Effect of Enzyme Activity and Frozen Storage on Jalapeño Pepper Volatiles by Selected Ion Flow Tube—Mass Spectrometry

Carolina Azcarate and Sheryl A. Barringer

Abstract: Samples of unblanched (fresh), stannous chloride-treated, or blanched jalapeño peppers were measured for real-time generation of lipoxygenase-derived volatiles during 10 min after tissue disruption. Volatiles were also measured before and after 1.5, 2.5, 3, 6, and 9 mo of frozen storage at −15 °C. The concentration of all lipoxygenase-derived compounds was significantly higher in unblanched jalapeño peppers compared to enzyme inhibited peppers. The maximum concentration of (Z)-3-hexenal, (E)-2-hexenal, and hexanal was detected at about 1.2, 1.5, and 1.5 min after tissue disruption, respectively. A decrease in (Z)-3-hexenal and an increase in dimethyl sulfide and methylbutanal occurred in blanched compared to stannous chloride-treated peppers due to heat. Frozen storage resulted in no major changes in the lipoxygenase-derived volatiles of whole and pureed blanched peppers after 9 mo. However, in whole unblanched peppers a gradual decrease of (Z)-3-hexenal, (E)-2-hexenal, hexanal, hexenol, and hexanol was observed over time; whereas in pureed unblanched peppers these compounds increased, reached maximum concentration, and then decreased. Similarly, the minor volatiles 2-pentenal, 1-penten-3-one, (E)-2-heptenal, (E)-2-octenal, and (E)-2-nonenal showed an initial increase followed by a decline in both whole and pureed unblanched peppers. Tissue disruption increased generation and degradation rates during frozen storage. The compounds (E,Z)-2,6-nonadienal, n-propyl aldehyde, 2-isobutyl-3-methoxypyrazine, and a mixture of terpenes decreased in unblanched and blanched frozen samples, while nonanal and methylbutanal increased only in unblanched samples.

Keywords: blanched, frozen storage, jalapeño pepper, lipoxygenase, volatiles

Practical Application: Data obtained in this study contribute to the understanding of the dynamics of lipoxygenase-derived volatile formation upon tissue disruption of jalapeño pepper. In addition, it contributes to generate knowledge on the effect of processing techniques, namely blanching and frozen storage, on the volatile profile of these peppers. This knowledge has applications in the manufacturing, product development, and quality control areas of the food industry as useful information to help in the designing and monitoring of processes aimed to obtain products with specific aroma characteristics. For instance, maximum levels of “fresh” and “green” aroma compounds are achieved rapidly during the first few minutes after pepper tissue is disrupted. The inhibition of enzyme activity shortly after this may help to maximize concentration of these aroma notes in the product. Frozen storage produces enzymatic and chemical changes in the volatile profile of unblanched peppers. The aroma profile of blanched peppers is more stable under frozen conditions, with a lower total volatile concentration.

Introduction

Jalapeño (Capsicum annuum L.), a capsicum pepper originating in Mexico, is the most popular chili cultivar in North America (Galicia-Cabrera 2006). It is greatly appreciated for its pleasant aroma and mild pungency.

Few studies have been conducted regarding the volatile compounds present in jalapeño pepper or the effect of processing on its flavor. Some compounds identified in this pepper are 2-isobutyl-3-methoxypyrazine (Huffman and others 1978; Chitwood and others 1983), (Z)-3-hexenol, and linalool (Keller and others 1981; Chitwood and others 1983).

More than 125 volatile compounds have been identified in other Capsicum peppers (Pino and others 2007). Some of the most important compounds are the group of 6-carbon (C6) aldehydes and alcohols generated by the lipoxygenase pathway. This oxidative pathway initiated by the lipoxygenase (LOX) enzyme is responsible for the formation of these aldehydes and alcohols from C18 polyunsaturated fatty acids in various fruits and vegetables upon tissue disruption (Wu and Liou 1986; Buttery and others 1987). The C6 volatile compounds hexanal, (Z)-3-hexenal, (E)-2-hexenal, hexanol, (Z)-3-hexenol, and (E)-2-hexenol are associated with the fresh and green notes of Capsicum peppers (Luning and others 1994).

Other major volatiles found in Capsicum peppers are the terpenes limonene, linalool, (E)-β-ocimene, and δ-3-carene; the
compound 2-methoxy-3-isobutylpyrazine; the aldehydes (E,Z)-
2,6-nonadienal and (E,E)-2,4-decadienal; the esters methyl sal-
cylate and ethyl acetate; and the ketones 1-penten-3-one and
2,3-butanedione (Buttery and others 1969; Chitwood and others
1983; Luning and others 1994).

Processing techniques generally result in some degree of al-
teration of the sensory characteristics of fresh capsicum peppers,
especially its flavor. Freezing, for instance, increases the content of
terpenes, pyrazines, and phenols of Padrón-type peppers; whereas
homogenization increases the content of aldehydes, esters,
pyrazines, and phenols; and produces a loss of ethers, pyrroles,
and sulfurous compounds (Oruña-Concha and others 1998).
Dried blanched green peppers resulted in reduced fresh green
pepper aroma, associated with the compound 2-isobutyl-3-
methoxyypyrazine, when compared to unblanched dried peppers
(Kuzmar and others 1983).

