Effect of Enzymes on Strawberry Volatiles During Storage, at Different Ripeness Level, in Different Cultivars and During Eating

Thesis

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ABSTRACT

Strawberry samples with enzyme activity and without enzyme activity (stannous chloride added) were measured for real time formation of lipoxygenase (LOX) derived aroma compounds after 5 min pureeing using selected ion flow tube mass spectrometry (SIFT-MS). The concentration of (*Z*)-3-hexenal and (*E*)-2-hexenal increased immediately after blending and gradually decreased over time while hexanal concentration increased for at least 5 min in ground strawberries. The formation of hexanal was slower than the formation of (*Z*)-3-hexenal and (*E*)-2-hexenal in the headspace of pureed strawberries. The concentration of LOX aldehydes and esters significantly increased during refrigerated storage. Damaging strawberries increased the concentration of LOX aldehydes but did not significantly affect the concentration of esters. The concentrations of many of the esters were strongly correlated to their corresponded acids and/or aldehydes. The concentration of LOX generated aldehydes decreased during ripening, while fruity esters increased. Different varieties had different aroma profiles and esters were the greatest percentage of the volatiles. The aroma release of some of the LOX derived aldehydes in the mouthspace in whole strawberries compared to chopped strawberries showed that these volatiles are formed in the mouth during chewing. The persistence of LOX derived compounds was higher than esters after swallowing. The MSas/MSbs persistence ratio of esters decreased as the chain length of the acid part of the ester compounds increased in whole strawberries.

PRACTICAL APPLICATIONS

The results of the storage study showed that the concentrations of fruity and fresh volatiles increased during ripening and storage. These results can be used to determine the best edible time of strawberries during storage based on their fruity and fresh flavor. More ripened strawberries are rich in fruity flavor which can show the best maturity to pick strawberries and market them. Maximizing the fruity flavor of strawberries in a product requires selecting the highest ester concentrated fruit variation, waiting until fruits ripen to harvest, trying to keep them from any damage to avoid off flavor formation during storage and storing them in a fresh condition until their consumption. The 4-41% of strawberry volatiles persist in the mouth after swallowing and the persistence of LOX derived compound was higher than esters. This knowledge can be used in flavor industry to improve the formula of natural strawberry flavor by considering human perception during eating.

DEDICATION

To my parents Nadire and Ömer

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CHAPTER 1

INTRODUCTION

Fragaria ananassa, the commercial strawberry genus, was first found in England in the 18th century (Wilhelm and Sagen 1974), and is the fifth most eaten fresh fruit after bananas, apples, oranges and grapes (Borris and others 2006). In production value, strawberries are the fourth highest ranked fruit in the US after grapes, apples and oranges (Borris and others 2006). Fresh strawberry consumption is 75% of total production while processed strawberries have 25% of overall production (Borris and others 2006). Strawberries are commonly used in the food industry as an important ingredient in jam, yogurt, syrup, tea, juice, ice cream and other food products. The flavor of strawberries is unique for its fresh and fruity notes. There are more than 360 volatile flavor compounds identified in strawberry flavor (Latrasse 1991).

Aldehydes and esters, which contribute to the fresh and fruity strawberry flavor, form through enzymatic oxidation of lipids and enzymatic biosynthesis of alcohols and acids. The lipid oxidation pathway is essential to C6 aldehydes formation (Leone and others 2006, Perez and others 1999a) via lipoxygenase (LOX) and hydroperoxide lyase (HPL) enzyme activity during ripening. The activity of LOX and HPL enzymes increases 25% and 200% and the total linolenic acid availability increases 200% when strawberries

are wounded, which encourages the formation of (*E*)-2-hexenal and (*Z*)-3-hexenal (Myung and others 2006).

Enzymatic formation of esters occurs during ripening and in the late ripening stages (Azodanlou and others 2004, Perez and others 1996, Perez and others 1992, Yamashita and others 1977). Enzymatic activity has an important role in changes in volatile composition. The release of compounds which are formed enzymatically increases after tissue maceration, because maceration increases enzymatic activity by rupturing of cell structure (Brauss and others 1998). Refrigerated storage is one of the ways to preserve the freshness of fruits (Wiley 1994). Esters are the main contributors to increases in the strawberry aroma during storage, increasing by 50 to 200% (Forney and others 1998). Ester formation was not affected by crushing or homogenizing strawberries (Yamashita and others 1975).

The activity of key enzymes for aroma formation may differ between various varieties of strawberries and these different enzymatic activities lead to diverse aroma profiles for consumers (Olias and others 2002). Also, the different activity of enzymes results in different flavor profile in each ripeness stage. Low alcohol acyltransferase enzyme activity may cause poor fruit flavor (Perez and others 1996).

Previous studies focused on flavor release and persistence in the mouth using solutions, which are less complex than food. These studies do not completely explain volatile composition changes and persistence in a real food during eating. Flavor release from different foods has been studied (Ingham and others 1995b, Clawson and others

1994, Van Ruth and others 1995), but there is only one study focused on strawberry aroma changes during eating. They found that the highest volatile concentration is observed after swallowing and the persistence of short chain esters are higher than longer chain esters in nosespace (Ingham and others 1995a).

The objectives of this study were to investigate the effect of enzyme inhibition on lipoxygenase generated compounds, the effect of refrigerated storage on fruity and fresh volatiles of strawberries, the volatile changes at different ripening stages, the volatile differences between strawberry varieties and to investigate volatile composition changes in the mouthspace and the nosespace during eating using SIFT-MS.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Strawberry Aroma

Strawberry aroma was previously studied by various researchers. Larsen and Poll (1992) focused on determining odor threshold values of 24 strawberry volatiles in the Senga Sengana variety. Odor threshold is the minimal required concentration of a compound to be detected by the human nose and the human olfactory system. When the concentration of a compound is higher than the threshold level, it is able to be perceived by the nose. One of the notable compounds in strawberries is ethyl butanoate which has the highest aroma value as compared to other compounds (concentration/threshold), because of the low threshold level (10^{-6} mg/kg) (Larsen and Poll 1992). Ethyl butanoate is one of the notable compounds in ripened strawberries and the concentration of ethyl butanoate is correlated with consumer satisfaction (Azodanlou and others 2004). Methyl and ethyl esters are the highest concentration compounds in the Senga Sengana variety (Schreier 1980), while 2,5-dimethyl-4-hydroxy-3(2H)-furanone is the most important aroma compound (Sundt 1970). 2,5-Dimethyl-4-methoxy-3(2H)-furanone is also found in strawberry aroma at a lower level (Pyysalo and others 1979). It has a higher threshold value than 2,5-dimethyl-4-hydroxy-3(2H)-furanone (Larsen and Poll 1992). 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, linalool and ethyl hexanoate are the most important compounds for strawberry aroma based on their aroma values (Larsen and others 1992).

A study has shown that desired strawberry flavor in wild species is substantially higher than in cultivated species (Ulrich and others 2007). The most important flavor compounds of "Annelie" and "Alaska Pioneer" cultivars, which are the backcrossed cultivars between wild and cultivated strawberries, are ethyl hexanoate, ethyl butanoate, 2,5-dimethyl-4-methoxy-3(2H)-furanone and (*E*)-2-hexenal (Hirvi and Honkanen 1982).

Strawberry flavor is affected by maturity of fruit. The amount of total volatile concentration increases over a very short time near to maturity (Azodanlou and others 2004). The most flavor and aroma alternation of strawberries occurs during the ripening stages and storage after harvesting (Yamashita and others 1975). Also various treatment methods have been applied to strawberries to understand aromatic changes due to treatments such as high pressure, heat treatment and freezing.

Esters, alcohols, aldehydes and acids have been identified in strawberry aroma (Perez and others 1997). A list of some compounds found in strawberry flavor is shown in Table 1.

Table 1 Some volatile compounds identified in different strawberry cultivars

Esters	3-Methylbutyl butanoate[a]	Furaneol[d]
Ethyl acetate[c, e]	Isopropyl hexanoate[a]	Geraniol[d]
Butyl acetate[d, e]	Hexyl hexanoate[a]	2-Pentanol[f]
Hexyl acetate[e, d]	2-Hydroxy methyl butanoate[d]	2-Heptanol[f]
Methyl butanoate[c, e]	Octyl butanoate[d]	2-Nonanol[f]
Ethyl butanoate[c,d, e]	3-Hydroxy methyl hexanoate[d]	1-Octanol[f]
Methyl hexanoate[c,d, e]		3-Methyl-2-botanol[b]
Ethyl hexanoate[c,d, e]	**Carbonyl compounds**	3-Phenyl-1-propanol[a]
Ethyl propaonate[f]	Hexanal[c,d, e]	Hexen-2-ol[d]
Ethyl pentanoate[f]	*(E)*-2-Hexenal[c, e]	
2-hexenyl acetate[d]	2-Pentanone[e]	**Acids**
Butyl hexanoate[f]	2-Heptanone[d, e]	Acetic acid[d, e]
Hexyl butanoate[f]	Hexen-2-al[d]	Propanoic acid[e]

Esters	**Carbonyl compounds**	**Acids**
2-phenylmethyl acetate[f]	Nonanal[d]	Butanoic acid[d, e]
Methyl acetate[c]	Furfural[d]	Hexanoic acid[d, e]
Mehyl 3-methylbutanoate[c]	*(Z)*-3-Hexenal[d]	2-Methyl propanoic acid[d, e]
Butyl butanoate[c]	2-Nonanone[f]	2-Methyl butanoic acid[d, e]
Hexyl formate[b]	Pentanal[b]	Sec-butyric acid[d]
(E)-2-Hexenyl acetate[b]	2-Undecanone[b]	Octanoic acid[d]
(Z)-3-Hexenyl acetate[b]	Diacetyl[b]	Cinnamic acid[d]
Benzyl acetate[b]	Acetoin[b]	Nonanoic acid[d]
Isoamyl acetate[a]	Benzaldehyde[b]	
Bornyl acetate[a]		**Others**
Mehyl 2-methylbutanoate[a]	**Alcohols**	(DHF) 2,5-Dimethyl-4-hydroxy-3-(2H)-furanone[e]
Propyl butanoate[a]	Linalool[d, e]	(DMF)2,5-Dimethyl-4-methoxy-3(2H)-furanone[d,e]
Isopropyl butanoate[a]	Nerolidol[e, d]	γ-Decalactone[d, e]
Ethyl-2-methyl butanoate[a]	Hexanol(1-hexanol) [d, e]	Methyl anthranilate[f]
Isobutyl butanoate[a]	(*E*)-2-Hexenol[e]	Vanilline[d]
2-Methylbutyl butanoate[a]	Benzene methanol[d]	α-Pinene[b]

[a]Azodanlou and others (2004), [b]Hirvi and Honkanen (1982), [c]Ingham and others (1995a), [d]Lambert and others (1999), [e]Larsen and Poll (1992), [f]Ulrich and others (2007)

2.1.1 Odor Characteristics of Some Strawberry Volatiles

Aroma discrimination can be made based on compounds' odor characteristics perceived by a trained sensory specialist. Some of these characteristics are green, grass, sweet, fruity and flower. Different odor features depend on the volatile compounds and chemical groups they belong to. Most of the esters have fruity, sweet and apple odor such as ethyl butanoate, methyl hexanoate, ethyl hexanoate and hexyl acetate (Larsen and Poll 1992). Ethyl acetate and butyl acetate have a glue odor (Larsen and Poll 1992). The alcohols hexanol and (*E*)-2-hexenol have a green odor while linalool has a lemon peel odor (Larsen and Poll 1992). Acetic acid has a vinegar odor, propanoic acid has a fruity and silage odor, butanoic acid has a sour odor, 2-methyl propanoic and butanoic acid

have sourish odor characteristics while hexanoic acid has a sour, sweet and stale odor (Larsen and Poll 1992, Lambert and others 1999). Hexanal and (*E*)-2-hexenal have green and grass odor characteristics which are associated with unripe fruit odor (Lambert and others 1999). Specific for some wild strawberry species, *Fragaria vesca* L. spp. Vesca 'Geising', *Fragaria vesca* L. spp. Vesca f. alba and *Fragaria moschata* L.'Cotta', methyl anthranilate has a strong sweetish odor and a wood strawberry like aroma (Ulrich and others 2007).

2.2 Breath

Typical aroma measurement methods such as headspace measurement, extraction or distillation give an idea about overall flavor of food but these overall results are not enough to explain changes in food during eating (Taylor 1996). Dilution or hydration with saliva, increasing surface area due to chewing and temperature are some of the factors that affect volatile composition changes in the breath (Taylor 1996). In dried food, the amount of hydration in the mouth is an important factor for aroma release (Taylor 1996). Measuring volatile flavor compounds in the mouth in real time may give an understanding about how volatile compounds change during eating.

2.2.1 Flavor perception

Flavor is the combination of taste in the mouth and odor in the nose. Taste occurs when food particles mix with saliva in the mouth and odor comes afterwards with air transmission to the nose either by chewing or swallowing (Land 1996) or by sniffing food via the nose (Diaz 2004). To perceive the flavor of food, volatile compounds need to travel through the olfactory epithelium by orthonasal or retronasal airways (Diaz

2004) (Figure 1). The sensed intensity of aroma is subject to the volatile concentration that reaches the receptor cells at the olfactory epithelium (Diaz 2004). Although, when flavor compounds transfer through the olfactory epithelium they also attach and release along the way, as a result the volatile compounds' concentration is not the same as originally released (Dattetreya and others 2002).

Some authors have different thoughts about the difference between aroma intensity sensed by sniffing and swallowing through the mouth. Research showed that sniffing citral solution and vanillin produced a lower perceived threshold value than inhaling the solution through mouth, which can be explained by the different flow rate in the nose and mouth (Voirol and Daget 1987). Achieving the same aroma intensity by sniffing requires a higher concentration than retronasal perception of the same solution (Diaz 2004). However, another study compared nasal and retronasal flavor perception of lemon aroma, rum aroma, ethyl butyrate, and amyl acetate and found no difference (Burdach and others 1987).

The flavor perception during eating depends on the amount of volatile compounds in the mouth and their interactions with the nose and mouth over time (Dattetreya and others 2002). Flavor release of food was found to be affected by some changes during eating. These changes are temperature alteration of the food after it is eaten, saliva penetration in to the food, mechanical movement which increases the surface area of the food, hydration of dry foods, phase changes (liquid to air or air to liquid) and enzyme activity of the food (Taylor 1996). Liquid foods may have little change in the mouth while dried foods have more interaction between saliva and non volatile compounds

which affect the partition of volatiles between food, air and saliva (Taylor 1996). Also some compounds may fasten to the mucous membranes and stay longer in the breath after swallowing (Taylor 1996).

There are two ways to transfer volatiles to the nose; retronasal and orthonasal. Swallowing is the main step to transfer volatiles to the nose retronasally. The intensity of a volatile compound decreases after swallowing. One study found that the first exhale after swallowing had more than 10 times higher ethyl butanoate concentration than the following exhales in an aqueous solution of this compound (Buettner and Schieberle 2000).

Strawberry volatiles have the highest concentration during swallowing (Ingham and others 1995a). Volatile compounds are conveyed to the nosespace from the mouthspace during exhalation (Buettner and others 2001). Most volatile transfer from the mouth (oral cavity) to the nose (nasal cavity) occurs with swallowing or unintentional exhales. Lowering the back of the tongue or active movement of the jaw may cause an opening which allows volatile transfer to the nasal cavity (Buettner and Schieberle 2000).

2.2.2 Persistence

The volatile compound's chemical structure is directly affected by the adsorption, thereby the persistence of odorant in the mouth (Buettner 2002b). The adsorbed compounds in the oral mucosa can be degraded by enzymes present in the saliva, which lower the persistence of these compounds (Buettner 2002b). The chemical and biological breakdown of ethyl butanoate, ethyl hexanoate and ethyl octanoate in the saliva decreases

as the chain length of the compounds increases (Buettner 2002b). When esters were held in saliva in a closed flask, ethyl butanoate had the most amounts remaining after 10 min, followed by ethyl hexanoate, then ethyl octanoate (Buettner 2002b). Highly water soluble compounds persist in the breath for a long time because of the interaction between mucus and highly water soluble compounds (Hodgson and others 2004). The air-water partition coefficient was the main factor to control the rate of decrease of dimethylpyrazine, carvone, linalool, anethole and menthone in the breath (Hodgson and others 2004).

All of the previous works used a solution of the specific compound to understand the persistence of specific volatile compound in the breath.

2.2.3 Headspace, Nosespace and Mouthspace

The mean percentage of the total peak area in both the headspace and the nosespace after 10-30sec and 1min in homogenized strawberry samples is similar for methyl acetate, ethyl acetate, methyl butanoate, methyl 3-methylbutanoate, ethyl butanoate, methyl hexanoate, butyl butanoate and ethyl hexanoate (Ingham and others 1995) (Table 2). Hexanal and (*E*)-2-hexenal have significantly higher mean percentage peak areas than esters in the headspace and the nosespace after 1 min homogenization, however homogenization for 10-30sec does not make any significant change between mean percentage peak area of hexanal and (*E*)-2-hexenal. Homogenization time, both 1min and 10-30sec homogenization does not have any significant effect on ester concentration (Ingham and others 1995). The increase in homogenization time from 30sec (Table 3) to 1min (Table 2) shows that the increase in concentration of (*E*)-2-

hexenal and hexanal depends on lipid oxidation. The esters' concentration may depend on volatile release during sampling, not volatile formation (Ingham and others 1995).

