Volatile changes caused by different factors in different types of chocolate

THESIS

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

By

Yi-Hsuan Lin, B.S.

Graduate Program in Food Science and Technology

The Ohio State University

2010

Master's Examination Committee:

Professor Dr. Sheryl A. Barringer, Advisor

Professor Dr. W. James Harper

Associate Professor Dr. V.M. (Bala) Balasubramaniam

Copyright by

Yi-Hsuan Lin

2010

ABSTRACT

The volatiles change caused by different factors in different chocolate was analyzed by using SIFT-MS (Selected Flow Tube-Mass Spectrometry). During conching, all volatiles initially increased then decreased in unsweetened dark (UDC), dark (DC), unsweetened milk chocolate (UMC). The greatest rate of volatile loss occurred during the first 28h of conching. The total volatile lost was 72-79% at 48h, and loss was mostly due to methanol. The percent lost of acetic acid was least, about 16-61%. Most volatiles lost 80-95% during conching, but a few volatiles had low percent lost. In storage study, unsweetened dark chocolate at 4°C, 25°C, and 50°C had higher total volatile concentration than unsweetened milk. Samples at 50°C showed less loss than at 25°C and 4°C in total volatile. The low loss of Strecker aldehydes, heptanal, and nonanal may indicate the occurrence of the Maillard reaction and lipid oxidation. In blooming measurement, cocoa mass, unsweetened dark chocolate, and dark chocolate were analyzed. Acetic acid in bloomed samples was significant lower than unbloomed in UDC, DC, and cocoa mass (CM). In the real time volatile release test, the volatiles decreased over time before swallowing and maintained a constant level after swallowing. The mouthspace before swallowing to headspace ratio (MSbs/HS) on average were 0.069. The mouthspace before to mouthspace after swallowing ratio (MSbs/MSas) varied from 0.042 to 49 between

different types of chocolate and volatiles. Benzaldehyde had the highest MSbs/MSas ratio in cocoa liquor and dark chocolate compared with other compounds. The nosespace to headspace ratio (NS/HS) were varied from 0.0012 to 0.17. No correlation was found between volatile lingering and air-water partition in this study.

ACKNOWLEDGEMENTS

I would like to express my appreciation to the following individuals:

My advisor, Dr. Sheryl A. Barringer, for her guidance, assistance, and support throughout my graduate studies. She has helped me solve many problems I have encountered, and it was a great experience working with her. Without her advice and persistent assistance, this thesis would not have been possible.

My parents, Mr. Pao-Chuan Lin and Ms. Hsiu-Fang Chou, for their love, encouragement, and financial support.

My committee members, Dr. W. James Harper and Dr. V.M. (Bala) Balasubramaniam, for serving on my graduate committee.

Cheryl Wick and Karen Fligner, for their support on SIFT-MS.

Yang Huang, for his advice and assistance.

Nutsuda Sumonsiri, Gulsah Ozcan, and all my lab mates for their encouragement and support.

VITA

January 1985	Taipei, Taiwan	
2007	B.S. Nutrition and Health Science	
	Taipei Medical University, Taiwan	
2007 to 2008	.Part-time student	
	University of Cincinnati	

FIELDS OF STUDY

Major Field: Food Science and Technology

TABLE OF CONTENTS

ABSTRACTii
ACKNOWLEDGEMENTS iv
VITAv
PRACTICAL APPLICATIONSxv
Chapter 1: Introduction
Chapter 2: Literature review
2.1 Chocolate4
2.2 Identification of volatile compounds
2.2.1 Important volatile compounds in dark chocolate
2.2.1.1 Aldehydes
2.2.1.2 Pyrazines
2.2.1.3 Acetic acid

2.2.1.4	Others	.9
2.2.2	Important volatile compounds in milk chocolate1	3
2.3	Volatile compound changes by different factor1	3
2.3.1	Sugar1	3
2.3.2	Milk solids1	5
2.3.3	Conching1	.6
2.3.3.1	Flavor changes during conching1	.7
2.3.3.2	2 Sensory properties influence by conching2	21
2.4	Selected ion flow tube-Mass spectrometry (SIFT-MS)2	.1
Chapte	er 3: Materials and Methods2	:4
3.1	Cocoa mass preparation2	24
3.2	Conching measurement2	:4
3.3	Storage measurement	:6
3.4	Blooming measurement	27

3.5	SIFT-MS analysis
3.5.1	Headspace analysis27
3.5.2	Mouthspace and nosespace analysis28
3.5.3	Performance condition
3.6	Statistical analysis methods
Chapte	er 4: Results and Discussion
4.1	Conching studies
4.1.1	Changes of top ten volatiles during conching
4.1.1.1	Methanol
4.1.1.2	2 Acetic acid
4.1.1.3	Other compounds in the top 1040
4.2	Storage
4.3	Blooming studies
4.3.1	Effect of blooming on different types of chocolate

4.4	Real time volatile release in chocolate	56
4.4.1	Headspace	56
4.4.2	Volatile release in the mouthspace during eating	62
4.4.3	Nosespace	71
4.5	Conclusion	73
Refere	ences	76
Apper	ndix A	83

List of Tables

Table 2.1 Compounds identified as important odorants by previous research 10
Table 2.2 Concentrations of identified compounds in chocolate A and B before
and after conching by Counet and others (2002) 19
Table 3.1 The compounds measured by SIFT-MS 32
Table 4.1 The percent lost of volatiles on day 56 at different temperature in dark
chocolate (DC) and unsweetened milk chocolate (UMC)
Table 4.2 Volatile concentration (ppb) in headspace (HS) in unsweetened dark
chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: O_2^+) 57
Table 4.3 Volatile concentration (ppb) in headspace (HS) in unsweetened dark
chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: NO ⁺)
Table 4.4 The mouthspace before swallowing to headspace (HS) ratio in
unsweetened dark chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC)
(precursor: O_2^+)
Table 4.5 The mouthspace before swallowing to headspace (HS) ratio in
unsweetened dark chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC)
(precursor: NO ⁺)

Table 4.6 The nosespace (NS) to headspace (HS) ratios in unsweetened dark

chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: O_2^+) 61

Table 4.7 The nosespace (NS) to headspace (HS) ratios in unsweetened dark chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: NO⁺)

|--|

Table 4.8 Volatile concentration in mouthspace before (MSb) and after	
swallowing (MSa) in unsweetened dark chocolate (UDC), dark (DC), and unsweetened	l
milk chocolate (UMC) (precursor: O_2^+)	65

Table 4.9 Volatile concentration in mouthspace before (MSb) and after
swallowing (MSa) in unsweetened dark chocolate (UDC), dark (DC), and unsweetened
milk chocolate (UMC) (precursor: NO ⁺)