Selected ion flow tube—mass spectrometry (SIFT-MS) is a rel-
atively new technique used initially for the analysis of trace gases
in air and for clinical breath analysis that has expanded its appli-
cation to the food industry for the analysis of aroma compounds.
The main advantages of this technique are that it requires minimal
sample preparation, it is fast, and allows for a real-time moni-
toring of selected volatile compounds providing differentiation
of some isomers. Some drawbacks include the requirement of previ-
ous identification of compounds present in the sample and their
product ions from the reaction with the chosen precursor ions,
and conflicts among product ions that make impossible the differ-
etiation of some compounds. It has been used to look at volatiles
in foods such as onions, bananas (Spanel and Smith 1999), olive
oil (Davis and McEwan 2007), chocolates (Huang and Barringer
2010), garlic (Hansungrum and Barringer 2010), and tomatillos
(Xu and Barringer 2010).

The objective of this study was to evaluate the effect of enzy-
matic activity, particularly the lipoygenase pathway, on the gen-
eration of aroma compounds; as well as the effect of frozen storage
on the volatile profile of whole and pureed jalapeño peppers using
SIFT-MS.

Materials and Methods

Materials
The mature-green jalapeño peppers used for this research were
bought fresh from a local store between March 2009 and January
2010.

Sample preparation
Blanching. To blanch peppers, whole fresh peppers were sub-
merged in boiling water for 2.5 min and then allowed to cool
at room temperature. Enzyme inactivation was confirmed by a
peroxidase test. For the peroxidase test, 2 g of blanched pepper
were crushed in a mortar, and then 0.5 mL of 0.5% guaiacol in
50% ethanol, and 0.5 mL 3% hydrogen peroxide solutions were
added. The crushed pepper and solutions were mixed and allowed
to react for 3.5 min. No reddish-brown color formation after
3.5 min indicated peroxidase inactivation.

Effect of enzyme activity on volatile formation. Whole
unblanched or blanched jalapeño peppers with the peduncles cut
off were sliced into 2 cm-thick slices and 50 g were put into a
blender (Waring Products Div., Dynamics Corp. of America,
New Hartford, Conn., U.S.A.) together with 150 mL of distilled
water or 0.2M stannous chloride solution (0.2M SnCl₂). Peppers
were blended for 30 s, and the puree obtained was transferred into
a 500-mL-glass bottle, capped, and tested for volatiles immediately.
The scan time was 10 min. Polybutylene terephthalate (PBT) open
top caps coupled to polytetrafluoroethylene (PTFE) faced silicone
septa were used. The 0.2M SnCl₂ solution was used to inhibit
enzyme activity and therefore inhibit LOX-derived compound
generation in unblanched peppers without the heating effect in-
herent to blanching. Inhibition in jalapeño peppers is evaluated in
terms of volatile generation in unblanched compared to blanched
samples.

Effect of frozen storage on volatile profile. Two sets of
samples of whole unblanched and blanched (450 g), and pureed
unblanched and blanched (120 g) jalapeño peppers were hand-
packed in plastic bags, sealed allowing some air in the head space,
and stored at −15 °C. One set was measured for volatiles before
and after 1.5, 2.5, and 3 mo of frozen storage only in unblanched
samples; whereas the other set was measured before and after 6
and 9 mo of frozen storage in both unblanched and blanched sam-
ple. Frozen whole and pureed peppers samples were thawed at
4 °C for 12 h, and then brought to room temperature in a 25 °C
water bath for ca. 1 h. Whole peppers samples were pureed prior
to analysis. Puree was obtained by blending 80 g of 2cm-thick
slices of pepper with 40 mL of water for 30 s, and then trans-
ferred immediately into 500-mL-glass bottles. Samples were tested
10 min after blending; or 10 min after transferring into bottles in
the case of thawed puree. Bottles were kept capped during the
10-min period. The scan time was 2 min.

Analysis of volatile compounds. The analysis of volatile
compounds was performed using a SIFT-MS instrument (SYFT
Voice100, Sift Technologies Ltd., Christchurch, New Zealand).
The SIFT-MS technology uses soft chemical ionization with 3
different precursor ions generated by microwave discharge. The
product ion count rates are determined by a quadrupole mass
spectrometer. The inlet port of the instrument was coupled with
an 18 ga 3.8 cm-long stainless steel passivated needle to facilitate
piercing and sampling volatiles from the glass bottle headspace.
Prepared samples were tested by inserting the needle through
the silicone septum fitted in the open top cap of the bottle. The
septum was also pierced by a 14 ga 15 cm-long syringe needle
to maintain atmospheric pressure inside the bottle. Headspace was
withdrawn at 120 cm³/min. The tip of the short and long needle
was located at ca. 12 cm and 1 cm from the surface of the sam-
ple, respectively. The background measured for the distilled water
or 0.2M SnCl₂ solution used for blending was subtracted from
the data. Measurements were performed using selected ion mode
(SIM) scans with H₃O⁺, NO₂⁻, or O₂⁻ as precursor ions, calcu-
lation delay time 5 s, product sample period 100 ms, precursor
sample period 20 ms, carrier gas argon pressure 280 kPa, helium
pressure 30 psi, capillary and atom temperature 120 °C, and flow
tube pressure 0.038 ± 0.003 Torr.

One requirement of the method is the previous identification
of the product ions formed by the reaction between each of the
volatile compounds present in the sample with the selected precu-
 sor ion (Spanel and Smith 1999). Reactions between the precursor
ions and numerous organic compounds have been studied in pre-
vious works, with the relevant ones cited in Table 1. The SIFT-MS
methods used include the main volatiles found in capsicum fruits
as reported by previous studies. Conflicts occur when product ions
for different compounds have the same m/z ratio and therefore
the compounds can not be differentiated. In this study, samples
of jalapeño peppers, with and without enzymes inhibited, were
measured in the SIFT-MS. All product masses produced by each
volatile compound were measured and the calculated compound
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concentrations compared. Any masses, which produced values significantly higher than the others were eliminated, as this indicates multiple compounds were being measured at the same mass. The exception was for compounds that are known to further react and thus produce inaccurately low values. Among the remaining masses, any mass with a known conflict with another compound in the sample was eliminated, when possible. If more than one mass remained, all but one was eliminated so the results would be comparable between samples. When the remaining mass has a conflict with another compound it was either reported as a mixture, or if the conflicting compound was calculated to contribute less than 5% to the final value, the conflict was ignored.