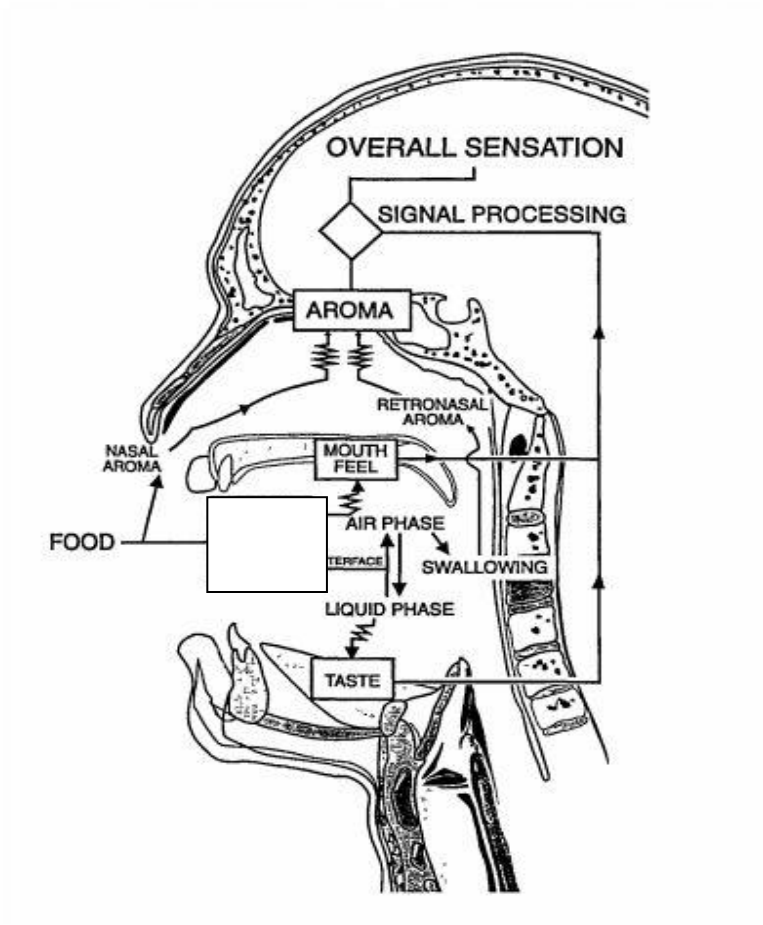


Figure 1 Flavor stimulation and perception locations in the brain (Figure modified from Taylor 1996)

Table 2 Mean percentage peak areas in nosespace of nine eaten strawberries over 3min and headspace of strawberries after 1min homogenization (Table modified from Ingham and others 1995)

Compounds	Nosespace	Headspace
Methyl acetate	47	41
Ethyl acetate	3.5	3.3
Methyl butanoate	41	38
Methyl 3-methylbutanoate	0.62	0.91
Hexanal	0.35	2.8*
Ethyl butanoate	1.7	1.9
(*E*)-2-Hexenal	0.0	4.1*
Methyl hexanoate	4.7	6.1
Butyl butanoate	0.09	0.06
Ethyl butanoate	0.49	0.37

*Significantly different ($P<0.01$)

Table 3 Mean percentage peak areas in nosespace of average concentration of 15 sec eaten strawberries and headspace of strawberries after 30 sec homogenization (Table modified from Ingham and others 1995)

Compounds	Nosespace	Headspace
Methyl acetate	27	19
Ethyl acetate	2.5	14
Methyl butanoate	61	51
Methyl 3-methylbutanoate	3.5	7.1
Hexanal	0.06	0.11
Ethyl butanoate	0.19	0.72
(*E*)-2-Hexenal	0.05	0.21
Methyl hexanoate	5.0	6.1
Butyl butanoate	0.02	0.02
Ethyl butanoate	0.01	0.01

2.2.4 Other Foods

In tomatoes, the mouthspace concentration of (*Z*)-3-hexenal, (*E*)-2-hexenal and hexanal increased during the 30sec after chewing, due to the time effect of chewing on enzymes responsible for forming these aldehydes (Xu and Barringer 2010). The concentration of tomato volatiles in the mouthspace is higher before swallowing than

after swallowing. The average ratio of concentrations before swallowing to after swallowing is between 2.8 and 73.9% (Xu and Barringer 2010). Swallowing decreases the volatile concentration. This may due to no new generation of lipoxygenase generated compounds after swallowing (Xu and Barringer 2010). Another probable reason may be the obvious removal of volatiles from the mouth or further dilution of volatiles in mouth with saliva after swallowing. The ratio of the mouthspace to headspace concentration varies between 0.4 and 59.2% in tomato and tomatillo, while nosespace to headspace concentrations are 50% lower than MS/HS in tomatillo (Xu and Barringer 2010).

Hexanal and (*E*)-2-hexenal are the characteristic compounds of unripe banana (Mayr and others 2003).The headspace and nosespace of (*E*)-2-hexenal are 400 and 40 ppb and the ratio of nosespace to headspace is 10 in unripe banana. NS/HS% of (*E*)-2-hexenal in ripe banana is 11.6, but the actual concentrations are 7 to 8 times lower than unripe banana (Mayr and others 2003). The NS/HS% ratio of hexanal is 8.4 for unripe banana and 4.5% for ripe banana while the actual concentration in unripe banana is 3 to 6 times higher than in ripe banana (Mayr and others 2003). As a result, green odor compounds, such as hexanal and (*E*)-2-hexenal, have higher concentrations in unripe banana compared to ripe banana (Mayr and others 2003).

Unlike dry foods, saliva easily mixes with juice and allows volatiles to increase in the mouth (Ingham and others 1995).

2.3 Enzymatic formation

Flavor formation is a result of breakdown of complex molecules into low molecular weight structures (Aharoni and others 2005). Some of the flavor compounds may be the products of non-enzymatic interactions such as the reaction of an alcohol and an acid (Zabetakis and Holden 1997). Precursors of the non-enzymatic reactants and some of the flavor compounds are formed by serial enzyme reactions such as the lipoxygenase pathway (Aparicio and others 2000, De Pooter and others 1989, Luning and others 1995, Zabetakis and Holden 1997).

2.3.1 Lipoxygenase generated aroma

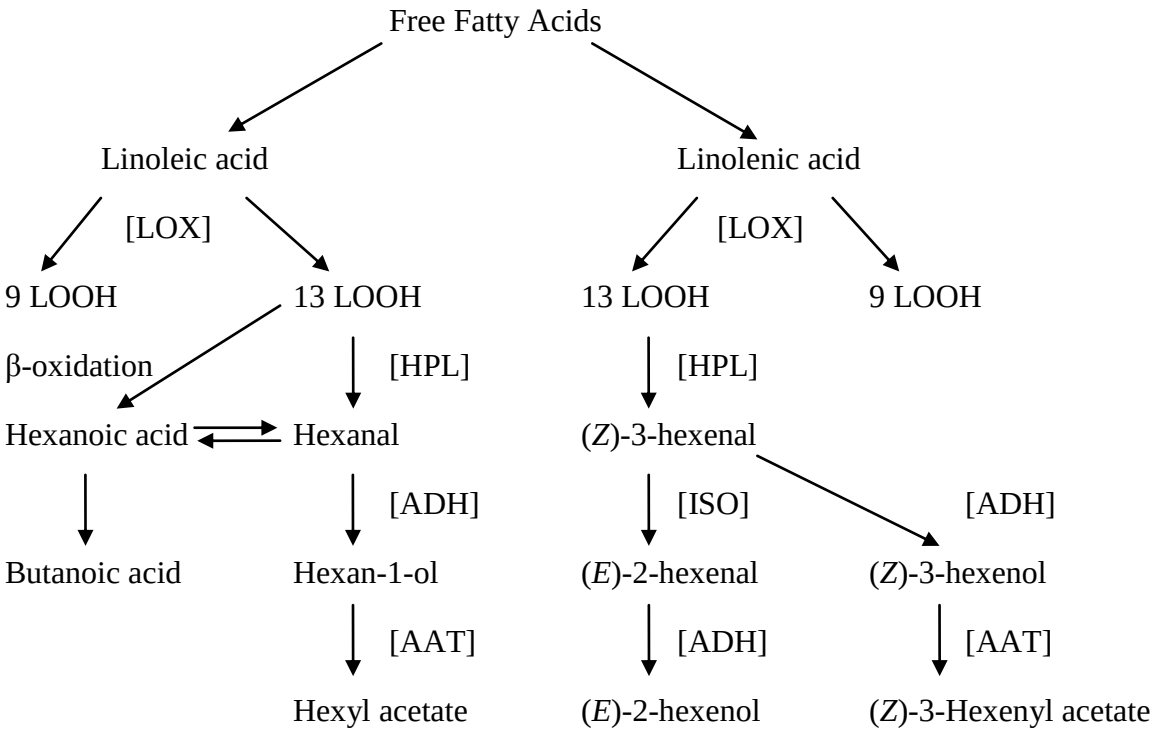
Lipids are one of the most important main substrates for flavor formation. Linoleic and linolenic acid are the precursors of most aldehydes, acids, alcohols and esters (Perez and others 1999a). Formation of the volatile compounds in these groups is a result of the lipid oxidation pathway with involvement of a series of enzymes such as lipase, lipoxygenase, lyase, isomerase and alcohol dehydrogenase (Brauss and others 1998).

Some strawberry volatiles are generated from strawberry lipids by enzymatic reactions. There are two lipid originated pathways for organic compound formation in strawberries, which are linoleic acid and linolenic acid pathways (Figure 6). Hexanal, hexanol and hexyl acetate are formed from linoleic acid by lipoxygenase, hydroperoxide lyase, alcohol dehydrogenase and alcohol acetyl transferase enzymes. Hexanoic acid is also a part of the linoleic acid pathway but formation from hexanal is an oxidation

reaction. (*Z*)-3-Hexenal, (*E*)-2-hexenal, (*Z*)-3-hexenol and (*E*)-2-hexenol are formed from linolenic acid by enzymatic reactions. Even though (*E*)-2-hexenal is formed from (*Z*)-3-hexenal, (*E*)-2-hexenal is at a higher concentration than (*Z*)-3-hexenal in strawberries. Due to the rapid isomerization process from (*Z*)-3-hexenal to (*E*)-2-hexenal, (*Z*)-3-hexenal has a lower concentration than (*E*)-2-hexenal (Perez and others 1999a). The threshold value of (*E*)-2-hexenal is more than 50 times higher than (*Z*)-3-hexenal (Ulrich and others 1997).

Strawberry volatiles are also classified by their odor descriptions. Generally, lipoxygenase generated compounds have green odor characteristics (Aparicio and others 2000). Hexanal, one of the most dominant green odor compounds, has a higher concentration in the green fruit than in the ripened fruit (Azodanlou and others 2004). In later maturity stages, this compound gradually decreases and in the mature fruits it becomes a minor compound (Azodanlou and others 2004). Also (*Z*)-3-hexenyl acetate and (*E*)-2-hexenol, which are also green odor compounds, show significant decrease during the transition between the unripe stage and the ripe stage in different strawberry varieties (Azodanlou and others 2004).

Hexanoic acid is one of the green odor compounds. Hexanoic acid is formed by hexanal from oxidation (DePooter and Schamp 1989).



LOX: Lipoxygenase
HPL: Hydroperoxide Lyase
ADH: Alcohol Dehydrogenase
ISO: Isomerase
AAT: Alcohol Acetyl Transferase

Reineccius 2005
De Pooter and others 1989
Aparicio and others 2000
Luning and others 1995

Figure 2 Volatile organic compound formation by lipoxygenase pathway

The research on formation of (*E*)-2-hexenal and (Z)-3-hexenal in damaged fruit concluded that (*E*)-2-hexenal and (Z)-3-hexenal formation is related to the availability of linolenic acid, and LOX and HPL enzyme levels in the fruit (Myung and others 2006). Wounding the strawberries increased the level of lipids in the fruit and lowered the free linolenic acid level 30% (Myung and others 2006). After 10 min, LOX activity had increased 25% and HPL activity increased 200%, thus (*E*)-2-hexenal and (Z)-3-hexenal levels increased in the fruit in the 15 minute after wounding (Myung and others 2006).

2.3.2 Esters

Methyl acetate and methyl butanoate are the most common esters in the strawberry fruit (Azodanlou and others 2004). Methyl butanoate has the highest concentration in Camarosa strawberries, and the methyl esters are present at twice the level of ethyl esters in Camarosa cultivar (Perez and others 1999b). Methanol is the major alcohol in strawberry and methyl esters form from methyl alcohol in strawberry (Ueda and others 1992). Strawberry alcohol acyltransferase (SAAT) is the enzyme responsible for ester formation in strawberries (Aharoni and others 2000). AAT accelerates the esterification reaction between acyl-CoA and alcohol.

Esters in strawberries are formed from alcohols and acids either due to enzymatic activity or chemical activity (Figure 3). Generation of methyl esters in the strawberry headspace is faster than ethyl esters during the first half of a 16 hr incubation (Yamashita and others 1975). However, ethyl ester formation continued until the end of a 16 hr incubation while methyl ester formation stopped after the 8^{th} hr. Limited methanol formation may have caused further ester formation, to stop at the 8^{th} hr (Yamashita and others 1975).

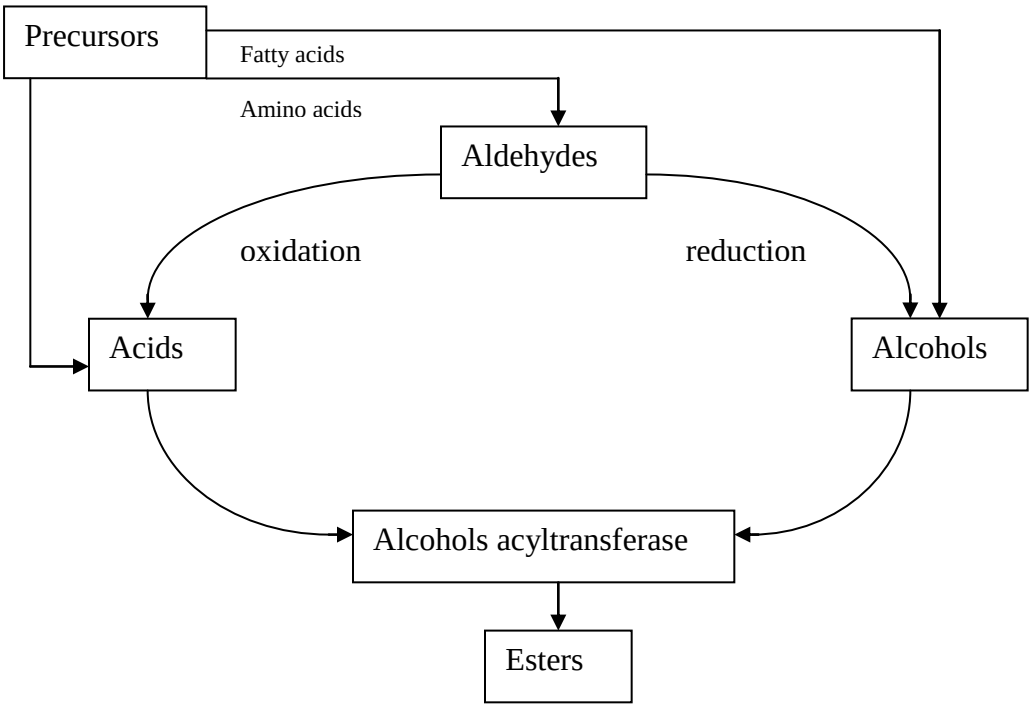


Figure 3. Biological pathway of volatile ester formation (Figure modified from Olias and others 1995)

Ester formation increases during ripening especially at the last stage of ripening (Azodanlou and others 2004). Ester formation in strawberries takes place only in the mature stages, because of the lack of ester forming enzyme activity in early stages (Yamashita and others 1977). Ester forming enzyme, AAT, activity is not observed in early maturity stages (Perez and others 1996). When strawberries become a mature fruit, volatile formation increases (Perez and others 1992). Ester presence at 36 days after blooming is 25% while this number decreases to 4.8% at the 46th day after blooming (Perez and others 1992).

Through the lipoxygenase pathway, 1-hexanol, (*E*)-2-hexen-1-ol and (*Z*)-2-hexen-1-ol produce their corresponding esters, which are hexyl acetate, (*E*)-2-hexenyl acetate and (*Z*)-2-hexenyl acetate. (Hamilton-Kemp and others 1996).