Table 4.13 Volatile concentration (ppb) in nosespace (NS) in unsweetened dark chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: O_2^+) 72

Table 4.14 Volatile concentration (ppb) in nosespace (NS) in unsweetened dark
chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: NO ⁺)
Table A.1 The contribution to total concentration at 1h and contribution to total
lost at 48h of top 10 volatiles in different types of chocolate
Table A.2 The top 10 volatiles and their concentration at 1 and 48h 87
Table A.3 Percent lost of volatiles in unsweetened dark chocolate (UDC),
unsweetened milk (UMC), and dark chocolate (DC) after conching 89
Table A.4 The volatile concentrations during storage in dark chocolate (DC) and
unsweetened milk chocolate (UMC)

List of Figures

Figure 2.1 The simplify structure of SIFT-MS (Syft Technologies Ltd. 2007) 23
Figure 3.1 the position of the needle in the conche
Figure 3.2 The position of the needles in the bottle
Figure 4.1 Volatile concentrations (above 1000ppb at 0h) during 48h conching in
unsweetened dark chocolate. (Acetic acid is plotted on the secondary axis)
Figure 4.2 Volatile concentrations (below 1000ppb at 0h) during 48h conching in
unsweetened dark chocolate
Figure 4.3 The total volatile in dark chocolate (DC) and unsweetened milk
chocolate (UMC) at 4 $^{\circ}$ C, 25 $^{\circ}$ C, and 50 $^{\circ}$ C
Figure 4.4 The change of Strecker aldehydes during storage in dark chocolate
(DC) and unsweetened milk chocolate (UMC) at 4 °C, 25 °C, and 50 °C
Figure 4.5 Volatile concentrations in unbloomed and bloomed cocoa mass 53
Figure 4.6 Volatile concentrations in unbloomed and bloomed unsweetened dark
chocolate
Figure 4.7 Volatile concentrations in unbloomed and bloomed dark chocolate 55
Figure 4.8 Real time concentration change of acetic acid between mouthspace
before and after swallowing
Figure A.1 Needle position of sampling volatiles in mouthspace or nosespace 83

PRACTICAL APPLICATIONS

This study provides better understanding of chocolate volatiles change effected by different factors. For flavor, conching can be finished in 28h since the volatile lost was greatest at this time. To minimize the volatiles change in chocolate, 25°C is the best temperature for storage, which has less chemical reactions occur than 50°C and lost similar level with chocolate at 4°C. The decrease of acetic acid in bloomed chocolate may apply to remove acetic acid from chocolate products. In mouthspace and nosespace, different chocolate provides similar level of lingering and transferring.

Chapter 1: Introduction

Chocolate, due to its attractive flavor, it is a very popular and common product in our daily lives. The volatile compounds in chocolate are responsible for the aroma of chocolate. So far, over six hundred compounds have been found in chocolate including aldehydes, pyrazines, acids, ketones, esters, furans, and phenols (Counet and others 2002). Many previous studies have reported the volatiles in cocoa and its products. 2-Methylpropanal, 2methylbutanal and 3-methylbutanal were analyzed in dark chocolate by gas chromatography-olfactometry and gas chromatography-mass spectrometry, and were reported as important volatiles with strong chocolate flavor (Counet and others 2002). In milk chocolate, 3-methylbutanal, (E)-2-octenal, 2, 3-diethyl-5-methylpyrazine, (E)-2nonenal, 2- and 3-methylbutanoic acid, vanillin, R- δ -decalactone, furaneol, and (E, E)-2, 4decadienal were reported as crucial volatiles (Schnermann and Schieberle 1997). The volatiles in chocolate are easily changed by many factors, such as cultivar, processing, including fermentation, roasting, conching, and other additional ingredients added in chocolate. Sugar and milk solids are common ingredients added in chocolate, and they may affect chocolate flavor. The high cocoa aroma intensity in milk chocolate was associated with low sugar content and high milk solid content (Guinard and Mazzucchelli 1999). However, the effects of added ingredients, such as milk solids and sugar, on chocolate flavor are still lacking investigation.

In chocolate manufacture, conching is an important process uses to improve texture and remove undesired volatiles, such acetic acid. However, although conching have been used for over a hundred years (Dimick and Hoskin 1981), there are not many studies investigated the volatile changes during the process. An overall reduction of headspace volatiles was reported in semi-sweet chocolate during 44h of conching at 82°C (Maniere and Dimick 1979). Low boiling flavor compounds decreased 26% during 60h of conching (Mohr 1959). Comparing the volatiles before and after conching in dark chocolate, most volatiles increased during conching at 70-80°C (Counet and others 2002). However, acetic, isobutyric, and isovaleric acid declined during 44h conching in a Suchard conche (Hoskin and Dimick 1979). The volatile acidity decreased in chocolate with different roasting temperature after 7h conching (Nebesny and Rutkowski 1998). In a sensory study, a preference was found for milk chocolate with a longer conching time (Prawira and Barringer 2009). Most studies on conching were performed with heat, and they provided the conflicting results which conching may either increase or decrease volatiles (Maniere and Dimick 1979; Counet and others 2002). The changes of volatiles during conching with heating may be complex because some chemical reactions may occur due to the high temperature, such as the Maillard reaction and caramelization. To eliminate these variable factors caused by heat, the conching process was performed at room temperature in this study.

Chocolate is a microbiologically stable product, and the sensory changes may occur before microbiological (De Melo and others 2009). Studies showed that chocolate can be stored at least 9 months without sensory properties change (De Melo and others 2009).

However, the volatiles may change during storage. A study showed that volatiles decreased at first two month and then increased in cocoa substitute flavor during six month measuring (Fadel and others 2006). Also, an increase in aldehydes, ketones, alcohols, and alkanes with a decrease in pyrazines was reported after 12 months storage of dark chocolate (Mexis and others 2010). Most of storage studies were focus on the shelf life, sensory, and few of them studied volatile change. Moreover, blooming is an appearance defect occurs during storage, but none of previous study has reported the volatile change during blooming.

Chocolate volatiles have been investigated in many studies and these studies were mostly based on the headspace analysis. However, the volatiles release during eating is more complex since it is affected by several factors, such as temperature, dilution effect, length of time the food in the mouth, melting of the fat, and inversion of emulsion phases (Taylor and Linforth 1996). The absorption of volatiles by mucus lining may also decrease the volatile concentration (Linforth and others 2002). Volatiles in the breath are lower than in the headspace, and breath exhaled by mouth contains 8-fold more volatiles than exhaled by nose (Linforth and others 2002). To obtain better represent of the volatiles reaching the olfactory epithelium, direct mouth and nose measurements are preferred (Piggott and Schaschke 2001). Few of previous research has determined the volatile changes during eating chocolate. Since sugar and milk solids may affect the flavor of chocolate, three types of chocolate (unsweetened dark, dark, and unsweetened milk chocolate) were compared.

