. After 3 exhale-and-inhale cycles, the sampling tube was quickly switched from the mouth into the left nose hole. The right nose hole was blocked by hand to avoid volatile compound leakage. Three exhales were made through the left nose hole in the following 10 sec period. Three swallows were made in this period in order to expel volatile compounds into the nose. Volatile compounds in the nose were tested in this period. The sampling tube was then quickly switched back to the mouth to collect volatile compounds in the mouth again.
every other 5 sec until the end of the 135 sec test. Mouth and nose volatile analysis was performed by the same male age 28, and 6 replicates were performed on different fruits.

The average concentration in the mouth at 10, 20, and 30 sec and the average concentration of the first 1 min in the headspace were used to calculate the mouthspace to headspace (MS/HS) ratio. The ratio of the average concentration at 55, 65, 75, and 85 sec to the average concentration at 10, 20, and 30 sec represented the ratio of mouthspace after swallowing (MSas) to mouthspace before swallowing (MSbs).

### 4.3.5 Statistical analysis

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

### 4.4 Results and Discussion

#### 4.4.1 Real-time release of volatiles in the mouthspace

Seven volatiles that were detected at relatively high levels in the headspace of tomatillo and tomato puree were measured in the mouthspace. The concentration of cis-3-hexenal, *trans*-2-hexenal, hexanal, and 1-penten-3-one in the mouthspace increased during chewing for the first 30 sec in all of the samples, indicating generation of these compounds during chewing (Figure 4.1-4.5). These volatiles are generated by the lipoxygenase pathway. The chewing movement breaks intact tissue cells to release a series of enzymes in the lipoxygenase pathway, which react with the fatty acids to form aromatic aldehydes. Linoleic acid and linolenic acid in tomatillo and tomato are quickly
degraded to hexanal and cis-3-hexenal respectively due to the action of lipoxygenase (LOX) and hydroperoxide lyase (HPL) (Stone and others 1975). cis-3-Hexenal is further transformed to trans-2-hexenal by cis-3/trans-2 isomerase (Z3/E2-ISO), and 1-penten-3-one is derived from linolenic acid through a branch reaction of the lipoxygenase pathway (Luning and others 1995). cis-3-Hexenal, trans-2-hexenal, and hexanal are key volatiles of tomatillo and tomato, and produce a fresh “green” note (McGorrin and Gimelfarb 1997; Petro-Turza 1987).

Isobutyl alcohol, nonanal, and methylbutanal (mixture of 2-methylbutanal and 3-methylbutanal) showed no significant change in concentration in the first 30 sec of chewing for any of the samples (Figure 4.1-4.5). These volatiles are formed in the intact fruit cell (Boukobza and others 2001). Since there was no significant generation of these volatiles in the mouth, the concentration should remain unchanged during chewing, or decrease due to dilution effects. Isobutyl alcohol produces an ethyl acetate-like aroma (Kiritsakis 1998), and it is not one of key volatiles for either tomatillo or tomato (McGorrin and Gimelfarb 2001; Buttery 1993). 3-Methylbutanal produces a caramel aroma (van Ruth and Roozen 2000). Although 3-methylbutanal is not a critical volatile for tomatillos, it is one of the key volatiles for the fresh aroma of tomato McGorrin and Gimelfarb; Buttery 1993). Nonanal is one of the important volatiles of tomatillos, producing a citrus like aroma (Reiners and Grosch, 1998).

Cherry tomatoes and grape tomatoes released more trans-2-hexenal, cis-3-hexenal, and 1-penten-3-one than tomatillos, roma tomatoes and vine-ripened tomatoes during chewing (Figure 4.4, Figure 4.5). It was previously reported that the generation of
hexenals (a mixture of cis-3-hexenal and trans-2-hexenal) during chewing of cherry tomatoes is higher than in plum tomatoes and delice tomatoes (Brauss and others 1998). These three volatiles are linolenic acid derived, so it is possible that the linolenic acid specific lipoxygenase activity in cherry tomatoes and grape tomatoes is higher than in tomatilloes, roma tomatoes and vine-ripened tomatoes. Hexanal, derived from linoleic acid, showed significantly higher levels in cherry tomatoes and tomatilloes (Figure 4.1, Figure 4.5). It is possible that the linoleic acid specific lipoxygenase activity in tomatillo is on par with cherry tomato, but higher than other tomato varieties. The nonanal level released in the mouth for tomatillo, cherry tomato, and grape tomato was higher than for roma tomato and vine-ripened tomato. Grape tomato released higher methylbutanal than tomatillo and any other tomato. There was no significant difference in isobutyl alcohol release between the different varieties.

The lingering of the volatiles after swallowing affects the aftertaste of tomatilloes and tomatoes. The concentration of volatiles in the mouthspace decreased after swallowing, but to a different extent depending on the volatile (Figure 4.1-4.5). The ratio of the average concentration of volatiles in the mouthspace after swallowing to the mouthspace before swallowing (MSas/MSbs) varied from 2.8 to 73.9% (Table 4.2). The MSas/MSbs ratio of methylbutanal and nonanal for roma tomato were not able to be calculated, because these volatiles were too low to be detected. The MSas/MSbs ratio showed that hexanal, methylbutanal, nonanal, and isobutyl alcohol had a higher retention than 1-penten-3-one, cis-3-hexenal, and trans-2-hexenal. The lingering of volatiles in the mouthspace has been reported to be related to the air/water partition coefficient, and a
volatile with a higher air/water partition coefficient decays faster than a compound with low air/water partition coefficient (Hodgson and others 2004). However, the coefficient obtained by examining a pure volatile solution in the mouthspace may not correspond to the more complicated tomatillo and tomato puree system, and showed no correlation in this study (Table 4.2). Since cis-3-hexenal, trans-2-hexenal, hexanal and 1-penten-3-one are lipoxygenase generated and are no longer being generated after swallowing, this causes a disproportionately large decrease in these volatiles, which may be the major reason for their low MSas/MSbs ratio.

