Volatile generation in bell peppers during frozen storage and thawing using selected ion flow tube mass spectrometry (SIFT-MS).

Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in
the Graduate School of The Ohio State University

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The Ohio State University
2012

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Abstract

Whole, pureed, blanched, and raw green and red bell peppers (*Capsicum annuum*) with and without SnCl₂ were frozen quickly or slowly then stored at -18°C for up to 7 mo and the volatiles were measured during storage. Headspace analysis of bell peppers was performed by a Selected Ion Flow Tube Mass Spectrometer (SIFT-MS). Lipxygenase (LOX) activity was determined by UV-Visible Spectrophotometry. As a direct effect of blanching, (*Z*)-3-hexenal had a large significant decrease in concentration since it is a heat labile compound while most other volatiles did not change in concentration. The freezing process increased volatile levels in the puree only. During frozen storage of blanched samples (*E*)-2-hexenal, hexen-1-ol, and (*E*)-2-pentenal increased because of nonenzymatic autooxidation of fatty acids into volatiles while other volatiles remained constant. In raw whole peppers (*Z*)-3-hexenal, hexanal, and 2-pentylfuran were generated during storage because the LOX enzyme is still active during frozen storage. However, blanched samples had higher concentrations of (*E*)-2-hexenal, hexen-1-ol, 1-penten-3-one, and (*E*)-2-heptenal because of enzymatic destruction of these volatiles in the raw samples. The levels of many of the volatiles in the raw samples, including (*Z*)-3-hexenal, (*E*)-2-hexenal, hexen-1-ol, hexanal, (*E*)-2-pentenal, and 2-pentylfuran, appeared to peak

around 34d after freezing. Significantly higher volatile formation occurred during thawing than it did during frozen storage. Slowly frozen peppers had higher levels of some LOX volatiles during storage. Pureed samples had significantly higher levels of volatiles than the whole samples which peaked earlier. Green bell pepper volatiles were always higher than red bell pepper volatiles.

Acknowledgements

First and foremost I would like to thank my advisor, Dr. Sheryl Barringer. She taught me a great deal about how to write an academic paper, how to plan and keep a research project on track, how to learn from mistakes, and most importantly for her overall help and support as I progressed through my project.

Secondly, I would like to thank my committee members Dr. James Harper and Dr. Luis Rodriguez-Saona for making food science interesting as an undergraduate and always being available to help with any questions I had and also for their help with my research.

I would like to thank Paul Courtright for his help in the pilot plant while I was processing my bell peppers. Dr. Steven Naber for all his help running my statistics and Dr. Kevin Goodner for helping me with specific aroma questions I had during my research.

I would like to give a big thank you to my dad, Doug Wampler and mom, Mindy Wampler for making me the individual I am today. They taught me to work hard for what I want in life, have confidence, to not let the hard times get you down, and most importantly to have faith. For all of these things and their constant love and support I cannot thank them enough.

Lastly, thank you to all my friends and lab mates for making all the long hours in the lab easier and for their support and encouragement.

Vita

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Practical Applications

This information will help manufacturers better understand how the aroma of frozen bell peppers change with time during storage and allow them to create a better shelf-life. In frozen products with bell peppers in them manufactures need to ensure they are not producing off-flavor aromas during storage. Studying and monitoring the headspace volatiles with a SIFT-MS can give the information needed on what the volatiles in bell peppers do during frozen storage to determine when the peppers should no longer be used to make products. This will give valuable information to processors on how to get the best aroma from their peppers and maintain that quality in all products.

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Dedicated to my family and friends

Chapter 1: Introduction

Bell peppers are grown in Asia, Africa, countries along the Mediterranean, and America and are widely used as a spice, coloring powder, vegetable, and external medicines (Matsui and others 1997). They have a distinct green and bitter flavor, which some people perceive as pleasant while others find unpleasant. They are also a source of vitamins A, C, and E.

Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) is a new and emerging analytical tool which can help to identify and quantify trace amounts of gases in many different fields of study. This piece of equipment has been used to measure environmental gas emission (Wilson and others 2003), medical diagnosis (Amann and others 2007), and recently food, flavor, and fragrances (Frank 2007). In the food industry it has been used to measure the real time formation of aromas related to spoilage and oxidation (Davis and McEwin 2007), breath analysis (Hansanugrum and Barringer 2009), enzyme activity (Azcarate and Barringer 2010), and roasting (Huang and Barringer 2011).

In bell peppers the major lipoxygenase (LOX) generated volatiles are (*Z*)-3-hexenal, (*E*)-2-hexenal, hexanal, (*Z*)-3-hexenol, (*E*)-2-hexenol, and hexanol which are created from two fatty acids, linoleic and linolenic acid. Upon rupturing of the cells the LOX enzyme combines with oxygen and forms hydroperoxides. Hydroperoxides are then

converted by hydroperoxide lyase and in linoleic acid this creates hexanal while in linolenic acid it creates (Z)-3-hexenal. Hexanal is then converted into hexanol by alcohol dehydrogenase while (Z)-3-hexenal is converted into two products one being (Z)-3-hexenol by alcohol dehydrogenase and the other product is (E)-2-hexenal by Z3/E2 isomerase. (E)-2-Hexenal is then converted into (E)-2-hexenol by alcohol dehydrogenase (Luning and others 1995a).

When jalapeno peppers, which are from the same *Capsicum annum* family as bell peppers, were frozen LOX generated volatiles changed throughout the storage period (Azcarate and Barringer 2010). In unblanched whole jalapeno pepper samples in the first 3mo of frozen storage (Z)-3-hexenal decreased in concentration most likely due to the conversion of (Z)-3-hexenal into (E)-2-hexenal. The volatile level concentration also decreased in the first 3mo of storage for hexanal, hexen-1-ol, and hexanol. (Z)-3-Hexenal, (E)-2-hexenal, hexanal, hexen-1-ol, and hexanol continued to decrease during 9mo of frozen storage (Azcarate and Barringer 2010). The disappearance of volatiles can be explained by the LOX pathway. (Z)-3-Hexenal is the first volatile created from linolenic acid and is then converted into (E)-2-hexenal by isomerization. (E)-2-Hexenal is also converted into other volatiles as well through other possible enzymatic reactions. Hexen-1-ol is then enzymatically acetylated into compounds such as hexyl acetate and (Z)-hexenyl acetate (Fall and others 1999). The disappearance of hexanal during frozen storage can be explained by the fact that it is the first LOX generated volatile created from linoleic acid which is then converted into hexanol by alcohol dehydrogenase (Luning and other 1995a). With the enzymes still active in these samples it was expected

that volatiles would be created and disappear or breakdown into other volatiles during storage.

In blanched jalapeno peppers since the enzyme was deactivated during the blanching process the volatiles did not significantly change except for (*E*)-2-hexenal which increased during storage because of natural isomerization during storage as well as during blanching. (*E*)-2-Hexenal was initially higher in the blanched jalapeno peppers because (*Z*)-3-hexenal is heat liable and will isomerize into (*E*)-2-hexenal during blanching (Azcarate and Barringer 2010).

In unblanched leek slices during long-term frozen storage, concentrations of saturated aldehydes increased during 12mo of storage (Nielsen and others 2003). Hexanal was the only aldehyde present in fresh leeks with the remaining aldehydes developing during storage. All of the (*E*)-2-monounsaturated aldehydes except (*E*)-2-hexenal increased during the 12mo frozen storage and only (*E*)-2-hexenal and (*E*)-2-nonenal were found in fresh leek with the others formed during storage (Nielsen and others 2003). The concentration of (*E*)-2-hexenal was high in fresh leeks and then declined over time which indicated further conversion to (*E*)-2-hexenol or (*Z*)-2-hexenal however no traces of those volatiles were found in any of the samples (Nielsen and others 2003).

There was a minor difference between the unblanched and blanched samples because six of the aldehydes (butanal, hexanal, heptanal, (*E*)-2-hexenal, (*E*)-2-heptenal, and 2-methyl-(*E*)-2-pentenal) decreased after blanching while pentanal and decanal increased (Nielsen and others 2004). After 12mo of frozen storage under a controlled atmosphere the differences between samples was greater because the total amount of

aldehydes was 7.69 mg/L in the unblanched 21% O₂ and 2.13 mg/L in blanched 21% O₂ samples (Nielsen and others 2004).

The objective of this study was to determine the extent to which lipoxygenase (LOX) volatiles are formed and destroyed during frozen storage and to determine how freezing rate and sample type effects volatile formation.

Chapter 2: Literature Review

Lipoxygenase Overview

Lipid peroxidation is common to every biological system and appears in both developmentally and environmentally regulated processes of plants. The hydroperoxy polyunsaturated fatty acids, synthesized by the action of various highly specialized forms of lipoxygenases are substrates of several different enzyme types. The composition and turnover of the intracellular lipids are frequently altered during a plant's development and are among the first targets of environmental changes. Aside from the fatty acid turnover in lipids the formation of oxidized polyenoic unsaturated fatty acids or PUFAs, collectively called oxylipins, is one of the main reactions in lipid alteration. The initial formation of hydroperoxides may occur by either autooxidation or by the action of enzymes such as lipoxygenases, or LOXs, or alpha-dioxygenase. The metabolism of PUFAs by the LOX-catalyzed step and the subsequent reactions are collectively named the LOX pathway. LOX catalyzes the region and stereo specific dioxygenation of PUFAs containing a (1Z,4Z)-pentadiene system, linoleic acid (LA), alpha-linolenic acid, or arachidonic acid (Feussner and Wasternack 2002). However, arachidonic acid is found only in low levels in plants so the main LOXs are classified with respect to their positional specificity of LA oxygenation (Feussner and Wasternack 2002).

Lipoxygenase Pathway for red and green bell peppers

Physical disruption of plant tissues will result in the production of volatile compounds from unsaturated fatty acids which are responsible for the characteristic aroma in some plants. The involvement of lipoxygenase in the formation of these volatile flavor compounds has been reported in many plants used for food products such as bananas, apples, cucumber, tomato, raw beans, mushrooms, and tea. The enzymatic formation of volatile compounds from unsaturated fatty acids depends on many different variables. One such variable is the type of plant since substrate specificity of wheat germ LOX isoenzymes demonstrated higher activity for linoleic acid compared to linolenic acid. Also there are several isoenzymes that are specific in the production of the C-9 and C-13 hydroperoxides. Then hydroperoxide lyases can specifically cleave C-9 and or C-13 hydroperoxides to make aldehyde fragments. Other enzymes like isomerases and alcohol dehydrogenases can change the primary formed volatile compounds. As a result of this a broad variety of volatile compounds can be created by the primary action of LOX (Luning and others 1995a).

This LOX activity has been observed in both green and red fruits of the *Capsicum annuum* varieties and the seeds of red bell peppers as well (Luning and others 1995a). The volatiles formed from the LOX activity starts with bell pepper lipids that are first broken down by acyl hydrolase enzymes which forms the fatty acids linoleic acid and linolenic acid. These fatty acids combine with oxygen and lipoxygenase to form hydroperoxides. Then the C-9, C-10, and C-12 hydroperoxides from linoleic acid all go through possible enzymatic transformation to form the volatile compounds of 2-pentylfuran and (*E*)-2-nonenal, (*E*)-2-octenal, and (*E*)-2-heptenal respectively. The C-13

hydroperoxides are transformed into the volatile compound hexanol by alcohol dehydrogenase (Luning and others 1995a).