The aim of this study is to investigate the chocolate volatile changes caused by different factors in different types of chocolate by SIFT-MS (Selected Flow Tube-Mass Spectrometry).

Chapter 2: Literature review

2.1 Chocolate

Cocoa beans, the seeds of *Theobroma cacao*, are the major ingredient of chocolate. Before being introduced to Europeans, cacao trees were grown by the Aztecs of Mexico who used the beans to produce a flavored drink named "chocolatl" (Beckett, 1994). In 1502, Christopher Columbus first brought cocoa beans to Spain. The Spaniards added sugar into the drink to cover the bitterness, and in this form it became well-liked and spread from Europe to America (Dimick and Hoskin, 1981). In 1828, Van Houten of Holland developed a method to extract fat from cocoa beans. The fat is known as cocoa butter which is generally added into chocolate to make chocolate melt easily so the mouth feel is enhanced. Daniel Peters, in 1876, developed the solid form of milk chocolate which nowadays has become the most popular chocolate product (Beckett, 1994).

In general, cocoa beans need to go through several processing steps including fermentation, drying, roasting, winnowing, grinding, conching, tempering, and packaging. During fermentation, the precursors of the Maillard reaction, free amino acids and reducing sugars, are formed. Those precursors react during roasting and form numerous flavor compounds in cocoa beans. After cocoa beans are made into cocoa mass, conching makes the cocoa mass smoother, removes undesired flavor and is an important process to both texture and flavor of chocolate. Tempering is the final process which can prevent fat blooming in chocolate.

2.2 Identification of volatile compounds

Generally, flavor studies are involved in researching the volatile compounds in food. Human beings can sense flavor by smell due to the stimulation of olfactory receptors in the nasal cavity by the volatiles. However, the human olfactory system is much more sensitive than analytical equipment, which causes difficulties in the volatile compound identification in the food. Flavor compound identification can be traced back more than a hundred years. Benzaldehyde was the first flavor compound identified by Robiquet and Boutron-Charlard from bitter almonds in 1832 (Pickenhagen 1999). Also, scientists have been investigating chocolate flavors for years. The study of the constituents of chocolate can be traced back approximately one hundred years. Bainbridge and Davies (1912) identified linalool, esters, and some fatty acids from 2000 kg of cocoa nibs.

The popularity of chocolate is due to its special attractive aroma contributed by numerous volatile aromatic compounds. Those volatile aromatic compounds are aldehydes, pyrazines, esters, acids, furans, ketones, hydrocarbons and other chemical compounds. So far, over 600 volatile compounds have been identified in chocolate products (Counet and others, 2002). However, since the composition of chocolate is complex, new volatile compounds are still being discovered. Chocolate flavor is affected by several factors including the cultivar of cocoa beans and the manufacturing processes, such as

fermentation, roasting, and conching. Those three processes are believed to have a significant influence on the chocolate aroma.

2.2.1 Important volatile compounds in dark chocolate

Important compounds are presented in Table 2.1 covering aldehydes, pyrazines, acids, esters, furans, ketones, phenols, pyrroles, alcohols, and also compounds reported with highest flavor dilution factors, including phenylacetaldehyde, 2-ethyl-3, 5dimethylpyrazine, 2- and 3-methylbutanoic acid, dimethyltrisulfide, 4-hydroxy-2, 5dimethyl-3(2H)-furanone, phenylacetaldehyde, and 3-hydroxy-4, 5-dimethyl-2(5H)furanone (sotolone) (Frauendorfer and Schieberle 2006).

2.2.1.1 Aldehydes

Aldehydes are one of the main groups formed from the roasting process in chocolate manufacture (Ramli and others 2005). Several aldehydes have been reported as crucial compounds in cocoa flavor including cocoa mass, chocolate, and cocoa beans, including (*E*)-2-nonenal, (*E*)-2-octenal, 2-furaldehyde, 2-methylbutanal, 2-methylpropanal, 3-methylbutanal, 5-methyl-2-phenyl-hexenal, acetaldehyde, benzaldehyde, hexanal, and phenylacetaldehyde (Counet and others 2002; Schnermann and Schieberle 1997; Praag and others 1968; Frauendorfer and Schieberle 2006).

Among these important aldehydes, some aldehydes contributed strong chocolate note, including 3-methylbutanal, 2-methylpropanal, 2-methylbutanal, acetaldehyde, isobutyraldehyde, benzaldehyde, phenylacetaldehyde, 5-methyl-2-phenyl-2-hexenal, and 2furaldehyde. 3-Methylbutanal have been reported as important volatile in cocoa beans, cocoa powder, cocoa mass, dark chocolate, and milk chocolate (Counet and others 2002; Schnermann and Schieberle 1997; Praag and others 1968; Frauendorfer and Schieberle 2006). The mixture of 3-methylbutanal and methyl disulfide was also observed to contribute a cocoa-like odor in roasted cocoa beans (Praag and others 1968). 5-Methyl-2-phenyl-2-hexenal was first identified in roasted cocoa beans, described as "a deep bitter persistent cocoa note" (Praag and others 1968).

Other aldehydes reported as key compounds were due to their high odor activities or flavor dilution factor, such as (E)-2-nonenal, (E)-2-octenal, hexanal in cocoa mass, (E,E)-2, 4-nonadienal, (E,E)-2, 4-decadienal, (Z)-2-decenal in milk chocolate identified by aroma extract dilution analysis (Schnermann and Schieberle 1997). Phenylacetaldehyde and 3-(Methylthio)propionaldehyde were strongly perceived at the sniffing port in milk chocolate, and phenylacetaldehyde was also reported as odor-active compound in dark chocolate (Frauendorfer and Schieberle 2006; Schnermann and Schieberle 1997).

2.2.1.2 Pyrazines

Pyrazine is one of major group formed in the Maillard reaction during roasting. Among 525 identified volatiles in roasted cocoa bean, one fifth of them were predominate pyrazine fractions (Schnermann and Schieberle 1997). Five pyrazines have been identified as important aroma compounds by previous studies (Table 2.1). 2-Ethyl-3, 5dimethylpyrazine had been reported as high odor-active and high flavor dilution factor contributing earthy and potato chip-like odors, and it was important to cocoa powder, cocoa mass, and milk chocolate (Schnermann and Schieberle, 1997; Frauendorfer and Schieberle, 2006). 2-Methoxy-3-isopropylpyrazine and 2, 3-diethyl- 5-ethylpyrazine were identified as important compounds, because of their high odor activities and flavor dilution factors from cocoa mass as well as milk chocolate (Schnermann and Schieberle 1997). 3 (or 2), 5-Dimethyl-2-ethylpyrazine, 3, 5(or 6)-diethyl-2-methylpyrazine, and trimethylpyrazines were reported to contribute praline and chocolate flavors, and the present of ethyl group in the first two pyrazines indicated the importance of alanine and its Strecker aldehyde, acetaldehyde, in chocolate flavor synthesis. Tetramethylpyrazines was the most plentiful pyrazine found in dark chocolate, and it provided milk coffee-mocha-roasted flavor (Counet and others 2002).