The SIFT-MS software uses the precursor and product ions count rates, product ion branching ratios, reaction rates, and controlled reaction conditions to calculate the volatile concentration in the headspace using the methods described in Spanel and others 2002. The instrument provides concentration readings every 5 s; then, for the real-time monitoring experiment, these individual concentration readings were plotted over the 10-min period test in order to observe generation or degradation trends for the main LOX-derived compounds over time, immediately after tissue disruption.

It is important to point out that the SIFT-MS technique assumes the main contributors to the masses measured are the compounds considered in the method; however, it is possible that unknown compounds are being measured at the same time. This is a limitation of the technique.

The kinetic parameters for the tested compounds are provided in Table 1. The compound hexenol represents a mixture of (Z)-3-hexenol and (E)-2-hexenol; the compound 2-pentenal is a mixture of (E)-2-pentenal and (Z)-2-pentenal; the compound methylbutanal is a mixture of 2-methylbutanal and 3-methylbutanal; and terpenes is a mixture of limonene, linalool, ocimene, and 2- and 3-carene.

Data from 6 and 9 mo whole unblanched peppers and 6 mo pureed unblanched peppers were normalized based on the 0 mo data on graphs that contain both this and the data from 1.5 to 3 mo of storage.

**Statistical analysis**

Data were analyzed using 1-way analysis of variance (ANOVA) and Tukey’s multiple comparison procedure. Significance was defined as $P \leq 0.05$. Five independent samples of peppers were prepared and analyzed for each treatment. Error bars in the graphs represent the standard error of the replicates.

**Results and Discussion**

Effect of enzyme activity on volatile formation

The concentrations of several volatiles were monitored in pureed samples of unblanched, unblanched with 0.2M SnCl$_2$, or blanched jalapeño pepper (Figure 1). Unblanched samples produced significantly higher concentrations of the compounds (Z)-3-hexenal, (E)-2-hexenal, hexenal, 2-pentenal, 1-penten-3-one, hexanal, hexanol, and (E)-2-heptenal. These compounds are known to be derived from polyunsaturated fatty acids by the action of the lipoxygenase pathway. Several LOX-derived compounds,