The hydroperoxides created from linolenic acid are C-13 and C-9 however the C-9 hydroperoxides can go through three different reaction pathways to form several different volatiles. One of the volatiles these C-13 hydroperoxides eventually produces is 1-penten-3-one after the hydroperoxide first goes through a possible enzymatic transformation. It also creates (*Z*)-2-pentenal after a possible enzymatic transformation which can be turned into (*E*)-2-pentenal through an enzymatic transformation or into (*Z*)-2-pentenol by alcohol dehydrogenase. (*E*)-2-Pentenal can be turned into (*E*)-2-pentenol by alcohol dehydrogenase. The last pathway for forming volatiles from the C-13 hydroperoxides is by hydroperoxide lyase which converts the hydroperoxide into (*Z*)-3-hexenal which can then be turned into (*Z*)-3-hexenol by alcohol dehydrogenase or into (*E*)-2-hexenol by (*Z*)-3/ (*E*)-2 isomerase. Finally, (*E*)-2-hexenal can be turned into (*E*)-2-hexenol by alcohol dehydrogenase or into (*Z*)-2-hexenal by an enzymatic transformation (Luning and others 1995a).

To start the LOX activity the tissues of the plant must be disrupted. It is this tissue disruption that starts the rapid hydrolytic and oxidative degradation of lipids inside the bell pepper to produce the various volatile compounds which can either be desirable or undesirable (Wu and Liou 1986). The enzymatic formation of hexanal, (*E*)-2-hexenal, hexanol, (*Z*)-3-hexen-1-ol, and (*E*)-2-hexen-1-ol are all created by the tissue disruption and subsequent LOX activity that is created by the tissue disruption. It is the composition and odor characteristics of C-6 aldehydes and alcohols that may be partly responsible for

the distinct aroma differences between the red and green bell pepper (Wu and Liou 1986).

In a study by Luning and others (1995a) it was found that the enzymatic formation of volatile flavor compounds from unsaturated fatty acids after tissue disruption created nine aldehydes, five alcohols, one ketone, and one furan. Of the compounds detected, hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-3-hexenol, (*E*)-2-hexenol, (*Z*)-2-hexenol, and hexanol were all present in different amounts depending on the stage of maturation of the bell pepper. Pattern A of volatile formation, is characterized by the significant decrease in volatiles between weeks 4 and 6 or from immature to mature green while between 6 (mature green) and 8 (turning) weeks no marked changes occurred (Table 1.1). However, after 8 weeks or after turning the level dropped significantly. This pattern is typical for the volatile compounds hexanal and hexanol.

In pattern B the volatile levels are high in the green stages (4-6 weeks) and decreased significantly between 7 and 8 weeks when the bell pepper turned from green to red. This pattern represented the development of (*Z*)-3-hexenal, (*Z*)-3-hexenol, (*Z*)-2-pentenal, (*Z*)-2-pentenol, (*E*)-2-pentenal, (*E*)-2-pentenol, and 1-penten-3-one during the maturation process.

Pattern C exhibits a significant increase between 7 and 8 weeks which is followed by a significant decrease after 9 weeks was observed. This represents the changes of (*E*)-2-hexenal, (*E*)-2-hexenol, and (*Z*)-2-hexenal during ripening. The other compounds of 2-pentylfuran, (*E*)-2-heptenal, (*E*)-2-octenal, and (*E*)-2-nonenal all had a maximum level at 8 weeks and declined in the following weeks.

In pattern D the remaining compounds of 2-pentylfuran, (*E*)-2-heptenal, (*E*)-2-octenal, and (*E*)-2-nonenal, showed a maximum level at 8 weeks and then declined sharply thereafter. When stannous chloride (SnCl₂) was added to the bell pepper homogenates there was a reduction in the amount of C-6 aldehydes and alcohols. SnCl₂ inhibits enzyme activity and therefore inhibits the formation of lipoxygenase derived compounds. This goes to prove that LOX activity is mainly responsible for the creation of the volatile compounds of bell peppers (Luning and others 1995a).

Table 1: Average GC peak areas collected to show changes in the volatile compounds of bell peppers collected at different maturation stages reported by Luning and others (1995).

	Harvest Time (weeks)							Pattern
	Immature Green		Mature Green		Turning		Red	
Compound	4	5	6	7	8	9	10	
Hexanal	194.2	123.9	81.8	72.9	62.6	35.4	12.9	A
1-Hexanol	10.8	8.4	4.2	4.6	5.7	4.2	2.1	A
(<i>Z</i>)-3-Hexenal	107.7	136.9	130.1	128.4	3.9	0.9	0.1	B
(<i>Z</i>)-3-Hexenol	17.0	24.9	19.1	17.4	2.2	0.5	0.3	B
(<i>Z</i>)-2-Pentenal	4.2	4.2	3.4	2.7	0.2	0.2	<0.1	B
(<i>Z</i>)-2-Pentenol	0.3	0.2	0.2	0.2	<0.1	<0.1	<0.1	B
(<i>E</i>)-2-Pentenal	0.1	0.1	0.1	0.1	<0.1	<0.1	<0.1	B
(<i>E</i>)-2-Pentenol	1.0	1.0	1.0	0.9	0.6	0.4	<0.1	B
1-Penten-3-one	5.9	7.4	7.0	6.8	2.3	1.8	1.0	B
(<i>E</i>)-2-Hexenal	53.6	44	34.8	105.9	301.2	279.9	170.9	C
(<i>Z</i>)-2-Hexenal	2.0	3.0	2.9	4.3	8.4	7.2	4.8	C
(<i>E</i>)-2-Hexenol	1.0	1.1	1.1	3.8	19.9	23.8	10.8	C
2-Pentylfuran	1.2	1.3	1.0	1.3	2.0	1.3	0.6	D
(<i>E</i>)-2-Heptenal	0.6	0.7	0.6	0.7	1.1	0.5	0.2	D
(<i>E</i>)-2-Octenal	0.5	0.5	0.4	0.5	0.6	0.3	0.2	D
(<i>E</i>)-2-Nonenal	0.4	0.4	0.3	0.2	0.5	0.2	0.1	D

A, B, C, and D describe patterns observed by the authors during the maturation of bell peppers starting from immature green and turning into red.

Water loss affects lipoxygenase activity

Water loss in commercial bell peppers has been identified as the principal physiological factor limiting the quality of the pepper and its prolonged storage (Maalekuu and others 2003). Water loss in fresh produce during postharvest stage has been linked to several factors such as loss of membrane integrity, lipoxygenase activity and membrane lipid peroxidation. Not only does LOX activity change volatile production and pigment degradation in peppers at different ripening stages but it can also change the membrane composition. This membrane change decreases the product quality and commercial value of the pepper. Increased LOX activity has been reported to be associated with increased water loss (Maalekuu and others 2006). Since lipids are the major structural blocks of membranes any change in their composition directly affects membrane stability and function. This can result in ion leakage and cellular decompartmentation (Marangoni and others 1996).

New Volatiles Found in Bell Peppers

Not only are the volatile compounds created by the LOX activity important but other volatiles are being researched and have been found to be important in the overall aroma of a bell pepper whether that is for the red or green stage of maturation. With only a few of these volatiles being characterized as having a bell pepper like odor such as 2-methylpropanal, 2- and 3-methylbutanal, 2,3-butanedione, 1-penten-3-one, hexanal, heptanal, beta-ocimene, (*E*)-3-hepten-2-one, dimethyl trisulfide, and beta-cyclocitral, it is important to identify new volatiles in bell peppers with the bell pepper aroma (Simian and others 2004). Bell pepper like volatile compounds like monoterpene delta-3-carene are particularly said to be abundant in red bell peppers (Simian and others 2004). This

bell pepper note has an increasing impact as the bell pepper matures especially in fresh as well as dried red, yellow, and white bell peppers. Also 2-heptanone/heptanal have both been reported as having bell pepper and cooked vegetable notes in rehydrated peppers (Simian and others 2004). There has been a correlation formed between gas chromatography and sensory data that shows green pepper aroma profiles are characterized as grassy, green bell pepper, and fruity which are closely related to 1-penten-3-one, (Z)-3-hexanal, (Z)-3-hexanol, 3-isobutyl-3-methoxypyrazine, and linalool (Simian and others 2004).

Sulfur volatile compounds that are created via enzymatic and chemical reactions are known to contribute to the aroma of many vegetables, fruits, and food products. Thiols and sulfides belong to the most intense and characteristic aroma substances with sulfury, vegetable-like, fruity notes perceived at low levels. However, there are only a few of these odor-active sulfur compounds in bell peppers such as methional and dimethyl trisulfide but no thiols have been reported as odorants. Recent research however has discovered 2-heptanethiol in red bell peppers. This volatile is described as having a sulfury and fruity aroma. This compound was found by running raw and cooked red and green bell peppers through a gas chromatography with a mass spectrophotometer and observing the profiles of the samples to confirm what the compound was thought to be. It was found that there are higher amounts of thiol in the cooked samples when compared to the raw samples. Furthermore, there are higher concentrations of the thiol in the red of bell pepper variety compared to the green variety. It is still detected at low concentrations in raw red and green bell peppers (Simian and others 2004).

With a large quantity of various unsaturated C-7 and C-9 compounds in bell pepper extracts there is a possibility that other sulfur compounds would contribute to their flavor either by the addition of a sulfur atom to the carbon to carbon double bonds or by replacing the oxygen with sulfur. The important volatiles that have been found present in bell peppers are linalool and beta-damascenone which have a fruity aroma. The three sulfur volatiles found present in the peppers are heptane-2-thiol, 3-methyl-5-propyl-1,2-dithiolane, and 1-(2-thienyl)-pentan-1-one. These sulfur compounds at lower concentrations have been described as bell pepper, fruity, and vegetable like all of which are desirable to have in a bell pepper. However, in higher concentrations these same sulfur compounds have been described as being sulfury, onion, and mushroom like (Naef and others 2008).

Effect of maturation on volatiles

Since bell peppers are consumed at many different stages of maturation it is important to understand how the volatile compounds in them change during the maturation process. Results from gas chromatography show that major volatiles decrease or even disappear during the maturation process of the bell peppers. This observation, of seeing volatile compounds disappear during maturation, was seen in samples that were sliced as well as homogenized samples. The composition of volatile compounds of bell peppers differs clearly from the immature green, to turning, and finally to mature red peppers. All of the volatiles found in green and red bell peppers except for (*E*)-2-hexenal decreased significantly in the levels present during maturation. The homogenized green bell peppers contained high levels of 1-penten-3-one, toluene, hexanal, (*Z*)- and (*E*)-2-pentenal, (*Z*)-3-hexenal, (*E*)-2-hexenal, (*E*)-beta-ocimene, 1-hexanol, (*Z*)-3-

hexenol, and 6-methylheptyl 2-propenoate, while (*Z*)-beta-ocimene and (*E,Z*)-3,4-dimethyl-2,4,6-octatriene were the major peaks found in sliced peppers along with those peaks found in homogenized peppers (Luning and others 1994). Yet as all those volatiles are significantly lower in turning and mature peppers (*Z*)-2-hexenal, (*E*)-2-hexenal, and (*E*)-2-hexenol were all significantly higher in homogenized peppers at the turning and mature stage (Luning and others 1994).