2.2.1.3 Acetic acid

Acetic acid is an important compound which has a significant effect on chocolate aroma, since it may provide undesired flavor, vinegar like odor. Acetic acid is the one of the most plentiful odorants in chocolate (Frauendorfer and Schieberle 2006). The volatile acids can be removed by processing, such as conching. A significant reduction (about 40%) of acetic acid was reported in both low and high roast chocolate at 24h in a Suchard conche with conching temperature 82°C, but discovered an increase in acetic acid at 44h in low roast chocolate (Hoskin and Dimick 1979). They assumed that the acetic acid was produced from oxidation of aldehydes and alcohols and released from esters by mechanical and/or thermal mechanisms during the long conching process. Acetic acid also was found a significant decrease after went through spinning disc reactor, an alternative process of conching, at 100 and 120°C of disc temperature (Akhtar and others 2010).

8

2.2.1.4 Others

Acids, esters, furans, ketones, phenols, and pyrroles also have been reported as important compounds, although not as much as aldehydes, pyrazines and acetic acid. 2-Methylbutanoic acid and 3-methylbutanoic acid were identified as important compounds due to their high odor intensities in cocoa mass (Schnermann and Schieberle 1997). They are the acidic form of 2-methylbutanal and 3-methylbutanal which are key aldehydes in chocolate flavor. Other than the acids mentioned above, butanoic acid, pentanoic acid, hexanoic acid, phenylacetic acid, propanoic acid, and methylpropanoic acid were reported as most odor-active compounds in milk chocolate, cocoa beans, and cocoa powder (Schnermann and Schieberle 1997; Frauendorfer and Schieberle 2008; Frauendorfer and Schieberle 2006). 2-Methyl-3-(methyldithio)furan was a key odorant in cocoa mass (Schnermann and Schieberle 1997).

Dark chocolate	Compound	Reference	Flavor dilution factor	Odor description
Aldehydes	(E)-2-nonenal	а	256	green, fatty
	(<i>E</i>)-2-octenal	a	512	fatty, waxy
	2-furaldehyde	b		
	2-methylbutanal	c, d	512 ^c , 1024 ^d	chocolate ^c , malty ^d
	2-methylpropanal (isobutyraldehyde)	b, c	2048 ^c	chocolate ^c
	3-methylbutanal (isovaleraldehyde)	a, b, c, d	1024 ^a , >4096 ^c , 1024 ^d	malty ^{a,d} , chocolate ^a
	5-methyl-2-phenyl- 2- hexenal	b		
	Acetaldehyde	b		
	benzaldehyde	b		
	Hexanal	a	512	green
	phenylacetaldehyde	b, c, d	>4096c, 1024 ^d	flowery ^c , honey ^{c,d}
Pyrazines	2, 3-diethyl- 5- methylpyrazine	a	256	potato chip-like
	2-ethyl-3, 5- dimethylpyrazine	a, c, d	32 ^c ,256 ^a , 2048 ^d	potato chip-like ^a , earthy ^d , roasted ^c , smoky ^c , praline ^c , rum ^c
	2-methoxy-3- isopropylpyrazine	a	512	earthy, bean
	trimethylpyrazines	с	256	cocoa, roasted, green
Acids	2-methylbutanoic acid	a, d	2048 ^a , 4096 ^d	sweaty ^{a,d}
	2-phenylacetic acid	d	1024	
	3-methylbutanoic acid	a, d	2048 ^a , 4096 ^d	sweaty ^{a,d}
	acetic acid	a, b, e		sour
Esters	isopentyl acetate	b		

Table 2.1 Compounds identified as important odorants by previous research

Continued

Table 2.1 continued

Furans	2-methyl- 3(methyldithio)furan	a	512	cooked meat-like
	4-hydroxy- 2, 5-dimethyl- 3(2H)- furanone	d	4096	
	furaneol	с		
Ketones	2-acetyl-1-pyrroline	d	1024	popcorn-like
Phenols	vanillin	c		
Pyrroles	acetylpyrrole	с	64	cocoa, chocolate, hazelnut, roasted
	furfurylpyrrole	с	128	roasted, chocolate, green
	methyl disulfide	b		
Milk chocolate		Reference	Flavor dilution factor	Odor description
Aldehydes	3-methylbutanal	а	1024	malty
	(E,E)-2, 4-nonadienal	a	512	fatty, waxy
	(E,E)-2, 4-decadienal	a	512	fatty, waxy
	(E,Z)-2, 6-nonadienal	a	512	cucumber-like
	(Z)-2-nonenal	a	512	green, tallowy
	(E)-2-nonenal	а	256	green, fatty
	(E)-2-nonenal (E)-2-octenal	a a	256 128	green, fatty fatty, waxy
	(E)-2-nonenal (E)-2-octenal phenylacetaldehyde	a a a	256 128 256	green, fatty fatty, waxy sweet, honey-like
Pyrazines	(<i>E</i>)-2-nonenal (<i>E</i>)-2-octenal phenylacetaldehyde 2-ethyl-3, 5- dimethylpyrazine	a a a a	256 128 256 1024	green, fatty fatty, waxy sweet, honey-like potato chip like
Pyrazines	 (E)-2-nonenal (E)-2-octenal phenylacetaldehyde 2-ethyl-3, 5- dimethylpyrazine 2, 3-diethyl-5- methylpyrazine 	a a a a	256 128 256 1024 512	green, fatty fatty, waxy sweet, honey-like potato chip like potato chip like

Continued

Table 2.1 continued

	2- and 3-methylbutanoic			
Acids	acid	a	512	sweaty
	ethyl cinnamate	0	256	sweet cinnamon like
	curyr chinainaic	a	250	sweet, emilamon-like
Esters	R-δ-decalactone	a	512	sweet, peach-like
	R-δ-octenolactone	а	256	sweet, coconut-like
	γ-decalactone	а	256	sweet, peach-like
	R- γ-nonalactone	а	128	sweet, coconut-like
	ethyl phenylacetate	а	128	sweet, waxy
Furans	4-hydroxy-2, 5- dimethyl- 3(2H)- furanone	a	128	caramel-like
	2-methyl-3- (methyldithio)-furan	a	512	cooked meat-like
	3-hydroxy-4, 5-dimethyl- 2(5H)-furanone (sotolone)	a	128	seasoning-like, spicy
Ketones	5-methyl-(<i>E</i>)-2- hepten-4- one	a	512	hazelnut-like
	1-octen-3-one	а	512	mushroom-like
	2, 3-butanedione	а	128	buttery
Phenols	vanillin	a	>1024	vanilla-like
Sulfur compounds	dimethyl trisulfide	a, d	256	sulfurous
Alcohol	2-Phenylethanol	a	128	sweet, yeast-like

a: Schnermann and Schieberle (1997). b: Praag and others (1968). c: Counet and others (2002). d: Frauendorfer and Schieberle (2006). e: Hoskin and Dimick (1979)