The MSas/MSbs ratio of cis-3-hexenal and trans-2-hexenal for roma tomato and vine-ripened tomato were higher than in tomatillo, cherry tomato, and grape tomato (Table 4.2). The concentrations of these two volatiles during chewing were lower in roma tomato and vine-ripened tomato, but the level after swallowing was similar for all the samples, thus the ratio was lower. Similarly, the MSas/MSbs ratio of hexanal for roma tomato was lower than tomatillo and other tomatoes and the concentration of hexanal was lower during chewing but similar after swallowing (Table 4.2). Since cis-3-hexenal, trans-2-hexenal and hexanal are lipid related volatiles, lipoxygenase activity may play an important part. There was no significant difference in MSas/MSbs ratio between tomatillo and the tomatoes for 1-penten-3-one, methylbutanal, nonanal, and isobutyl alcohol (Table 4.2). Most of these volatiles are not lipid related, so are not affected by differences in enzyme activity. Although 1-penten-3-one is lipid related, the generation is low, thus differences in enzyme activity have a smaller effect.
4.4.2 Comparison of mouthspace (MS) and headspace (HS) volatiles

The average concentration of each volatile in the mouthspace during chewing relative to the headspace concentration (MS/HS) of tomatillo and tomatoes was between 0.4 and 59.2% (Table 4.3). Clearly, the release of volatiles in the mouth is different from in the headspace. The release of volatiles in the mouthspace occurs under dynamic conditions, and is affected by many factors, such as temperature, hydration, surface area, enzymes, dilution, interaction with salivary components, volatile loss during mouth movement, physicochemical properties of volatiles, and different food matrices (Taylor and Linforth 1996; Diaz 2004). The amount of volatile in the mouthspace relative to the headspace varied considerably between volatiles, especially for tomatillos (Table 4.4). It was previously reported that the MS/HS ratio varies from 0.17 to 70% for a wide range of volatile compounds in aqueous solution (Linthor and others 2002). Volatiles with a low partition coefficient have a higher MS/HS ratio (Linthor and others 2002). However, a correlation was not found in this study. Linforth and others (2002) found the $R^2$ for the correlation between MS/HS and partition coefficient was 0.72, so other factors are also important. Interaction with salivary components may be one possible reason for the variation. Some volatiles have a higher interaction with salivary components in the mouth, whereas, certain volatiles have a low or no affinity to saliva. For example, adding mucin into aqueous solutions of some volatiles, such as decanal and heptanal, decreased the volatiles in the headspace, but had no effect on other volatiles, such as linalool and 2-methyl butanol (Friel and Taylor 2001). The difference in how volatiles are generated in the mouth versus a jar is another possible factor. It is possible that enzymatically
generated volatiles build up more or less in the headspace than mouthspace, resulting in a different MS/HS ratio.

The MS/HS ratio of 1-penten-3-one, hexanal, methylbutanal, and nonanal was significantly higher in tomatilloes than in any of the tomato varieties (Table 4.3). The concentration of 1-penten-3-one, hexanal, and methylbutanal was much lower in the headspace of tomatillo than the other tomatoes in this study, while the mouthspace values were similar. Thus, the higher MS/HS ratio in tomatillo may be due to its low pectin content. The presence of hydrocolloids affects the rate and the intensity of volatile release through two mechanisms: reduction of the diffusion of volatile compounds as viscosity of the solution increases, and reduction of volatile release caused by the specific binding interactions of volatile compounds with hydrocolloids through adsorption, entrapment and hydrogen bonding (Bylaite and Stahnke 2002). For example, the addition of pectin decreases the flavor intensity of strawberry jam (Guichard and others 1991). The mature tomatillo contains 1.1% pectin (Cantwell and others 1992), whereas, the pectin content in tomato ranges between 5-7% depending on variety and maturity (Hobson 1963). Tomato puree also visually showed a higher viscosity than tomatillo, which supports the hypothesis that the high pectin content in tomatoes increases the viscosity, traps more volatiles and slows the release rate, resulting in a lower MS/HS ratio than in the tomatillo. The MS/HS ratio of cis-3-hexenal and trans-2-hexenal were not higher in the tomatillo. These volatiles are generated at high levels, so enzyme activity may be more important than pectin content for these volatiles.
The MS/HS ratio of cis-3-hexenal, trans-2-hexenal, nonanal, and isobutyl alcohol was significantly higher in cherry tomatoes than in the other tomato varieties (Table 4.3). Since the concentrations of volatiles in the headspace were similar, more cis-3-hexenal and trans-2-hexenal were released from cherry tomatoes in the mouthspace than from grape, roma and vine-ripened tomatoes, which caused the high MS/HS ratio. It has been previously reported that the concentration of cis-3-hexenal and trans-2-hexenal in cherry tomatoes is higher than in plum and delice tomatoes during chewing, while hexanal and methylbutanal are not higher (Brauss and others 1998). Since both cis-3-hexenal and trans-2-hexenal are key volatiles responsible for a fresh “green” aroma, cherry tomato may produce a fresher and better aroma for consumers than other varieties. As to nonanal and isobutyl alcohol, the combination of higher level of volatiles in the mouth and lower level in the headspace were the cause of the high MS/HS ratio.

4.4.3 Comparison of nosespace (NS) and headspace (MS) volatiles

The nosespace to headspace (NS/HS) ratios for volatiles in tomatillo were below 50%, lower than the mouthspace to headspace (MS/HS) ratios (Table 4.4). The volatiles in the nosespace are what is actually perceived by the consumer, and is the closest measurement to actual perception. The difference between the NS/HS ratio and the MS/HS ratio indicates the loss of volatiles during the transportation of volatiles from the mouthspace to the nosespace. The volatiles pass through the posterior nares of the nasopharynx to reach the receptors located in the rear of the nasal cavity to be perceived during respiratory exhalation or after swallowing (Diaz 2004). Along the way, the volatile are absorbed and desorbed by olfactory mucosa, resulting in reduced delivery efficiency,
moreover, volatiles are also transported through the respiratory tract, which also causes loss (Dattatreya and others 2002).

The NS/HS ratio for 1-penten-3-one, hexanal, cis-3-hexenal, and nonanal was significant higher in tomatilloes than in tomatoes (Table 4.4). The volatiles in the nosespace are directly related to the volatiles in the mouthspace. Since the MS/HS ratio of 1-penten-3-one, hexanal and nonanal was higher in the mouthspace of tomatillo than in tomatoes, higher NS/HS ratio in tomatillo was expected. There was no significant difference in NS/MS ratio for volatiles between tomatoes of different varieties (Table 4.4). The volatiles are delivered into the nasal cavity by the chewing movement of the mouth, which injects flavored air into the exhaling air subsequently passing through the nasal cavity (Dattatreya and others 2002). In preliminary tests, volatiles were detected in the nosespace after swallowing, but not during chewing. There is a barrier between the mouth and pharynx, which functions to avoid food entering airways during eating (Hodgson and others 2003). The barrier is closed during chewing and prevents the volatiles entering into the nosespace. The barrier between mouthspace and nosespace opened during swallowing, which is in accord with previous studies (Buettner and others 2001).

4.5 Conclusion

The real-time volatile release from tomatilloes and tomatoes was measured and compared. The volatile release patterns were similar for the tomatillo and tomatoes. Although previous sensory studies revealed stronger green note in the tomatillo than in tomatoes, the “green” aldehydes, such as cis-3-hexenal, trans-2-hexenal, and hexanal,
released during chewing were not significant higher than most tomato varieties, and just higher than roma tomatoes. Cherry tomato released relatively more volatiles during chewing, whereas, roma tomato were generally poor in volatiles in the mouth. The lingering of volatiles in the mouth after swallowing was different for different volatiles and varieties, which may appear as a sensory difference detected by consumers. The information obtained on the dynamic generation of volatile compounds provided a better understanding of volatile release in the mouth.

4.6 References


Table 4.1 Information for SIFT-MS analysis of selected volatile compounds.