A number of the volatile compounds such as 2-ethylfuran, (*Z*)- and (*E*)-2-pentenal, 3-penten-2-one, (*Z*)-3-hexenal, (*Z*)-2-hexenal, (*E,E*)-2,4-hexadienal, (*E*)-2-octenal, 1-octen-3-ol, and (*E,Z*)-2,6-nonadienal were only detectable in homogenized samples and not the cut bell pepper samples (Luning and others 1994). The average peak areas of 1-penten-3-one, hexanal, (*E*)-2-hexenal, hexanol, (*Z*)-3-hexenol, and (*E*)-2-hexenol were also significantly larger for the homogenized samples (Luning and others 1994). These results are in line with those of Wu and Liou (1986) that demonstrated that tissue disruption of green bell peppers favored the formation of 2-ethylfuran, hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, hexanol, (*Z*)-3-hexanol, and (*E*)-2-hexenol (Luning and others 1994). With the levels of (*Z*)-3-hexenal and (*Z*)-3-hexenol decreasing during bell pepper maturation and levels of (*E*)-2-hexenal and (*E*)-2-hexenol increasing this shows that the activities of several enzymes changed during the ripening of bell peppers and particularly the ones involved with the formation of lipid degraded products (Luning and others 1994). Only the turning and red bell peppers had an additional compound of (*E*)-2-hexenol, but overall the peppers had the same compounds just at different levels which can explain the difference in the aromas between green, turning, and red bell peppers. Table 1.2 shows the differences in volatile levels in green, turning, and red bell peppers.

Table 2: Identified compounds and their average peaks for homogenized (HG) and cut bell peppers at different ripening stages reported by Luning and others (1994).

Compound	GC Peak Area					
	Green		Turning		Red	
	HG	Cut	HG	Cut	HG	Cut
3-Buten-2-one	1.04	1.59	0.9	1	0.89	0.96
2-Ethylfuran	3.38	e	0.53	e	0.81	e
2,3-Butanedione	0.04	0.16	0.04	0.07	0.03	0.05
3-Pentanone	8.49	1.91	7.02	1.62	4.41	1.28
Pentanal	3.61	1.02	2.46	0.8	2.08	0.87
2-Butanol	0.66	1.62	0.53	0.87	0.08	0.34
1-Penten-3-one	17.34	1.25	5.24	0.58	5.54	0.57
α -Pinene	0.95	1.56	0.78	1.27	0.58	1.00
Toluene	15.14	14.72	7.09	10.7	7.46	10.22
2,3-Pentanedione	e	0.17	e	0.17	e	0.14
Dimethyl disulfide	1.77	1.45	0.35	1.23	0.29	0.41
Hexanal	7.83	0.28	4.88	0.17	5.31	0.19
(Z)-2-Pentenal	14.87	e	0.19	e	0.09	e
1-Methoxy-2-propanol	1.01	0.86	0.74	0.54	0.86	0.63
β -Pinene	0.05	0.06	0.06	0.05	0.05	0.03
3-Penten-2-one	1.69	e	0.07	e	0.05	e
(E)-2-Pentenal	14.73	e	1.06	e	1.42	e
1-Butanol	7.94	4.41	5.29	4.55	3.12	2.18
p-Xylene	2.14	2.19	2.33	2.36	1.97	1.54
m-Xylene	1.72	4.84	3	5.22	2.73	3.39
1-Penten-3-ol	6.98	3.22	2.58	2.24	2.44	2.6
(Z)-3-Hexenal	219.43	e	8.54	e	6.73	e
3-Carene	0.6	0.46	0.19	0.32	0.31	0.49
Myrcene	1.39	1.98	0.25	1.43	0.19	0.73
2-Heptanone	2.36	6.91	3.59	4.04	2.02	1.98
Heptanal	1.18	2.62	1.73	2.3	1.35	2.3
o-Xylene	2.42	2.17	2.5	4.82	2.6	2.53
2,3-Dihydro-4-methylfuran	0.85	0.57	0.46	0.13	0.34	0.17
(Z)-2-Hexenal	9.61	e	11.71	e	13.78	e
Limonene	2.25	3.82	3.36	3.27	2.61	2.57
(E)-2-Hexenal	5.76	0.06	>13.9	0.21	>15.3	0.25
2-Pentylfuran	g	g	g	g	g	g
1-Pentanol	g	g	g	g	g	g

Continued

Table 2 continued

(Z)- β -Ocimene	6.94	23.1	2.22	4.17	0.87	1.32
3-Octanone	g	g	g	g	g	g
(E)- β -Ocimene	180.14	379.63	44.79	67.62	10.83	28.44
Octanal	0.07	0.05	0.04	0.04	0.03	0.05
(Z)-2-Pentenol	0.89	0.1	0.05	0.06	0.06	0.04
(E)-2-Pentenol	3.57	1.1	0.67	0.74	0.62	0.45
2-Heptanal	2.48	0.31	1.43	0.14	1.1	0.11
4-Nonanone	0.18	0.27	0.47	0.33	0.47	0.22
6-Methyl-5-hepten-2-one	0.42	0.75	0.42	0.47	0.35	0.4
1-Hexanol	40.69	11.42	29.82	10.66	23.72	4.87
(E)-3-Hexenol	2.13	0.68	0.54	0.17	0.83	0.16
(Z)-3-Hexenol	2.88	1.74	0.05	0.06	0.02	0.01
(E,Z)-3,4-Dimethyl-2,4,6-octatriene	6.95	15.24	2.1	1.83	0.44	0.95
Dimethyl Trisulfide	0.12	0.32	0.07	0.25	0.09	0.14
(E,E)-2,4-Hexadienal	0.58	e	e	e	e	e
(E)-2-Hexenol	0.11	0.02	2.34	0.15	1.95	0.09
Nonanal	11.28	3.35	0.73	2.67	0.32	2.72
(E)-2-Octenal	1.30	e	1.04	e	0.6	e
1-Octen-3-ol	1.51	e	0.41	e	0.23	e
1,2,4,5-Tetramethylbenzene	1.35	0.86	0.41	0.32	0.35	0.19
2-Furancarboxyaldehyde	0.45	0.18	0.18	0.13	0.16	0.14
1,3,8-p-Menthatriene	1.12	4.33	0.44	0.63	0.3	0.27
2-Pentylthiophene	1.42	1.16	0.67	0.53	0.27	0.13
2-Ethyl-1-hexanol	4.87	5.88	2.52	1.76	2.24	1.46
6-Methylheptyl 2-propenoate	21.8	18.81	16.54	20.83	16.56	18.17
Decanal	1.63	2.00	0.55	1.40	0.36	1.19
2-Sec-butyl-3-methoxypyrazine	<0.01	<0.01	e	e	e	e
Benzaldehyde	4.48	3.64	2.07	1.94	2.49	3
2-Isobutyl-3-methoxypyrazine	0.18	0.03	0.13	0.01	0.07	0.01
Linalool	0.15	0.09	0.06	0.01	0.03	0.01
(E,Z)-2,6-Nonadienal	<0.01	e	<0.01	e	e	e

e= not detected

g= peaks were insufficiently separated from neighboring compounds.

Effect of frozen storage on volatiles

Azcarate and Barringer (2010) did a study on the effect of freezing on jalapeno pepper LOX volatiles. Two sets of peppers were stored at -15°C and one set of

unblanched samples was measured at 0, 1.5, 2.5, and 3 mo of storage. The second set was measured at 0, 6, and 9 mo of storage and had both blanched and unblanched samples. The peppers in this study were also stored both whole and pureed.

In the blanched samples the expected results were that no major changes during frozen storage should occur since enzymes are inactivated during the blanching process and the LOX formed volatiles cannot form as a result. This was found to be true since during frozen storage no major changes in the LOX volatiles were observed however some minor chemical changes were observed. These changes were more present in the pureed pepper samples than the whole pepper samples. Similar results were observed in the aroma profile of blanched and frozen leeks due to inhibited enzymatic activity (Nielsen and Poll 2004).

A decrease in (*Z*)-3-hexenal was accompanied by an increase in (*E*)-2-hexenal over time in the blanched peppers. This was explained by the gradual chemical isomerization of (*Z*)-3-hexenal into the more stable (*E*)-2-hexenal. The blanched pureed samples which were stored for only 6 mo had greater isomerization than the whole blanched peppers that were stored for 9 mo. This was explained by the high degree of cell rupture that has occurred in the pureed samples which favors better interaction and exposure of components to allow isomerization. The sum of (*Z*)-3-hexenal and (*E*)-2-hexenal concentrations did not change in this study for any of the samples which indicates a direct conversion of (*Z*)-3-hexenal to (*E*)-2-hexenal during frozen storage. All other LOX volatiles maintained their initial concentration during storage except for hexanal, 2-pentenal, and (*E*)-2-heptenal. These volatiles slightly increased in concentration during the 6 mo frozen storage. The reason for this was thought to be

caused by autooxidation of linoleic and linolenic acid occurring in the pureed blanched samples. A similar trend was also found by Nielsen and others (2004) that saw a significant increase in the aldehydes in frozen blanched leeks. They also concluded that the development of the aldehydes was due to autooxidation of polyunsaturated fatty acids.

When observing the volatiles in unblanched samples Azcarate and Barringer (2010) found that in the first 3 mo of frozen storage for the whole pepper samples, (*Z*)-3-hexenal decreased in concentration most likely due to the conversion of (*Z*)-3-hexenal into (*E*)-2-hexenal. They also observed that hexanal, hexen-1-ol, and hexanol decreased in concentration during this same timeframe. The previously mentioned volatiles along with (*E*)-2-hexenal reportedly continued to decrease throughout the 9 mo storage study when compared to their initial measurements. The aldehydes in the peppers then convert to their alcohol form which then gets further degraded into other compounds. In whole unblanched jalapeno peppers the rate at which these products degrade is higher than the rate at which they are being formed so they are decreasing over time with little accumulation.

For pureed samples a similar trend as the whole peppers was observed. Only in the pureed samples the concentration of (*Z*)-3-hexenal, (*E*)-2-hexenal, and hexanal all reach maximum levels sooner before decreasing. This is an expected result due to the increased interaction between components and oxygen which favors enzymatic and chemical reaction. In whole pepper samples this same interaction is harder to occur which limits the reaction.

Minor LOX derived volatiles in whole pepper sample such as 1-penten-3-one, (*E*)-2-heptenal, (*E*)-2-octenal, and (*E*)-2-nonenal gradually increased over the first few months reaching a maximum concentration at 2.5 mo and then started decreasing (Azcarate and Barringer 2010). While the pureed pepper samples were similar to that of the whole peppers only the maximum concentration of these minor volatiles occurred earlier in the pureed samples.

Blanching effect on volatiles

Before freezing can occur in many frozen vegetables they must be blanched. Blanching is a thermal process that is used to reduce the microbial load and inactivate enzymes since many of the quality changes that occur during distribution and storage are caused by enzymes such as peroxidase, polyphenols oxidase, and pectin methylesterase (Castro and others 2007). However this thermal treatment causes a loss of many sensory attributes such as texture, taste, flavor, and color as well as nutritional quality such as the reduction of ascorbic acid content (Castro and other 2007).

Since blanching inactivates enzymes that change the volatiles it is expected that they will not change drastically during frozen storage. In a study conducted by Azcarate and Barringer (2010) it was found that no significant difference in the concentration of any volatiles between blanched and stannous chloride treated samples. Stannous chloride has been shown to chemically inhibit enzyme activity in bell peppers and other products. Differences in the concentrations of (*Z*)-3-hexenal, methylbutanal, and dimethyl sulfide were found. The concentration of (*Z*)-3-hexenal was higher in the stannous chloride treated samples when compared to the blanched samples. This difference could be due to the fact that (*Z*)-3-hexenal is a heat labile compound which was reported in blended

tomatoes when they were exposed to heat (Kazeniak and Hall 1970). Heat promotes the isomerization of (*Z*)-3-hexenal to (*E*)-2-hexenal.