2.2.2 Important volatile compounds in milk chocolate

Literature has identified important volatile compounds in milk chocolate, and the key odorants which had significant differences in flavor dilution factor in milk chocolate and cocoa mass were compared (Schnermann and Schieberle 1997). From the literature, nine compounds are important odorants in milk chocolate but not in cocoa mass, and they are vanillin, R- δ -decalactone, 5-methyl-(*E*)-2-hepten-4-one, 1-octen-3-one, (*E*,*E*)-2, 4- nonadienal, dimethyl trisulfide, 4-hydroxy-2, 5-dimethyl-3(2H)-furanone, (*E*,*E*)-2, 4- decadienal, (*Z*)-2-decenal. In both milk chocolate and cocoa mass, 3-methylbutanal, (*E*)-2- octenal, 2-ethyl-3, 5-dimethylpyrazine, 2, 3-diethyl-5-methylpyrazine, (*E*)-2-nonenal, 2- methyl-3-(methyldithio)-furan, and 2- and 3-methylbutanoic acid were identified as crucial compounds (Schnermann and Schieberle 1997). Acetic acid, butanoic acid, pentanoic acid, hexanoic acid, and phenylacetic acid were reported as most odor-active compounds in milk chocolate (Schnermann and Schieberle 1997). Vanillin is a common odorant additional added in chocolate. Although it does not appear in chocolate naturally, it is still an important contributor to milk chocolate (Schnermann and Schieberle 1997).

2.3 Volatile compound changes by different factor

2.3.1 Sugar

In chocolate manufacture, sucrose is the most common sugar used. The main purpose of adding sugar in the chocolate is to provide the sweetness and cover the bitter taste. The research showed that when sucrose content increased, the chocolate flavor and the

acceptability of chocolate were significant increased; in contrast, the bitterness was decreased (Prawira and Barringer 2009). Sugar may affect the volatiles in food by retaining, increasing or decreasing volatiles. In dehydrated food, sucrose has been demonstrated effective in volatile retention. In a freeze-dried model system, sucrose had highest ability to retain the organic volatiles including acetone, methyl acetate, 2-propanol, *n*-butanol, and tert-butanol compared with other sugars (Flink and Karel 1970). In headspace analysis, sucrose also has been shown to influence the volatility of flavor compounds. Generally, the addition of a solute may increase the volatility of volatile substance in solution because of the salting-out effect. Increasing sucrose may have considerable effect on the volatility of compounds (Godshall 1995). A significant increasing of menthone was reported in the headspace of 40% sucrose solution compared to 5 and 20% sucrose solution and to water. Also, a significant elevated headspace concentration values was observed in 20 and 40% sucrose with 10 and 20 ppm isoamyl acetate solutions; however, there was no significant effect of sucrose in the perceived aroma intensity results (Ebeler and others 1988). A increasing of acetone in headspace was observed as the sucrose concentration was increased from 0 to 60% in aqueous solutions (Nawar 1971). The increasing of volatility of *n*-hexyl acetate was also observed when sucrose concentration increased (Massaldi and King 1973). However, sucrose may also depress volatile compounds in the headspace. The partial vapor pressure of some compounds decreased in the strawberry aroma solution with 73.1% invert sugar compared with non-sugar solution while other compounds increased (Wientjes 1968). Heptanone and heptanal were found a significant decrease in headspace when sucrose concentration

increased in aqueous solutions (Nawar 1971). *d*-Limonene and *n*-butylbenzene decreased in volatility when sucrose concentration increased. The studies on the volatility of flavors affected by sucrose gave the conflict results. The probability of volatiles decrease by sucrose may due to the intermolecular associations or complexation between the volatiles and the solute which is sucrose (King 1983). The polarity of the volatiles may also be a factor drives the results of volatile concentration changes. A pattern was concluded that the more polar volatiles, such as acetone, isopentyl acetate, and ethyl acetate, increase when sucrose concentration increases whereas nonpolar volatiles, such as 2-heptanone, 2-heptanol, and butylbenzene, were depressed (Roberts and others 1996).

2.3.2 Milk solids

Milk chocolate is the most popular chocolate product. The added milk solids in chocolate provide milky flavor, as well as caramel flavor if the Maillard reaction occurred. Not only improves the flavor of chocolate, but adding milk solids also improved the texture due to the additional milk fat by smooth the chocolate. Research has shown that higher milk solids content was preferred by consumers (Prawira and Barringer 2009). The major compounds in non fat milk solids are proteins (36%), mainly casein. Proteins in the food may interact with flavor compounds and cause perceived taste and aroma to be reduced (Ebeler and others 1988). Caseinates has been proved that has higher ability to retain volatiles compared with whey protein in yoghurt (Saint-Eve and others 2006). In 5% soy protein solution, aldehydes and ketones showed significant interaction with protein and retention of volatiles (Gremli 1974). A reduction of headspace concentration of menthone solution with increasing albumin from 12-50% was reported (Ebeler and others 1988). In

perceived aroma intensity results, a significant decrease of menthone in egg albumin solution was discovered compared to water. A decrease of volatilities of diacetyl, ethyl butanoate, and ethyl hexanoate were discovered at 50g.litre⁻¹ of sodium caseinate aqueous solution compared with 5g.litre⁻¹ (Landy and others 1995).The interactions between proteins and flavors are varied by the type of protein and flavors.

The fat in the milk may also affect the flavor of chocolate. Research showed that the increasing of milk fat in chocolate ice cream may decrease the volatile compounds (Welty and others 2001). In the research, 0.6% milk fat ice cream had higher concentration of benzaldehyde and phenylacetaldehyde than the 9.0% milk fat ice cream, and released more mixture of 2-ethyl-3, 5-dimethyl and tetramethylpyrazines than 6.0 and 9.0% milk fat chocolate ice cream.