<table>
<thead>
<tr>
<th>Volatile Compound</th>
<th>Molecular formula</th>
<th>Precursor Ion</th>
<th>k (10⁻⁹ cm³ s⁻¹)</th>
<th>m/z</th>
<th>Ion Responsible</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Penten-3-one</td>
<td>C₅H₈O</td>
<td>NO⁺</td>
<td>2.5</td>
<td>144</td>
<td>[C₅H₈O·NO]⁺</td>
<td>Syft Technologies 2009</td>
</tr>
<tr>
<td>Hexanal</td>
<td>C₆H₁₂O</td>
<td>NO⁺</td>
<td>2.5</td>
<td>99</td>
<td>[C₆H₁₁O]⁺</td>
<td>Spanel and others 1997</td>
</tr>
<tr>
<td>cis-3-Hexenal</td>
<td>C₆H₁₀O</td>
<td>H₂O⁺</td>
<td>4.2</td>
<td>81</td>
<td>[C₆H₉]⁺</td>
<td>Spanel and others 1997</td>
</tr>
<tr>
<td>trans-2-Hexenal</td>
<td>C₆H₁₀O</td>
<td>H₂O⁺</td>
<td>4.6</td>
<td>99+117+135</td>
<td>[C₆H₁₁O+nH₂O]⁺</td>
<td>Spanel and others 1997</td>
</tr>
<tr>
<td>Methylbutanal</td>
<td>C₅H₁₀O</td>
<td>NO⁺</td>
<td>3.0</td>
<td>85</td>
<td>[C₅H₉]⁺</td>
<td>Spanel and others 2002</td>
</tr>
<tr>
<td>Nonanal</td>
<td>C₆H₁₃O</td>
<td>O₂⁺</td>
<td>3.2</td>
<td>110</td>
<td>[C₆H₁₄]⁺</td>
<td>Syft Technologies 2009</td>
</tr>
<tr>
<td>Isobutyl alcohol</td>
<td>C₄H₁₀O</td>
<td>O₂⁺</td>
<td>2.5</td>
<td>42</td>
<td>[C₄H₆]⁺</td>
<td>Spanel and Smith 1997</td>
</tr>
<tr>
<td>MSas/MSbs ratio</td>
<td>1-Penten-3-one</td>
<td>Hexanal</td>
<td>cis-3-Hexenal</td>
<td>trans-2-Hexenal</td>
<td>Methylbutanal</td>
<td>Nonanal</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------</td>
<td>---------</td>
<td>---------------</td>
<td>----------------</td>
<td>---------------</td>
<td>---------</td>
</tr>
<tr>
<td>Tomatillo</td>
<td>4.3&lt;sub&gt;a&lt;/sub&gt;</td>
<td>30.9&lt;sub&gt;abc&lt;/sub&gt;</td>
<td>10.8&lt;sub&gt;b&lt;/sub&gt;</td>
<td>5.8&lt;sub&gt;c&lt;/sub&gt;</td>
<td>41.8&lt;sub&gt;a&lt;/sub&gt;</td>
<td>64.5&lt;sub&gt;a&lt;/sub&gt;</td>
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<td>Cherry tomato</td>
<td>2.8&lt;sub&gt;a&lt;/sub&gt;</td>
<td>39.0&lt;sub&gt;a&lt;/sub&gt;</td>
<td>12.7&lt;sub&gt;b&lt;/sub&gt;</td>
<td>12.4&lt;sub&gt;b&lt;/sub&gt;</td>
<td>34.6&lt;sub&gt;a&lt;/sub&gt;</td>
<td>42.0&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>Grape tomato</td>
<td>9.4&lt;sub&gt;a&lt;/sub&gt;</td>
<td>22.4&lt;sub&gt;bc&lt;/sub&gt;</td>
<td>11.6&lt;sub&gt;b&lt;/sub&gt;</td>
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</tr>
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<td>Roma tomato</td>
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<td>19.8&lt;sub&gt;a&lt;/sub&gt;</td>
<td>17.8&lt;sub&gt;a&lt;/sub&gt;</td>
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<td>N/A</td>
</tr>
<tr>
<td>Vine-ripened tomato</td>
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<td>33.1&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>17.3&lt;sub&gt;a&lt;/sub&gt;</td>
<td>16.1&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>83.5&lt;sub&gt;a&lt;/sub&gt;</td>
<td>46.7&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>Air/water partition coefficient</td>
<td>1.29*10&lt;sup&gt;-2&lt;/sup&gt; (Reiners and Grosch 1998)</td>
<td>37*10&lt;sup&gt;-2&lt;/sup&gt; (van Ruth and others 2001)</td>
<td>N/A</td>
<td>2.13*10&lt;sup&gt;-2&lt;/sup&gt; (Buttery and others 1971)</td>
<td>1.8<em>10&lt;sup&gt;-2&lt;/sup&gt; &amp; 1.6</em>10&lt;sup&gt;-2&lt;/sup&gt; (Jouquand and others 2004)</td>
<td>3*10&lt;sup&gt;-2&lt;/sup&gt; (van Ruth and others 2001)</td>
</tr>
</tbody>
</table>

Values in the same column with different subscripts are significantly different.
### Table 4.3 The ratio of volatiles in the mouthspace (MS) to the headspace (HS) (%).

<table>
<thead>
<tr>
<th>MS/HS ratio</th>
<th>1-Penten-3-one</th>
<th>Hexanal</th>
<th>cis-3-Hexenal</th>
<th>trans-2-Hexenal</th>
<th>Methylbutanal</th>
<th>Nonanal</th>
<th>Isobutyl alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomatillo</td>
<td>42.3&lt;sub&gt;a&lt;/sub&gt;</td>
<td>13.1&lt;sub&gt;a&lt;/sub&gt;</td>
<td>2.5&lt;sub&gt;b&lt;/sub&gt;</td>
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<td>59.2&lt;sub&gt;a&lt;/sub&gt;</td>
<td>4.2&lt;sub&gt;c&lt;/sub&gt;</td>
</tr>
<tr>
<td>Cherry tomato</td>
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<td>3.4&lt;sub&gt;b&lt;/sub&gt;</td>
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<td>42.4&lt;sub&gt;b&lt;/sub&gt;</td>
<td>13.0&lt;sub&gt;a&lt;/sub&gt;</td>
</tr>
<tr>
<td>Grape tomato</td>
<td>2.7&lt;sub&gt;b&lt;/sub&gt;</td>
<td>2.3&lt;sub&gt;b&lt;/sub&gt;</td>
<td>2.1&lt;sub&gt;b&lt;/sub&gt;</td>
<td>3.3&lt;sub&gt;b&lt;/sub&gt;</td>
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<td>15.6&lt;sub&gt;c&lt;/sub&gt;</td>
<td>6.3&lt;sub&gt;bc&lt;/sub&gt;</td>
</tr>
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<td>Roma tomato</td>
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<td>1.1&lt;sub&gt;b&lt;/sub&gt;</td>
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<td>14.2&lt;sub&gt;c&lt;/sub&gt;</td>
<td>10.6&lt;sub&gt;ab&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

Values in the same column with different subscripts are significantly different.
Table 4.4 The ratio of volatiles in the nosespace (NS) to the headspace (HS) (%).