Dimethyl sulfide has a cooked cabbage like aroma and is a key volatile in the aroma of several cooked vegetables (Scherb and others 2009). When combined with other volatile sulfur compounds have been reported to be responsible for sulfurous off flavors of many heat processed foods (Vazquez-Landaverde and others 2005, 2006; Lozano and others 2007). Methylbutanal represents the mixture of 2- and 3-methylbutanal which was found to be 86% higher in blanched peppers than unblanched peppers (Azcarate and Barringer 2010). These two compounds have been characterized as having a cacao, sweaty, and cooked vegetable odors and have been reported having increased concentration in heated products (Luning and other 1995b). 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 odors in fried chili paste, and their concentration increased as heating time increased (Rotsatchakul and others 2008).

Dimethyl sulfide can be formed from the amino acid methione by Strecker degradation or from S-methylmethionine by heat induced breakdown (Azcarate and Barringer 2010). When methione is broken down in Strecker degradation the methional that is produced from methionine is decomposed to methanethiol that then oxidizes to dimethyl sulfide (Lozano and others 2007). While 2- and 3-methylbutanal are formed by Strecker degradation of isoleucine and leucine respectively during Maillard reactions (Vazquez-Landaverde and other 2005; Rotsatchakul and others 2008).

Bell peppers contain the amino acids methionine, leucine, and isoleucine in similar amounts to those found in jalapeno peppers, 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 jalapeno pepper samples in Azcarate and Barringer (2010). They also reported that the heat treatment 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 when compared to unblanched peppers.

Changes in Bell Pepper Volatiles when made into an Oil

Not only is the aroma of bell peppers found in the fruit but it can also be found in the oil obtained by vacuum steam-distillation-continuous-extraction, which had an aroma similar to that of fresh bell peppers (Buttery and others 1969). During this process the temperature of the bell peppers was never higher than 45 to 50°C. The oil that was captured from this process was then run on gas-liquid chromatography and was determined to have high levels of limonene, trans-beta-ocimene, 2-methoxy-3-isobutylpyrazine, and methyl salicylate. When the steam-distillation-continuous-extraction was run at atmospheric pressure and the temperature of the bell peppers reached 100°C gave an oil with volatiles different than the oil obtained under vacuum conditions. From the atmospheric pressure process it produced for the first time to be reported in bell peppers the compound 2-methoxy-3-isobutylpyrazine as well as C-9 ketones non-1-en-4-one, non-trans-2-en-4-one, and nona-trans,trans-2,5-dien-4-one. These C-9 ketones found during this process are somewhat related to oct-1-en-3-one which has been reported as a metallic or mushroom flavored compound of dairy products

(Buttery and others 1969). It was also found that two C-7 ketones, 2-heptanone and hept-trans-3-en-one, also were found in the oil. The concentration of the C-9 ketones is very small in the oil from using the steam-distillation-extraction under a vacuum. However, after heating these same compounds increased in concentration rapidly, so under the atmospheric pressure the monounsaturated C-9 ketones and linalool form the major volatiles in the oil. While the C-9 ketones increase at the atmospheric conditions all the other major compounds from the vacuum conditions become minor contributors to the aroma. Other compounds that increased but not as much as the C-9 ketones are hexanal, furfural, 2-heptanone, benzaldehyde, 2-pentylfuran, phenylacetaldehyde, nona-(*E*),-(*Z*)-2,6-dienal, and deca-(*E*),(*E*)-2,4-dienal.

Enzyme Activity in Bell Peppers during maturation and freezing

When analyzing the volatile compounds in bell pepper fruits the six carbon aldehydes and alcohols along with other volatiles such as 2-isobutyl-3-methoxypradine and (*E*)-beta-ocimene have been determined as the major compounds. Luning and others (1994) showed that the amounts of six carbon compound rapidly decreases during maturation in bell peppers. During this maturation process the bell pepper fruit's color is changing. The color is changing largely due to the chloroplast-to-chromoplast transition that occurs in the fruit cells. Lipoxygenase (LOX) and fatty acid hydroperoxide lyase (HPO lyase) are involved in the formation of six carbon volatiles. It is assumed that they are responsible for the change in flavor of the peppers upon maturation (Matsui and others 1997).

In a study done by Matsui and others (1997) they studied how the LOX and HPO activity both changed during the maturation process of bell peppers. HPO lyase activity

was determined spectrophotometrically by using 13-hydroperoxy-(9Z, 11E)-octadecadienoic acid as a substrate in 0.1M MES-KOH at pH 5.5. Lipoxygenase activity was determined polarographically by using an YSI 5331 oxygen probe with linoleic acid as a substrate. The bell peppers used in this experiment were harvested at 60, 69, 72, 75, 78, 81, and 90 days after flowering (DAF). The peppers were green until 69 DAF and then began to turn red 72 DAF. At 81 DAF the peppers had turned completely red. These findings are similar to those in Luning and others (1995a).

The HPO lyase and LOX activities showed the highest values in the peppers collected 60 DAF (Matsui and others 1997). Both the enzyme activities decreased rapidly between 69 DAF and 81 DAF. After 81 DAF, about 30% of the maximum activities of both the enzymes still remained and they remained constant thereafter (Matsui and others 1997). Changes in both LOX and HPO lyase activities closely resembled one another which means they are cooperatively regulated. Matsui and others (1997) believe that since a rapid decrease in both enzyme activities was at the breaker stage of the pepper's maturation, which is when the disappearance of chloroplasts started, and because there remained substantial and constant activities even after that stage means there are two types of each enzyme, one of which is associated with the chloroplastic function and the other which does not. It has been demonstrated by Blee and Joyard (1996) that LOX and HPO lyase are associated in the envelope membrane of spinach chloroplast. Furthermore, Sekiya and others (1984) used cultured cells of tobacco to demonstrate that there exists two types of HPO lyases, a chloroplastic and non-chloroplastic. In Matsui and others (1997) the HPO lyase activity was always about three times higher than the LOX activity. Since the products of LOX, fatty acid hydroperoxides, are highly toxic to plant cells, it

should be degraded to less-toxic substances. This is why it is reasonable to believe that LOX rather than HPO lyase determined the rate of formation of aldehydes from fatty acids (Matsui and others 1997). From Matsui and others (1997) it was found that HPO lyase and LOX activities decreased rapidly as the color of the peppers changed from green to red. The changes in enzyme activities are attributed to the degradation of the enzymes. It is also important to note that the changes occurred when rapid conversion of chloroplasts to chromoplasts occurred in the peppers.

Enzymes are active in unblanched peppers but their activity can be slowed by reducing the temperature however their activity is never completely stopped. LOX in leeks gradually decreased in activity during frozen storage at -20°C but 25% of the initial activity was still detected after 12mo of storage (Nielsen and others 2003). During the freezing process the 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 (Azcarate and Barringer 2010). During frozen storage reactions can take place in the liquid water phase (Nielsen and others 2003, 2004). This can explain why Azcarate and Barringer (2010) saw slow but continued LOX initiated reactions during frozen storage of unblanched jalapeno peppers.

Aroma of Bell Pepper Powder

Drying bell peppers is known to significantly change their overall aroma profile. It has been reported that drying green, white, yellow or red bell pepper pieces results in an increase in the caramel like, cocoa-like and rancid or hay like odors. These changes can be attributed to 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal in the dried samples (Zimmermann and Schieberle 2000). From these dried samples 4-hydroxy-2,5-

dimethyl-3(2H)-furanone was identified for the first time in bell peppers. Other important odorants in bell pepper powder are 2- and 3-methylbutanoic acids, phenylacetic acid, 4-vinyl-2-methoxyphenol, 2,3-dihydro-5-hydroxy-6-methyl-4(H)-pyran-4-one, and propionic acid. Even though 3-isobutyl-2-methoxypyrazine and 3-sec-butyl-2-methoxypyrazine have both been reported as an important odorant for fresh bell peppers they do not have a major role in the aroma of bell pepper powder (Zimmerman and Schieberle 2000). Not all bell pepper powders have the same aroma. This can be attributed to the origin of the bell peppers used to make the powder. When comparing two powders one made from Hungarian sweet bell peppers to one made from Moroccan sweet bell peppers, the two samples had different aroma profiles. The Moroccan powder was described as more musty with a weak fishy note, while the Hungarian samples was described as being spicier and sweeter than the Moroccan powder. With the exception of propionic acid all the compounds detected in the Hungarian sample were found in the Moroccan sample with an additional nine compounds that could explain the difference in aroma profiles between the two samples (Zimmermann and Schieberle 2000).

Selected Ion Flow Tube Mass Spectrometry (SIFT-MS)

Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) is a relatively new technique used initially for the analysis of trace gases in air and breath analysis that has expanded its application to the food industry for the analysis of volatile or aroma compounds. The main advantages of this technique are that it requires minimal sample preparation, it is fast, and allows for the real-time monitoring of selected volatile compounds providing differentiation of some isomers. Some of the disadvantages to this are the requirement of previous identification of compounds present in the sample and

their product ions which make it impossible to differentiate some of the compounds (Syft Technologies 2009).

Principles of SIFT-MS

The general principles of SIFT-MS can be explained with the help of figure 3 located in Appendix A. Precursor ions are generated by a microwave discharge source. These precursor ions then pass into an upstream chamber where a quadrupole mass filter selects only the reagent ions of H_3O^+ , NO^+ , and O_2^+ , which are chosen on their ability to not react with air, continue on to react with volatile compounds (Spanel and Smith 1999). At a known velocity the reagent ions pass through a venturi inlet to inert carrier gases helium and argon. The head space gases from the sample enter the reaction chamber at a controlled rate and react with reagent ions forming product ions. These product ions then enter a downstream chamber and are filtered by a second quadrupole mass filter. Lastly, a particle multiplier detects the ions at the selected mass and count rate is transferred to the instrument computer for interpretation (Smith and Spanel 2005).

Kinetic studies of ion reactions using selected ion flow tubes have been done for many years (Adams and Smith 1976) which has provided a large kinetics database for thousands of ion-neutral reaction (Smith and Spanel 2005). The database holds information on how rapidly an analyte reacts with a precursor ion, the products of the reaction, and their relative abundances. The determination of individual reaction rate coefficients and product ion branching ratios are necessary when quantifying volatile compounds in the samples. For the reaction $\text{A}^+ + \text{B} \rightarrow \text{D}^+ + \text{E}$, a rate law is defined in the following equation where k is the bimolecular rate coefficient, t is the time and $[\text{A}^+]$ and $[\text{B}]$ are respective concentrations of the precursor ion and the volatile compound (Frank

2007).

$$\frac{-d[A^+]}{dt} = -[A^+][B]$$

With known reaction rates volatile concentrations $[M]$ can be determined from the ratios of the product to precursor ion count rates (I_p/I) and time (t), which is defined by the known flow velocity of the helium carrier gas according to the following equation (Spanel and Smith 1999):

$$[M] = \frac{I_p}{Ikt}$$

SIFT-MS uses soft chemical ionization, whereas a GC-MS uses electron impact ionization, which results in less compound fragmentation and simpler mass spectrum. This combined with the three precursor ions reduces compound interference because only one or two product ions results from each volatile in the sample (Spanel and Smith 1999).

Chapter 3: Methods

Type of peppers

Green and red bell peppers (*Capsicum annuum*) were obtained from Gordon Food Service (Hilliard, OH) on January 18th and May 17th, 2011 and Fremont Farm September 22nd, 2011. Peppers were washed before use to remove possible contamination from the outside of the pepper.