2.3.3 Conching

In chocolate manufacture, conching is a process involved mixing chocolate ingredients, such as cocoa mass, with aeration and heat in a tank for hours or days. During conching process, chocolate is rolled and kneaded by the rollers and rotational mechanisms. The first conching method was originated by Rudolf Lindt in 1879, and since then, finely ground, fat-based chocolate has been developed (Dimick and Hoskin, 1981). Conching is named after the original sea shell like machine, the conche. Typically, conching involved physical and chemical changes, such as reduction of viscosity and moisture content, volatile evaporations, oxidations of volatile compounds (Pratt-Johnson 1987; Hoskin and Dimick 1979). Those changes improve the texture and flavor of chocolate, which are two main purposes to conch chocolate. The flavor changes can be affected by the degree of agitation, conching temperature, and aeration exposure (Robert and Martin 1987). Conched chocolate is regarded as mellower than unconched and has less bitterness and acidity (Stauffer 2000). Researchers believe that conching improves the flavor of chocolate by reducing the undesirable volatile flavors, such as acids. Conching not only removes the undesired volatile compounds but also retains desired flavors (Stauffer 2000).

2.3.3.1 Flavor changes during conching

Research showed that the chocolate flavor was altered by conching process. The volatile acidity was shown to decrease during 8h conching process in cocoa mass with different roasting temperature (110°C, 135°C, and 150°C) and subjected to secondary fermentation. The volatile acidities were from acidity equal to 0.156% of acetic acid to 0.132%, 0.162% to 0.120%, 0.138% to 0.120%, and 0.120% to 0.100%, respectively (Nebesny and Rutkowski 1998). The de-fatted Accra and Arriba chocolate were analyzed by gas chromatography, and a 26% reduction of low boiling flavors in plain Arriba chocolate during 60h conching was reported (Mohr 1959). The odor intensity was also found to be reduced 20% during conching measured by STOD, the differential olfactometer (stimulateur olfactometre differentiel) (Plumas and others 1996). An overall reduction of volatile compounds was reported in semi-sweet chocolate (Maniere and Dimick 1979). The chocolate made from high and low temperature roasted cocoa beans lost 66% and 79%, respectively, of the volatile compounds during conching.

On the other hand, another study conducted by Counet and others (2002) shows that the volatile compounds may increase during conching. Except Strecker aldehydes, over half of the volatile compounds which they identified increased in concentration during conching (Table 2.2). The reduction of Strecker aldehydes is due to volatile evaporation and chemical reactions (Counet and others 2002). The conching temperature they used was around 70-80 °C. The Maillard reaction can occur during conching with protein in milk chocolate and form volatiles (Pontillon 1995). The significant increase of 2-phenyl-5methyl-2-hexenal may due to aldol condensation of phenylacetaldehyde and 3methylbutanal followed by dehydration (Counet and others 2002).

		concentration(ppb)			
Compounds	Concentration changes after conching	sample A		Sample B	
Alcohols		Before conching	After conching	Before conching	After conching
1-pentanol	-	121	145	67	58
2-heptanol	-	-	-		
benzyl alcohol	↑	119	190	81	108
3, 7-dimethyl-1, 6- octadien-3-ol	Ļ	8516	5698	9630	5720
2-phenylethanol	↑	839	1728	800	7277
Aldehydes					
2-methylpropanal	\downarrow	18787	13030	10450	9500
3-methylbutanal	\downarrow	19371	12454	11700	8400
2-methylbutanal	\downarrow	11951	10800	7800	7660
2-methyl-2-butenal					
3-(methylthio) propionaldehyde	Ļ			49	45
heptanal					
benzaldehyde	↑ (2098	2500	710	970
phenylacetaldehyde	\downarrow	7228	4468	3380	1660
nonanal	\downarrow	247	124	215	150
2-phenyl-2-butenal					

Table 2.2 Concentrations of identified compounds in chocolate A and B before and after conching by Counet and others (2002)

Continued

Table 2.2 continued

2-phenyl-5-methyl-2- hexenal	¢	251	496	307	546
Esters					
ethylbenzoylformate					
ethylbenzoate					
ethyloctanoate	↑	32	100	8	15
2-phenylethyl acetate	\downarrow	198	102	53	41
Furans					
dihydro-2-methyl-3(2 <i>H</i>)- furanone	↑	172	215	125	162
furancarboxaldehyde	<u>↑</u>	1292	1398	457	566
furfuryl alcohol	<u>↑</u>	1247	1972	920	1238
1-(2-furanyl)ethanone	<u>↑</u>	218	230	40	46
5-methyl-2- furancarboxaldehyde	↑	215	325	104	184
5-ethenyltetrahydro-R, R 5- trimethyl- <i>cis</i> -2- furanmethanol					
3-phenylfuran					
2, 5-dimethyl-4-hydroxy- 3(2H) furanone	↑	584	1034	1680	5670
Hydrocarbons					
methylbenzene		-	-	-	-
Ketones					
2, 3-butanedione	Ļ	857	170	1093	400
2.3.3.2 Sensory properties influence by conching

Generally, conched chocolate provides smoother texture, less acidity and bitterness. Sensory test has shown that longer conching time results in smoother texture and smaller particle size, and panelists preferred longer conched samples (21h and 76h) compared with short conching time samples (8h). However, there was no significant difference in preference between 21 and 76h (Prawira and Barringer 2009). The results showed the similar trends with the results reported by Maniere and Dimick (1979), which has shown that no significant difference between 24h and 44h samples. Their results have also shown that 24h and 44h were significant different to 0h in preference; however, there was no significant difference found between 0h and 6h or 0 and 12h. The results suggested that there was no significant flavor change in the first 12h but flavor showed to be refined after 24h or 44h (Maniere and Dimick 1979).

2.4 Selected ion flow tube-Mass spectrometry (SIFT-MS)

Nowadays, most flavor identifications are analyzed by gas chromatography-mass spectrometry (GC/MS) due to the good ability of separation and sensitivity. However, sample preparation, such as extraction and concentration of aroma, are required when using gas chromatography-mass spectrometry. During sample preparation, chemical changes may occur, such as fermentation, enzyme or microbially induced changes, and oxidative changes in long isolation procedures (Reineccius 2006). A method, selected ion flow tube mass spectrometry (SIFT-MS), does not require sample preparations has been developed since 1976 (Adams and Smith 1976). The principle of SIFT-MS is reacting volatiles in the

sample with selected precursor ions and forms the product ions. The volatiles are identified and quantified by analyzing these product ions using a quadrupole mass spectrometer and particle multiplier. The selected precursors usually used are H_3O^+ , NO^+ , and O_2^+ , because they can react rapidly with most volatiles and numerous inorganic molecules, but they do not react with the major compounds in the air (N₂, O₂, H₂O, Ar and CO₂) (Spanel and Smith 1999). The SIFT-MS process contains five major steps (Syft Technologies Ltd. 2007). (1) SIFT-MS produces positive ions by using a microwave discharge or radio frequency source. (2) Other than selected precursor ions, all ions are removed by a quadrupole mass filter in an upstream chamber. (3) After selecting ions, the precursor ions pass through a venturi and react with sample volatiles in a reaction chamber (flow tube). (4) The product ions are selected in a downstream chamber by a second quadrupole mass filter. (5) The selected product ions are detected and quantified by a particle multiplier. The advantage of SIFT-MS is that it can measure the sample in real time without sample preparations. It can also quantify trance volatiles to ppb levels (Spanel and Smith 1999). However, the machine required established data base to quantified volatile compounds, so compounds are not in the data base cannot be identified. Since different volatiles can generated the same mass/charge (m/z) value, conflicts between compounds are usual and need to be removed.