<table>
<thead>
<tr>
<th>NS/HS ratio</th>
<th>1-Penten-3-one</th>
<th>Hexanal</th>
<th>cis-3-Hexenal</th>
<th>trans-2-Hexenal</th>
<th>Methylbutanal</th>
<th>Nonanal</th>
<th>Isobutyl alcohol</th>
</tr>
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<tr>
<td>Tomatillo</td>
<td>2.38&lt;sub&gt;a&lt;/sub&gt;</td>
<td>1.63&lt;sub&gt;a&lt;/sub&gt;</td>
<td>0.78&lt;sub&gt;a&lt;/sub&gt;</td>
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<td>Cherry tomato</td>
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<td>0.37&lt;sub&gt;b&lt;/sub&gt;</td>
<td>0.16&lt;sub&gt;c&lt;/sub&gt;</td>
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<tr>
<td>Vine-ripened tomato</td>
<td>0.31&lt;sub&gt;b&lt;/sub&gt;</td>
<td>0.21&lt;sub&gt;b&lt;/sub&gt;</td>
<td>0.47&lt;sub&gt;b&lt;/sub&gt;</td>
<td>0.68&lt;sub&gt;a&lt;/sub&gt;</td>
<td>0.79&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>6.15&lt;sub&gt;b&lt;/sub&gt;</td>
<td>2.04&lt;sub&gt;ab&lt;/sub&gt;</td>
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</tbody>
</table>

Values in the same column with different subscripts are significantly different.
Figure 4.1 Real-time release of volatiles from tomatillo in the mouthspace.
Figure 4.2 Real-time release of volatiles from roma tomato in the mouthspace.
Figure 4.3 Real-time release of volatiles from vine-ripened tomato in the mouthspace.
Figure 4.4 Real-time release of volatiles from grape tomato in the mouthspace.
Figure 4.5 Real-time release of volatiles from cherry tomato in the mouthspace.
CHAPTER 5

A COMPARISON OF TOMATILLO AND TOMATO VOLATILE COMPOUNDS IN THE HEADSPACE BY SELECTED ION FLOW TUBE MASS SPECTROMETRY (SIFT-MS)

5.1 Abstract

The concentration of 31 volatiles were measured in the headspace of tomatillo using selected ion flow tube-mass spectrometry (SIFT-MS), and were compared with those in vine-ripened tomato, roma tomato, cherry tomato and grape tomato. None of volatiles was higher in the headspace of tomatillos than of tomatoes. *trans*-2-Octenal and *trans*-2-pentenal, 2-isobutylthiazole, 6-Methyl-5-hepten-2-one and phenylacetaldehyde were significantly lower in tomatillo than in the tomato varieties in the headspace. After blending, volatiles in the headspace increased, and then decreased after reaching a maximum concentration, due to further degradation or depletion. *trans*-2-Pentenal and 1-Penten-3-one reached a maximum concentration later than *trans*-2-hexenal, *cis*-3-hexenal and hexanal for tomatillo and tomatoes. The slope of the ratio of *trans*-2-hexenal and *cis*-3-hexenal was not significantly different for any of the samples, implying that the activity of cis/trans isomerase was not different between tomatillos and tomatoes.
5.2 Introduction

The tomatillo (*Physalis ixocarpa Brot*) is a solanaceous fruit with a similar appearance to the green tomato, except for featuring a paper-like husk formed from the calyx. It originates in Mexico, and is also called husk tomato and Mexican tomato. The tomatillo is a major ingredient in preparation of green sauces and salsas in Mexican cuisine, and is used to make jam in Australia (Morton 1987). Tomatillo has been adopted in a variety of recipes to create Latin American flavor themes (McGorrin and Gimelfarb 1998).

Tomatillo has a unique flavor profile, although it exhibits a similar appearance to a green tomato. Tomatillo produces a strong green flavor note, which is similar to tomato, but lacks the typical tomato viney and sulfur notes (McGorrin and Gimelfarb 1998). The flavor profile of tomato has been well studied analytically, and there are over 400 volatile compounds identified in tomatoes, of which 30 have been shown to contribute to the characteristic aroma of tomatoes (Petro-Turza 1987). In contrast to extensive studies which have been done on tomato volatiles, there is limited information about the flavor profile of tomatillos. Only a total of 52 volatile compounds have been identified in tomatillos, and of these, 6 volatiles, *cis*-3-hexenal, *trans*,*trans*-2,4-decadienal, nonanal, hexanal, hexanol, and *cis*-3-hexenol, are reported to be important to tomatillo aroma (McGorrin and Gimelfarb 2001). A comparison of the volatile profile between tomatillo and red plum tomato using GC/MS revealed that some characteristic key tomato volatiles, such as isobutythiazole, nitrophenylethane, and phenylacetonitrile, are missing in tomatillos (McGorrin and Gimelfarb 1998). Compounds unique to tomatillo flavor are
hydroxy esters, aromatic esters, 8- to 12-carbon aldehydes, decanoic acid and terpenes (McGorrin and Gimelfarb 1998).

A variety of analytical techniques have been developed for volatile identification and quantification, such as gas chromatography-mass spectrometry (GC/MS), atmospheric pressure ionization mass spectrometry (API-MS), and proton transfer reaction mass spectrometry (PTR-MS) (Brauss and others 1998; Masanobu and Hisakatsu 2005; Beltran and others 2006; Carbonell-Barrachina and others 2006; Markovic and others 2007). However, these techniques either have difficulty monitoring real-time release of volatiles (GC/MS) or cannot differentiate isomers (API-MS and PTR-MS), such as cis-3-hexenal and trans-2-hexenal in tomatoes (Boukobza and others 2001). Selected ion flow tube mass spectrometry (SIFT-MS) allows real-time analysis of complex mixtures of volatile compounds without trapping or pre-concentration, and also provides differentiation of some isomers (Spanel and Smith 1999). SIFT-MS has been applied in the analysis of breath volatiles for medical diagnosis and therapeutic monitoring (Senthilmohan and others 2000; Smith and others 2003), detection of bacterial metabolites (Scotter and others 2005; Allardyce and others 2006), monitoring air pollution (Smith and Spanel 1996), monitoring real-time release of volatiles in cut onion, crushed garlic and ripe banana (Spanel and Smith 1999) and monitoring olive oil oxidation (Davis and McEwan 2007).

The objective of this study is real-time detection of volatile compounds released from tomatillo or tomatoes in the headspace using SIFT-MS. The volatile release from tomatillo is compared with tomatoes.
5.3 Materials and methods

5.3.1 Materials

Samples of tomatillos, vine-ripened tomatoes, roma tomatoes, cherry tomatoes, and grape tomatoes were purchased from local food supermarkets in Columbus OH, USA. Samples were washed and stored for 5 h at room temperature before testing. All testing was carried out at 23 °C.