Wild and cultivated strawberries have different fruit size, flavor and aroma composition (Aharoni and others 2005). These flavor difference are caused by a few genes (Aharoni and others 2005). These genes characterize the alcohol acyltransferase enzyme both in wild and cultivated strawberry. The AAT enzyme in cultivated strawberry (SAAT) and the wild strawberry (VAAT) show a similarity in their sequence, but difference in substrate choice (Aharoni and others 2005). These enzymes are important for ester formation in the soft fruits. Both enzyme availability and substrate availability were investigated and it was concluded that substrate availability had the greater importance on flavor composition, especially on the ester profile (Aharoni and others 2005).

The acetate esters of high molecular weight alcohols such as heptyl, octyl and nonyl alcohol were detected in low amounts by GC, because of their low volatility (Yamashita and others 1975).

No butyl ester formation occurs when strawberry is incubated alone, but addition of 1µl butanol to strawberry results in formation of butyl esters from alcohol by enzymatic activity (Yamashita and others 1975). Increasing the butanol addition to 2µl increased the butyl ester formation, but additional alcohol did not further increase ester formation (Yamashita and others 1975). Butyl formate, butyl isobutyrate and butyl *n*-valerate are not formed with butanol addition, however ester formation is also observed

after incubation of formic acid, isobutyric acid and *n*-valeric acid at 30°C for 1 hr in a strawberry and butanol containing headspace flask (Yamashita and others 1975).

The concentration of acetate esters in whole strawberry increased very little when cutting the strawberry into 2 or 4 pieces with addition of alcohol; however, increasing the slice number to 8 decreased ester concentrations (Yamashita and others 1975). No ester formation occurred when crushed or homogenized strawberries were incubated with butanol (Yamashita and others 1975).

Strawberries have the ability to use volatiles from the air and metabolize them to produce volatile metabolites. This ability can be used to react alcohols with acid to produce esters, reduce aliphatic aldehydes or reduce carbon-carbon double bonds in the carbonyl part of aldehydes and ketones (Hamilton-Kemp and others 1996).

2.4 Heat

Heat has an important effect on volatile concentration changes. Sterilization treatment at 120°C for 20 min caused a significant increase in the concentration of butyl acetate, hexanal, heptan-2-one, hexen-2-al, linalool, 2-methyl propanoic acid, butanoic acid, sec-butyric acid, hexanoic acid, benzene methanol, furaneol, nerolidol, octanoic acid and γ-decalatone compared to raw strawberry (Lambert and others 1999). Sterilization treatment also increases the concentration of acetaldehyde (Sloan and others 1969). However, the flower-scented strawberry flavor is lost due to heat treatment, with a significant decrease in nerolidol and furaneol concentrations (Lambert and others 1999).

At sterilization treatment (120°C, 20 min) geraniol and vanillin are formed (Lambert and others 1999). 120°C heat treatment also helps to form dimethyl sulfide and isobutyl aldehyde which are not present in raw strawberry puree. Also isobutyraldehyde may be formed by Strecker degradation of valine (Sloan and others 1969). 2-Furaldehyde, 2-acetyl furan and ethyl furoate are the heat generated compounds which are found in strawberry jam (Sloan and others 1969).

Heating effects the odor characterization of strawberries; a sweet caramel like odor turns into a dominant odor in heated strawberries while green and fruity odors are the most desirable odor in the fresh strawberry (Schieberle 1994).

In strawberries, ethyl esters and ethanol are not formed during 5 min at 100°C (Yamashita and others 1975).

2.4.1 Volatility and Partition Coefficient

In every environment, low boiling point compounds are more volatile, because they are more polar. Due to polarity effects, low boiling point compounds are more persistent than high boiling point compounds in the breath (Ingham and others 1995).

2.5 High Pressure

High pressure treatments at 200MPa and 500MPa produce no significant volatile concentration changes, while 800MPa treatment produces a significant difference in concentration of strawberry volatiles as compared with untreated, 200MPa and 500MPa pressure treated samples (Lambert and others 1999). While concentrations of ethyl

hexanoate, butyl acetate, hexanol, linalool, hexanoic acid, furaneol and nerolidol decreased, concentrations of hexyl acetate, hexanol, nonanal, furfural, benzene methanol, octanoic acid and cinnamic acid increased. During the same time, γ-lactone and 3, 4-dimethoxy-2-methyl furan or 2, 5-dimethyl-4-methoxy furan-3-one appeared after 800MPa pressure treatment (Lambert and others 1999)

Comparing untreated, high pressure treated and heat treated samples based on total mass of volatile compounds, high pressure treated samples are not significantly different from untreated samples while sterilized samples have significantly higher total mass of volatiles compared to untreated and high pressure treated samples (Lambert and others 1999).

2.6 Storage and Different Varieties

Storage is one of the important steps for maintaining the fruit's quality attributes such as freshness, desired flavor, hardness or softness. During 5 days of storage, hardness of strawberries is not changed while color of strawberries became more red (Forney and others 1998).

The concentration of methyl butanoate, ethyl butanoate, methyl hexanoate and ethyl hexanoate are the highest of the esters in strawberries after 9 d storage (Perez and others 1996). Concentrations of ethyl acetate, ethyl butyrate, acetaldehyde and ethanol are increased in strawberries which are stored under controlled atmosphere conditions for 7 days (Ke and others 1994). Esters help to elevate the strawberry aroma 50 to 200% during storage (Forney and others 1998). Controlled atmosphere storage increases the

activity of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH), therefore ethanol concentration accumulates and leads to formation of ethyl esters (Ke and others 1994).

Different storage temperatures have different effect on volatile composition change during storage. Higher strawberry volatile production is observed during storage at 5°C and 10°C than 0°C (Ayala-Zavala and others 2004). Off flavor formation is increased with an increase in storage temperature. AAT enzyme activity is higher at the 7- 9 days storage at 17 °C, while AAT activity is low in strawberries stored at 1°C (Perez and others 1996). Chitosan coated strawberries show lower off flavor formation than uncoated strawberries (Almenar and others 2009).

Cultivar is one of the important factors that contribute the volatile profile of strawberries (Forney and others 2000).

2.7 Ripening

Fruit maturity is directly related with volatile composition of strawberries and so aroma profiles (Forney and others 2000). The activity of corresponded enzymes during ripening is directly related with the flavor of the fruit (Perez and others 1999a). LOX and HPL activities are developed during ripening with the color changes of fruit from green to dark red (Perez and others 1999a). The activity of these enzymes and the amount of C_6 aldehydes present are shown in unripe strawberries (Perez and others 1999a, Tressl and Drawert 1973). Maximum HPL activity is observed in green strawberry fruit (Leone and

others 2006). The high amount of HPL activity in early mature strawberries is an evidence further formation of aldehydes (Perez and others 1999a).

(*E*)-2-Hexenal is the main aldehyde in strawberries during ripening, while (*Z*)-3-hexenal has a minor place (Perez and others 1999a). Perez and others (1999a) found that the proportion of (*E*)-2-hexenal is not significantly changed during fruit maturation while Leone and others (2006) stated that trace amount of (*E*)-2-hexenal presence in unripe fruit increased during maturation. Hexanal presence is observed in all stages of ripening (Leone and others 2006) and an increase along with maturation (Perez and others 1999a, Leone and others 2006). The amount of (*E*)-2-hexenal and hexanal depends on the substrate availability and enzyme presence in maturity stages (Leone and others 2006).

2.8 Other Processes

The effect of ozone treatment on strawberry quality was investigated by measuring off flavor formation, overall flavor composition and enzyme activity (Perez and others 1999b). Ethanol, acetaldehyde and ethyl acetate were chosen as an indicator of off flavor formation by Perez and others (1999b). There is no significant difference in ethyl acetate and acetaldehyde presence in ozone treated samples compared to non treated samples (Perez and others 1999b). Ethanol concentration is significantly lower in ozone treated samples than control samples; therefore this may be an indicator for preventing off flavor formation in strawberries (Perez and others 1999b).

2.9 Analytical methods for analyzing flavor compounds

The traditional analytical methods for the flavor profile mostly required an isolation step before identify the compounds. Some of the isolation methods used in previous strawberry studies are extraction, distillation, dynamic headspace and solid phase micro extraction (Holt 2002, Fischer and Hammerschmidt 1992, Zhang and others 2009, Perez and others 1992, Larsen and Poll 1995). Gas chromatography-mass spectrometry GC-MS) was used for separation and identification of strawberry flavor compounds (Holt 2002, Zhang and others 2009); as well as Gas chromatography-flame ionization detector (GC-FID) (Azodanlou and others 2004, Perez and others 1992). Because of these analytical methods require sample preparation, they are not suitable for monitoring the real time changes of volatiles.

Proton transfer reaction mass spectrometry (PTR-MS) is a newly developed technique which allows rapid detection of aroma compounds in alcoholic beverages (Aprea and others 2007) and breath analysis of banana aroma during eating (Mayr and others 2003) based on chemical ionization. Atmospheric pressure chemical ionization mass spectrometry (APCI-MS) is another technology which was developed to monitor aroma compounds in the breath during eating (Harvey and Barra 2003).These two techniques use soft chemical ionization with the H_3O^+ as a reagent ion to monitor volatile organic compounds.

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

Selected ion flow tube mass spectrometry (SIFT-MS) (Syft Inc. Christchurch, New Zealand) is an emerging technique that provides rapid identification and

quantification of trace amount of gases in air and human breath in real time (Spaněl and others 1996). Distinctively from other real time monitoring methods, SIFT-MS uses 3 precursor ions H_3O^+, NO^+ and O_2^+ to ionize volatile compounds (Spaněl and Smith 1996).

The basic principle of the SIFT-MS is soft chemical ionization (Spaněl and Smith 1996). An external ion source produces selected ions and forwards them to the ion injection orifice (Figure 4). Selected ions, H_3O^+, NO^+ and O_2^+, move forward through a flow tube carried by Helium as a carrier gas (Spaněl and Smith 1996). Sampled air or reactant gas is injected into the flow tube via the inlet port and travels in the flow tube with the inert carrier gas (Spaněl and Smith 1996). Detection by the quadrupole mass spectrometer counts product ions and the target gas concentration can be calculated by using the k value and product to precursor rate of the specific volatile compounds (Spaněl and Smith 1996, Spaněl and Smith 1999).

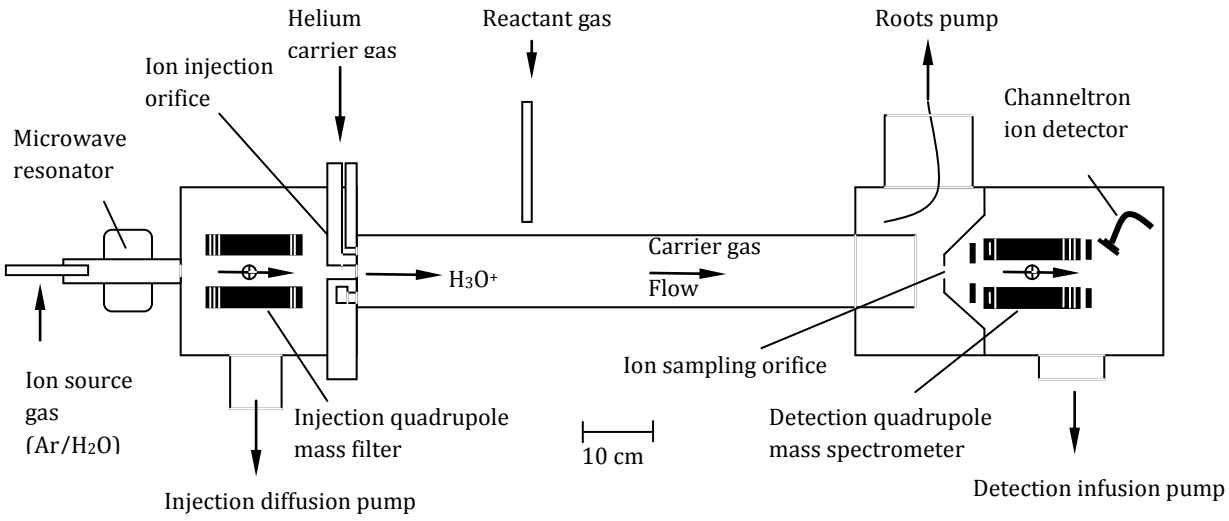


Figure 4 Schematic of SIFT-MS (Figure modified from http://www.transspectra.com/about.htm)

CHAPTER 3

MATERIALS AND METHODS

3.1. Raw Material

Strawberries were obtained from a local market (Kroger, Columbus, OH) and stored at 4°C until use or from the Ohio State University student farm (Columbus, OH) on the morning of the experiment. Caps were removed and strawberries were washed.

3.2 Headspace: with and without enzyme inhibition

Whole strawberries, saturated $SnCl_2$ solution and water were kept in a water bath (Fisher Scientific, USA) at 24°C until equilibrium temperature was achieved, approximately 30 min. A 40 g sample was placed in a blender (Waring, New Hartford, Conn., USA) with 120g saturated $SnCl_2$ solution or water and blended at high speed for 30 sec. Pureed strawberries were placed in 500ml Pyrex bottles (Fisher Scientific, USA) with silicon septum at the same temperature, 24°C, and the sample was immediately analyzed with the SIFT-MS. Two needles were used during measurement. The short passivated needle (5.5cm), which was connected to the SIFT-MS, was placed in the middle of the septum with the tip of the short needle 3 cm below the septum. The long needle (27cm), which allows air inside the bottle, was placed at the edge of the septum with the tip of the long needle10cm below the septum (5cm above the sample).

All measurements were 350 sec and results were graphed as time versus average concentration of five different samples.

The partition coefficients of the volatiles presented in this study were taken from http://www.thegoodscentscompany.com.

3.3 Refrigerated Storage

Sweet Charlie variety strawberries were picked on the morning of the experiment from the Ohio State University student farms. Undamaged and two differently damaged strawberries were measured for the storage study. For undamaged, strawberries were kept in the bottle as whole undamaged fruit. For punctured, strawberries were damaged with a needle 6 times with 4 horizontal and 2 vertical punctures all the way through. For bruised, strawberries were shaken for 2 min in a sieve shaker (Tyler Inc. Ohio, USA). After all treatments, strawberries were held in the bottles for 1 h to equilibrate under refrigeration condition (4°C) and then measured by SIFT-MS and recorded as Day 0. Bottled strawberries were kept in the refrigerator and the same bottles measured every day for 9 d. Lids were removed and the bottles were left for 30 min to remove the accumulated volatiles from the headspace, the lids were replaced and the bottles were held at 4°C to equilibrate for 1 h. After equilibrium, five different bottles for each treatment were measured by SIFT-MS.

3.4 Unripe and ripe fruit

Chandler variety strawberries were picked on the morning of the experiment from the Ohio State University student farms. Strawberries were selected to have a range of ripeness, based on their color. Ripening levels of strawberries were divided into the

following categories: 100% greenish white, 80% white and 20% pink, 70% white and 30% pink, 40% white and 60% pink, 30% white and 70% pink, 25% white and 75% pink, 20% pink and 80% red, 10% pink and 90% red and 100% red. A 40 g sample of strawberries was chopped in a 355ml closed chopper for 10 sec. After chopping, samples were directly measured using SIFT-MS. Total preparation time before measurement was 30 sec. and one replicate was measured.

3.5 Different Varieties

Five different strawberry varieties; Albion, Camarosa, Chandler, Festival and Sweet Charlie were measured to identify differences between varieties. All varieties were picked from The Ohio State University student farms on the morning of the experiment. A 40 g sample of strawberries were chopped in a 355ml closed chopper (Chefmate, Minneapolis, USA) for 10 sec, then headspace volatiles were directly measured using a VOC 100 Selected Ion Flow Tube- Mass Spectrometer. The chopper cup was connected to the SIFT-MS with a passivated needle (5.5 cm). There were two holes in the lid, 1 cm from the edge of the two sides of the lid. The needle was placed in one of the holes and the second hole was left open to allow air entry during measurement. The lid of the container was not removed anytime during the experiment. The holes were closed with labeling tape (Fisher Scientific, USA) before headspace measurement and opened during measurement. The values were reported as the percentage of average concentrations of three different samples.

3.6 Headspace measurements for whole and chopped strawberries

The headspace measurement of a whole mid size strawberry was measured, after holding the strawberry in a 355ml closed chopper for 5 min to allow equilibration of the volatiles released from the whole fruit. For the headspace measurement of chopped strawberries, 55g of strawberries was chopped in the chopper for 20 sec. A new lid was used after chopping to prevent water dripping into the inlet of SIFT-MS. The strawberries used in this experiment were kept at room temperature and all experiments were carried out at room temperature. Five different samples were measured.