- a. Generation of ions
- b. Ion selection
- c. Sample introduction and reaction
- d. Selection of reaction products
- e. Detection

Figure 2.1 The simplify structure of SIFT-MS (Syft Technologies Ltd. 2007).

Chapter 3: Materials and Methods

3.1 Cocoa mass preparation

Cocoa mass was made from 2kg of fermented cocoa beans from Panama (Trinatario, 2008/2009, Chocolate Alchemy, Oakland, OR, USA). The cocoa beans were roasted for 18 min at 149°C in a convection oven (Doyon model JA 14, Dayton, Ohio, U.S.A.) and cooled at room temperature for 10 min. The cooled cocoa beans were cracked by a cocoa mill (Crankandstein, Atlanta, GA, USA), and an air gun was used to remove the shells of the cracked cocoa beans. The cracked cocoa nibs were ground (Champion, Plastaket Meg. Co., Inc., California, USA) at 1725 R.P.M. to produce cocoa mass. The cocoa mass was cooled and solidified at room temperature for 3h and cut into small cubes. The solid chocolate was preserved in plastic bags in the freezer at -18°C until testing.

3.2 Conching measurement

Before conching, the frozen cocoa mass and cocoa butter were melted using a water bath (Circulating bath model 260, Precision Inc., Winchester, Va.) at 50 °C for 30 min. The melted cocoa mass and cocoa butter (Chocolate Alchemy, Oakland, OR, USA) were added first, and sugar and milk solids were added after the machine started. The formulation of the chocolate was: 83% cocoa mass and 17% cocoa butter for unsweetened dark chocolate (UDC), 50% cocoa mass, 40% sugar, 10% cocoa butter for dark chocolate (DC), and 50% cocoa mass, 40% milk solids, 10% cocoa butter for unsweetened milk chocolate (UMC). The same cocoa mass to cocoa butter ratio in all types of chocolate, and the same percent of cocoa mass in dark chocolate and unsweetened milk chocolate were used.

In each batch, 1kg total chocolate was made. This 1kg chocolate was conched by a chocolate melanger (Alchemist's stone chocolate melanger, SantaUSA, Hickory, NC) for 48 h. During conching, the volatile concentrations were measured by Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) (VOC 100, Syft Ltd, Christchurch, New Zealand). The volatile compounds were collected by a 14ga (15cm) needle. The needle entered through a hole in the center of the lid and the tip of the needle was 3 cm under the lid in the top of headspace of the conche (Figure 3.1). For the first 8 h, the volatile concentration was measured every hour, and then the chocolate was left to conch overnight. After the first 8 h, the volatile concentration was measured at 24, 28, 32 and 48 h. Four measurements at each time were recorded. The first measurement was discarded because it was always significantly lower than the others. The room air was measured at the same position of the conche before conching started to provide a background level to be subtracted from the measured results. The 1h measurement was assumed as the start point.



Figure 3.1 the position of the needle in the conche

3.3 Storage measurement

Dark chocolate and unsweetened milk chocolate were conched for 24h, and 50g placed into a 250mL storage bottle. These bottles were stored at 4°C, 25°C, and 50°C, and 5 bottles were stored at each temperature for each sample. One day before measuring, samples were moved to a hood overnight to remove the accumulated volatiles in the bottles. Samples were transferred to a 50°C water bath for 2h with top screw caps and polytetrafluoroethylene-faced silicone septa (Pyrex 1395-45TS, Corning, N.Y.) on to make the headspace volatiles achieve equilibrium condition. The samples were measured weekly for 8 weeks.

3.4 Blooming measurement

One batch of chocolate was divided in half to make the tempered and untempered chocolate. Seed chocolate was added to help crystal formed. For the untempered chocolate, 30g seed chocolate (Hershey's special dark, Hershey Co., Hershey, PA) was melt first in the tempering machine (Revolution 2, Choco-vision Corp., Poughkeepsie, NY) and then 265g cocoa mass was added and melt. The end temperature of the chocolate was 42°C. For the tempered chocolate, 265g cocoa mass was placed in the tempering machine and heated from room temperature to reach 42°C, and then the 30g seed was added. After adding the seed, the temperature dropped gradually, and the final temperature was 31.5°C. The samples were transferred 50g in each 250ml storage bottle and then sat at room temperature for few days to wait for blooming occurred. Each bottle was put on an open top screw cap with a polytetrafluoroethylene-faced silicone septum before headspace analysis. Five measurements were measured for each batch.

3.5 SIFT-MS analysis

3.5.1 Headspace analysis

Headspace volatile compounds in each bottle were measured by SIFT-MS (Voice 100, Syft Technologies). The SIFT-MS instrument has been described by Smith and Spanel (2005). An 18ga (3.8cm) passivated collection needle was used to collect volatiles in the headspace, and a 14ga (15cm) needle was used to create circulation. The short needle pierced the center of the septum and was placed in the top of the headspace (about 5 cm

below the cap). The long needle was placed in the middle of the headspace which was about 7 cm under the cap (Figure 3.2). Samples were placed in a 250ml Pyrex brand storage bottle during headspace analysis.



Figure 3.2 The position of the needles in the bottle

3.5.2 Mouthspace and nosespace analysis

Volatiles in mouthspace and nosespace were collected by piercing the 18ga passivated collection needle into a 7cm plastic straw at 1.5cm from the end, and exhaling volatiles into straw (Figure A.1).

Samples (unsweetened dark, dark, unsweetened milk chocolate) in bottles (2.5g) were put in a 37°C water bath for 1h, and measured headspace. After measuring the headspace, sample was placed into the mouth with minimal delay, and then 3 exhales were made, 10s for each. Volatiles in the mouth before swallowing (MSb) were measured at this time. During this period, no swallowing was allowed. After 3 exhales, the sample was swallowed and the straw was changed from mouth to right nostril, and the left nostril was blocked by hand in order to prevent volatile leakage. Three exhales from nose were made, and volatiles in the nosespace (NS) were measured at this time. After examining volatiles in nosespace, the straw was moved to mouth and measured 3 exhales again. Volatiles in the mouth after swallowing (MSa) were test in this period. Before measuring samples, volatiles in the mouth were examined to give a background level to be subtracted from the measured values. Three replicates were analyzed for each type of chocolate. The mouthspace and nosespace analysis was conducted by an age 25 female.