Sample preparation

Whole green peppers were blanched in boiling water for 6min to inactivate lipooxygenase. Peroxidase activity was determined by crushing green peppers with a mortar and pestle, adding ½ml of 0.5% guaiacol in 50% ethanol followed by ½ml of 3% hydrogen peroxide, mixing and waiting 3.5 min. After this time if there was no color then the green peppers were negative for peroxidase but if they were reddish-brown then they were peroxidase positive. This test was conducted in order to ensure that the bell peppers had been properly blanched to inactivate the lipooxygenase enzyme. The pureed samples were pureed for 30sec using a chopper (Magic Bullet, Homeland Housewares, Pacoima, California). Quick freeze samples were frozen quickly in a blast freezer at -40°C for 1.5h. Slow freeze samples underwent a slow freeze in the -18°C freezer to freeze overnight. All samples were stored in a -18°C freezer in Ziploc freezer bags. The pureed samples were

stored in 3lb batches with nothing added to them and little headspace. The whole peppers were stored completely whole and contained 6 peppers per bag with as little headspace as possible. Samples were only pulled out on the day that they were tested for volatiles in the Selected Ion Flow Tube Mass Spectrometer (SIFT-MS).

Lipoxygenase Activity Measurement

For whole samples, two peppers were cut in half and half of each pepper was pureed quickly in a chopper. The green raw whole was pureed with a silicone anti-foaming agent (Trans – 10 K, Trans-Chemco Inc, Bristol, Wisconsin). The puree (2g) was mixed in a mortar and pestle with 2g of sodium phosphate buffer pH 7 with 0.2% Triton. After mixing, 1mL was transferred to a 1.5mL centrifuge tube and centrifuged at 13,200g for 7.5min. Sodium phosphate buffer at pH 7 (3mL), 60 μ L of substrate solution (consisting of 155 μ L of linoleic acid, 257 μ L of Tween-20 added to 5mL of water, and 0.6mL of 1N NaOH, brought up to 25mL total with sodium phosphate buffer), and 75 μ L of homogenate liquid from the centrifuge were combined into a cuvette. The cuvette was put into a UV-Visible spectrophotometer (UV-2450, Shimadzu Scientific Instruments, Columbia, Maryland) and the absorbance was measured at 234nm for 240sec. The average slope of the line for two replicates per samples was used to calculate LOX activity. The average slope was multiplied by 3 since the assay volume was 3.0mL then divided by 0.023 which is the molar extinction coefficient of LOX (Anthon 2011). This converted absorbance to nmoles and was then multiplied by 60 to convert seconds into minutes. That was then divided by 37.5 which accounts for the dilution of the added homogenization buffer, 12.5 per 25 μ L since 75 μ L of homogenate was added to the assay

it was multiplied by 3, and the whole answer was then multiplied by 100 to give the final activity in nmol/g fwt/min.

Volatile Measurement

Whole bell peppers were cut into 5g pieces for a total of 50g per sample to ensure proper pureeing. Each sample contained no seeds, stems or inner membrane of the pepper. Before being blended in a chopper for 30 sec, 10g of NaCl (Morton Extra Fine 325 salt, Chicago, Illinois) was added to each sample. Raw whole green bell peppers were prepared both with and without stannous chloride (SnCl_2) (Stannous chloride, Ricca Chemical Company, Arlington, Texas). To the samples prepared with SnCl_2 , 5g of SnCl_2 was added to the chopper with the 50g of pepper sample and 10g of NaCl and then blended for 30 sec. Each sample was prepared in replicates of five to ensure reproducibility.

Frozen samples were allowed to thaw in 500ml Pyrex bottles, sealed with polybutylene terephthalate (PBT) open caps coupled with polytetrafluoroethylene (PTFE) faced silicone septa, in a Precision water bath for 30min at 50°C. Only two variables at a time were prepared to ensure no sample was in the water bath for longer than 30min. A SIFT-MS (V-100, Syft Technologies, Christchurch, New Zealand) was used to measure volatiles. Measurements were performed on this piece of equipment by using selected ion mode (SIM) scans with H_3O^+ , NO^+ , or O_2^+ as precursor ions, calculation delay time 5s, product sample period 100ms, precursor sample period 20ms, carrier gas argon pressure 200kPa, helium pressure 30psi, capillary and arm temperature 120°C, and flow pressure 0.038 ± 0.003 Torr. The SIFT-MS method used was a 2 min run to prevent the exhausting of the headspace that occurred in longer runs. Two needles were inserted into

the bottle. One was an 18 ga 3.8cm long stainless steel passivated needle used to sample volatiles from the glass bottle headspace placed 12cm from the surface of the sample. The other needle was a 14 ga 15cm long syringe needle to maintain atmospheric pressure inside the bottle and was 1cm from the sample surface inserted along the edge of the septa. The SIFT-MS uses H_3O^+ , O_2^+ , and NO^+ to react with compounds in the samples. These ions are generated in a microwave. The ions then travel to an upstream chamber where a quadrupole mass filter removes all ions but the preferred reagent ions. The reagent ions pass through a venturi to the flow tube where they react with the sample. The reaction products enter a downstream chamber where they are filtered by another quadrupole mass filter. A particle multiplier detects and counts the selected products. The SIFT-MS has a set of internal calibrations and validations that are run prior to the samples being run. These ensure that the machine is working properly and detecting mass peaks at the proper time and correct amounts (Spanel and others 2002).

Samples were tested before and after freezing to see if freezing had any immediate effect on the volatiles and their formation. During storage, samples were tested at weekly or monthly intervals.

Table 3: Kinetics parameter for SIFT-MS analysis of selected volatile compounds.

Volatile Compound	Molecular formula	Precursor ion	(k) ($10^{-9}\text{cm}^3\text{s}^{-1}$)	<i>m/z</i>	Product ion	Ref
(<i>E</i>)-2-Heptenal	C ₇ H ₁₂ O	NO ⁺	3.9	111	[C ₇ H ₁₁ O] ⁺	[7]
(<i>E</i>)-2-Hexenal	C ₆ H ₁₀ O	NO ⁺	3.8	71	[C ₄ H ₇ O] ⁺	[5]
(<i>E</i>)-2-Nonenal	C ₉ H ₁₆ O	NO ⁺	4.5	139	[C ₉ H ₁₅ O] ⁺	[7]
(<i>E</i>)-2-Octenal	C ₈ H ₁₄ O	NO ⁺	4.1	125	[C ₈ H ₁₃ O] ⁺	[7]
(<i>E</i>)-2-Pentenal	C ₅ H ₈ O	NO ⁺	4.0	83	[C ₅ H ₇ O] ⁺	[7]
(<i>E,E</i>)-2,4-Decadienal	C ₁₀ H ₁₆ O	NO ⁺	4.2	182	[C ₁₀ H ₁₆ O.NO] ⁺	[7]
(<i>E,Z</i>)-2,6-Nonadienal	C ₉ H ₁₄ O	NO ⁺	2.5	137	[C ₉ H ₁₃ O] ⁺	[8]
(<i>Z</i>)-3-Hexenal	C ₆ H ₁₀ O	H ₃ O ⁺	4.2	81	[C ₆ H ₉] ⁺	[4]
1-Butanol	C ₄ H ₁₀ O	H ₃ O ⁺	2.8	57	[C ₄ H ₉] ⁺	[5]
1-Hexanol	C ₆ H ₁₄ O	NO ⁺	2.4	101	[C ₆ H ₁₃ O] ⁺	[5]
1-Octanol	C ₈ H ₁₈ O	NO ⁺	2.3	129	[C ₈ H ₁₇ O] ⁺	[5]
1-Octen-3-one	C ₈ H ₁₄ O	NO ⁺	2.5	156	[C ₈ H ₁₄ O.NO] ⁺	
1-Pentanol	C ₅ H ₁₂ O	NO ⁺	2.5	87	[C ₅ H ₁₁ O] ⁺	[5]
1-Penten-3-one	C ₅ H ₈ O	NO ⁺	2.5	114	[C ₅ H ₈ O.NO] ⁺	[8]
2- and 3-Methylbutanoic acid	C ₅ H ₁₀ O ₂	NO ⁺	2.5	132	[C ₅ H ₁₀ O ₂ .NO] ⁺	[8]
2 or 3-Isopropyl-2 or 3-methoxypyrazine	C ₈ H ₁₂ N ₂ O	H ₃ O ⁺	3.0	167	[C ₈ H ₁₂ N ₂ O.H] ⁺	[8]
2,3-Butanediol	C ₄ H ₁₀ O ₂	NO ⁺	2.3	89 107	[C ₄ H ₉ O ₂] ⁺ , [C ₄ H ₉ O ₂ ⁺ .H ₂ O]	[8]
2,3-Butanedione	C ₄ H ₆ O ₂	NO ⁺	1.3	86	[C ₄ H ₆ O ₂] ⁺	[4]
2,3-Dimethylpyrazine	C ₆ H ₈ N ₂	NO ⁺	2.8	108	[C ₆ N ₂ H ₈] ⁺	
2-Heptanol	C ₇ H ₁₆ O	NO ⁺	2.4	115	[C ₇ H ₁₅ O] ⁺	[9]
2-Heptanone	C ₇ H ₁₄ O	NO ⁺	3.4	144	[C ₇ H ₁₄ O.NO] ⁺	[3]
2-Hexanone	C ₆ H ₁₂ O	NO ⁺	3.6	130	[NO ⁺ .C ₆ H ₁₂ O]	[4]
2-Isopropyl-3-methoxypyrazine	C ₈ H ₁₂ N ₂ O	H ₃ O ⁺	3.0	153	[C ₈ H ₁₂ N ₂ O.H] ⁺	[8]
2-Methyl-1-butanol	C ₅ H ₁₂ O	NO ⁺	2.4	87	[C ₅ H ₁₁ O] ⁺	[10]
2-Methyl-2-butanol	C ₅ H ₁₂ O	O ₂ ⁺	2.6	59	[C ₃ H ₇ O] ⁺	[5]
2-Methylbutanal	C ₅ H ₁₀ O	NO ⁺	3.2	85	[C ₅ H ₉ O] ⁺	[1]
2-Methylpropanal	C ₄ H ₈ O	NO ⁺	3.1	71	[C ₄ H ₇ O] ⁺	[7]
2-Octanone	C ₈ H ₁₆ O	NO ⁺	3.3	158	[C ₈ H ₁₆ O.NO] ⁺	[3]
2-Pentylfuran	C ₉ H ₁₄ O	NO ⁺	2.0	138	[C ₉ H ₁₄ O] ⁺	
3-Hexen-1-ol	C ₆ H ₁₂ O	NO ⁺	2.5	72	[C ₄ H ₈ O] ⁺	[8]
3-Methylbutanal	C ₅ H ₁₀ O	H ₃ O ⁺	3.6	66	[C ₅ H ₆] ⁺	[7]
3-Pentanone	C ₅ H ₁₀ O	NO ⁺	3.4	116	[NO ⁺ .C ₅ H ₁₀ O]	[4]
5-Methylfurfural	C ₆ H ₆ O ₂	NO ⁺	3.1	110	[C ₆ H ₆ O ₂] ⁺	[10]
6-Methyl-5-hepten-2-one	C ₈ H ₁₄ O	O ₂ ⁺	2.5	126	[C ₈ H ₁₄ O] ⁺	[8]
Acetophenone	C ₈ H ₈ O	NO ⁺	3.6	150	[NO ⁺ .C ₈ H ₈ O]	[4]
Benzaldehyde	C ₇ H ₆ O	NO ⁺	2.8	105	[C ₇ H ₅ O] ⁺	[4]