**Table 1—Kinetics parameters for SIFT-MS analysis of selected volatile compounds.**

<table>
<thead>
<tr>
<th>Volatile compound</th>
<th>Molecular formula</th>
<th>Precursor ion</th>
<th>$k$ ($10^{-3}$cm$^3$·s$^{-1}$)</th>
<th>$m/z$</th>
<th>Product ion</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E)-2-heptenal</td>
<td>C$_7$H$_8$O</td>
<td>NO$^+$</td>
<td>3.9</td>
<td>111</td>
<td>[C$_8$H$_8$O]$^+$</td>
<td>[4]</td>
</tr>
<tr>
<td>(E)-2-hexenal</td>
<td>C$_6$H$_9$O</td>
<td>NO$^+$</td>
<td>3.8</td>
<td>71</td>
<td>[C$_8$H$_8$O]$^+$</td>
<td>[2]</td>
</tr>
<tr>
<td>(E)-2-nonenal</td>
<td>C$_8$H$_9$O</td>
<td>NO$^+$</td>
<td>3.8</td>
<td>139</td>
<td>[C$_8$H$_8$O]$^+$</td>
<td>[4]</td>
</tr>
<tr>
<td>(E)-2-octenal</td>
<td>C$_9$H$_10$O</td>
<td>NO$^+$</td>
<td>4.1</td>
<td>125</td>
<td>[C$_8$H$_8$O]$^+$</td>
<td>[2]</td>
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<tr>
<td>(E)-2,4-decadienal</td>
<td>C$<em>{10}$H$</em>{11}$O</td>
<td>H$_2$O$^+$</td>
<td>4.9</td>
<td>167</td>
<td>[C$_8$H$_8$O]$^+$</td>
<td>[3]</td>
</tr>
<tr>
<td>(E)-2,5-nonenal</td>
<td>C$<em>{10}$H$</em>{12}$O</td>
<td>NO$^+$</td>
<td>2.5</td>
<td>137</td>
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<td>(Z)-3-hexenal</td>
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<td>H$_2$O$^+$</td>
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<td>1-penten-3-one</td>
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<td>NO$^+$</td>
<td>2.5</td>
<td>114</td>
<td>[C$_8$H$_8$O$\cdot$NO]$^+$</td>
<td>[5]</td>
</tr>
<tr>
<td>2,3-butanedione</td>
<td>C$_6$H$_8$O$_2$</td>
<td>NO$^+$</td>
<td>1.3</td>
<td>86</td>
<td>[C$_8$H$_8$O$^+$]</td>
<td>[1]</td>
</tr>
<tr>
<td>2-hydroxy-3-methylpentaenoic acid</td>
<td>C$<em>{11}$H$</em>{14}$O$_2$</td>
<td>NO$^+$</td>
<td>2.5</td>
<td>152</td>
<td>[C$_8$H$_8$COOH$\cdot$NO]$^+$</td>
<td>[5]</td>
</tr>
<tr>
<td>2-isobutyl-3-methoxyazine</td>
<td>C$<em>{11}$H$</em>{15}$NO</td>
<td>H$_2$O$^+$</td>
<td>3.0</td>
<td>167</td>
<td>[C$_8$H$_8$NO$\cdot$H$^+$]</td>
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<td>C$_8$H$_8$O</td>
<td>NO$^+$</td>
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<td>116</td>
<td>[C$_8$H$_8$O$\cdot$NO]$^+$</td>
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<td>NO$^+$</td>
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<td>Acetaldehyde</td>
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<td>O$_2^+$</td>
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<td>44</td>
<td>[C$_8$H$_4$O]$^+$</td>
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<tr>
<td>Dimethyl disulfide</td>
<td>C$<em>{10}$H$</em>{16}$S$_2$</td>
<td>O$_2^+$</td>
<td>2.3</td>
<td>94</td>
<td>[C$_8$H$_6$S$^+$]</td>
<td>[3]</td>
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<td>NO$^+$</td>
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<td>118</td>
<td>[C$_8$H$_6$COOC$_2$H$_5$]$^+$</td>
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<td>Guaiacol</td>
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<td>NO$^+$</td>
<td>2.5</td>
<td>124</td>
<td>[C$_8$H$_8$O$^+$]</td>
<td>[5]</td>
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<td>C$_7$H$_8$O</td>
<td>NO$^+$</td>
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<td>99</td>
<td>[C$_8$H$_8$O]$^+$</td>
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<td>Hexenal</td>
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<td>NO$^+$</td>
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<td>Hexenol</td>
<td>C$_8$H$_8$O</td>
<td>NO$^+$</td>
<td>2.5</td>
<td>72</td>
<td>[C$_8$H$_8$O]$^+$</td>
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<tr>
<td>Isobutanal</td>
<td>C$_5$H$_8$O</td>
<td>O$_2^+$</td>
<td>3.0</td>
<td>72</td>
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<td>[4]</td>
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<tr>
<td>Isobutyl alcohol</td>
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<td>O$_2^+$</td>
<td>2.5</td>
<td>42</td>
<td>[C$_8$H$_7$]$^+$</td>
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<td>Methylalcohol</td>
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<td>[C$_8$H$_7$O]$^+$</td>
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<td>Methyl salicylate</td>
<td>C$_6$H$_7$O$_2$</td>
<td>O$_2^+$</td>
<td>2.7</td>
<td>152</td>
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<td>Methylbutanal</td>
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<td>NO$^+$</td>
<td>3.0</td>
<td>85</td>
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<td>Methylbutanoic acid</td>
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<td>NO$^+$</td>
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<td>Nonanal</td>
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<td>112</td>
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<td>N-propyl alcohol</td>
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<td>NO$^+$</td>
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<td>Phenylacetaldehyde</td>
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<td>Terpenes</td>
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<td>NO$^+$</td>
<td>2.2</td>
<td>136</td>
<td>[C$_8$H$_8$O]$^+$</td>
<td>[6]</td>
</tr>
</tbody>
</table>
such as C₆ aldehydes and alcohols have been reported to be responsible for the fresh and green aroma of various fruits and vegetables including capsicum peppers (Luning and others 1994). The tissue disruption of unblanched jalapeño peppers increases the rate of generation of these compounds due to the activity of enzymes involved in the lipid oxygenase pathway such as LOX, hydroperoxide lyase (HPOL), (Z)-3/(E)-2 isomerase (Z3/E2 ISO), and alcohol dehydrogenase (ADH) on their substrates. In contrast, the unblanched sample blended with 0.2M SnCl₂ and the blanched sample showed significantly lower concentration of all LOX-derived volatiles compared to unblanched samples. The stannous chloride solution was used to chemically inactivate LOX and other enzymes, while blanching thermally denatures the enzymes. Therefore, the generation of LOX-derived volatile compounds was prevented in both stannous chloride-treated and blanched samples due to enzyme inactivation. Wu and Liou (1986) confirmed the inhibition effect of stannous chloride on the enzymatic generation of C₆ aldehydes and alcohols upon tissue disruption of bell peppers.

In unblanched samples, the concentration of (Z)-3-hexenal accounted for 75% of the total concentration of all LOX-derived volatiles tested, while hexanal accounted for 16%, as measured during the first 10 min after tissue disruption. (Z)-3-Hexenal and hexanal are produced from the 13-hydroperoxide (13-HPO) of linolenic and linoleic acid, respectively (Luning and others 1995a). These aldehydes are the first compounds generated in their respective pathways by the action of LOX and HPOL enzymes.

The ratio of (Z)-3-hexenal to (E)-2-hexenal was 10:1 over the first 10 min after blending, suggesting a faster rate for the generation of (Z)-3-hexenal than for its isomerization into (E)-2-hexenal. This ratio is extremely time dependent as the concentration of both compounds vary greatly according to their generation/degradation reactions. In grape tomatoes, the (Z)-3-hexenal to (E)-2-hexenal ratio is 1:3:1 over the first 10 min after blending (Xu and Barringer 2009). However, at about 1 h after blending the ratio is 1.2:1 and 1.28.5 in green and red bell peppers, respectively (Luning and others 1995a); while at about 2 h after tissue disruption the ratio is 1.34 in bell peppers (Wu and Liou 1986). (Z)-3-Hexenal is generated immediately after tissue disruption, and then it starts isomerizing into (E)-2-hexenal. Both compounds are converted into their corresponding alcohols. Consequently, the relative concentration of each compound is constantly changing over time; therefore, it is expected that the ratios will be different for these volatiles when measured at different times. If care is not taken, (Z)-3-hexenal can isomerize to (E)-2-hexenal by enzymes present in the medium during the volatile isolation phase in tomatoes (Buttery and others 1987).