5.3.2 Instrument and sampling

The selected ion flow tube-mass spectrometry (SIFT-MS) instrument (SYFT Voice100, Syft Ltd, Christchurch, New Zealand) was used for headspace analysis of tomato and tomatillo volatiles, using the method described by Xu and Barringer (2009). Analysis was performed using selected ion mode (SIM) scans, and the concentration of volatile compounds was calculated using known kinetic parameters (Table 5.1). Using the pre-determined reaction rate constant for the volatile with that precursor ion, and accounting for dilution of the sample gas into the carrier gas, the absolute concentration of the volatile was calculated (Smith and Spanel 1996). Numerous studies have quantified concentrations, but in this study it is the comparison of values over time and between samples that is most important. The soft chemical ionization used in SIFT-MS yields a smaller range of product ions than is common in electron impact mass spectrometry (as used by gas chromatography mass spectrometry (GC-MS), for example). Hence the need for gas chromatographic separation of the sample is circumvented. However, the measured product mass produced by reaction with one of the three reagent ions must be carefully chosen. Many of the masses produced are
produced by several different volatiles, which creates an interference that must be removed or the results must be reported as a mixture. *trans*-2-Hexenal has an interference with *cis*-3-hexenal at *m/z* 99, 117, and 135. At these *m/z*, 35% of *cis*-3-hexenal and 100% of *trans*-2-hexenal are measured (Spanel and others 1997). However, since *cis*-3-hexenal has no interference at the *m/z* at which it was measured, 35% of *cis*-3-hexenal was subtracted from the concentration of *trans*-2-hexenal. Also 2-methylbutanal and 3-methylbutanal, and *trans*-2-hexenol and *cis*-3-hexenal have irresolvable interferences and are reported as mixtures (methylbutanal and hexenol). Other compounds with interferences were not included in the paper. Concentrations are reported in parts-per-billion by volume (ppb).

### 5.3.3 Headspace volatile analysis

The headspace volatile compounds were sampled directly by piercing the septa on a 500 ml Pyrex bottle with a 14-gauge passivated needle connected to the SIFT-MS. The needle point was 16 cm above the bottom of the bottle. A second long 14-gauge syringe needle pierced the septa to touch the bottom to maintain the pressure in the bottle at atmosphere pressure and also to provide oxygen for lipid oxidation. Tomatoes or tomatillos were blended for 30 sec at the highest speed using a blender (Waring, Dynamics corp, USA). Puree (60 ml) was transferred into a 500 ml pyrex bottle with minimal delay (typically 30 sec). NaCl (3 g) (Alberger flake salt, Cargill Incorporated, Minneapolis, MN USA) was added into the pyrex bottle to increase the amount of volatile compounds in the headspace. A magnetic stirrer (PC-351, Corning Incorporated, Corning, NY, USA), was used at the highest speed to stir the puree during sampling. The
volatile compounds in the headspace above the puree were analyzed continuously for 60 min after blending in triplicates. Room air was tested before each test and subtracted from the data. The highest concentrations during 60 min of tomatillo and tomatoes were compared.

5.3.4 Statistical analysis

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

5.4 Results and discussion

5.4.1 Comparison of volatiles in the headspace between tomatillos and tomatoes

Thirty one volatiles that were previously reported in tomatillo or tomato were measured and compared for their maximum concentration above the headspace of the fruit puree (Table 5.2) (Kazeniac and Hall 1970; Petro-Turza 1987; McGorrin and Gimelfarb 2001). The volatiles measured were mostly aldehydes and alcohols with a small number of acids, ketones, esters, hydrocarbons, and sulfur and nitrogen containing heterocyclic compounds. In general grape tomato had higher levels of volatiles in the headspace than any other tomato variety. None of the volatiles in the headspace of tomatillo were higher than in grape tomatoes, while several were lower than in any of the tomatoes. McGorrin and Gimelfarb (1998) found a number of aldehydes were higher in the tomatillo than in the tomato, which may be due to differences in varieties tested or preparation method.
Lipid related saturated and unsaturated 6-carbon aldehydes are known for their contribution of a green and leafy aroma (Petro-Turza 1987). *cis*-3-Hexenal and *trans*-2-hexenal were present in significant concentrations in the headspace of tomatillos and tomatoes. The maximum concentration of *cis*-3-hexenal was 4527 ppb in the headspace of tomatillo, which was not significantly different from that detected in the cherry tomato and roma tomato, but lower than in the grape tomato and vine-ripened tomato. The maximum concentration of *trans*-2-hexenal was 4846 ppb in the headspace of the tomatillo, significantly lower than in the cherry tomato and roma tomato, but not significantly different from the grape tomato and the vine-ripened tomato. These volatiles are formed from linolenic acid by the action of a series of enzymes in the lipoxygenase pathway (Tressl and others 1981). *cis*-3-Hexenal is the decomposition product of 13-hydroperoxides of linolenic acid by lipoxygenase, and then is further converted to *trans*-2-hexenal by *cis/trans*-isomerase (Stone and others 1975). These volatiles likely produce the characteristic “green” aroma for tomatillo and tomatoes due to their low threshold of 0.25 ppb for *cis*-3-hexenal and 17 ppb for *trans*-2-hexenal in water (Buttery 1993).

Another important lipid derived aldehyde, hexanal, had a maximum concentration of 1216 ppb (Table 5.2). Hexanal is formed from linoleic acid through the lipoxygenase pathway, produces a “green” aroma at 4.5 ppb and above (Buttery 1993), and is also important to the characteristic aroma of tomatillo. The concentration of hexanal in tomatillo was not significantly different from the vine-ripened tomato, but was lower than in other tomatoes. Since the linoleic acid content in tomatillos is typically higher than in tomatoes (USDA 2009), this implies that the enzyme activity is lower in tomatillos.
trans-2-Octenal, trans-2-pentenal, 2-isobutylthiazole, 6-methyl-5-hepten-2-one and phenylacetaldehyde were significantly lower in the tomatillo than in the tomato varieties in the headspace (Table 5.2). trans-2-Octenal and trans-2-pentenal reached a maximum of 30 ppb and 50 ppb respectively in the headspace of tomatillo, which was significantly lower than in any of the tomato varieties. trans-2-Octenal produces a sweet and phenolic aroma with a threshold of 3 ppb, whereas trans-2-pentenal provides a fresh fruit odor with a threshold of 1500 ppb (Stevens and others 1977, Maga 1981, Ullrich and Grosch 1988). The enzymatic generation of trans-2-octenal is from the decomposition of 10-hydroperoxides produced by the rearrangement of hydroperoxides of linoleic acid, which is an alternate pathway for the precursors to hexanal (Luning and others 1995). trans-2-Pentenal is derived from 13-hydroperoxides of linolenic acid, which are the precursors of cis-3-hexenal (Luning and others 1995).