Continued

Table 3 continued

Beta-ionone	C ₁₃ H ₂₀ O	O ₂ ⁺	2.5	177	[C ₁₂ H ₁₇ O] ⁺	
Butanal	C ₄ H ₈ O	NO ⁺	3.5	71	[C ₄ H ₇ O] ⁺	[4]
Butanone	C ₄ H ₈ O	NO ⁺	2.8	102	[NO ⁺ .C ₄ H ₈ O]	[4]
Cyclohexanone	C ₆ H ₁₀ O	NO ⁺	3.3	128	[C ₆ H ₁₀ O.NO] ⁺	[10]
Decanal	C ₁₀ H ₂₀ O	NO ⁺	3.3	155	[C ₁₀ H ₁₉ O] ⁺	[7]
Dimethyl disulfide	C ₂ H ₆ S ₂	NO ⁺	2.4	94	[(CH ₃) ₂ S ₂] ⁺	[6]
Ethanol	C ₂ H ₆ O	NO ⁺	1.2	45 63 81	[C ₂ H ₅ O] ⁺ , [C ₂ H ₅ O ⁺ .H ₂ O], [C ₂ H ₅ O ⁺ .2H ₂ O]	[5]
Ethyl acetate	C ₄ H ₈ O ₂	NO ⁺	2.1	118	[NO ⁺ .CH ₃ COOC ₂ H ₅]	[6]
Eugenol	C ₁₀ H ₁₂ O ₂	NO ⁺	2.5	164	[C ₁₀ H ₁₂ O ₂] ⁺	[8]
Furfural	C ₅ H ₄ O ₂	NO ⁺	3.2	95	[C ₅ H ₃ O ₂] ⁺	[10]
Heptanal	C ₇ H ₁₄ O	NO ⁺	3.3	113	[C ₇ H ₁₃ O] ⁺	[7]
Hexanal	C ₆ H ₁₂ O	O ₂ ⁺	2.0	44	[C ₂ H ₄ O] ⁺	[4]
Hexanoic acid	C ₆ H ₁₂ O ₂	NO ⁺	2.5	146	[C ₆ H ₁₂ O ₂ .NO] ⁺	
Linalool	C ₁₀ H ₁₈ O	NO ⁺	2.6	96	[C ₇ H ₁₂] ⁺	
Methanol	CH ₄ O	H ₃ O ⁺	2.7	33 51 69	[CH ₃ O] ⁺ , CH ₃ OH ₂ ⁺ .H ₂ O], [CH ₃ OH.H ⁺ .(H ₂ O) ₂]	[5]
Methyl salicylate	C ₈ H ₈ O ₃	O ₂ ⁺	2.7	138	[C ₇ H ₆ O ₃] ⁺	
Naphthalene	C ₁₀ H ₈	O ₂ ⁺	1.8	128	[C ₁₀ H ₈] ⁺	[2]
Nonanal	C ₉ H ₁₈ O	O ₂ ⁺	3.2	112	[C ₈ H ₁₆] ⁺	
Octanal	C ₈ H ₁₆ O	NO ⁺	3.0	127	[C ₈ H ₁₅ O] ⁺	[7]
Octane	C ₈ H ₁₈	H ₃ O ⁺	.090	133	[H ₃ O ⁺ .C ₈ H ₁₈]	[6]
Phenylacetaldehyde	C ₈ H ₈ O	NO ⁺	2.5	150	[C ₈ H ₈ O.NO] ⁺	[8]
Propanal	C ₃ H ₆ O	NO ⁺	2.5	57	[C ₃ H ₅ O] ⁺	[4]
Terpenes		NO ⁺	2.2	136	[C ₁₀ H ₁₆] ⁺	
Terpinolene	C ₁₀ H ₁₆	O ₂ ⁺	2.2	136	[C ₁₀ H ₁₆] ⁺	
Tetramethylpyrazine	C ₈ H ₁₂ N ₂	O ₂ ⁺	2.5	136	[C ₈ H ₁₂ N ₂] ⁺	
Toluene	C ₇ H ₈	H ₃ O ⁺	2.2	93	[C ₇ H ₁₀ N ₂] ⁺	[6]
Trimethylpyrazine	C ₇ H ₁₀ N ₂	NO ⁺	2.5	122	[C ₇ H ₁₀ N ₂] ⁺	
Xylene	C ₈ H ₁₀	H ₃ O ⁺	2.4	107	[C ₈ H ₁₀ .H] ⁺	[6]

References: [1] Michel and others (2005); [2] Milligan and others (2002); [3] Smith and others (2003); [4] Spanel and others (1997); [5] Spanel and Smith (1997); [6] Spanel and Smith (1998); [7] Spanel and others (2002); [8] Syft Technologies (2009); [9] Wang and others (2003); [10] Wang and others (2004).

Statistics Method

When comparing blanched, SnCl₂, whole, pureed, and red peppers a MANOVA was performed on the concentrations for 13 volatiles using two factors: product (pepper type) and time (day 0, day 1 or overall). In the portion of the study that examined

freezing rates, a MANOVA was performed for the volatiles using the three factors product, time, and speed (freezing rate). When examining the relationship between LOX activity and volatile level formation an ANCOVA was performed with two factors (product and time) and with LOX as the covariate. Correlations were also calculated between volatiles and LOX activity. The significance level for all analyses performed was set of 5%. Significant differences between the various products, days, freeze rates, and their interactions was determined by using a Tukey multiple comparisons with a family-wise error rate of 5%. All statistics performed was done using a SAS 9.2 program.

Chapter 4: Results and Discussion

Six carbon aldehydes and alcohols formed by the lipoxygenase pathway (LOX), such as 2-hexenals, 2-hexenols, (Z)-3-hexenal, and (Z)-3-hexenol, are responsible for much of the distinct aroma and flavor of plant material such as green bell peppers. Fruity, sweet, and fresh odor notes are dominant for the 2-hexenals and 2-hexenols, while (Z)-3-hexenal and (Z)-3-hexenol have a strong spicy and grassy green aroma (Luning and others 1995a). Since red bell peppers, which have lower LOX activity, have lower concentrations of the LOX generated volatiles, 2-hexenals, 2-hexenols, (Z)-3-hexenal, and (Z)-3-hexenol, they have a very different and distinct aroma profile when compared to green bell peppers (Luning and others 1994, 1995a). These volatiles have also been shown to decrease during frozen storage in jalapeno peppers (Azcarate and Barringer 2010).

Effect of Blanching and the Freezing Process

The volatile levels of most compounds did not change significantly during blanching (Table 2). However, (Z)-3-hexenal had a large significant decrease. (Z)-3-Hexenal is a heat labile compound thus blanching greatly reduces its concentration in the samples. In the fresh green bell pepper before freezing (day 0), the (Z)-3-hexenal levels for raw whole and blanched were 924 and 31 ppb respectively (Table 2). (Z)-3-Hexenal was also shown to have poor stability in blended tomatoes and jalapeno peppers that were

exposed to heat (Kazeniak and Hall 1970, Azcarate and Barringer 2010). A few of the other ketones and aldehydes also decreased in concentration. Two volatiles that increased due to blanching were 2-methylbutanal and 2,3-butanedione. Methylbutanal was also found to be 86% higher in blanched compared to unblanched jalapeno peppers (Azcarate and Barringer 2010). Methylbutanal has cacao, sweaty, and cooked vegetable odor notes (Luning and others 1995b) and has been reported to increase in concentration in heated products (Cremer and Eichner 2000). Methylbutanal is formed by Strecker degradation of isoleucine and leucine during Maillard reactions (Vazquez-Landaverde and others 2005; Rotsachakul and others 2008). Another Maillard browning product is 2,3-butanedione (Yaylayan and Keyhani 1999) which was also higher in concentration in blanched bell peppers than unblanched.

Comparing day 0 and 1, blanched and red bell pepper volatiles levels did not significantly change as a result of freezing while raw with and without SnCl_2 , and pureed had some changes (Table 2). In raw whole with and without SnCl_2 , volatile concentrations did not change significantly except for a decrease in a handful of volatiles. Most raw pureed volatiles significantly increased due to the freezing process. The increase in volatile levels must be due to the increased tissue disruption allowing for enzyme, substrate, and oxygen to mix more readily and increasing the volatile concentrations in only one day. Hexanal doubled from 402 to 830 ppb after freezing (Table 2). This increase was observed not only for LOX generated volatiles but for most volatiles found in pureed bell peppers. However five volatiles decreased during freezing: 1-penten-3-one, 6-methyl-5-hepten-2-one, acetophenone, phenylacetaldehyde, and

terpenes, all of which are reactive compounds that are oxidized due to cell rupture during pureeing.

Table 4: Volatile concentrations (ppb) for bell peppers before freezing (Day 0) and after freezing (Day 1) shows how freezing changes volatile concentrations.

	Compound	Blanched Whole		Raw Whole w/ SnCl ₂ *		Raw Whole*	Raw Pureed*	Red Raw Whole	
		Day 0	Day 1	Day 0	Day 1	Day 1	Day 1	Day 0	Day 1
Linolenic Acid Volatiles	(Z)-3-Hexenal	31 ^c	42 ^c	925 ^{ab}	369 ^{bc}	471 ^{bc}	1438 ^a	17 ^c	183 ^{bc}
	(E)-2-Hexenal	64 ^{bcd}	106 ^b	74 ^{bc}	65 ^{bcd}	45 ^{cd}	159 ^a	34 ^{cd}	24 ^d
	Hexen-1-ol	3 ^b	7 ^b	11 ^b	4 ^b	5 ^b	619 ^a	1 ^b	1 ^b
	(E)-2-Pentenal	5 ^b	6 ^b	18 ^b	4 ^b	10 ^b	209 ^a	2 ^b	3 ^b
	1-Pentanol	6 ^{bc}	10 ^b	9 ^b	1 ^{cd}	7 ^b	31 ^a	1 ^{cd}	0 ^d
	1-Penten-3-one	11 ^{bc}	9 ^{bc}	75 ^a	3 ^c	46 ^{ab}	31 ^{bc}	4 ^c	1 ^c
Linoleic Acid Volatiles	Hexanal	132 ^{bc}	181 ^{bc}	402 ^b	176 ^{bc}	259 ^{bc}	830 ^a	30 ^c	102 ^{bc}
	1-Hexanol	2 ^b	3 ^b	6 ^b	1 ^b	2 ^b	74 ^a	1 ^b	0 ^b
	(E)-2-Heptenal	8 ^b	14 ^{ab}	15 ^a	9 ^b	10 ^{ab}	13 ^{ab}	2 ^c	2 ^c
	(E)-2-Octenal	3 ^b	5 ^b	11 ^a	4 ^b	6 ^{ab}	12 ^a	2 ^b	1 ^b
	(E)-2-Nonenal	5 ^{ab}	4 ^{bc}	3 ^{bc}	2 ^{bc}	2 ^{bc}	9 ^a	0 ^c	1 ^{bc}
	2-Pentylfuran	1 ^{bc}	2 ^{abc}	5 ^{ab}	3 ^{abc}	1 ^{bc}	6 ^a	1 ^c	0 ^c
	(E,E)-2,4-Decadienal	0 ^b	1 ^b	4 ^b	1 ^b	1 ^b	14 ^a	1 ^b	0 ^b
	(E,Z)-2,6-Nonadienal	10 ^{cd}	5 ^{cd}	27 ^b	17 ^{bc}	10 ^{cd}	49 ^a	1 ^d	1 ^d
	1-Butanol	333 ^{bcd}	337 ^{bcd}	356 ^{ab}	346 ^{bc}	343 ^{bcd}	382 ^a	315 ^{cd}	312 ^d
	1-Octanol	1 ^a	1 ^a	11 ^a	3 ^a	2 ^a	10 ^a	2 ^a	2 ^a
	1-Octen-3-one	4 ^{abc}	5 ^{abc}	8 ^a	2 ^c	4 ^{abc}	6 ^{ab}	2 ^{bc}	1 ^c
	2- and 3-Methylbutanoic acid	1 ^b	2 ^b	4 ^b	4 ^b	4 ^b	31 ^a	3 ^b	2 ^b
	2 or 3-Isopropyl-2 or 3-methoxypyrazine	24 ^{de}	15 ^{def}	71 ^a	47 ^{bc}	36 ^{cd}	60 ^{ab}	4 ^{ef}	2 ^f
	2,3-Butanediol	5 ^b	7 ^b	19 ^{ab}	7 ^b	9 ^b	43 ^a	6 ^b	5 ^b
	2,3-Butanedione	31 ^b	52 ^a	18 ^c	14 ^{cde}	18 ^{cd}	50 ^a	4 ^c	5 ^{de}
	2,3-Dimethylpyrazine	0 ^b	1 ^b	2 ^{ab}	2 ^b	1 ^b	3 ^a	0 ^b	1 ^b
	2-Heptanol	2 ^a	1 ^a	20 ^a	1 ^a	5 ^a	12 ^a	0 ^a	1 ^a
	2-Heptanone	12 ^{abc}	9 ^{bc}	15 ^{ab}	16 ^{ab}	17 ^a	12 ^{abc}	5 ^c	4 ^c
	2-Hexanone	3 ^b	1 ^b	14 ^b	3 ^b	2 ^b	41 ^a	0 ^b	1 ^b
	2-Isopropyl-3-methoxypyrazine	3 ^b	3 ^b	16 ^b	6 ^b	12 ^b	314 ^a	3 ^b	4 ^b
	2-Methyl-1-butanol	5 ^{bc}	9 ^b	8 ^b	1 ^{cd}	6 ^b	28 ^a	1 ^{cd}	0 ^d
	2-Methyl-2-butanol	40 ^b	52 ^b	50 ^b	34 ^b	37 ^b	121 ^a	31 ^b	26 ^b
	2-Methylbutanal	102 ^b	185 ^a	53 ^c	12 ^d	56 ^c	128 ^b	7 ^d	7 ^d
	2-Methylpropanal	12 ^{bcd}	19 ^b	14 ^{bc}	12 ^{bcd}	8 ^{cd}	29 ^a	6 ^{cd}	4 ^c
	2-Octanone	1 ^b	1 ^b	2 ^{ab}	4 ^a	1 ^b	2 ^{ab}	0 ^b	0 ^b
	3-Methylbutanal	1 ^b	4 ^b	16 ^b	5 ^b	6 ^b	293 ^a	1 ^b	7 ^b