The ratio of the total amount of unsaturated to saturated C₆ aldehydes and alcohols was 5:1 over the first 10 min after blending. Hexanal and hexanol are the only saturated C₆ volatiles in the reported pathway and they are both primarily produced from linoleic acid, while the unsaturated C₆ volatiles (Z)-3-hexenal, (E)-2-hexenal, and hexenal are produced from linolenic acid. Thus, the ratio obtained indicates a higher rate of generation of C₆ aldehydes and alcohols from linolenic acid than from linoleic acid in jalapeño peppers. Similarly, a ratio of 3:1 in bell peppers at about 2 h after tissue disruption (Wu and Liou 1986); 2:1 and 5:1 in green and red bell peppers, respectively, at about 1 h after

![Figure 1](https://example.com/figure1.png)

Figure 1—Effect of enzyme activity on the concentration of LOX-derived volatiles in the headspace of jalapeño pepper. Columns with different letters within the same compound are significantly different. Standard error for (Z)-3-hexenal and hexanal in unblanched samples was 384 and 73, respectively.
Effect of enzyme activity... 

blending (Luning and others 1995a); and 2:5:1 in tomatoes over the 1st 10 min after blending (Xu and Barringer 2009) has been reported. Unlike the (Z)-3-hexenal to (E)-2-hexenal ratio, the ratio of the total amount of unsaturated to saturated C6 aldehydes and alcohols is not considerably affected by time. The ratio of linolenic to linoleic acid is ca. 1:20, 1:7, and 1:27 in jalapeno pepper, bell pepper, and tomato, respectively (USDA National Nutrient Database 2010). Thus, there is much more linoleic acid present, but a higher concentration of volatiles are being formed from linolenic acid.

Real-time monitoring of LOX-derived volatiles generation

The concentration of some LOX-derived volatiles was monitored in real time for 10 min starting 1 min after initiation of blending the peppers.

(Z)-3-hexenal and (E)-2-hexenal

The compounds (Z)-3-hexenal and (E)-2-hexenal showed a similar trend (Figure 2). The concentration of these compounds was significantly lower in SnCl2-treated and blanched samples compared to unblanched samples because enzyme activity was either chemically or thermally inactivated. Thus, the concentrations detected in the SnCl2-treated and blanched samples correspond to the levels of these volatiles inside the tissue before its disruption. Any increase above these levels is most probably due to formation by enzymes upon tissue disruption.

For unblanched samples, high levels of (Z)-3-hexenal and (E)-2-hexenal were generated immediately after tissue disruption, with the highest rate of formation by 1.3 min and 1.5 min after the start of blending for (Z)-3-hexenal and (E)-2-hexenal, respectively. Since the measurements started 1 min after initiation of blending, there is already a high concentration of these compounds at the beginning of the testing period. It may be possible that peak concentration occurred within the first minute after tissue disruption. A similar trend in the production of (Z)-3-hexenal and (E)-2-hexenal was observed for tomatoes; where the maximum level was reached at 3.3 and 3.7 min after blending for (Z)-3-hexenal and (E)-2-hexenal, respectively (Xu and Barringer 2009). Another study with tomatoes reported the maximum concentration of (Z)-3-hexenal at approximately 3 min after tissue disruption (Buttery and others 1987). This implies a faster LOX activity in jalapeno peppers compared to tomatoes.

The rapid increase in (Z)-3-hexenal and (E)-2-hexenal is due to a very rapid reaction between enzymes and substrates present in jalapeno pepper. After reaching its peak level, concentration of both compounds declined over time. This can be explained by 2 factors: the conversion of primary volatiles into different compounds and the depletion of sample headspace. LOX-derived volatiles are constantly changing to other compounds during the testing period. (Z)-3-Hexenal is converted to (E)-2-hexenal by Z3/E2 ISO and these aldehydes are reduced to their respective alcohols by ADH (Luning and others 1995a; Xu and Barringer 2009). These changes in the concentrations of the compounds constitute the major cause of the decline over the 10 min. However, this is a dynamic system. Volatiles are generated within the tissue based on enzyme reaction rates, volatilized into the air based...
on Henry's Law, and withdrawn from the headspace by the SIFT. The SnCl₃-treated and blanched samples serve as something of a control because all conditions are present except generation of the volatiles. Thus, the difference in results between the untreated and enzyme-inhibited samples is due to the enzyme activity.

Hexanal

Similarly, SnCl₃-treated and blanched samples represent the levels of hexanal in the intact tissue before disruption, and the concentration of hexanal in both samples was significantly lower than in the unblanched sample. In unblanched samples, the generation of hexanal occurs immediately upon tissue disruption (Figure 2). Since the measurements started 1 min after initiation of blending, in unblanched samples there is already a high concentration of hexanal at the beginning of the testing period due to fast enzymatic activity. The concentration of hexanal reached a maximum level at or before 1.5 min after the start of blending. The concentration remained constant for ca. 7 min and then began to decrease. In tomato puree, the headspace concentration of hexanal increased after blending, reached a peak level at 4.7 min, and then decreased over time (Xu and Barringer 2009). The decline in hexanal concentration after reaching a maximum level is due to a decrease in the generation rate and to a slow reduction to hexanol.

Hexenol and hexanol

Hexenol represents the mixture of (Z)-3-hexenol and (E)-2-hexenal, which are the corresponding alcohols formed from (Z)-3-hexenal and (E)-2-hexenal by the action of the enzyme ADH. These isomers cannot be differentiated by SIFT-MS; therefore, they are reported together as a mixture. Hexanol is the corresponding alcohol generated from hexanal also by the ADH enzyme (Luning and others 1995a; Xu and Barringer 2009). The results for hexenol and hexanol were similar thus only 1 is shown (Figure 2). For unblanched samples, the concentration was low, around 10 ppb for hexenol and 8 ppb for hexanol; however, it was significantly higher than in SnCl₃-treated and blanched sample, indicating enzyme activity. The concentration was roughly constant over the 10-min period tested. Xu and Barringer (2009) also reported a low concentration of hexanol and hexenol in tomato puree over a 60-min period after blending. The aldehydes decreased over time; then following the pathway, alcohols should be correspondingly forming. It is possible that the alcohols are quickly converting into other compounds such as acids, and therefore little accumulation is detected.