The maximum concentration of 2-isobutylthiazole in the headspace of tomatilloes was significantly lower than in any of the tomato varieties (Table 5.2). McGorrin and Gimelfarb (1998) also reported a lower level of 2-isobutylthiazole in the headspace of tomatillo than in red plum tomato. 2-Isobutylthiazole produces a spoiled vine-like and slightly horseradish type flavor with a threshold of 2 ppb (Kazeniac and Hall 1970). It is regarded as one of the characteristic fresh aroma volatiles of tomato because adding 2-isobutylthiazole increases the fresh tomato aroma of canned tomato paste (Kazeniac and Hall 1970). Since addition of oxygen and use of different blending methods showed no effect on 2-isobutylthiazole (Kazeniac and Hall 1970), the majority of 2-isobutylthiazole should be formed in the intact fruit. The formation of 2-isobutylthiazole may be related to
thiamine biogenesis with methionine providing the sulfur precursor (Kazeniac and Hall 1970).

6-Methyl-5-hepten-2-one was detected at a maximum concentration of 7 ppb, at least 5 times lower than in tomatoes (Table 5.2). 6-Methyl-5-hepten-2-one produces a fruit-like aroma with a threshold of 50 ppb in the water (Buttery and others 1990). Because 6-methyl-5-hepten-2-one is formed from the degradation of lycopene (Kazeniac and Hall 1970), it is reasonable that the red tomatoes, with higher lycopene content, produce higher concentrations of 6-methyl-5-hepten-2-one than the green tomatillo. McGorrin and Gimelfarb (1998) also detected higher levels of 6-methyl-5-hepten-2-one in red plum tomato than in tomatillo.

Phenylacetaldehyde reached a maximum of 28 ppb in the headspace of tomatilloes, slightly lower than in tomatoes (Table 5.2). Phenylacetaldehyde produces a floral note with a threshold of 4 ppb in the water (Buttery and others 1988), so it may be important to the aroma of tomatillo and tomatoes. Phenylacetaldehyde is amino acid related and formed from phenylalanine.

5.4.2 The real-time release of volatiles in the headspace

Volatile in the headspace of tomatilloes and tomatoes increased then decreased after the tomatilloes or tomatoes were blended (Figure 5.1). Volatiles are released after tissue disruption, and then partition into the air of the headspace. The concentration of volatiles reach a maximum level, and then decrease by degradation and removal by SIFT-MS. Lipid related volatiles were generated through sequential reactions along the lipoxygenase pathway, resulting in different times to reach the maximum concentration.
In tomatilloes, cis-3-hexenal reached the maximum concentration in the headspace at 3 min after blending (Table 5.3). Hexanal reached the maximum concentration at 6 min, which was not statistically different from cis-3-hexenal (Table 5.3). cis-3-Hexenal and hexanal are derived from linoleic and linolenic acid respectively by the activity of hydroperoxide lyase (HPL) (Stone and others 1975). Since enzymes act on free fatty acids simultaneously, there is no interaction in the formation of these volatiles. trans-2-Hexenal reached a maximum level at 12 min in tomatillo, which was significantly later than cis-3-hexenal. trans-2-Hexenal is formed from cis-3-hexenal by cis-3/trans-2 isomerase (Z3/E2-ISO) (Stone and others 1975). The extra enzyme step delayed the release of trans-2-hexenal. trans-2-Pentenal and 1-penten-3-one peaked later than trans-2-hexenal, cis-3-hexenal, and hexanal at 28 min and 35 min respectively. They are formed from branch reactions of enzymatic oxidation of linolenic acid (Luning and others 1995). The trans-2-hexenal, cis-3-hexenal, and hexanal were further degraded to alcohols by alcohol dehydrogenase (ADH). However, the acidic pH of tomatillo and tomato puree causes low ADH activity (Longhurst and others 1990; Prestage and others 1999), thus no significant maximum concentration for hexanol and hexenol were measurable in this study.

The release of cis-3-hexenal and hexanal for tomatoes of different varieties was similar to tomatilloes with no significant difference in time to reach the maximum level (Table 5.3). Also similar to tomatilloes, trans-2-pentenal and 1-penten-3-one showed a significant delay in time to reach the maximum level compared to trans-2-hexenal, cis-3-
hexenal, and hexanal in all of the tomatoes. However, the peaks occurred earlier than in tomatilloes.

The ratio of the concentration of trans-2-hexenal to cis-3-hexenal in the headspace of tomatilloes and tomatoes increased with time, because the conversion from cis-3-hexenal to trans-2-hexenal continued for at least the 60 min of this study (Figure 5.2). cis-3-Hexenal forms first, and then is isomerized to trans-2-hexenal by Z3/E2-ISO. As the isomerization continued, the trans-2-hexenal accumulated in the headspace, increased the ratio. The slopes of the ratio for tomatilloes and tomatoes were not significantly different, implying that the activity of Z3/E2-ISO in tomatilloes and tomatoes is not significantly different. There is some cis-3-hexenal and trans-2-hexenal formed in the intact fruit, and the y axis intercept of the ratio for tomatilloes was significantly higher than for tomatoes, but only the ratio and not the concentrations of these two volatiles for tomatilloes were different from tomatoes.

5.5 Conclusion

The concentration of most volatiles in tomatilloes is not significant different from the range found in tomatoes. trans-2-Octenal, trans-2-pentenal, 2-isobutylthiazole, 6-methyl-5-hepten-2-one and phenylacetaldehyde was significantly lower in the tomatillo than other tomato varieties, which may one of the causes for sensory difference perceived by consumer. In general, grape tomatoes had the highest volatile levels of all tomato varieties. The real-time release of lipid related volatiles showed difference in time to reach the peak concentration in the headspace for tomatilloes and tomatoes, which imply the sequencial release of volatiles of lipoxygenase pathway. The information obtained on
the dynamic generation of volatile compounds provided a better understanding of volatile release in the headspace.

5.6 References


Table 5.1 Information for SIFT-MS analysis of selected volatile compounds.