Continued

Table 4 continued

3-Pentanone	7 ^{bcd}	11 ^{bc}	14 ^b	3 ^{cd}	5 ^{bcd}	37 ^a	1 ^{cd}	1 ^d
5-Methylfurfural	1 ^a	1 ^a	1 ^a	1 ^a	2 ^a	1 ^a	1 ^a	0 ^a
6-Methyl-5-hepten-2-one	12 ^{bcd}	11 ^{bcd}	36 ^a	24 ^{ab}	11 ^{bcd}	17 ^{bc}	10 ^{cd}	3 ^d
Acetophenone	1 ^b	0 ^b	3 ^a	1 ^b	2 ^{ab}	1 ^b	1 ^b	0 ^b
Benzaldehyde	26 ^{bc}	42 ^b	32 ^b	8 ^{cd}	27 ^{bc}	76 ^a	3 ^d	3 ^d
Beta-ionone	0 ^b	1 ^b	1 ^b	0 ^b	0 ^b	7 ^a	0 ^b	0 ^b
Butanal	10 ^{bcd}	17 ^b	12 ^{bc}	11 ^{bcd}	7 ^{cd}	26 ^a	5 ^{cd}	4 ^d
Butanone	9 ^{bc}	12 ^b	10 ^b	13 ^b	12 ^b	17 ^a	6 ^c	6 ^c
Cyclohexanone	2 ^a	2 ^a	129 ^a	5 ^a	4 ^a	75 ^a	5 ^a	1 ^a
Decanal	3 ^a	2 ^a	8 ^a	5 ^a	2 ^a	4 ^a	3 ^a	3 ^a
Dimethyl disulfide	1 ^{cd}	2 ^{cd}	4 ^c	15 ^a	3 ^{cd}	10 ^b	1 ^d	1 ^{cd}
Ethanol	33 ^b	42 ^b	629 ^b	157 ^b	319 ^b	1532 ^a	10 ^b	284 ^b
Ethyl acetate	3 ^b	2 ^b	14 ^b	5 ^b	4 ^b	257 ^a	4 ^b	4 ^b
Eugenol	1 ^a	0 ^a	0 ^a	0 ^a	1 ^a	1 ^a	0 ^a	0 ^a
Furfural	5 ^b	5 ^b	13 ^b	10 ^b	4 ^b	99 ^a	3 ^b	2 ^b
Heptanal	4 ^b	2 ^b	5 ^{ab}	5 ^{ab}	4 ^b	10 ^a	3 ^b	2 ^b
Hexanoic acid	1 ^{cd}	3 ^{bcd}	4 ^b	3 ^{bc}	3 ^{bc}	8 ^a	2 ^{cd}	1 ^d
Linalool	4 ^b	2 ^b	19 ^b	29 ^b	8 ^b	110 ^a	2 ^b	2 ^b
Methanol	2996 ^b	4160 ^b	4002 ^b	1690 ^b	1850 ^b	50320 ^a	303 ^b	148 ^b
Methyl salicylate	6 ^b	5 ^b	29 ^b	17 ^b	21 ^b	172 ^a	3 ^b	3 ^b
Naphthalene	1 ^b	1 ^b	4 ^{ab}	2 ^b	2 ^b	9 ^a	2 ^b	0 ^b
Nonanal	20 ^{ab}	27 ^a	31 ^a	23 ^{ab}	18 ^{ab}	31 ^a	10 ^b	10 ^b
Octanal	4 ^a	5 ^a	8 ^a	7 ^a	4 ^a	5 ^a	14 ^a	2 ^a
Octane	32 ^b	21 ^b	46 ^b	34 ^b	34 ^b	545 ^a	10 ^b	9 ^b
Phenylacetaldehyde	5 ^b	2 ^b	18 ^a	5 ^b	9 ^{ab}	6 ^b	5 ^b	0 ^b
Propanal	30 ^{cd}	20 ^{de}	74 ^a	16 ^{de}	42 ^{bc}	60 ^{ab}	7 ^c	7 ^c
Terpenes	22 ^{bc}	13 ^c	103 ^a	74 ^{ab}	27 ^{bc}	34 ^{bc}	4 ^c	3 ^c
Terpinolene	9 ^{ab}	3 ^b	29 ^a	30 ^a	7 ^{ab}	32 ^a	1 ^b	2 ^b
Tetramethylpyrazine	2 ^{ab}	1 ^b	8 ^a	8 ^a	2 ^{ab}	9 ^a	0 ^b	1 ^b
Toluene	39 ^{bc}	33 ^{bc}	60 ^b	63 ^b	62 ^b	199 ^a	9 ^c	14 ^c
Trimethylpyrazine	2 ^{ab}	2 ^{ab}	3 ^a	2 ^{ab}	1 ^{ab}	3 ^a	1 ^{ab}	0 ^b
Xylene	23 ^{bc}	33 ^b	37 ^b	10 ^c	23 ^{bc}	137 ^a	5 ^c	6 ^c

* Indicates that volatile concentrations are the same on Day 0.

a, b, c, d, e, f in the same row indicates significance of that volatile level concentration.

Volatile Changes During Frozen Storage

In blanched samples the majority of lipoxygenase (LOX) generated volatiles remain constant throughout storage (Figure 1). Only (*E*)-2-hexenal, hexen-1-ol, and (*E*)-2-pentenal increased during storage compared to their day 0 levels. The increase in both (*E*)-2-hexenal and hexen-1-ol during frozen storage is caused by nonenzymatic

autooxidation of fatty acid into these volatiles. In jalapeno peppers (*E*)-2-hexenal also increased in blanched samples during storage (Azcarate and Barringer 2010). In frozen blanched leeks a small but significant increase in aldehydes including hexanal, (*E*)-2-pentenal, (*E*)-2-hexenal, and (*E*)-2-heptenal also occurred due to autooxidation of polyunsaturated fatty acids (Nielsen and others 2004).

Significant volatile formation occurred during frozen storage of raw, unblanched green bell peppers due to enzymatic activity, as shown by comparing blanched whole and raw whole with SnCl₂ added after storage (Figure 1). The blanched green bell peppers had no enzymatic activity, as verified experimentally, because blanching denatured the enzymes preventing their ability to form volatiles through the enzymatic pathways. SnCl₂ is an enzyme inhibitor (Wu and Liou 1986) and was added after storage to prevent enzyme activity during thawing. The SnCl₂ samples only had enzyme activity inhibited during thawing, allowing the enzymes to remain active to create volatiles during storage but not during thawing.

The raw with SnCl₂ samples had higher levels of (*Z*)-3-hexenal, hexanal, and 2-pentylfuran than the blanched samples (Figure 1). (*Z*)-3-Hexenal is produced from linolenic acid being hydrolyzed by lipoxygenase (LOX) and oxygen to create an 18:3 13-hydroperoxide which is converted to (*Z*)-3-hexenal by hydroperoxide lyase. Hexanal is produced from linoleic acid being hydrolyzed by LOX and oxygen to create an 18:2 13-hydroperoxide which hydroperoxide lyase converts into hexanal. 2-Pentylfuran is formed from linoleic acid interacting with LOX and oxygen to form an 18:2 9-hydroperoxide which then goes through an enzymatic conversion to 2-pentylfuran. In all three cases, this is the first volatile formed in that pathway. The higher concentration of (*Z*)-3-hexenal,

hexanal, and 2-pentylfuran in the SnCl_2 samples when compared to the blanched is due to enzymatic conversion of the fatty acids into volatiles during frozen storage.

Figure 1: Concentrations (ppb) during frozen storage of lipoxygenase created volatiles that are important to the fresh aroma of green bell peppers.

In contrast, the blanched samples had higher volatile levels than the raw with SnCl_2 samples for (*E*)-2-hexenal, hexen-1-ol, 1-penten-3-one, and (*E*)-2-heptenal (Figure

1). These volatiles were likely higher in the blanched peppers due to enzymatic destruction of these volatiles in the raw with SnCl_2 samples. These are all minor volatiles or volatiles formed from other volatiles, which are then further degraded. (*E*)-2-Hexenal is formed from (*Z*)-3-hexenal by *Z*-3/*E*-2 isomerase but then is converted by alcohol dehydrogenase into hexen-1-ol. Hexen-1-ol is enzymatically acetylated into hexyl acetate and (*Z*)-3-hexenyl acetate (Fall and others 1999). 1-Penten-3-one and (*E*)-2-heptenal are also converted enzymatically into other volatiles. The nonLOX generated volatiles remained constant throughout frozen storage for all samples.

The levels for many of the volatiles in the raw with SnCl_2 samples, including (*Z*)-3-hexenal, (*E*)-2-hexenal, hexen-1-ol, hexanal, (*E*)-2-pentenal, and 2-pentylfuran, appeared to peak around 34 days after freezing. (*Z*)-3-Hexenal is the first major LOX generated volatile from linolenic acid which is then isomerized into (*E*)-2-hexenal and both are acted on by alcohol dehydrogenase to form hexen-1-ol. Once (*Z*)-3-hexenal decreases it will be accompanied by a decrease in (*E*)-2-hexenal and hexen-1-ol as both are degrading into other volatiles. Hexanal is the first major LOX generated volatiles created from linoleic acid which is then converted into other volatiles. In frozen jalapeno peppers, (*Z*)-3-hexenal, (*E*)-2-hexenal, hexanal, hexenol, hexanol, and 1-penten-3-one also peaked at 1.5mo and then declined in the following months of storage (Azcarate and Barringer 2010). Hexanal also increased in sliced leeks until 9mo of frozen storage where it peaked and then started to decline (Nielsen and others 2004).