3.7 Mouthspace and Nosespace

The same strawberries from the headspace measurement were used for the breath tests to reduce variability. After headspace analysis, the background breath was measured. Background measurements of the mouth were taken before each sample. A 25 year old adult woman chewed a strawberry 5 sec and exhaled three times through a 6 cm straw, which was connected to the SIFT-MS. Mouthspace and nosespace measurement procedures and time were the same for both background and fruit tests. Total measurement time was 140 sec. There were three tests in the 140 sec time interval, as follows; mouthspace before swallowing, nosespace and mouthspace after swallowing. Each measurement took 45 sec with three 10 sec exhalations and a 5 sec inhalation. Between before and after swallowing mouthspace measurements, the nosespace was measured. The straw was moved to the right nostril directly after the first mouthspace measurements and the same procedure applied to obtain nosespace concentration. The left nostril was plugged to avoid volatile loss during nose exhalation. The mouthspace

before swallowing measurements were used to determine mouthspace and nosespace ratio (MS_{bs}/NS). MS_{as}/MS_{bs} was determined by mouthspace after swallowing and before swallowing measurements. Results were reported as ratio of mouthspace after and before swallowing (MS_{as}/MS_{bs}), nosespace to mouthspace (NS/MS_{bs}), mouthspace to headspace (MS_{bs}/HS) and nosespace to headspace (NS/HS).

There were two methods used in the breath analysis. First method include (*Z*)-3-hexenal, methyl acetate, methyl hexanoate and ethyl hexanoate and second method was include (*E*)-2-hexenal, hexanal, ethyl acetate and methyl butanoate. The same sample was used for both methods and methods were run consecutively after background.

Using breath results from just one person is preferable, because there are variations between people regarding chewing frequency, swallowing and breathing (Linforth and others 1994). The release rate of volatiles between people varies due to the chewing technique and swallowing time (Ingham and others 1995c).

3.8 Method Information

The concentration of volatile compounds in this study was calculated using known kinetic parameters (Table 4). The concentration [M] of selected volatiles was calculated using the product count rate (Ip), reaction rate constant (k), precursor ions count rate (I) and reaction time (t) as follows: [M]=Ip/Ikt (Spaněl and Smith 1999). Some compounds produce the same mass for a given precursor ion, in which case the interfering compounds have to be reported as a mixture. In this study 2-pentenal is a mixture of (*E*)-2-pentenal and (*Z*)-2-pentenal, 3-hexen-1-ol is a mixture of (*E*)-3-hexen-1-ol and (*Z*)-3-hexen-1-ol, and methyl butanoic acid is a mixture of 2-methylbutanoic acid

and 3-methylbutanoic acid. Compounds with irresolvable conflicts or low concentrations are not reported. Other conflicts were removed by selecting the different masses or the different precursor ions in the method.

Table 4 Detailed SIFT-MS information of measured volatile compounds

Volatile compound	Molecular formula	Precursor ion	k (10^9 cm^3s^{-1})	*m/z*	Product ion	ref
(*E*)-2-heptenal	$C_7H_{12}O$	O_2^+	3.6	112	$C_7H_{12}O^+$	4
(*E*)-2-hexenal	$C_6H_{10}O$	O_2^+	3.7	69	$C_5H_9^+$ or $C_4H_4O^+$	1
(*E*)-2-nonenal	$C_9H_{16}O$	NO^+	3.8	139	$C_9H_{15}O^+$	4
(*E*)-2-octenal	$C_8H_{14}O$	NO^+	4.1	156	$C_8H_{14}O.NO^+$	4
2-pentenal	C_5H_8O	NO^+	4.0	83	$C_5H_7O^+$	4
3-hexen-1-ol	$C_6H_{12}O$	NO^+	2.5	82	C_6H10^+	6
(*E,E*)-2,4-decadienal	$C_{10}H_{16}O$	NO^+	4.2	151	$C_{10}H_{15}O^+$	4
(*E,Z*)-2,6-nonadienal	$C_9H_{14}O$	NO^+	2.5	168	$C_9H_{14}O.NO^+$	6
(*Z*)-3-hexenal	$C_6H_{10}O$	NO^+	3.1	70	$C_4H_6O^+$	1
1-butanol	$C_4H_{10}O$	NO^+	2.2	73	$C_4H_9O^+$	2
1-hexanol	$C_6H_{14}O$	NO^+	2.4	101	$C_6H_{13}O^+$	2
1-pentanol	$C_5H_{12}O$	NO^+	2.5	87	$C_5H_{11}O^+$	2
1-penten-3-one	C_5H_8O	NO^+	2.5	114	$C_5H_8O.NO^+$	6
2-heptanone	$C_7H_{14}O$	NO^+	3.4	144	$C_7H_{14}O.NO^+$	5
methylbutanoic acid	$C_5H_{10}O_2$	NO^+	2.5	85	$C_5H_9O^+$	6
2-pentanone	$C_5H_{10}O$	NO^+	3.1	116	$NO^+.C_5H_{10}O$	1
2-pentylfuran	$C_9H_{14}O$	NO^+	2.0	138	$C_9H_{14}O^+$	6

Volatile compound	Molecular formula	Precursor ion	k (10^9 cm^3s^{-1})	*m/z*	Product ion	ref
acetic acid	$C_2H_4O_2$	NO^+	0.9	90	$NO^+.CH_3COOH$	3
acetoin	$C_4H_8O_2$	NO^+	2.5	118	$C_4H_8O_2.NO^+$	6
acetone	C_3H_6O	NO^+	1.2	88	$NO^+.C_3H_6O$	1
butanoic acid	$C_4H_8O_2$	H_3O^+	2.9	71	$C_3H_7CO^+$	3
butyl acetate	$C_6H_{12}O_2$	H_3O^+	2.9	61	$C_2H_3O_2H_2^+$	7
butyl hexanoate	$C_{11}H_{22}O_2$	NO^+	2.2	73	$C_4H_9O^+$	6
ethanol	C_2H_6O	NO^+	1.2	45,63, 81	$C_2H_5O^+$	2
ethyl 2-methylbutanoate	$C_7H_{14}O_2$	O_2^+	2.5	130	$C_4H_9O^+$	6
ethyl acetate	$C_4H_8O_2$	O_2^+	2.4	43	CH_3CO^+	3
ethyl butanoate	$C_6H_{12}O_2$	O_2^+	2.5	71	$C_4H_7O^+$	7
ethyl hexanoate	$C_8H_{16}O_2$	NO^+	2.5	174	$C_8H_{16}0_2.NO^+$	6
gamma-decalactone	$C_{10}H_{18}O_2$	NO^+	2.5	200	$C_{10}H_{18}O_2.NO^+$	6
hexanal	$C_6H_{12}O$	NO^+	2.5	99	$C_6H_{11}O^+$	1
hexanoic acid	$C_6H_{12}O_2$	NO^+	2.5	146	$C_6H1_2O_2.NO^+$	6
hexenyl acetate	$C_8H_{14}O_2$	NO^+	2.5	172	$C_8H_{14}O_2.NO^+$	6
hexyl acetate	$C_8H_{16}O_2$	H_3O^+	3.0	85	$C_6H_{13}^+$	6
isobutanoic acid	$C_4H_8O_2$	$O2^+$	2.5	88	$(CH_3)_2CHCOOH^+$	3
linalool	$C_{10}H_{18}O$	NO^+	2.6	96	$C_4H_8O_2$	6
methanol	CH_4O	H_3O^+	2.7	33,51, 69	CH_5O^+	6
methyl acetate	$C_3H_6O_2$	NO^+	1.6	104	$CH_3COOCH_3.NO^+$	3
methyl butanoate	$C_5H_{10}O_2$	O_2^+	2.4	74	$C_3H_6O_2^+$	3

Volatile compound	Molecular formula	Precursor ion	k (10^9 cm^3s^{-1})	*m/z*	Product ion	ref
methyl hexanoate	$C_7H_{14}O_2$	NO^+	2.5	160	$C_7H_{14}O_2.NO^+$	6
methyl methacrylate	$C_5H_8O_2$	O_2^+	2.3	100	$C_5H_8O_2^+$	6
propanoic acid	$C_3H_6O_2$	NO^+	1.5	57	$C_2H_5CO^+$	3

[1] Spaněl and others (1997), [2] Spaněl and Smith (1997), [3] Spaněl and Smith (1998), [4] Spaněl and others (2002), [5] Smith and others (2003), [6] Syft 2009 [7]Francis and others (2007)

3.9 Statistical Analysis

All statistical analyses were performed using PASW Statistic 18 software (SPSS Inc.). Samples were subject to one way analysis of variance (ANOVA) with an alpha (α) level of 0.05 and all samples were compared using Tukey HSD for an alpha (α) level of 0.05. The samples in the storage study were subjected to independent samples t- test with 95% confidence interval. The headspace concentration of chopped strawberries was not included for the statistical analysis of table 6, because their concentration was found quite high compared to other treatments in the same table. The concentration of the nosespace and mouthspace after swallowing in chopped and whole strawberries was not calculated for statistical analysis in table 6, because their concentration was not compared any other treatment in the same table. Correlation between compounds was found using the statistical analysis mentioned above and correlation coefficient (R^2) values were reported.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Headspace concentration of pureed strawberries with and without enzyme activity

(*Z*)-3-Hexenal is one of the important C6 aldehydes which is responsible for the green odor in strawberries. It is also the precursor of the intense green odor compound (*E*)-2-hexenal (Larsen and Poll 1992, Perez and others 1997, Ulrich and others 1997). The headspace concentration of (*Z*)-3-hexenal, (*E*)-2-hexenal and hexanal was measured in the pureed strawberry samples with addition of 0.2M $SnCl_2$ solution or water to produce samples with and without enzyme inhibition respectively (Figure 5). Saturated $SnCl_2$ addition inhibits enzyme activity (Wu and Liou 1986), specifically lipoxygenase (LOX) (German and Kinsella 1985), preventing the formation of enzymatically formed compounds via lipid oxidation. LOX generated volatiles were present in the fruit before the cells were damaged, as shown in the $SnCl_2$ treated samples. The concentrations of the volatile compounds in the $SnCl_2$ treated strawberries indicate the amount of these volatiles enzymatically formed during ripening rather than after tissue maceration. The LOX volatiles are already present in unripe strawberries (Perez and others 1999a, Azodanlou and others 2004).

The concentration of (*Z*)-3-hexenal peaked within 2 min of tissue disruption in non enzyme inhibited samples (Figure 5). Strawberries were blended for 30 sec and

transferring to the bottles took 30 sec, so measurements started 1 min after tissue disruption began. Linolenic acid is converted into 13- and 9-hydroperoxides and 13-hydroperoxide is converted into (*Z*)-3-hexenal by strawberry LOX and hydroperoxide lyase (HPL) (Perez and others 1999a). The concentration of C_6 aldehydes increases with increasing cell damage in strawberries, because the activity of the enzymes increases when the cells are disrupted (Latrasse 1991). In cucumbers, when the fruit tissues are mechanically ruptured the formation of carbonyl compounds occurs within 5 min (Fleming and others 1968). In tomatoes, (*Z*)-3-hexenal reached its highest concentration 3 to 3.3 min after blending (Buttery and others 1987, Xu and Barringer 2009).

After formation, (*Z*)-3-hexenal isomerizes to (*E*)-2-hexenal by the isomerase enzyme. The concentration of (*Z*)-3-hexenal decreased quickly and its concentration was much lower than (*E*)-2-hexenal (Figure 5). In strawberries, the concentration of (*Z*)-3-hexenal is two times lower than the concentration of (*E*)-2-hexenal (Myung and others 2006). This is different from some other fruits, such as tomatoes, that show a higher concentration of (*E*)-2-hexenal than (*Z*)-3-hexenal (Buttery and others 1987, Xu and Barringer 2010).

The level of volatile compounds in the headspace is determined by the amount of sample, the rate of formation in the sample, rate of volatilization into the air and the rate of removal from the headspace during measurement. The amount of sample and the rate of air removal by the SIFT-MS from the headspace during measurement are the same for all samples. The partition coefficient measures the volatilization into the air, but the partition coefficients of (*E*)-2-hexenal, (*Z*)-3-hexenal and hexanal are close to each other

(1.58, 1.58, 1.78). Thus the rate of formation of the volatile compounds explains the difference between volatile levels in the headspace.

(*E*)-2-Hexenal is one of the green odor compounds which gives a fresh odor to strawberries. The concentration of (*E*)-2-hexenal reached its highest level in the headspace within 2 min and then decreased (Figure 5). This fast increase is due to the quick conversion of (*Z*)-3-hexenal to (*E*)-2-hexenal. In grape tomatoes, (*E*)-2-hexenal reached its highest concentration 3.7 min after blending (Xu and Barringer 2009). The decrease of (*E*)-2-hexenal after 2 min may be due to the slower conversion of (*E*)-2-hexenal into (*E*)-2-hexenol while there is little additional formation from (*Z*)-3-hexenal.

Hexanal is one of the green odor compounds which is formed from linoleic acid via the LOX pathway. The concentration of hexanal continued to increase during the 5 min measurement (Figure 5). Thus, the rate of formation of hexanal is slower than (*Z*)-3-hexenal and (*E*)-2-hexenal which peaked within 2 min of pureeing (Figure 5). The activity of alcohol dehydrogenase on linoleic acid, which forms hexanal, is only 13% of its activity on linolenic acid, which forms (*E*)-2-hexenal and (*Z*)-3-hexenal, which may explain the slower formation of hexanal (Perez and others 1999a). The next step is the conversion of hexanal to hexanol. The concentration of hexanol was only 1-2ppb during the first 5 min after tissue disruption (data not shown).

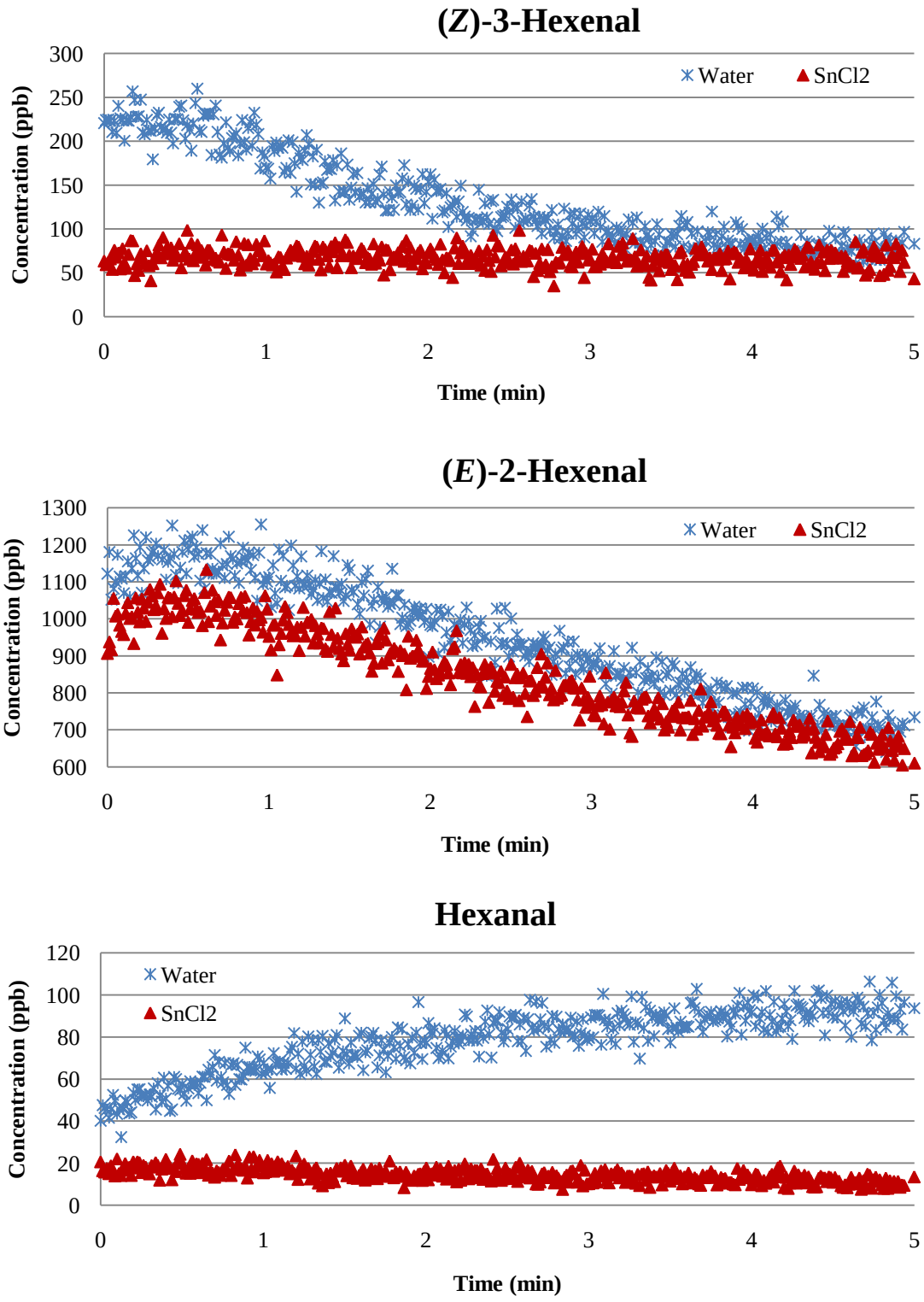


Figure 5.Concentration of (*Z*)-3-hexenal, (*E*)-2-hexenal and hexanal with enzyme inhibition ($SnCl_2$) and without enzyme inhibition (water). Measurements started 1 min after tissue disruption.