<table>
<thead>
<tr>
<th>Volatile compounds</th>
<th>Molecular formula</th>
<th>Precursor Ion</th>
<th>k ( \text{(10^{-9}\text{cm}^3\text{s}^{-1})} )</th>
<th>m/z</th>
<th>Ion Responsible</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
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<td><strong>Acids</strong></td>
<td></td>
<td></td>
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<td>2-Methylbutyric acid</td>
<td>C(<em>5)H(</em>{10})O(_2)</td>
<td>NO(^+)</td>
<td>2.5</td>
<td>132</td>
<td>[C(<em>5)H(</em>{10})O(_2)\text{NO}] (^+)</td>
<td>Syft Technologie s 2009</td>
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<tr>
<td><strong>Aldehydes</strong></td>
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<td>Acetaldehyde</td>
<td>C(_2)H(_4)O</td>
<td>NO(^+)</td>
<td>60</td>
<td>43</td>
<td>[CH(_3)CO](^+)</td>
<td>Spanel and others 1997</td>
</tr>
<tr>
<td>cis-3-Hexenal</td>
<td>C(<em>6)H(</em>{10})O</td>
<td>H(_2)O(^+)</td>
<td>4.2</td>
<td>81</td>
<td>[C(_6)H(_9)](^+)</td>
<td>Spanel and others 1997</td>
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<tr>
<td>Decanal</td>
<td>C(<em>{10})H(</em>{20})O</td>
<td>NO(^+)</td>
<td>3.3</td>
<td>155</td>
<td>[C(<em>{10})H(</em>{19})O](^+)</td>
<td>Spanel and others 2002</td>
</tr>
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<td>Furfuraldehyde</td>
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<td>3.2</td>
<td>96</td>
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<td>Hexanal</td>
<td>C(<em>6)H(</em>{12})O</td>
<td>NO(^+)</td>
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<td>99</td>
<td>[C(<em>6)H(</em>{11})O](^+)</td>
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<td>[C(_3)H(_9)O](^+)</td>
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<td>O(_2)(^+)</td>
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<td>[C(<em>8)H(</em>{14})](^+)</td>
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<td>Octanal</td>
<td>C(<em>8)H(</em>{16})O</td>
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<td>[C(<em>8)H(</em>{15})O](^+)</td>
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<td>Phenylacetaldehyde</td>
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<td>trans-2-Heptenal</td>
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<td>NO(^+)</td>
<td>3.9</td>
<td>111</td>
<td>[C(<em>7)H(</em>{11})O](^+)</td>
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<th>Volatile compounds</th>
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<th>( m/z )</th>
<th>Ion Responsible</th>
<th>Reference</th>
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<td>[C₈H₁₄O·NO]⁺</td>
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<td><em>trans</em>-2-pentenal</td>
<td>C₅H₈O</td>
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<td>3.9</td>
<td>83</td>
<td>[C₅H₇O]⁺</td>
<td>Spanel and others 2002</td>
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</tbody>
</table>

**Alcohols**

| 2-Pentanol         | C₅H₁₂O       | NO⁺           | 2.3             | 87       | [C₅H₁₁O⁺]       | Syft Technologies 2009 |
| Hexanol            | C₆H₁₄O       | NO⁺           | 2.4             | 101      | [C₆H₁₃O⁺]       | Syft Technologies 2009 |
| Ethanol            | C₂H₆O        | NO⁺           | 1.2             | 45+63+81 | [C₂H₅O+nH₂O⁺]   | Spanel and Smith 1997 |
| Hexenol            | C₆H₁₂O       | NO⁺           | 2.5             | 72       | [C₆H₈O⁺]        | Syft Technologies 2009 |
| Methanol           | CH₄O         | H₂O⁺          | 2.7             | 33+51+65+69+83 | [CH₃O+nH₂O⁺]   | Spanel and Smith 1997 |
| Isobutyl alcohol   | C₄H₁₀O       | O₂⁺           | 2.5             | 42       | [C₃H₆⁺]         | Syft Technologies 2009 |
| n-Propyl alcohol   | C₃H₈O        | NO⁺           | 2.3             | 59       | [C₃H₇O]⁺        | Spanel and Smith 1997 |

**Esters**

| Hexyl acetate      | C₈H₁₆O₂      | NO⁺           | 2.5             | 174      | [C₈H₁₆O₂·NO⁺]   | Syft Technologies 2009 |
| Methyl salicylate  | C₈H₈O₃       | O₂⁺           | 2.7             | 152      | [C₈H₈O₃⁺]       | Syft Technologies 2009 |

Continued
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<th>Volatile compounds</th>
<th>Molecular formula</th>
<th>Precursor Ion</th>
<th>$k$ (10^{-9} \text{cm}^3 \text{s}^{-1})$</th>
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<td>6-Methyl-5-hepten-2-one</td>
<td>C_{8}H_{14}O</td>
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<td>Acetone</td>
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<td>1-Penten-3-one</td>
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<tr>
<td>2-carene</td>
<td>C_{10}H_{16}</td>
<td>NO$^+$</td>
<td>2.3</td>
<td>136</td>
<td>[C_{10}H_{16}]$^+$</td>
<td>Syft Technologies 2009</td>
</tr>
<tr>
<td>2-isobutylthiazole</td>
<td>C_{7}H_{11}NS</td>
<td>H_{3}O$^+$</td>
<td>3</td>
<td>142</td>
<td>[C_{7}H_{12}NS]$^+$</td>
<td>Syft Technologies 2009</td>
</tr>
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</table>
Table 5.2 Volatiles in the headspace of tomatillo and tomatoes.