Effect of blanching on volatiles

Blanched samples represent the cumulative result of enzymatic inhibition and heating; therefore, differences between blanched and stannous chloride-treated samples can be attributed to the effect of heat. There was no significant difference in the concentration of any volatiles between blanched and stannous chloride-treated samples, except for (Z)-3-hexenal (Figure 1), methylbutanal, and dimethyl sulfide (Figure 3). The concentration of (Z)-3-hexenal was higher in the stannous chloride-treated sample compared to the blanched sample. This difference may be attributed to heat degradation during the blanching process. (Z)-3-Hexenal is a heat labile compound. Kazeniac and Hall (1970) reported a poor stability of (Z)-3-hexenal when blended tomatoes were exposed to heat. Heat promotes the isomerization of this compound to (E)-2-hexenal. In order to recover (Z)-3-hexenal from samples, an isolation technique involving minimal heating is required (Kazeniac and Hall 1970).

Dimethyl sulfide and methylbutanal had higher concentrations in the blanched sample compared to the stannous chloride-treated sample (Figure 3). Dimethyl sulfide was 760% higher in blanched compared to unblanched peppers. This compound has a cooked, cabbage-like aroma and it is considered to be a key volatile in the aroma of several cooked vegetables (Scherb and others 2009). Together with other volatile sulfur compounds, dimethyl sulfide has been reported to be responsible for the sulfurous off-flavors of several heat-processed foods (Vázquez-Landaverde and others 2005, 2006; Lozano and others 2007). Methylbutanal that represents the mixture of 2- and 3-methylbutanal, was 86% higher in blanched compared to unblanched peppers. These compounds have cacao, sweaty, and cooked vegetable odor notes (Luning and others 1995b), and have also been reported to increase concentration in heated products. An increase in 2- and 3-methylbutanal was found in bell peppers after hot-air drying (Luning and others 1995b). 2- and 3-Methylbutanal and dimethyl sulfide were predominant odorants in fried chili paste, and their level increased as the heating time increased (Rotsatchakul and others 2008).

Dimethyl sulfide can be formed from the amino acid methionine by Strecker degradation or from S-methylmethionine by heat-induced breakdown. By Strecker degradation, methional that is produced from methionine, is decomposed to methanethiol that then oxidizes to dimethyl sulfide (Lozano and others 2007). Likewise, 2- and 3-methylbutanal are formed by Strecker degradation of isoleucine and leucine, respectively, during Maillard reactions (Vázquez-Landaverde and others 2005; Rotsatchakul and others 2008).

Jalapeño pepper contains the amino acids methionine, leucine, and isoleucine in similar amounts to those found in cabbage and leeks (USDA National Nutrient Database 2010). The heat treatment involved in blanching causes an increase in dimethyl sulfide and methylbutanal in the blanched sample, and may also be responsible for the characteristic aroma that was perceived in blanched peppers during sample preparation, which had less green and fresh notes and more cooked notes compared to unblanched peppers.

Effect of frozen storage on LOX-derived volatiles

The concentration of LOX-derived volatiles was measured in 2 sets of samples of frozen stored (−15 °C) jalapeño peppers. In

Figure 3–Effect of blanching on methylbutanal and dimethyl sulfide. Columns with different letters within the same compound are significantly different.
Effect of enzyme activity . . .

1 set, volatiles were measured at 0 (initial measurement), 1.5, 2.5, and 3 mo of storage in unblanched samples; whereas in the other set, volatiles were measured at 0, 6, and 9 mo of storage in both unblanched and blanched samples. Peppers were stored whole or pureed.

**Blanched peppers**

Since enzymes are inactivated, no major changes were expected in the concentration of LOX-derived volatiles during the frozen storage of blanched samples. There were no major changes in these volatiles during storage time; however, some minor chemical changes were observed, especially in pureed pepper samples (Figure 4). In frozen stored leeks, blanching minimized changes in the aroma profile due to inhibited enzymatic activity (Nielsen and Poll 2004).

A decrease in (Z)-3-hexenal accompanied by an increase in (E)-2-hexenal over time was found in blanched peppers. This is probably due to a gradual chemical isomerization of (Z)-3-hexenal into the more stable (E)-2-hexenal. A higher degree of isomerization occurred in the pureed sample compared to the whole pepper sample at the same storage time (6 mo), which was even greater than in whole pepper stored 3 mo longer (9 mo). In pureed samples, extensive cell rupture has occurred, favoring greater interaction and exposure of components. The sum of (Z)-3-hexenal and (E)-2-hexenal concentration did not significantly change for any of the samples tested. This indicates a direct conversion of (Z)-3-hexenal into (E)-2-hexenal throughout the frozen storage period.

The rest of the LOX-generated volatiles maintained their initial concentration during storage, as expected. However, hexanal, 2-pentenal, and (E)-2-heptenal slightly increased concentration in the 6 mo pured samples. This increase may be due to some autoxidation of linoleic and linolenic acid occurring in the pureed samples where the interaction between the components and oxygen is maximized by tissue breakage and air incorporation resulting from blending. Nielsen and others (2004b) also found a small but significant increase in aldehydes including hexanal, (E)-2-pentenal, (E)-2-hexenal, and (E)-2-heptenal in frozen stored blanched leeks. The development of aldehydes in the blanched leeks was attributed to autoxidation of polyunsaturated fatty acids.