4.2 Strawberry volatile changes during refrigerated storage

Volatile concentration changes in the headspace of whole undamaged, whole punctured and whole bruised strawberries were investigated under refrigerated storage conditions for 8 days (Figure 6). The undamaged strawberries, which show the effect of storage without damage, significantly increased in volatile levels of (*E*)-2-hexenal, (*Z*)-3-hexenal, hexanal and hexanoic acid during storage (Figure 6A). These compounds increase due to the continuing activity of enzymes and biosynthesis of precursor compounds. The HPL enzyme is active in all ripening stages of strawberries and is more active than LOX in ripened fruit (Perez and others 1999a). The concentration of hexanoic acid is significantly increased during 10°C storage for a week in undamaged strawberries (Almenar and others 2009).

In punctured strawberries, the concentration of volatiles was not significantly different from the undamaged strawberries (Figure 6B). In bruised strawberries, the concentration of (*E*)-2-hexenal and hexanoic acid was significantly higher than undamaged strawberries at the end of storage (Figure 6C). Bruised strawberries had more severe damage than punctured strawberries which allows more enzymatic activity by disrupting cells in the fruit, so the volatile concentrations were significantly higher. An increase in enzymatic activity would include LOX, HPL and isomerase enzyme rapidly converting linolenic acid into (*Z*)-3-hexenal and (*Z*)-3-hexenal to (*E*)-2-hexenal. The activity of LOX and HPL enzymes increases 25% and 200%, respectively, and the total linolenic acid availability increases 200% when strawberries are wounded, which encourages the formation of (*E*)-2-hexenal and (*Z*)-3-hexenal (Myung and others 2006).

Hexanoic acid is formed from linoleic acid either from the 13-hyroperoxide via β-oxidation or LOX oxidation of hexanal (Reineccius 2005, De Pooter and others 1989).

Esters are very important to strawberry flavor, because they give a fruity odor note. The concentration of the fruity esters methyl butanoate, ethyl butanoate, methyl hexanoate and ethyl hexanoate, but not hexyl acetate, significantly increased during storage in undamaged strawberries (Figure 7A). The increase in fruity ester concentration may indicate that the fruity odor was maintained during storage. The concentration of methyl butanoate, ethyl butanoate, ethyl hexanoate and hexyl acetate is significantly increased during 10°C storage for a week (Almenar and others 2009) while ethyl hexanoate shows a decrease after the first week of storage at 3°C (Almenar and others 2006). However, Perez and others (1996) found that the ester concentrations decreased during 9 d modified atmosphere storage at 1°C. Similar to Perez and others (1996) findings, ethyl and methyl hexanoate is decreased at the end of 14 d storage at 0°C (Ayala-Zavala and others 2004). The ester concentration of undamaged, punctured and bruised strawberries was the same at the end of storage. It is clear that damaging did not have any effect on ester formation in strawberries. No ester formation occurred when crushed or homogenized strawberries are incubated with an alcohol (Yamashita and others 1975).

Methyl butanoate had the highest concentration among the fruity esters in all treatments (Figure 7). Similar to our finding, methyl butanoate has the highest concentration among the esters in strawberries after nine days modified atmosphere storage at 1°C (Perez and others 1996). Ethyl acetate and ethanol correlate to off flavor

formation in strawberries (Sanz and others 1999, Larsen and Watkins 1995). The concentration of ethyl acetate and ethanol significantly increased (from 1430 and 730ppb to 12500 and 5400ppb) in bruised strawberries but not undamaged and punctured strawberries at the end of 8 d storage (Figure 11). Personal observation showed that the fruity and fresh strawberry odor was maintained in strawberries during the entire storage and no off odor was detected even in the bruised strawberries.

During storage, the LOX volatiles increased in undamaged strawberries, and even more so in bruised strawberries. Thus for LOX volatiles severe damage (bruised but not punctured) creates more volatile increase than storage. However, the volatile esters increased due to storage but showed no effect of damage, except ethyl acetate. Even in bruised strawberries where was severe damage applied and cell rupture occurred which may increase the availability of substrate to form more ester.

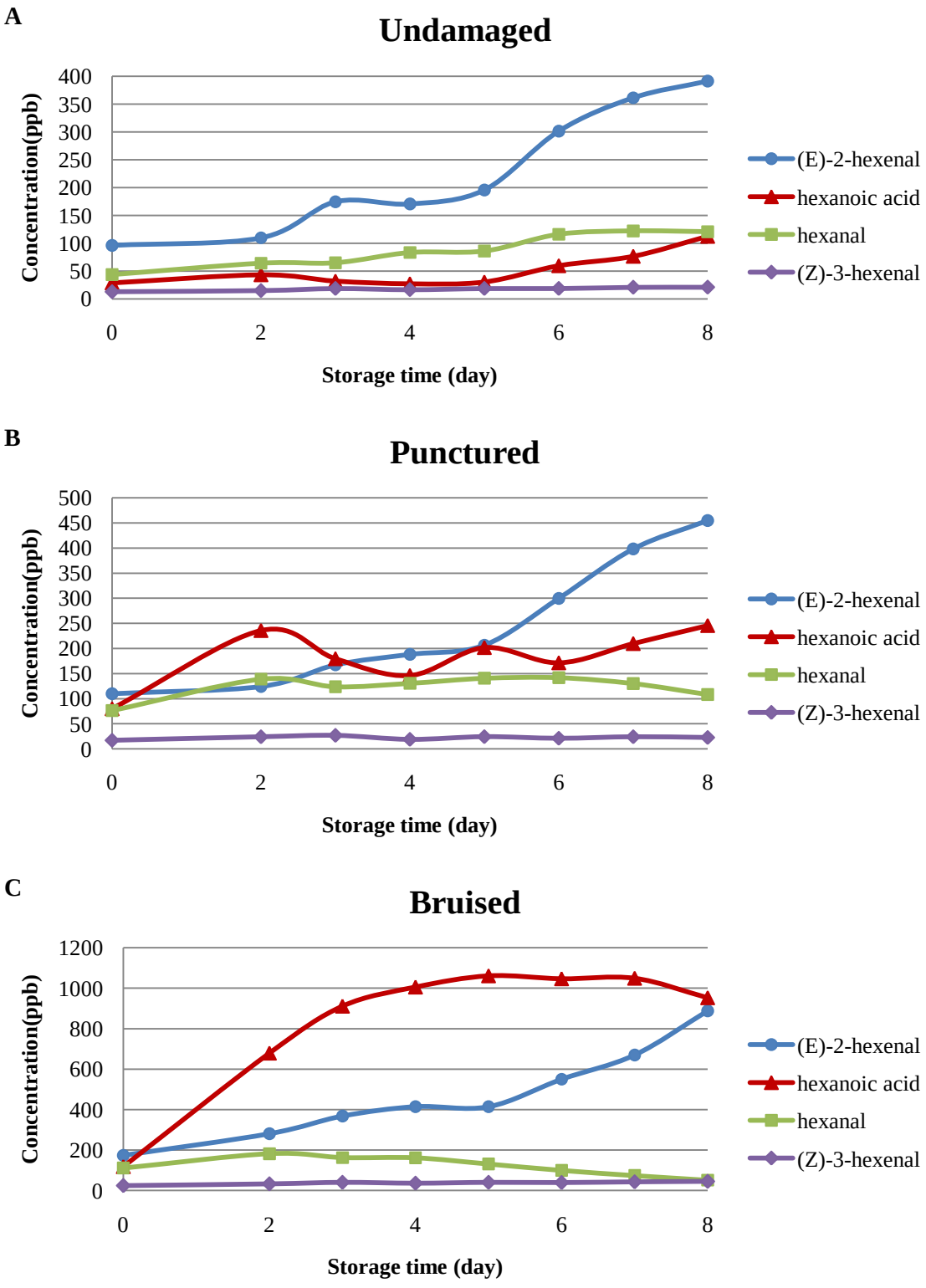


Figure 6. Concentration changes of LOX generated compounds in the headspace of whole strawberries under refrigerated storage conditions

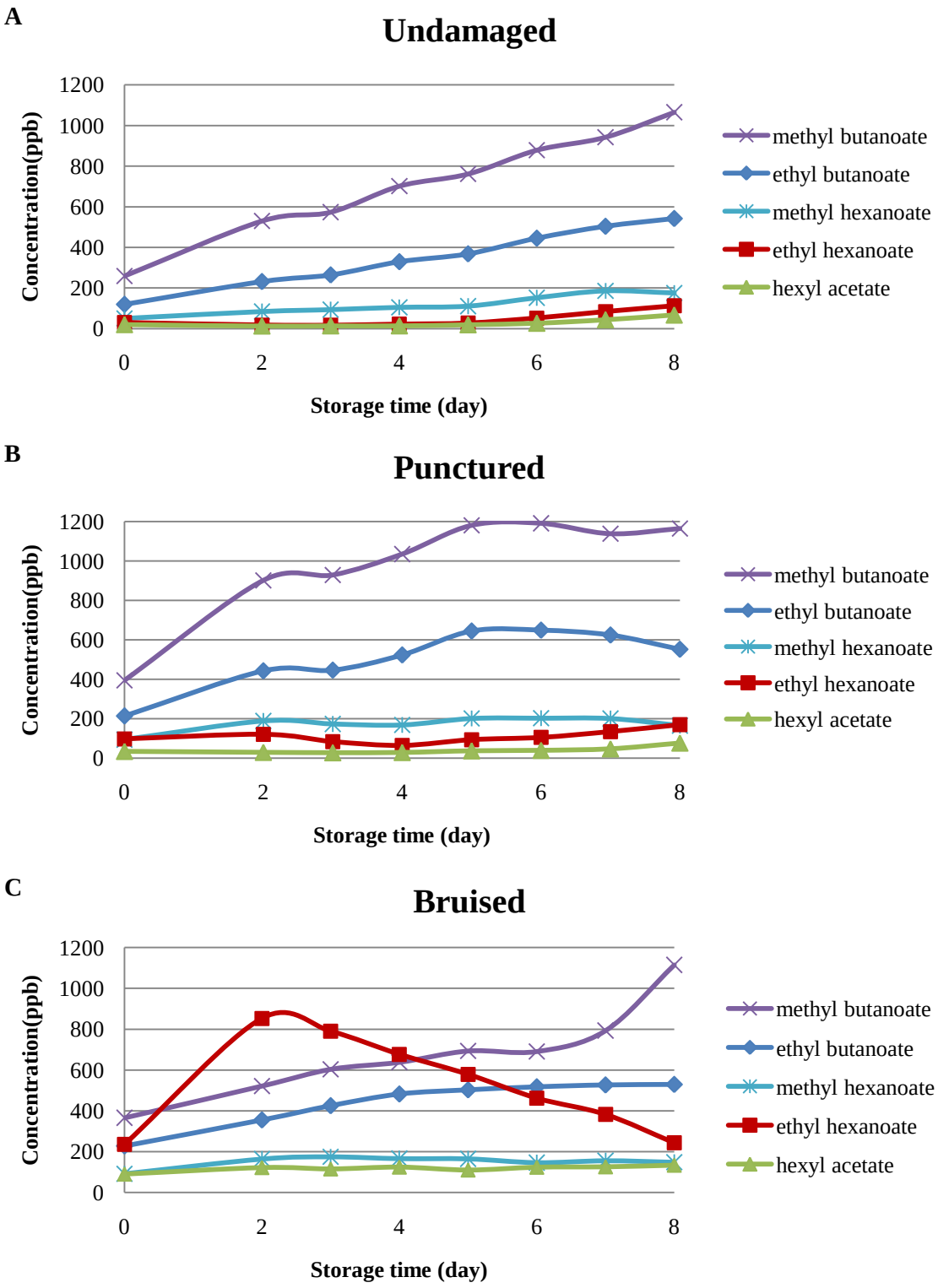


Figure 7. Concentration changes of fruity esters in the headspace of whole strawberries during refrigerated conditions

Volatile esters are formed by biosyntheses of an acid and an alcohol by alcohol acyltransferase enzyme activity (Olias and others 1995). During storage the ester concentration on each day usually correlated with the concentration of the alcohols and acids from which they were formed. Ethyl acetate, formed from ethanol and acetic acid, increased as ethanol increased in all treatments (R^2=0.95, 0.87, 0.94) for undamaged, punctured and bruised respectively (Figure 12). There was no correlation with acetic acid concentration. Ethyl butanoate increased as butanoic acid increased in all treatments (R^2=0.97, 0.90, 0.87) (Figure 13), but there was no correlation with ethanol concentration. Ethyl hexanoate correlated to both ethanol (R^2=0.74 and 0.62) and hexanoic acid (R^2=0.94 and 0.40) in undamaged and punctured strawberries, however there was no correlation in bruised strawberries.

Methyl acetate correlated with methanol in all treatments (R^2=0.87, 0.82, 0.81) (Figure 14), but not acetic acid. Methyl butanoate correlated with butanoic acid (R^2= 0.97, 0.94, 0.82) in all treatments (Figure 15) and correlated with methanol (R^2=0.86 and 0.86) in undamaged and bruised strawberries. Methyl hexanoate had no good correlation with methanol or hexanoic acid.

4.3 Strawberry volatile changes during ripening

The volatile concentrations of strawberries were investigated at different levels of ripeness, as indicated by the percent white, pink and red color in the fruit. (*Z*)-3-Hexenal, (*E*)-2-hexenal and hexanal were in high concentrations in unripe fruit and in the early stages of ripening (Figure 8). The concentration of these green odor compounds gradually decreased as the stage of ripeness progressed. The activity of LOX and HPL is high in

unripe and turning fruit and decreases in ripe fruit (Leone and others 2006). Azodanlou and others (2004) found a gradual decrease in hexanal as strawberries ripen, however Perez and others (1999a) found that the concentration increases with ripening. For (*E*)-2-hexenal, Azodanlou and others (2004) found either an increase or decrease depending on variety while Perez and others (1999a) found no change.

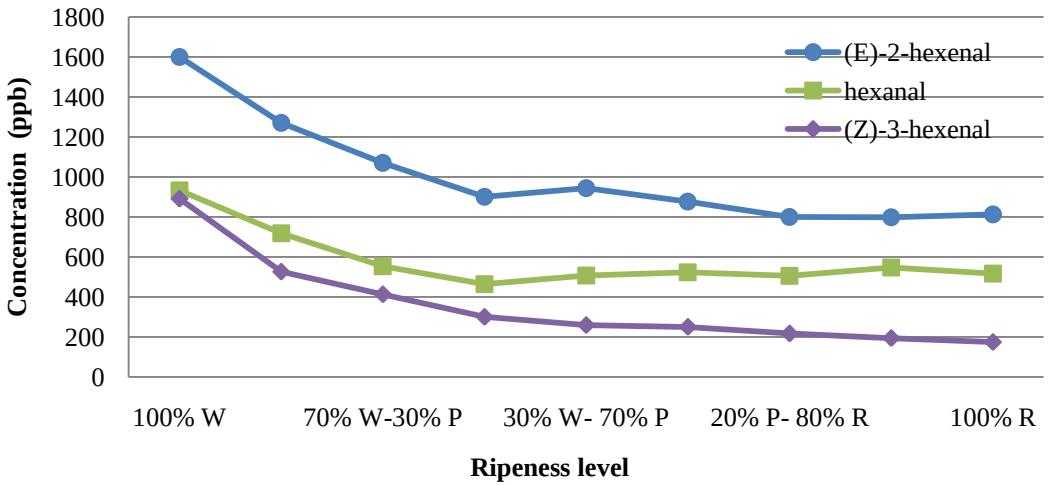


Figure 8. (*E*)-2-Hexenal, (*Z*)-3-hexenal and hexanal concentration in the headspace of strawberries at different stages of ripeness. W: white, P: pink, R: red

In contrast to the aldehydes, formation of fruity esters increased during ripening (Figure 9). The concentrations of all of the esters were lower than 100ppb in the early stages of ripening and the concentrations increased as ripeness increased. Methyl butanoate increased as soon as any pink color appeared on the berries and continued to increase with ripeness. The concentration of ethyl butanoate, ethyl hexanoate and methyl hexanoate was constant until 50% pink color when reached and then began to increase in ripe and fully ripened fruit (Figure 9). AAT increases with ripening (Perez and others

1996). Previous studies also shown that ester concentration increases during ripening, especially at the last stage of ripening (Azodanlou and others 2004, Forney and others 2000, Yamashita and others 1977) Forney and others (1998) also found that the concentration of methyl esters in ripened strawberries was increased 1.3 to 5.7 fold after 7 d storage.