Significant volatile formation also occurred during thawing, as seen by comparing the raw whole to the raw whole with SnCl_2 (Figure 1). The volatile concentration in raw whole was higher than in raw whole with SnCl_2 for all LOX generated volatiles. Volatile

formation during thawing was greater than it was during storage for all LOX generated volatiles. During thawing the higher temperature and greater tissue disruption as the samples are pureed during sample preparation, both of which increase enzymatic activity, allow for more oxygen to interact with LOX to create hydroperoxide compounds that are then converted into volatiles. In contrast, the formation of the nonLOX generated volatiles were greater during storage than they were during thawing. This result was expected since oxidation and other nonenzymatic changes can occur during storage while only enzymatic reactions occur rapidly during thawing.

Peppers were quickly frozen in a blast freezer at -40°C for 1.5h or slowly frozen in a -18°C walk-in freezer and the volatiles monitored during storage. It was expected that there would be a difference in the volatiles produced between the freezing rates because in a quick freeze smaller ice crystals form within the cells, causing less tissue disruption and therefore less enzyme activity during storage, while samples frozen slowly form larger ice crystals between the cells and the greater tissue disruption should cause more enzyme activity. A comparison of quick and slow freezing rates showed a significant increase during storage in hexanal, 1-hexanol, (*E*)-2-heptenal, (*E*)-2-pentenal, and hexen-1-ol in the slowly frozen peppers while there was no difference for the other volatiles (Table 3). The increased tissue disruption apparently increased conversion of linoleic acid to hexanal, which converted into 1-hexanol by alcohol dehydrogenase. There were also a few minor volatiles formed by secondary pathways that were also higher in the slowly frozen peppers. The major linolenic derived volatiles were not different. Table 3 shows the volatile levels for some of the volatiles on one day of storage, which is representative

of the results throughout storage. The nonLOX generated volatiles showed no significant difference between the two freezing rates.

Table 5: Volatile concentrations (ppb) for bell peppers frozen quickly and frozen slowly 40 and 47 days after freezing.

Compound	Raw Whole		Raw Whole SnCl ₂		Pureed		Pureed SnCl ₂		Blanched Whole		Blanched pureed	
	Day 40		Day 40		Day 40		Day 40		Day 47		Day 47	
	Quick	Slow	Quick	Slow	Quick	Slow	Quick	Slow	Quick	Slow	Quick	Slow
(Z)-3-Hexenal	713 ^{ab}	790 ^a	464 ^b	632 ^{ab}	153 ^c	508 ^{ab}	93 ^c	81 ^c	116 ^c	87 ^c	71 ^c	85 ^c
(E)-2-Hexenal	141 ^{de}	14 ^{de}	157 ^{cde}	237 ^{bcd}	345 ^{ab}	273 ^{abc}	36 ^e	39 ^e	337 ^{ab}	351 ^{ab}	269 ^{abc}	373 ^a
<u>Hexen-1-ol</u>	7 ^c	7 ^c	7 ^c	9 ^c	223 ^b	343 ^a	13 ^c	7 ^c	16 ^c	16 ^c	10 ^c	16 ^c
<u>(E)-2-Pentenal</u>	12 ^c	9 ^c	8 ^c	6 ^c	70 ^b	96 ^a	8 ^c	8 ^c	6 ^c	6 ^c	4 ^c	5 ^c
1-Pentanol	11 ^{cd}	10 ^{cd}	8 ^{de}	5 ^{efg}	15 ^{bc}	19 ^{ab}	3 ^{fg}	1 ^g	10 ^{cd}	19 ^{ab}	7 ^{def}	22 ^a
1-Penten-3-one	65 ^a	53 ^a	3 ^b	2 ^b	58 ^a	64 ^a	3 ^b	2 ^b	15 ^b	16 ^b	22 ^b	17 ^b
<u>Hexanal</u>	471 ^a	477 ^a	359 ^{abc}	389 ^{ab}	349 ^{abc}	497 ^a	68 ^d	78 ^d	224 ^{bcd}	260 ^{bc}	202 ^{dc}	206 ^{cd}
<u>1-Hexanol</u>	2 ^c	3 ^c	2 ^c	2 ^c	31 ^b	51 ^a	1 ^c	1 ^c	3 ^c	3 ^c	2 ^c	2 ^c
<u>(E)-2-Heptenal</u>	16 ^{acd}	13 ^{cde}	9 ^{de}	8 ^{de}	27 ^a	19 ^{abc}	7 ^{de}	6 ^e	27 ^a	26 ^a	24 ^{ab}	22 ^{abc}
(E)-2-Octenal	14 ^b	14 ^b	4 ^b	5 ^b	26 ^a	26 ^a	3 ^b	3 ^b	8 ^b	9 ^b	8 ^b	6 ^b
(E)-2-Nonenal	13 ^{ab}	16 ^a	5 ^c	7 ^{bc}	8 ^{bc}	13 ^{ab}	2 ^c	2 ^c	6 ^c	3 ^c	2 ^c	4 ^c
2-Pentylfuran	3 ^{bc}	4 ^{abc}	6 ^{ab}	7 ^a	7 ^{ab}	6 ^{abc}	4 ^{abc}	5 ^{abc}	4 ^{abc}	3 ^{bc}	2 ^c	2 ^c

a, b, c, d, e, f, g in the same row indicates of that volatile level concentration

Underlined volatiles show a significance difference between quick and slow freezing throughout storage.

For most LOX and nonLOX generated volatiles, the pureed samples had higher volatile concentrations than the whole samples (Figure 1). Pureed bell peppers should be higher in volatiles than whole peppers due to the increase in tissue disruption which is known to increase volatile level concentrations (Luning and others 1994). An increase in cell disruption by homogenizing the bell peppers allows for more oxygen to interact with the lipoxygenase enzyme and convert fatty acids like linoleic and linolenic into volatiles. The only two volatiles where pureed was lower than whole are (Z)-3-hexenal and hexanal. (Z)-3-Hexenal and hexanal are the first volatiles created in the LOX pathway, which then convert into other volatiles, thus in the pureed samples they are being more rapidly converted into secondary volatiles than they are being formed. LOX was

measured in pureed and whole and was not significantly different between the two samples. The increased volatile levels in pureed samples therefore was due to increased oxygen availability and increased substrate availability not a change in the enzyme itself. Frozen pureed jalapenos were also higher in volatile concentrations than whole jalapenos (Azcarate and Barringer 2010).

Pureeing also causes the enzymes to react faster and therefore the pureed samples peak earlier than the green raw whole samples (Figure 1). The pureed volatiles peak in the first month of frozen storage for (*Z*)-3-hexenal, hexanal, hexen-1-ol, and 1-hexanol while (*E*)-2-hexenal and (*E*)-2-octenal increase throughout frozen storage. In pureed frozen jalapeno (*Z*)-3-hexenal peaks at 1.5mo with all other volatiles remain constant throughout storage (Azcarate and Barringer 2010).

All LOX and nonLOX generated volatiles in green bell peppers were higher than those in red bell peppers (Figure 1). During the ripening process, volatiles in bell peppers decrease or even disappear completely (Luning and others 1994). In frozen stored red bell peppers the LOX enzyme activity was significantly lower (41 nmol/g fwt/min) than that of the green bell peppers (186 nmol/g fwt/min). It is believed that LOX enzyme activity declines because of the rapid conversion of chloroplasts to chromoplasts, which happens between 69 and 81 days after flowering (Matsui and others 1997). Bell peppers also change from green to red between 69 and 81 days after flowering. With lower enzymatic activity there will be lower LOX volatiles forming during both storage and thawing in the red bell peppers than in the green.

Chapter 5: Conclusions

Blanching had little effect on volatile levels, except for a significant decrease in (Z)-3-hexenal. The freezing process also had little effect on volatile levels, except for an increase in volatile levels in raw pureed samples. During frozen storage blanched samples showed autooxidation as some volatiles were created. Raw samples had higher levels of (Z)-3-hexenal, hexanal, and 2-pentylfuran than the blanched samples due to enzyme activity during frozen storage. Blanched samples had higher volatile levels than the raw samples for (E)-2-hexenal, hexen-1-ol, 1-penten-3-one, and (E)-2-heptenal because of enzymatic destruction of these volatiles in the raw samples. LOX was active both during frozen storage and during thawing, but was greater in thawing due to higher temperature and greater tissue disruption during thawing. Slow freezing produced higher levels of some volatiles during storage, than quick freezing. Pureeing produced significantly higher levels LOX generated volatiles than whole samples during storage. Green bell peppers were significantly higher in LOX activity and LOX generated volatiles than red bell peppers.

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Appendix A: Additional Figures

Figure 4. Biochemical pathways proposed for the formation of volatile compounds from linoleic and linolenic acids in fresh bell peppers. Abbreviations: LOOH, hydroperoxide; [LOX], bell pepper lipoxygenase; [HPOL], hydroperoxide lyase; [ADH], alcohol dehydrogenase; [Z3/E2-ISO], *cis-3/trans-2* isomerase; [E?], possible enzymatic transformation.

Figure 2: Schematic diagram of the lipoxygenase pathway (Luning and others 1995a)

Figure 3: Principles of Selected Ion Flow Tube Mass Spectrometry (Smith and Spanel 2005)

Figure 4: Sample preparation method for sample runs on SIFT-MS

Table 6: Blanching effect on lipoxygenase generated volatile concentrations (ppb) in green bell peppers prior to freezing

		Blanched Whole	Raw Whole
	Compound	Day 0	Day 0
Linolenic Acid Volatiles	(Z)-3-Hexenal	31 ^c	925 ^{ab}
	(E)-2-Hexenal	64 ^{bcd}	74 ^{bc}
	Hexen-1-ol	3 ^b	11 ^b
	(E)-2-Pentenal	5 ^b	18 ^b
	1-Pentanol	6 ^{bc}	9 ^b
	1-Penten-3-one	11 ^{bc}	75 ^a
Linoleic Acid Volatiles	Hexanal	132 ^{bc}	402 ^b
	1-Hexanol	2 ^b	6 ^b
	(E)-2-Heptenal	8 ^b	15 ^a
	(E)-2-Octenal	3 ^b	11 ^a
	(E)-2-Nonenal	5 ^{ab}	3 ^{bc}
	2-Pentylfuran	1 ^{bc}	5 ^{ab}

a, b, c, d in the same row indicates significance of that volatile level concentration

Table 7: The effect of freezing on lipoxygenase generated volatile concentrations (ppb) for raw pureed samples.

	Compound	Raw Pureed	
		Day 0	Day 1
Linolenic Acid Volatiles	(Z)-3-Hexenal	925 ^{ab}	1438 ^a
	(E)-2-Hexenal	74 ^{bc}	159 ^a
	Hexen-1-ol	11 ^b	619 ^a
	(E)-2-Pentenal	18 ^b	209 ^a
	1-Pentanol	9 ^b	31 ^a
	1-Penten-3-one	75 ^a	31 ^{bc}
Linoleic Acid Volatiles	Hexanal	402 ^b	830 ^a
	1-Hexanol	6 ^b	74 ^a
	(E)-2-Heptenal	15 ^a	13 ^{ab}
	(E)-2-Octenal	11 ^a	12 ^a
	(E)-2-Nonenal	3 ^{bc}	9 ^a
	2-Pentylfuran	5 ^{ab}	6 ^a

a, b, c, d in the same row indicates significance of that volatile level concentration

Figure 5: Autooxidation of fatty acids during frozen storage generated (*E*)-2-hexenal and hexen-1-ol in blanched green bell peppers