<table>
<thead>
<tr>
<th>Volatile compounds (ppb)</th>
<th>Vine-ripened tomato</th>
<th>Cherry tomato</th>
<th>Grape tomato</th>
<th>Roma tomato</th>
<th>Tomatillo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acids</td>
<td></td>
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</tr>
<tr>
<td>2-Methylbutyric acid</td>
<td>21 (^b)</td>
<td>15 (^c)</td>
<td>27 (^a)</td>
<td>24 (^{ab})</td>
<td>11 (^c)</td>
</tr>
<tr>
<td>Aldehydes</td>
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<td></td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>538 (^b)</td>
<td>970 (^b)</td>
<td>2826 (^a)</td>
<td>1078 (^b)</td>
<td>639 (^b)</td>
</tr>
<tr>
<td>cis-3-Hexenal</td>
<td>6059 (^b)</td>
<td>4600 (^c)</td>
<td>10466 (^a)</td>
<td>4622 (^c)</td>
<td>4527 (^c)</td>
</tr>
<tr>
<td>Decanal</td>
<td>9 (^b)</td>
<td>10 (^b)</td>
<td>13 (^a)</td>
<td>9 (^b)</td>
<td>9 (^b)</td>
</tr>
<tr>
<td>Furfuraldehyde</td>
<td>7 (^{ab})</td>
<td>2 (^{bc})</td>
<td>8 (^a)</td>
<td>3 (^{abc})</td>
<td>2 (^c)</td>
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<tr>
<td>hexanal</td>
<td>1151 (^c)</td>
<td>4289 (^a)</td>
<td>3593 (^a)</td>
<td>2713 (^b)</td>
<td>1216 (^c)</td>
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<tr>
<td>Methylbutanal</td>
<td>113 (^b)</td>
<td>95 (^{bc})</td>
<td>168 (^b)</td>
<td>286 (^a)</td>
<td>21 (^c)</td>
</tr>
<tr>
<td>Nonanal</td>
<td>265 (^{bc})</td>
<td>177 (^c)</td>
<td>367 (^a)</td>
<td>158 (^c)</td>
<td>179 (^c)</td>
</tr>
<tr>
<td>Octanal</td>
<td>10 (^b)</td>
<td>10 (^b)</td>
<td>21 (^a)</td>
<td>9 (^b)</td>
<td>10 (^b)</td>
</tr>
<tr>
<td>Phenylacetaldehyde</td>
<td>73 (^b)</td>
<td>57 (^c)</td>
<td>97 (^a)</td>
<td>48 (^c)</td>
<td>28 (^d)</td>
</tr>
<tr>
<td>trans-2-Heptenal</td>
<td>25 (^{bc})</td>
<td>36 (^b)</td>
<td>59 (^a)</td>
<td>27 (^{bc})</td>
<td>14 (^c)</td>
</tr>
<tr>
<td>trans-2-Hexenal</td>
<td>4408 (^{bc})</td>
<td>2869 (^d)</td>
<td>5956 (^a)</td>
<td>3239 (^{cd})</td>
<td>4846 (^{ab})</td>
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<tr>
<td>trans-2-Octenal</td>
<td>51 (^{bc})</td>
<td>50 (^b)</td>
<td>88 (^a)</td>
<td>47 (^b)</td>
<td>30 (^c)</td>
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<tr>
<td>trans-2-Pentenal</td>
<td>204 (^b)</td>
<td>163 (^{bc})</td>
<td>295 (^a)</td>
<td>154 (^c)</td>
<td>50 (^d)</td>
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<td>Alcohols</td>
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<tr>
<td>2-Pentanol</td>
<td>30 (^a)</td>
<td>25 (^a)</td>
<td>37 (^a)</td>
<td>43 (^a)</td>
<td>87 (^a)</td>
</tr>
<tr>
<td>Hexanol</td>
<td>17 (^c)</td>
<td>32 (^a)</td>
<td>37 (^a)</td>
<td>23 (^b)</td>
<td>15 (^c)</td>
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<tr>
<td>Ethanol</td>
<td>250 (^{b})</td>
<td>1054 (^b)</td>
<td>2481 (^a)</td>
<td>450 (^b)</td>
<td>763 (^b)</td>
</tr>
<tr>
<td>Hexenol</td>
<td>21 (^a)</td>
<td>29 (^a)</td>
<td>39 (^a)</td>
<td>30 (^a)</td>
<td>104 (^a)</td>
</tr>
<tr>
<td>Methanol</td>
<td>164630 (^a)</td>
<td>134626 (^{ab})</td>
<td>10466 (^c)</td>
<td>105745 (^b)</td>
<td>33611 (^c)</td>
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<tr>
<td>Isobutyl alcohol</td>
<td>258 (^{a})</td>
<td>226 (^a)</td>
<td>365 (^a)</td>
<td>247 (^a)</td>
<td>399 (^a)</td>
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<tr>
<td>n-Propyl alcohol</td>
<td>43 (^{b})</td>
<td>29 (^c)</td>
<td>53 (^a)</td>
<td>36 (^{bc})</td>
<td>60 (^a)</td>
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<td>Esters</td>
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<tr>
<td>Hexyl acetate</td>
<td>4 (^b)</td>
<td>5 (^{ab})</td>
<td>9 (^a)</td>
<td>4 (^{ab})</td>
<td>8 (^{ab})</td>
</tr>
<tr>
<td>Methyl salicylate</td>
<td>84 (^b)</td>
<td>95 (^{ab})</td>
<td>149 (^a)</td>
<td>59 (^b)</td>
<td>40 (^b)</td>
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<td>Ketones</td>
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<tr>
<td>2-Pentanone</td>
<td>94 (^b)</td>
<td>64 (^c)</td>
<td>142 (^a)</td>
<td>75 (^c)</td>
<td>99 (^b)</td>
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<tr>
<td>6-Methyl-5-hepten-2-one</td>
<td>62 (^b)</td>
<td>44 (^b)</td>
<td>106 (^a)</td>
<td>47 (^b)</td>
<td>8 (^c)</td>
</tr>
<tr>
<td>Acetone</td>
<td>2065 (^{ab})</td>
<td>1426 (^{cd})</td>
<td>2531 (^a)</td>
<td>1208 (^d)</td>
<td>1720 (^{bc})</td>
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<tr>
<td>1-Penten-3-one</td>
<td>526 (^{b})</td>
<td>317 (^c)</td>
<td>935 (^a)</td>
<td>242 (^{cd})</td>
<td>96 (^d)</td>
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<tr>
<td>Phenols</td>
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</tr>
<tr>
<td>Eugenol</td>
<td>4 (^a)</td>
<td>5 (^a)</td>
<td>10 (^a)</td>
<td>4 (^a)</td>
<td>6 (^a)</td>
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<tr>
<td>Guaiacol</td>
<td>35 (^{bc})</td>
<td>26 (^c)</td>
<td>45 (^{ab})</td>
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<td>50 (^a)</td>
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<tr>
<td>Sulfer compounds and hydrocarbon compounds</td>
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<tr>
<td>2-carene</td>
<td>51 (^b)</td>
<td>120 (^a)</td>
<td>107 (^a)</td>
<td>69 (^b)</td>
<td>55 (^b)</td>
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<td>2-isobutylthiazole</td>
<td>66 (^a)</td>
<td>72 (^a)</td>
<td>35 (^b)</td>
<td>19 (^c)</td>
<td>7 (^d)</td>
</tr>
</tbody>
</table>

Values with different superscripts for the same compound (row) are significantly different.
Table 5.3 Time (min) to reach maximum level for lipid related volatiles in tomatoes and tomatillo after blending.

<table>
<thead>
<tr>
<th>Volatiles</th>
<th>Tomatillo</th>
<th>Vine-ripened tomato</th>
<th>Cherry tomato</th>
<th>Grape tomato</th>
<th>Roma tomato</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-3-Hexenal</td>
<td>$3^c_d$</td>
<td>$3^{bc}_c$</td>
<td>$6^{a_cd}$</td>
<td>$6^{a_b}$</td>
<td>$5^{ab}_c$</td>
</tr>
<tr>
<td>trans-2-Hexenal</td>
<td>$12^a_c$</td>
<td>$4^{c_c}$</td>
<td>$7^{b_bc}$</td>
<td>$7^{b_b}$</td>
<td>$7^{b_bc}$</td>
</tr>
<tr>
<td>Hexanal</td>
<td>$6^{ab_d}$</td>
<td>$3^{c_c}$</td>
<td>$5^{bc_d}$</td>
<td>$6^{a_b}$</td>
<td>$5^{ab_c}$</td>
</tr>
<tr>
<td>1-Penten-3-one</td>
<td>$35^{a_a}$</td>
<td>$6^{b_b}$</td>
<td>$8^{b_b}$</td>
<td>$9^{b_b}$</td>
<td>$10^{b_ab}$</td>
</tr>
<tr>
<td>trans-2-Pentenal</td>
<td>$28^{a_b}$</td>
<td>$16^{bc_a}$</td>
<td>$20^{b_a}$</td>
<td>$14^{cd_a}$</td>
<td>$11^{d_a}$</td>
</tr>
</tbody>
</table>

Values with different superscripts for the same compound (row) are significantly different.
Values with different subscripts for the same fruit (column) are significantly different.
Figure 5.1 The headspace concentration of cis-3-hexenal, trans-2-hexenal and hexanal after blending of tomatillos.
Figure 5.2 The increase in the trans-2-hexenal/cis-3-hexenal ratio with time.
REFERENCES