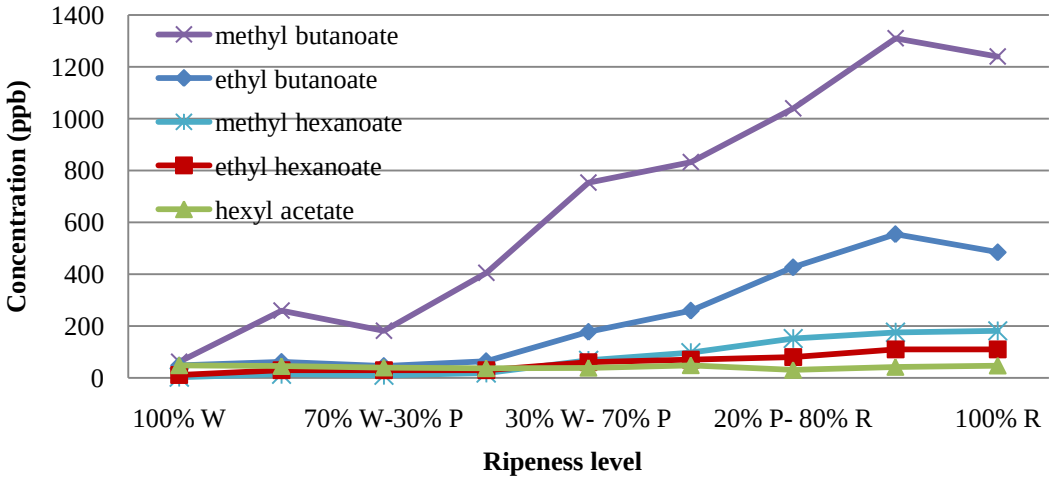


Figure 9. Concentration of fruity esters in the headspace of strawberries at different stages of ripeness. W: white, P: pink, R: red

4.4 Strawberry volatile concentration differences between cultivars

The concentration of volatiles in five strawberry varieties, Festival, Albion, Chandler, Sweet Charlie and Camarosa were measured to identify the difference in aroma profile (Table 5). (*Z*)-3-Hexenal, (*E*)-2-hexenal and hexanal were at a higher percentage of the headspace volatiles in Festival while hexanoic acid was the highest percentage in Chandler and Sweet Charlie. There were no significant differences between varieties in the relative percentage of ethyl esters. Percentages of methyl esters varied between 0.6-

31% dependent on cultivar. Larsen and Poll (1995) found cultivar dependent difference based on the concentrations of methyl and ethyl esters in strawberries.

Esters were greater than 36% of the total volatiles in all strawberry varieties, though the exact percent varied by variety type (Table 11). Similar to the storage study, the concentrations of the esters correlated with the concentration of their corresponding alcohols and acids. For the ethyl esters, the esters correlated with the same acids and alcohols as in the storage study. For the methyl esters, methyl acetate correlated with acetic acid (R^2=0.74), methyl butanoate correlated with butanoic acid (R^2=0.54), and methyl hexanoate correlated to methanol and hexanoic acid (R^2=0.94, 0.64). The total amount of esters was significantly lower in Chandler and higher in Camarosa varieties. Similarly, the sum of the relative proportion of ethyl and methyl esters and total volatile concentration was found to vary by variety in previous studies (Forney and others 1998, Hakala and others 2002). The total amount of aldehydes was significantly higher in Festival followed by Albion, Camarosa and Sweet Charlie, then Chandler. The total amount of acids was significantly higher in Chandler followed by Sweet Charlie, Camarosa, Albion and Festival. The total amount of alcohols was significantly higher in Chandler, Festival and Sweet Charlie followed by Albion and Camarosa. The total amount of ketones was significantly higher in Albion followed by other varieties (Table 11).

Table 5 Percentage (%) values of overall volatile compounds in the headspace of different strawberry varieties

	Festival	Albion	Chandler	Sweet Charlie	Camarosa
methyl acetate	27.7^{ab}	23.1^{ab}	14.4^{b}	16.0^{b}	31.5^{a}
methyl butanoate	10.5^{ab}	11.1^{ab}	5.8^{c}	7.4^{bc}	12.0^{a}
(*E*)-2-hexenal	9.1^{a}	6.3^{b}	4.3^{b}	4.1^{b}	6.3^{b}
acetone	9.0^{b}	19.4^{a}	11.8^{ab}	16.1^{ab}	10.4^{b}
methanol	6.3^{a}	5.1^{ab}	3.5^{b}	5.1^{ab}	4.1^{ab}
hexanal	6.2^{a}	4.2^{b}	2.6^{c}	3.9^{bc}	4.3^{b}
ethyl butanoate	6.1^{a}	6.7^{a}	4.3^{a}	4.9^{a}	7.0^{a}
butanoic acid	5.6^{a}	6.9^{a}	4.8^{a}	5.0^{a}	6.9^{a}
ethyl acetate	3.1^{b}	2.0^{b}	9.6^{a}	5.5^{ab}	2.1^{b}
isobutanoic acid	3.0^{b}	2.5^{b}	10.6^{a}	9.5^{a}	3.0^{b}
(*Z*)-3-hexenal	2.9^{a}	1.7^{bc}	1.1^{c}	1.2^{c}	2.2^{ab}
acetoin	2.0^{b}	1.1^{b}	12.4^{a}	7.9^{ab}	1.5^{b}
propanoic acid	1.5^{a}	1.4^{ab}	1.1^{b}	0.7^{c}	1.6^{a}
hexanoic acid	0.8^{b}	1.4^{ab}	3.5^{a}	3.6^{a}	1.0^{ab}
methyl hexanoate	0.8^{a}	1.5^{a}	1.0^{a}	1.9^{a}	0.6^{a}
methylbutanoic acid	0.7^{c}	1.3^{ab}	1.6^{a}	0.8^{bc}	1.7^{a}
ethanol	0.6^{b}	0.3^{b}	4.1^{a}	1.9^{ab}	0.3^{b}
linalool	0.6^{a}	0.4^{ab}	0.3^{b}	0.3^{b}	0.5^{a}
acetic acid	0.5^{a}	0.4^{b}	0.3^{b}	0.3^{b}	0.6^{a}
methyl methacrylate	0.5^{a}	0.4^{ab}	0.3^{b}	0.3^{b}	0.4^{ab}

	Festival	Albion	Chandler	Sweet Charlie	Camarosa
hexyl acetate	0.4^{a}	0.4^{a}	0.3^{a}	0.3^{a}	0.4^{a}
3-hexen-1-ol	0.4^{a}	0.3^{ab}	0.2^{ab}	0.2^{b}	0.4^{ab}
ethyl hexanoate	0.3^{a}	0.7^{a}	1.0^{a}	1.7^{a}	0.4^{a}
butyl acetate	0.3^{ab}	0.3^{b}	0.3^{ab}	0.3^{b}	0.4^{a}
butanol/butyl hexanoate	0.2^{a}	0.2^{b}	0.1^{bc}	0.1^{c}	0.2^{b}
ethyl 2-methylbutanoate	0.1^{ab}	0.2^{ab}	0.2^{ab}	0.3^{a}	0.1^{b}
2-pentenal	0.1^{a}	0.1^{b}	0.0^{b}	0.0^{b}	0.0^{b}
(*E*)-2-heptenal	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}	0.1^{a}
Total concentration (ppb)	8623	11882	18767	19191	12213

Different superscripts on the same volatile are significantly different ($p<0.05$).

4.5 Change of Volatiles in the Breath

The headspace concentration of whole and chopped strawberries was measured. The headspace concentration of all volatile compounds was significantly higher in chopped strawberries than whole strawberries, as expected (Table 6). The increase of the volatiles is due to both the mechanical release of volatiles from ruptured strawberry cells and the enzymatic activity as a result.

The mouthspace of chopped strawberries was measured in the breath after the same batch of chopped strawberries was measured for headspace. In chopped strawberries, the headspace concentration of all volatiles was higher than the mouthspace

concentration, since there is a longer equilibration time in the headspace than mouthspace (Table 6). Similarly in whole strawberries, the headspace concentrations of ethyl hexanoate, methyl hexanoate, methyl acetate, methyl butanoate, ethyl acetate and hexanal were two to nine times higher than the mouthspace concentrations. However, the results for (*Z*)-3-hexenal and (*E*)-2-hexenal were different.

Unlike the other volatiles, the mouthspace concentration of (*Z*)-3-hexenal and (*E*)-2-hexenal were two times higher than the headspace in whole strawberry (Table 6). Similarly, the concentration of (*E*)-2-hexenal was significantly higher in the mouthspace of whole strawberries than chopped strawberries unlike the other volatiles (Table 6). Chewing increased the concentration of LOX generated volatiles present (Table 6). These compounds are formed by the lipoxygenase pathway and this reaction is apparently fast enough that it occurs during maceration in the mouth during chewing. (*Z*)-3-Hexenal and (*E*)-2-hexenal compounds are the first volatiles formed in the lipid oxidation pathway via linolenic acid (Brauss and others 1998). The headspace measurements were performed on a whole strawberry, so no cellular damage occurred during measurement unlike the mouthspace tests where volatiles were measured during maceration. The mouthspace concentrations of chopped strawberries show just the effect of saliva and motion in the mouth but the mouthspace concentration of whole strawberries also shows enzyme formation in the mouth. During chewing the cells are disrupted, enzymes are released from the cells and enzymatic activity increases. (*Z*)-3-Hexenal, and (*E*)-2-hexenal are formed very quickly (Figure 5). Since there was three to five min between chopping and mouthspace measurements, the measurements of chopped strawberries are beyond the peak time of these LOX volatiles. In tomatoes the mouthspace concentration of (*Z*)-3-

hexenal and (*E*)-2-hexenal continued to increase from the first exhale to the third exhale due to enzymatic formation in the mouth (Xu and Barringer 2010).

Unlike the other LOX generated volatiles, the concentration of hexanal in the mouthspace after consuming whole and chopped strawberries was not significantly different. Hexanal formation was slower than the formation of (*Z*)-3-hexenal and (*E*)-2-hexenal thus significant formation did not occur in the mouth (Figure 5). The mouthspace before swallowing (Mbs) concentration of esters in chopped versus whole strawberries is not significantly because there is no enzymatic formation of esters due to damage (Table 6).

Table 6 Headspace (HS) concentration (ppb) of whole (W) and chopped (C) strawberries and their mouthspace (MSbs, MSas) and nosespace (NS) concentration (ppb)

	W HS	C HS	W MSbs	C MSbs	W NS	C NS	W MSas	C MSas
(*Z*)-3-hexenal	8.0[b]	210	17.7[a]	10.6[a]	3.19	1.52	6.27	3.95
(*E*)-2-hexenal	109[b]	950	207[a]	143[b]	46.9	31.6	62.3	58.5
methyl acetate	373[a]	2100	116[b]	74.2[b]	25.2	29.9	15.7	11.7
ethyl acetate	63.4[a]	414	26.7[b]	26.6[b]	9.29	7.74	4.12	3.74
methyl butanoate	165[a]	1800	19.0[b]	22.2[b]	4.98	3.35	0.79	1.18
hexanal	40.4[a]	342	15.1[b]	17.7[b]	4.66	4.8	3.68	1.28
methyl hexanoate	65.4[a]	600	6.9[b]	8.3[b]	1.11	2.8	0.18	0.35
ethyl hexanoate	25.8[a]	215	1.6[b]	5.9[a]	0.39	1.2	0.15	0.58

Different superscripts for the same chemical compound are significantly different ($p<0.05$).

4.5.1 Mouthspace concentrations before and after swallowing

Measurements were taken before and after swallowing, to determine how volatile chemistry effects persistence of the volatiles after swallowing, MSas/MSbs. The MSas/MSbs persistence ratio of esters decreased as the chain length of the acid part of the ester compounds increased in whole strawberries (Table 7). Methyl acetate had the highest MSas/MSbs ratio of the methyl esters with 14%, followed by methyl butanoate with 4% and methyl hexanoate with 3% in whole strawberries. The same order was also observed in the ethyl esters. While ethyl acetate had 15% persistence, ethyl hexanoate had 9% in whole strawberries. Similar results were seen in chopped strawberries. The longer the chain length, the more degradation occurs in the saliva due to the action of the salivary enzymes on the esters (Buettner 2002b). Thus, the longer the chain length of the acid parts of the esters, the more degradation in the mouthspace due to the salivary enzymes and the lower the persistence of the esters.

The LOX generated aldehydes (*Z*)-3-hexenal, (*E*)-2-hexenal and hexanal had higher retention (24-30%) than the esters (3-15%) in the mouthspace of a whole strawberry after swallowing (Table 7). The retention of (*Z*)-3-hexenal, (*E*)-2-hexenal and hexanal was similar to what was found in tomatoes (Xu and Barringer 2010).

Chopping increased the enzyme activity, increased volatile release in headspace and lowered the MS/HS ratio. Similarly, Xu and Barringer (2010) found that the MS/HS ratio of (*Z*)-3-hexenal and (*E*)-2-hexenal varied 1.1 to 9.0% in tomatoes when the headspace was measured on pureed tomatoes. As a result, maceration is the main factor

to allow enzyme activity and increase the concentration of lipid oxidation pathway generated compounds in fruits.

Table 7 Mouthspace after swallowing (MSas), mouthspace before swallowing (MSbs), nosespace (NS) and headspace (HS) percentage ratios in whole and chopped strawberry

	Whole				Chopped			
	MSas/ MSbs	NS/ MSbs	MSbs/ HS	NS/ HS	MSas/ MSbs	NS/ MSbs	MSbs/ HS	NS/ HS
(*E*)-2-hexenal	30.1	23.0	190	43.0	41.0	22.0	15.0	3.3
(*Z*)-3-hexenal	35.0	18.0	221	40.0	37.0	14.0	5.1	0.7
hexanal	24.0	30.8	37.0	12.0	7.2	27.0	5.2	1.4
methyl acetate	14.0	22.0	31.0	6.7	16.0	40.2	3.5	1.4
methyl butanoate	4.1	26.0	12.0	3.0	5.3	15.0	1.2	0.2
methyl hexanoate	2.6	16.0	10.5	1.7	4.3	34.0	1.4	0.5
ethyl acetate	15.0	35.0	42.0	15.0	14.0	29.0	6.4	1.9
ethyl hexanoate	9.3	24.0	6.3	1.5	9.9	20.6	2.7	0.6

4.5.2 Nosespace

The concentration of all volatile compounds was higher in the mouthspace than in the nosespace (Table 6). The transfer of volatile compounds from mouthspace to nosespace is the NS/MS ratio. The nosespace to mouthspace ratios in whole strawberry are similar to tomatoes for (*Z*)-3-hexenal and (*E*)-2-hexenal (Xu and Barringer 2010).

The nosespace to headspace, NS/HS, ratio of LOX generated aldehydes was higher than esters in whole strawberry just as the MS/HS ratios are higher (Table 7). The

high ratio of LOX generated compounds may be due to the formation of volatiles in mouth during eating. In chopped strawberries, the NS/HS ratio of volatiles was only 0.2-3.3%, much lower than in whole strawberries. Because of higher headspace concentration of chopped strawberries, NS/HS ratio decreased.

In the whole strawberries, the nosespace concentration of all compounds except (*Z*)-3-hexenal and (*E*)-2-hexenal was correlated to their log P values like mouthspace (Figure 17). Persistence of a compound is related with the polarity of that compound (Ingham and others 1995a). Methyl acetate is much polar than methyl hexanoate, so higher persistence of methyl acetate was observed in the mouthspace which is a polar environment. Low polar compounds such as methyl hexanoate release more but persist less in the mouthspace.

CHAPTER 5

CONCLUSION

The formation of hexanal was slower than the formation of (*Z*)-3-hexenal and (*E*)-2-hexenal in the headspace of pureed strawberries. During refrigerated storage there was a progressive increase in LOX derived aldehydes and fruity esters. The concentration of LOX derived aldehydes was higher in bruised strawberries than undamaged and punctured strawberries. Damaging strawberries is an important factor to elevate volatile levels in LOX derived aldehydes but not esters.

Strawberry volatile profiles changed distinctively during ripening. LOX derived compounds had high concentrations in unripe and early to middle stages of ripening while esters had high concentrations at middle and late stages of ripening. The initial presence of LOX generated aldehydes in the headspace of enzyme inhibited strawberries was due to the formation of these compounds in the unripe fruit and the early stages of ripening. Strawberry samples from different varieties showed a different aroma profile based on the level of volatile compounds they contain.

Chewing strawberries allows enzymatic formation of LOX derived volatiles in the mouthspace. The concentration of volatiles transferred to the nosespace was lower than mouthspace. Swallowing decreased the concentration of volatile compounds present in

the mouthspace and the persistence of LOX derived compounds was higher than esters in the mouthspace. The nosespace to mouthspace ratio (NS/MS) of volatile compounds varied between 14-40% in both whole and chopped strawberries.