**Unblanched peppers**

**Lipoxygenase-derived major volatiles.** During the 1st 3 mo of frozen storage of the whole pepper samples, (Z)-3-hexenal gradually decreased concentration over time, most probably due to conversion of (Z)-3-hexenal into (E)-2-hexenal (Figure 5). Hexanal, hexenol, and hexanol decreased in concentration as well. All of these volatiles, as well as (E)-2-hexenal, continued to decrease at 6 mo and even further at 9 mo of storage compared to the initial measurement (Figure 6). Differences in compound’s concentrations between the 2 set of samples are due to natural variations among different batches of peppers.

Thus, in the whole unblanched jalapeño peppers (Z)-3-hexenal is generated and then rapidly isomerized to (E)-2-hexenal. (E)-2-Hexenal maintained its level over the 1st 3 mo due to (Z)-3-hexenal converting into (E)-2-hexenal, but after that it decreases. These aldehydes are then converted to hexenol. In the same way, hexanal is formed and then converted to hexanol. The alcohols, hexenol and hexanol are further degraded into other compounds.

![Figure 4–Effect of frozen storage on LOX volatiles in the headspace of whole and pureed blanched jalapeño peppers. Columns with different letters within the same compound are significantly different.](C716 Journal of Food Science • Vol. 75, Nr. 9, 2010)
However, in whole unblanched jalapeño peppers the degradation rate of these compounds is higher than their generation rate; therefore, they are continuously decreasing over time and not much accumulation occurs.

Enzymes are active in unblanched peppers and even though their activity is reduced at low temperatures it is not completely stopped. LOX in leeks gradually decreases activity during frozen storage at −20 °C; however, 25% of the initial activity was still detected after 12 mo of storage (Nielsen and others 2003). Furthermore, during the freezing process, cell structure is damaged by growing ice crystals allowing interaction between LOX enzymes and its substrate polyunsaturated fatty acids that are located in the cytosol and the cell membrane, respectively. During frozen storage, reactions can take place in the liquid water phase (Nielsen and others 2003, 2004a). Hence, slow but continued LOX-initiated reactions were expected to occur during the frozen storage of unblanched jalapeño peppers.

The behavior of the pureed pepper samples was similar to that of whole pepper samples, except that an early peak occurred in all compounds (Figure 7). The concentration of (Z)-3-hexenal, (E)-2-hexenal, and hexanal increased, reached a maximum level and then decreased over time. Again, (Z)-3-hexenal is generated and reached a maximum at 1.5 mo, then gradually converted into (E)-2-hexenal that maintained its maximum from 1.5 to 3 mo. (Z)-3-Hexenal and (E)-2-hexenal are further transformed into hexenol. Hexanal increases concentration and then it is converted to hexanol. Similarly, in unblanched sliced leeks hexanal considerably increased concentration during frozen storage, most probably due to polyunsaturated fatty acids oxidation by the lipoxygenase pathway (Nielsen and others 2003). Hexenol and hexanol followed the same trend; they were generated and then gradually degraded into other compounds.

In pureed peppers, all reactions should occur faster and to a greater extent than in whole peppers due to an increased interaction between components and oxygen, which favors enzymatic and chemical reactions; whereas in whole peppers this interaction is limited. Therefore, during frozen storage, generation of the LOX-derived compounds occurs at a higher rate compared to whole samples that explain the initial increase in concentration for all these compounds in the pureed samples. In frozen stored leeks, LOX activity and consequently LOX-derived aldehydes and alcohols generation was more favorable in 4-mm slices than in 15-mm slices due to a higher degree of cell disruption per weight unit (Nielsen and others 2004a).

**Lipoxygenase-derived minor volatiles.** In whole peppers, the minor LOX-derived volatiles 1-penten-3-one, (E)-2-heptenal, (E)-2-octenal, and (E)-2-nonenal gradually increased over the first months of storage reaching a maximum concentration at about 2.5 mo, depending on the volatile, and then started decreasing (Figure 8). 2-Pentenal and 1-penten-3-one are formed from the 13-HPO of linolenic acid by an alternate pathway proposed in a previous study on bell peppers (Luning and others 1995a). (E)-2-Heptenal, (E)-2-octenal, and (E)-2-nonenal are formed from the 12-HPO, 10-HPO, and 9-HPO of linoleic acid, respectively. The 12-HPO and 10-HPO are formed by a rearrangement of the 13-HPO and 9-HPO of linoleic acid (Luning and others 1995a). Results suggest that under the frozen storage conditions tested, there is a slow but continued activity of the alternative 9-HPO, 10-HPO, and

![Figure 5](image_url) Effect of frozen storage on major LOX aldehydes and their corresponding alcohols in the headspace of whole unblanched jalapeño peppers from 0 to 3 mo. Columns with different letters within the same compound are significantly different.
Figure 6—Effect of frozen storage on major LOX aldehydes and their corresponding alcohols in the headspace of whole unblanched jalapeno peppers at 6 and 9 mo. Columns with different letters within the same compound are significantly different.

Figure 7—Effect of frozen storage on major LOX aldehydes and their corresponding alcohols in the headspace of pureed unblanched jalapeno peppers. Columns with different letters within the same compound are significantly different. Data from 6 mo were normalized based on the 0 mo data.
12-HPO pathway for linoleic acid and 13-HPO alternative pathway for linolenic acid proposed by Luning and others (1995a) in previous studies with bell peppers. In tomato puree at 37 °C, an increase in the generation rate of (E)-2-pentenal, (E)-2-heptenal, and (E)-2-octenal also occurred, suggested an increase in the activity of alternate pathways at that temperature (Xu and Barringer 2009).