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APPENDIX

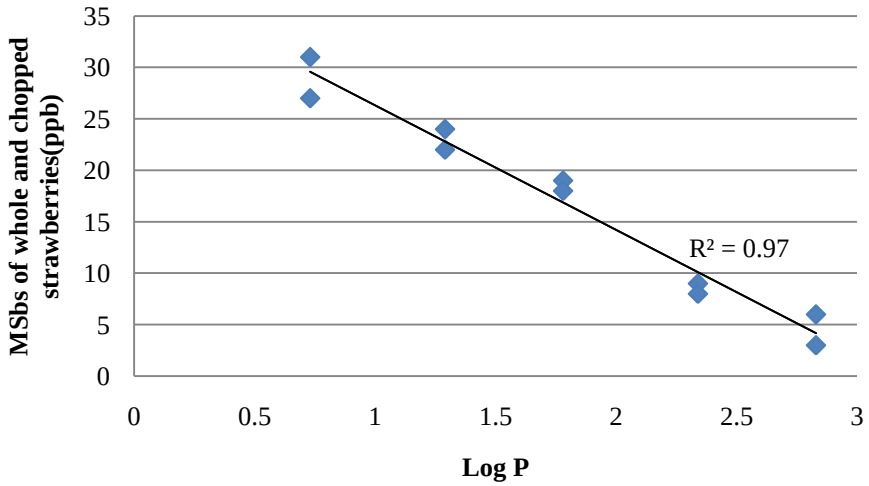

Figure 10. Relationship between Log P values and mouthspace (MSbs) concentrations of selected volatiles of whole and chopped strawberries

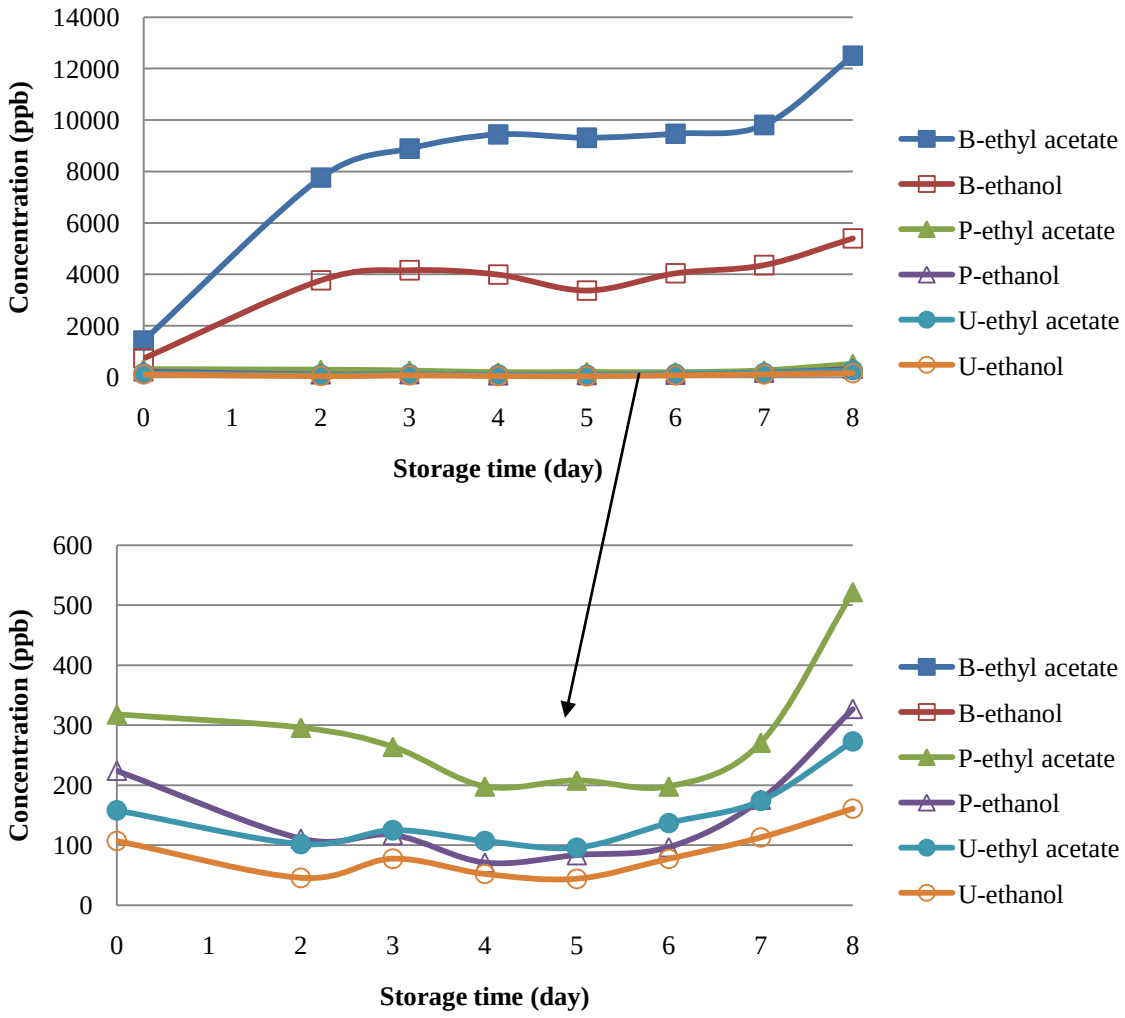

Figure 11. Concentration change of ethyl acetate and ethanol in the headspace of whole strawberries during refrigerated storage B: bruised, P: punctured, U: undamaged