These minor LOX-derived compounds are continuously generating and degrading during storage; however, the relative rate of these reactions throughout storage results in a net increase or decrease in concentration. Similarly, in unblanched leeks, an increase in (E)-2-monounsaturated aldehydes including (E)-2-pentenal, (E)-2-heptenal, (E)-2-octenal, and (E)-2-nonenal during frozen storage was also reported. Most of these compounds were not present in the fresh leek but were formed during storage (Nielsen and others 2003).

The behavior of the pureed pepper samples was similar to that of whole pepper samples (Figure 9). However, for some compounds the maximum concentration tended to occur a little earlier in pureed peppers than in whole peppers. The maximum concentration of (E)-2-heptenal and (E)-2-nonenal was reached after 1.5 to 2.5 mo of frozen storage compared to about 3 mo for whole pepper samples. This may be explained again by the higher rate of generation and degradation reactions in tissue disrupted peppers.

Effect of frozen storage on other volatiles

The concentration of other volatiles in jalapeño pepper also changed during frozen storage (Table 2). Levels of 2-isobutyl-3-methoxypyrazine, n-propyl aldehyde, and a mixture of terpenes decreased over time in both unblanched and blanched peppers indicating a chemical degradation of these compounds under frozen storage conditions. Aldehydes may be converted into alcohols or even further into acids. Terpenes may be attacked by HPOs to form terpene-related ketones and alcohols (Kazeniac and Hall 1970). Conversely, an increase in terpenes and 2-isobutyl-3-methoxypyrazine was found in frozen Padron-type peppers; however, lot variability made it difficult to identify a characteristic volatile profile for the frozen peppers (Oruña-Concha and others 1998). 2-Isobutyl-3-methoxypyrazine has been associated with the aroma of fresh green bell pepper, and the terpenes analyzed in jalapeño pepper samples, which are a mixture of limonene, linalool, ocimene, and 2- and 3-carene, are related to citrus, floral, and sweaty odor notes (Buttery and others 1969; Chitwood and others 1983).

The compounds nonanal and methylbutanal increased concentration only in unblanched samples suggesting enzymatic formation of these volatiles. An increase in nonanal in frozen stored unblanched leeks was attributed to enzyme activity (Nielsen and others 2003). Similarly, the formation of 3-methylbutanal from the amino acid L-leucine using enzyme extracts from tomatoes was reported (Yu and others 1968). Nonanal has painty and turpentine-like odor notes; while methylbutanal have cacao and cooked vegetable odor notes (Luning and others 1995b; Nielsen and Poll 2004).

Thus, results suggest an effect of frozen storage on the volatile profile of both unblanched and blanched jalapeño peppers, independent of that produced by the LOX pathway. Differences in volatile concentrations between unblanched and blanched samples may be due to both enzymatic formation and heat degradation.

![Figure 8–Effect of frozen storage on minor LOX volatiles in the headspace of whole unblanched jalapeño peppers. Columns with different letters within the same compound are significantly different. Data from 6 and 9 mo were normalized based on the 0 mo data.](image)
Effect of enzyme activity...

Conclusions
Results confirmed that enzyme activity in jalapeño peppers is essential for the generation of volatile compounds related to fresh and green aromas. The formation of these compounds occurs very fast, reaching a maximum concentration within the 1st 1.5 min after tissue disruption. The ability of SIFT-MS to measure volatiles in real time directly from the headspace of samples is useful to determine the generation and transformation trends of these compounds immediately after the reactions are initiated.

Blanching affected the volatile profile of jalapeño pepper by both enzyme inactivation and a thermal effect. It markedly prevented the generation of LOX-derived compounds due to enzyme

Figure 9–Effect of frozen storage on minor LOX volatiles in the headspace of pureed unblanched jalapeño peppers. Columns with different letters within the same compound are significantly different. Data from 6 and 9 mo were normalized based on the 0 mo data.

Table 2–Effect of frozen storage on some volatiles in whole and pureed, unblanched and blanched jalapeño peppers.

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<thead>
<tr>
<th>Compound</th>
<th>Concentration (ppb)</th>
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<tr>
<td>(E,E)-2,4-decadienal</td>
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<tr>
<td>(E,Z)-2,6-nonalidinal</td>
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<tr>
<td>2,3-butanedione</td>
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<tr>
<td>2-hydroxy-3-methylpentanoic acid</td>
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<td>2-pentanone</td>
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<td>Acetaldehyde</td>
<td>154</td>
</tr>
<tr>
<td>Dimethyl disulfide</td>
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<tr>
<td>Dimethyl sulfide</td>
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<tr>
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<tr>
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<tr>
<td>Isobutanal</td>
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<td>Isobutyl alcohol</td>
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<td>Phenylacetaldehyde</td>
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<td>Terpenes</td>
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Concentration (ppb)

Unblanched

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Blanched

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Table 2–Effect of frozen storage on some volatiles in whole and pureed, unblanched and blanched jalapeño peppers.
inactivation; and at the same time, it resulted in an increased concentration of compounds with “cooked” and “cabbage-like” notes due to heating.

Frozen storage at −15 °C resulted in changes in the LOX-derived volatiles of unblanched jalapeño peppers due to a continued enzymatic activity. In contrast, no major enzymatic changes were observed in the volatiles of blanched jalapeño peppers. Pureed pepper samples showed an increased rate of generation and degradation reactions than whole pepper samples due to a greater degree of tissue disruption.

Similar changes in nonenzymatic volatiles in both unblanched and blanched peppers suggest an effect of frozen storage on the aroma profile of jalapeño pepper additional to that produced by enzymatic activity.

References