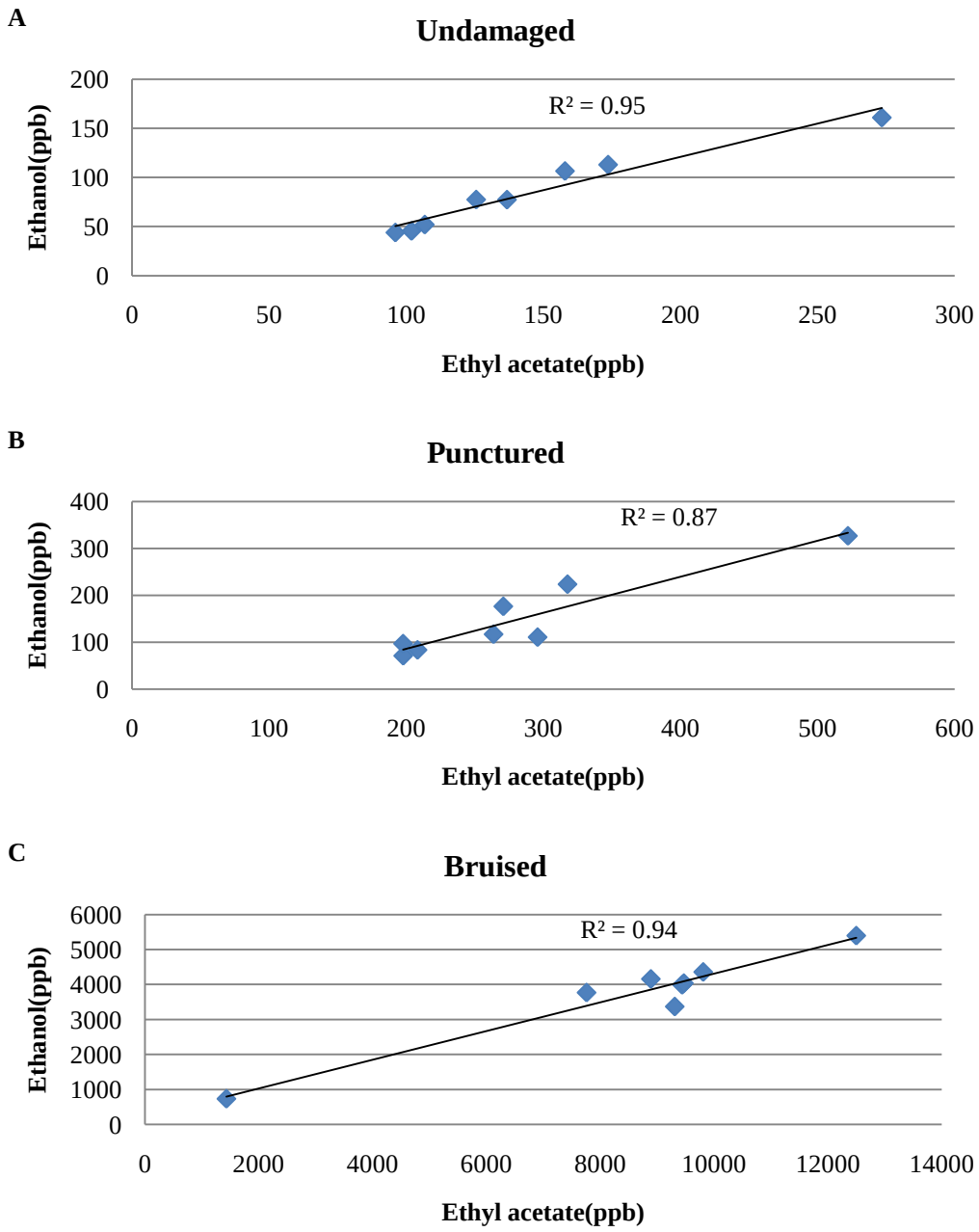

Figure 12. Correlation of ethanol and ethyl acetate

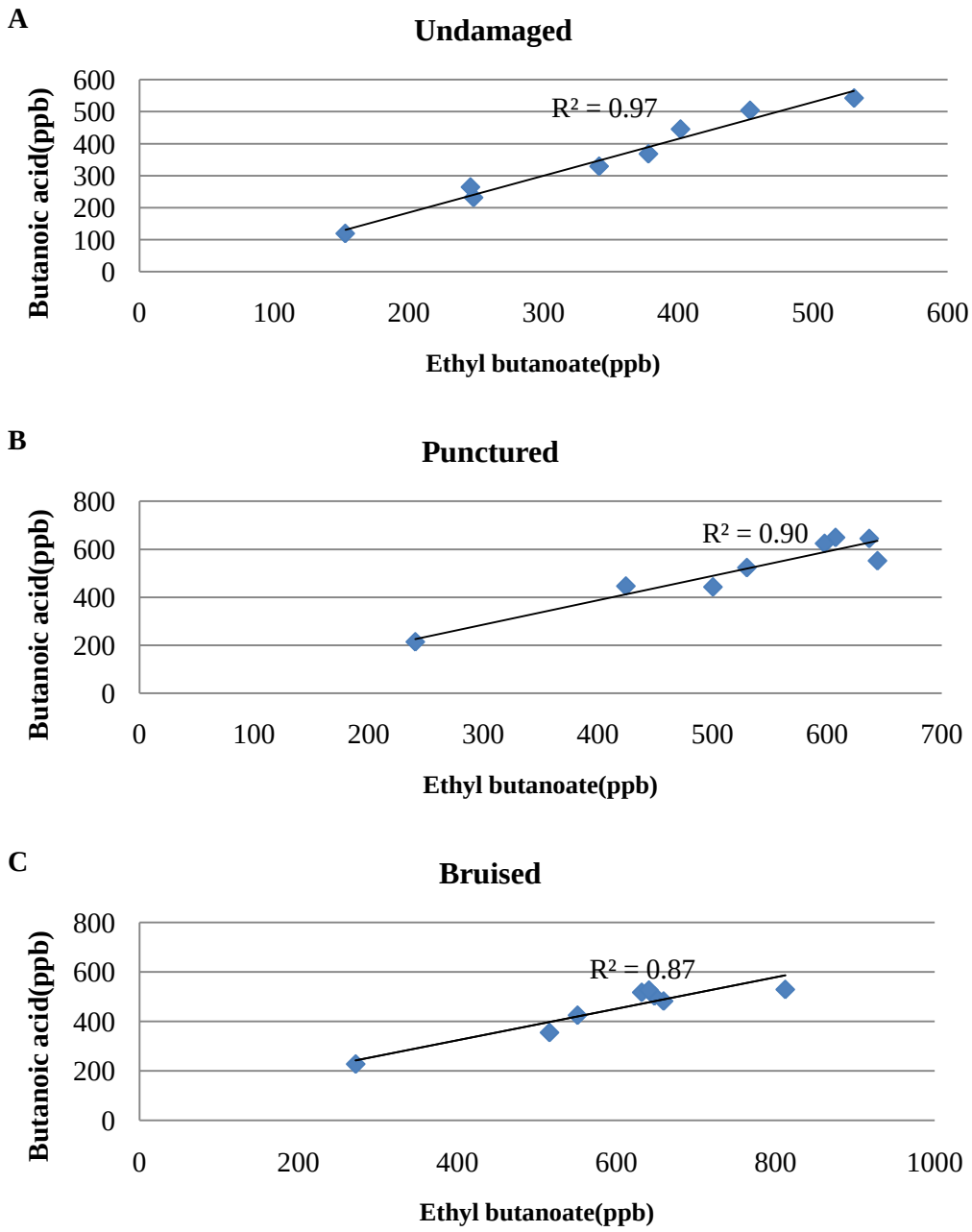

Figure 13.Correlation of butanoic acid and ethyl butanoate

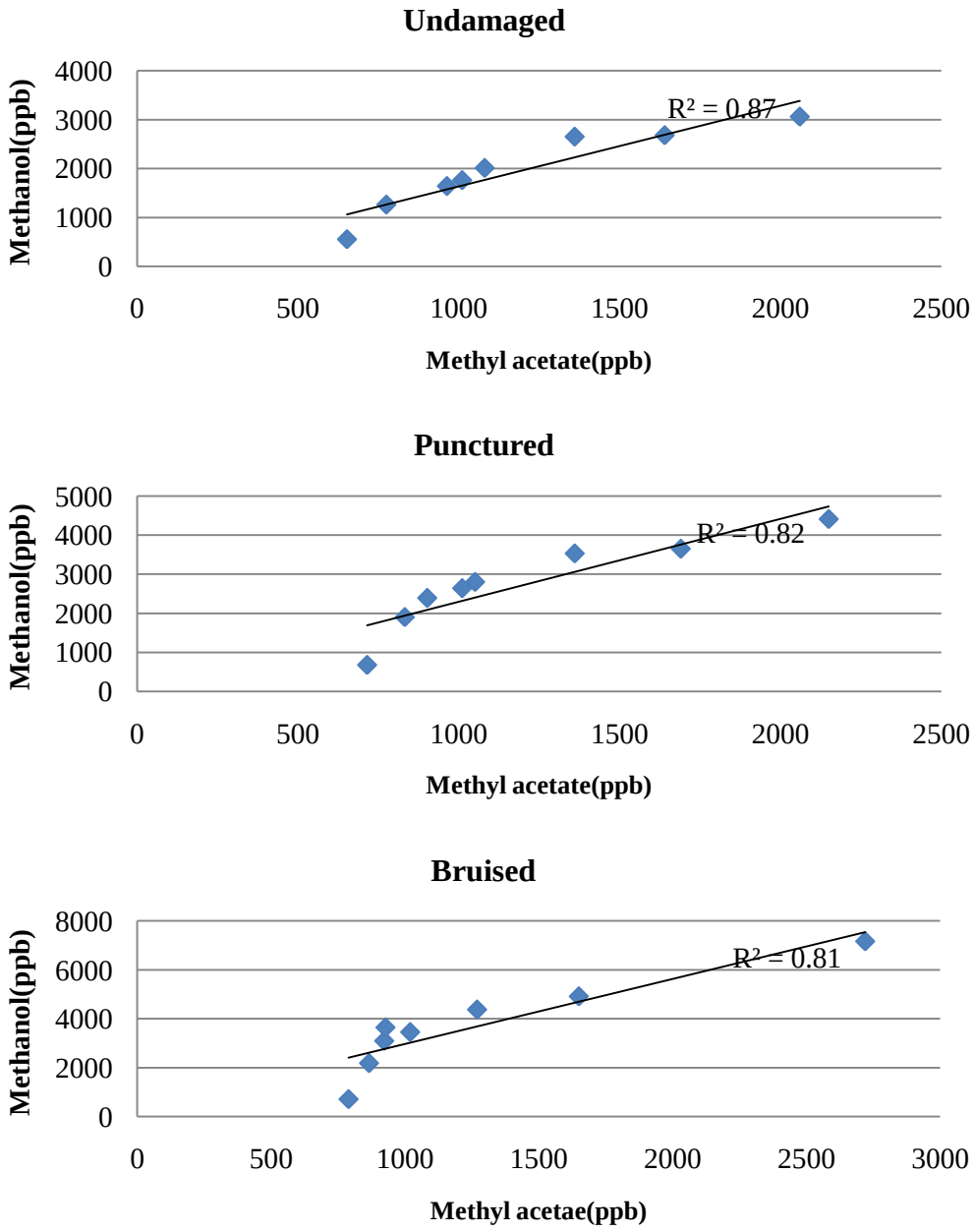

Figure 14. Correlation of methanol and methyl acetate

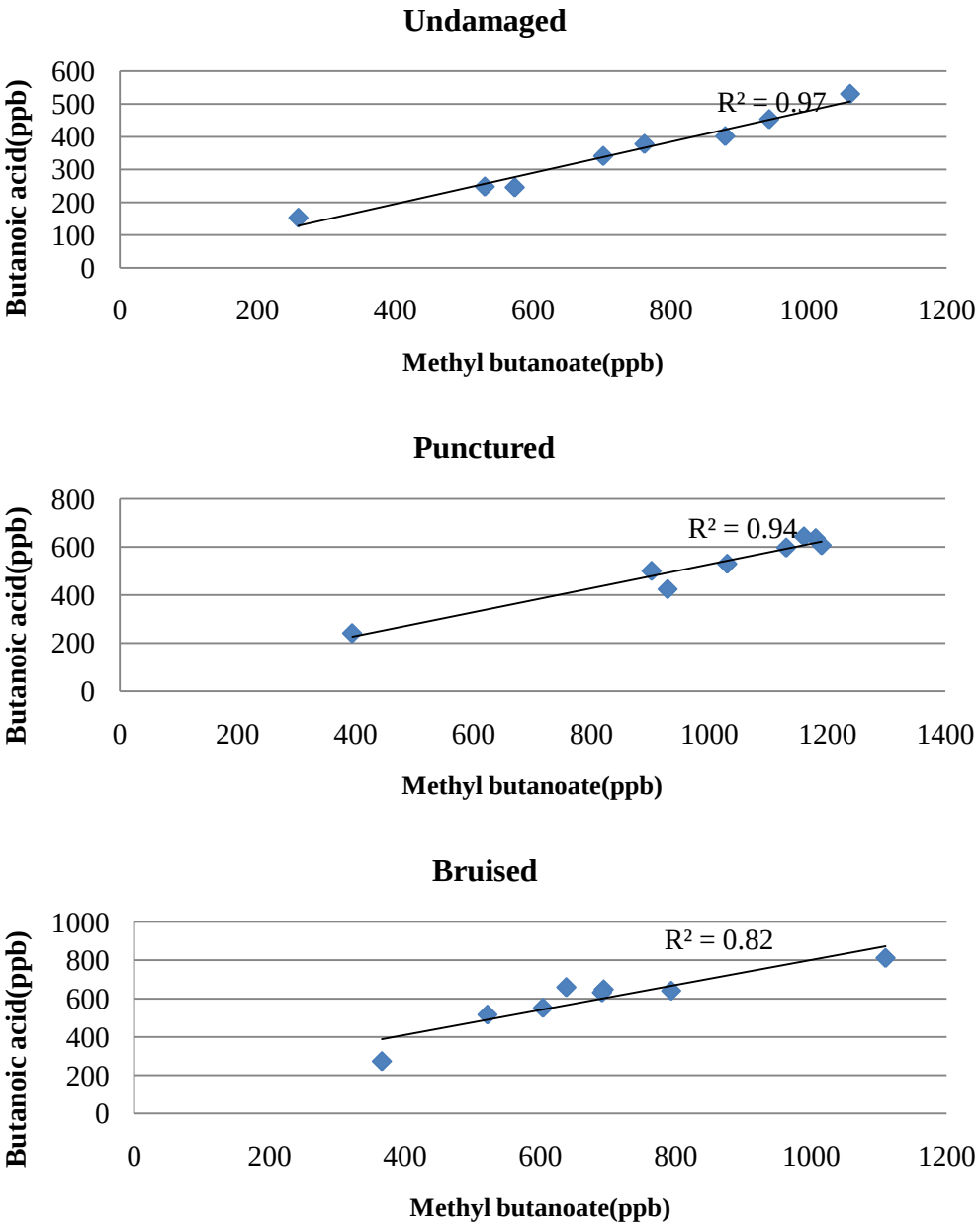

Figure 15.Correlation of butanoic acid and methyl butanoate

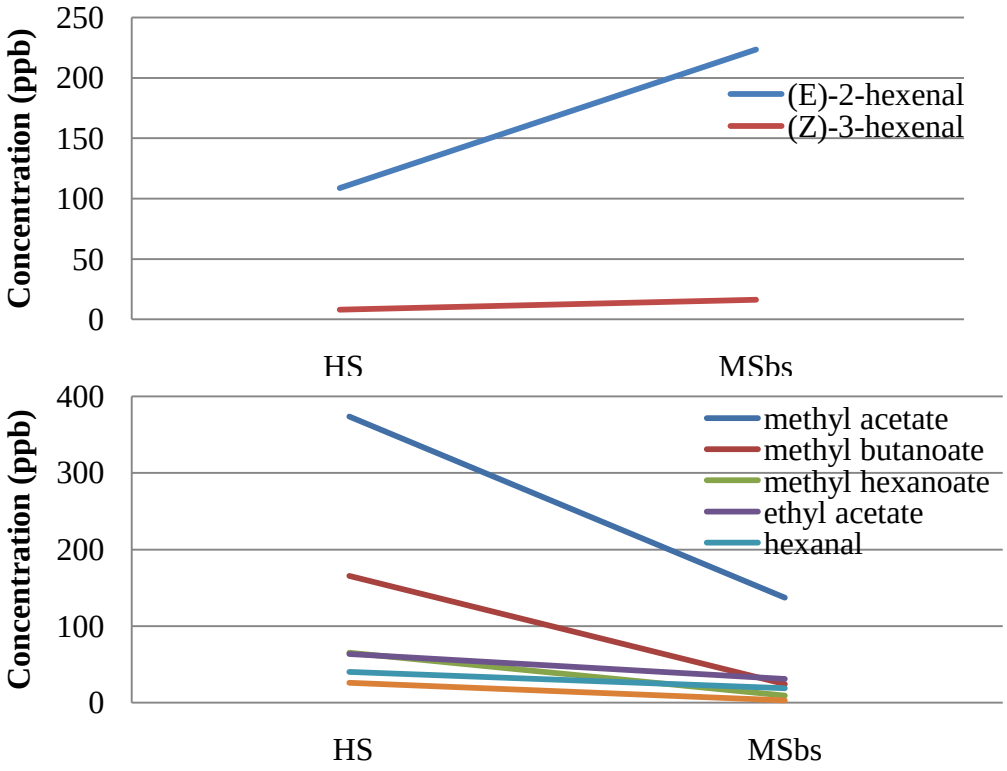

Figure 16. Volatile compounds increase (A) and decrease (B) in mouthspace compared to headspace

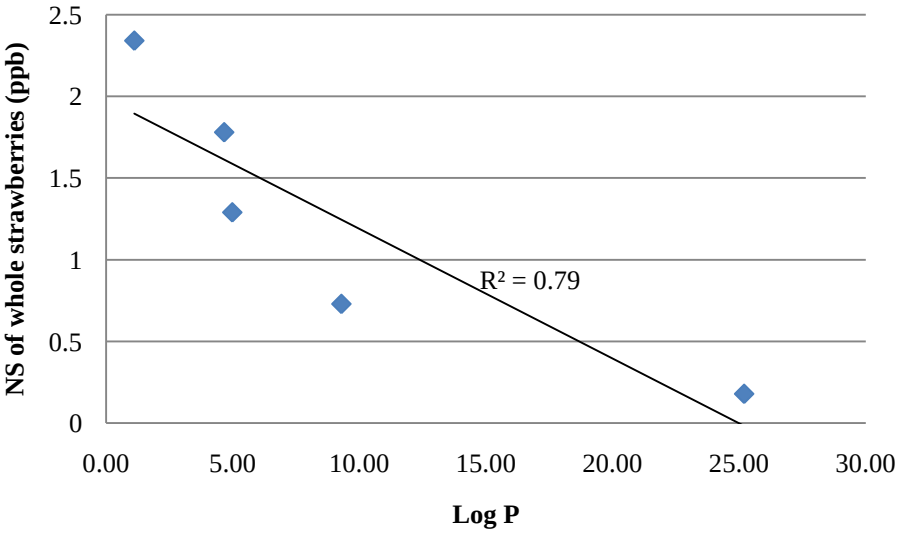

Figure 17. Relationship between Log P values and nosespace (NS) concentrations of selected volatiles of whole strawberries

Table 8 Average volatile concentration of undamaged strawberries in refrigerated storage

	Undamaged D0	Undamaged D2	Undamaged D3	Undamaged D4	Undamaged D5	Undamaged D6	Undamaged D7	Undamaged D8
methanol	652	774	963	1010	1080	1360	1640	2060
acetone	610	801	1012	1020	1041	1220	1330	1340
methyl acetate	551	1260	1640	1760	2010	2650	2680	3060
methyl butanoate	259	530	573	702	762	879	943	1060
ethyl acetate	158	102	125	107	96	137	174	273
butanoic acid	153	248	246	341	378	402	453	530
ethyl butanoate	119	231	264	329	368	445	504	542
ethanol	107	45.6	77.5	52.1	44.0	77.4	113	161
(*E*)-2-hexenal	96.2	110	175	171	196	302	361	391
isobutanoic acid	74.4	106	90	91	93	152	171	242
methyl hexanoate	50.6	84.3	93.6	105	111	152	186	175
hexanal	43.8	64.2	65.0	83.4	86.0	116	122	121
acetoin	30.7	58.0	56.0	50.6	58.4	105	127	210
ethyl hexanoate	30.1	17.7	16.7	22.0	27.3	52.3	83.9	112
hexanoic acid	28.8	43.3	32.0	27.2	30.4	59.6	76.6	112
methylbutanoic acid	27.0	24.6	24.7	24.2	27.7	31.2	31.3	35.0
3-hexen-1-ol	24.0	4.45	3.75	3.95	4.88	5.18	7.42	6.31
hexyl acetate	20.5	13.5	14.3	14.3	19.7	27.2	43.9	67.2
propanoic acid	19.4	27.8	33.9	35.2	40.7	48.6	54.1	63.9
butanol/butyl hexanoate	14.5	14.4	18.1	18.8	19.2	24.4	25.0	22.3
(*Z*)-3-hexenal	12.9	15.0	18.7	16.6	18.7	18.8	20.9	21.1
acetic acid	11.1	28.1	18.4	15.2	16.3	17.8	20.3	18.9
butyl acetate	8.92	23.9	13.3	15.0	15.8	16.1	18.6	26.7
ethyl 2-methylbutanoate	7.97	12.5	12.2	15.0	16.1	22.1	25.6	25.3

Table 9 Average volatile concentration of punctured strawberries in refrigerated storage

	punctured D0	punctured D2	punctured D3	punctured D4	punctured D5	punctured D6	punctured D7	punctured D8
methanol	714	831	901	1010	1050	1360	1690	2150
acetone	698	899	1030	1060	1210	1400	1470	1430
methyl acetate	676	1900	2390	2640	2800	3530	3650	4410
methyl butanoate	394	902	929	1030	1180	1190	1130	1160
ethyl acetate	318	296	264	198	208	198	271	522
butanoic acid	241	500	424	530	637	607	598	644
ethanol	224	111	117	70.9	83.4	96.7	176	327
ethyl butanoate	214	443	446	523	644	649	624	552
isobutanoic acid	170	534	388	331	379	316	349	470
(*E*)-2-hexenal	110	124	167	188	206	300	398	455
ethyl hexanoate	97.6	121	83.4	63.9	93.3	105	135	170
methyl hexanoate	93.7	188	173	168	201	202	201	165
hexanoic acid	80.3	236	180	146	202	172	210	246
hexanal	76.4	139	124	131	141	142	130	108
acetoin	64.8	331	301	261	280	226	246	453
3-hexen-1-ol	60.8	15.4	9.31	7.41	6.51	6.13	6.31	6.65
methylbutanoic acid	36.8	58.3	49.5	47.2	49.8	47.2	38.3	39.4
hexyl acetate	34.0	29.1	27.2	28.5	36.9	39.2	46.9	76.4
propanoic acid	27.9	45.5	51.4	58.4	72.5	81.1	81.5	89.7
(*Z*)-3-hexenal	17.0	24.1	26.9	18.9	24.3	21.1	24.2	22.7
methyl methacrylate	16.5	13.1	10.2	11.4	10.5	10.9	11.1	11.0
acetic acid	16.0	30.7	19.8	19.1	20.0	22.8	23.7	22.4
butyl acetate	13.1	36.3	22.8	22.8	26.2	24.1	25.3	37.6
butanol/butyl hexanoate	13.0	12.7	17.4	18.1	19.8	23.8	25.8	22.7
ethyl 2-methylbutanoate	10.6	26.1	25.4	24.8	28.9	28.9	27.8	21.6

Table 10 Average volatile concentration of bruised strawberries in refrigerated storage

	bruised D0	bruised D2	bruised D3	bruised D4	bruised D5	bruised D6	bruised D7	bruised D8
ethyl acetate	1430	7760	8890	9440	9310	9470	9810	12500
acetone	1010	1320	1610	1620	1540	1720	1690	1720
methanol	790	866	923	1020	928	1270	1650	2720
ethanol	730	3770	4160	3990	3370	4040	4360	5400
methyl acetate	709	2180	3090	3450	3640	4370	4910	7160
isobutanoic acid	383	5420	7740	9240	9720	10500	11400	12400
methyl butanoate	366	522	604	638	694	691	793	1110
acetoin	316	8480	14700	17800	19300	22400	23500	27900
butanoic acid	272	516	551	659	648	632	641	812
ethyl hexanoate	236	853	790	677	579	462	382	243
ethyl butanoate	228	356	426	483	503	518	527	530
3-hexen-1-ol	197	152	122	104	73	62	48	32
(*E*)-2-hexenal	174	281	368	415	414	550	670	888
hexanoic acid	118	679	911	1000	1060	1040	1040	953
hexanal	111	181	162	161	131	99.0	73.6	51
methyl hexanoate	91.2	164	175	166	165	146	155	148
hexyl acetate	89.9	122	115	124	110	123	125	133
propanoic acid	59.8	75.8	82.4	87.2	87.7	91.8	103	127
methyl methacrylate	57.1	52.5	40.9	33.3	26.4	19.5	16.7	14.3
methylbutanoic acid	46.5	171	218	210	198	167	150	142
acetic acid	41.3	90	102	118	110	136	152	184
butyl acetate	26.1	179	196	218	246	214	230	310
(*Z*)-3-hexenal	24.3	32.7	40.0	35.9	40.0	39.0	42.4	44.8
butanol/butyl hexanoate	18.9	15.8	18.2	20.2	19.3	26.4	27.7	31.5
ethyl 2-methylbutanoate	14.5	25.7	30.6	32.3	27.5	24.8	27.6	20.9
(*E*)-2-pentenal	14.0	15.8	17.0	15.2	13.3	15.0	16.7	19.7
1-penten-3-one	12.9	9.53	6.40	5.19	4.14	1.88	2.03	1.38
2-pentanone	12.7	43.5	66.7	89.3	111	168	206	242

	bruised D0	bruised D2	bruised D3	bruised D4	bruised D5	bruised D6	bruised D7	bruised D8
gamma-decalactone	12.7	10.2	20.5	20.1	16.0	21.4	16.7	14.4
1-pentanol	9.46	20.3	32.7	21.1	23.7	24.3	31.8	37.6

Table 11 Total percentage values of chemical groups of different strawberry varieties

	Festival	Albion	Chandler	Sweet Charlie	Camarosa
esters	49.2^{ab}	44.9^{bc}	36.2^{d}	36.7^{cd}	54.2^{a}
aldehydes	18.5^{a}	12.5^{b}	8.2^{c}	9.3^{bc}	13.0^{b}
acids	12.1^{b}	13.9^{ab}	21.9^{a}	19.9^{ab}	14.7^{ab}
alcohols	7.9^{a}	6.3^{ab}	8.2^{a}	7.6^{a}	5.4^{b}
ketones	9.2^{b}	19.6^{a}	12.0^{ab}	16.6^{ab}	10.5^{b}
others	3.0	2.8	13.5	9.9	2.2

Different superscripts at the same chemical group are significantly different ($p<0.05$).

Table 12 Nosespace (NS) concentration (ppb) change over three exhales in whole and chopped strawberries

	whole			chopped		
	1^{st} exhale	2^{nd} exhale	3^{rd} exhale	1^{st} exhale	2^{nd} exhale	3^{rd} exhale
(*Z*)-3-hexenal	5.30^{a}	3.20^{a}	1.10^{a}	1.56^{a}	2.30^{a}	0.71^{a}
(*E*)-2-hexenal	63.00^{a}	43.00^{a}	35.00^{a}	35.00^{a}	33.00^{a}	26.00^{a}
hexanal	7.90^{a}	4.30^{ab}	1.70^{b}	6.90^{a}	4.10^{a}	3.40^{a}
methyl acetate	39.00^{a}	20.50^{b}	16.00^{b}	57.00^{a}	23.00^{b}	10.20^{b}
ethyl acetate	17.00^{a}	6.70^{ab}	3.80^{b}	12.00^{a}	6.40^{a}	5.30^{a}
methyl butanoate	11.00^{a}	3.60^{ab}	0.70^{b}	5.50^{a}	2.60^{a}	1.90^{a}
methyl hexanoate	2.30^{a}	0.60^{b}	0.40^{b}	6.50^{a}	1.20^{b}	0.65^{b}
ethyl hexanoate	0.74^{a}	0.41^{a}	0.00^{a}	1.70^{a}	1.30^{a}	0.69^{a}

Different superscripts at the same compounds are significantly different ($p<0.05$).