Two methods with different precursor ions (O_2^+ and NO^+) were used to analyze volatiles. The compounds reacted with O_2^+ were including 2, 3-diethyl-5-methylpyrazine, vanillin, methanol, acetone, benzaldehyde, 2, 5-dimethylpyrazine, tetramethylpyrazines, 2-phenylethanol, nonanal, 2-methylbutanal, 2-methylpropanal, 2-nonanone, 3-methylbutanal. The compounds used NO⁺ as the precursor ion were including benzyl alcohol, acetone, methional, 3-methylbutanoic acid, phenylacetic acid, acetic acid, 2-heptanol, acetophenone, 2, 3-butanedione, benzonitrile.

The average concentration of volatiles in the mouth before swallowing (MSb) was divided by the average concentration of volatiles in the headspace (HS) to calculate the ratio of mouthspace before swallowing to headspace (MSb/HS). The ratio of mouthspace before swallowing to mouthspace after swallowing (MSb/MSa) and nosespace to headspace (NS/HS) were also calculated by their average concentrations. The log P values were calculated by an online program, ALOGPS 2.1, from Virtual Computational Chemistry Laboratory.

3.5.3 Performance condition

Analysis of SIFT-MS was performed using selected ion mode (SIM). The quantification of volatile concentration is based on known kinetic parameters (Table 3.1). Utilizing the rate coefficients, k, of the volatile with the precursor ion, and the known ratios of product to precursor ion count rates, the concentrations of the sample volatiles can be calculated (Spanel and Smith 1999). Each morning, the instrument was calibrated with a given concentration of benzene, toluene, ethylbenzene, and xylene.

The SIFT-MS quantifies the volatile concentration by counting the masses of the product ions. If compounds generate different product ions with the same mass after reaction (Table 3.1), the SIFT-MS can not quantify the concentration for each compound separately. In this study, to avoid conflicts, the precursor ion for each volatile was carefully chosen, and compounds with conflicts were eliminated or reported as mixture, including methylpropanals (mixture of 2- and 3-methylpropanal), mixture of 2- phenylethanol and phenylacetaldehyde, decadienals (mixture of (E,Z)-, (E,E)-, 2, 4-, 2, 6-decadienal), dimethylpyrazines (mixture of 2, 3-, 2, 5-, 2, 6-dimethylpyrazine), mixture of furaneol and sotolone, mixture of isobutanoic and butanoic acid, methylbutanals (mixture of 2-, 3-methylbutanal), nonadienal (mixture of (E,Z)-,(E,E)- 2, 6-, 2, 4- nonadienal), nonenal (mixture of (E)-,(Z)-2-nonenal), mixture of phenylacetaldehyde and acetophenone, mixture of phenylacetic acid and phenylacetaldehyde, and mixture of γ -octalactone and 2-nonanone.

The parameters of the SIFT-MS were set as following: sample scan time 110s, calculation delay time 5s, product sample period, 100ms, precursor sample period 20ms, heated inlet temperature 120°C, carrier gas argon pressure 200kPa, helium pressure 30 psi, inlet flow rate 120 cm³/min. The flow tube vacuum pressure was 0.038 ± 0.003 Torr.

3.6 Statistical analysis methods

Data was analyzed by One-Way ANOVA and Student's t-test using SPSS, version 18.0 (SPSS Inc., Chicago, IL, USA). The significant difference appears when p < 0.05.

Compound	Reagent	Branching ratio (%)	m/z	k (10 ⁻⁹ cm ³ /s)	Product ion	Reference
(E)-2-octenal	NO ⁺	80	125	4.1	$C_8H_{13}O^+$	Spanel and others 2002
2, 3-butanedione	NO ⁺	65	86	1.3	$C_4H_6O_2^+$	Spanel and others 1997
2, 3-diethyl-5- methylpyrazines	O ₂ ⁺	100	150	2.5	$C_9H_{14}N_2^+$	Syft 2009
2-acetylpyrrole	H ₃ O ⁺	100	110	3.3	C ₆ H ₇ NO.H ⁺	Syft 2009
2-heptanol	NO ⁺	100	115	2.4	$C_7H_{15}O^+$	Wang and others 2004a
2-heptanone	NO ⁺	100	144	3.4	$C_7H_{14}O.NO^+$	Smith and others 2003
2-nonanone	O ₂ ⁺	30	142	3.2	$C_9H_{18}O^+$	Smith and others 2003
2-phenylethanol and phenylacetaldehyde	O ₂ +	75	92	2.4 2.5	C ₇ H ₈ ⁺	Wang and others 2004c
3-methylbutanoic acid	NO ⁺	70	132	2.5	$C_5H_{10}O_2.NO^+$	Syft 2009
3-methylindole	H ₃ O ⁺	100	132	3.3	$C_9H_{10}N^+$	Wang and others 2004b
5-methylfurfural	NO ⁺	100	110	3.1	$C_{6}H_{6}O_{2}^{+}$	Wang and others 2004b
acetic acid	NO ⁺	100	90+108	0.9	NO ⁺ .CH ₃ COOH, NO ⁺ .CH ₃ COOH.H O	Spanel and Smith 1998a
acetone	NO ⁺	100	88	1.2	NO ⁺ .C ₃ H ₆ O	Spanel and others 1997
azine	H ₃ O ⁺	100	80	3.3	$\mathrm{C_5H_5N.H^+}$	Spanel and others 1998b

Table 3.1 The compounds measured by SIFT-MS

Continued

Table 3.1 continued

benzaldehyde	O ₂ ⁺	65	106	2.4	$C_7H_6O^+$	Spanel and others 1997
benzonitrile	NO ⁺	100	133	2.2	NO ⁺ .C ₆ H ₅ CN	Spanel and Smith 1998b
benzyl alcohol	NO^+	50	107	2.3	$C_7H_7O^+$	Wang and others 2004c
decadienals	NO^{+}	80	151	4.2	$C_{10}H_{15}O^{+}$	Spanel and others 2002
dimethylpyrazines	O ₂ ⁺	100	108	2.7	$C_6 N_2 H_8^+$	Syft 2009
furaneol and sotolone	NO ⁺	50	128	2.5	$C_{6}H_{8}O_{3}^{+}$	Syft 2009
furfural	NO ⁺	20	95	3.2	$C_{5}H_{3}O_{2}^{+}$	Wang and others 2004b
furfuryl pyrrole	NO ⁺	100	147	2.7	C ₉ H ₉ NO ⁺	Syft 2009
guaiacol	NO ⁺	100	124	2.5	$C_7H_8O_2^+$	Syft 2009
heptanal	NO^+	100	113	3.3	$C_7H_{13}O^+$	Spanel and others 2002
indole	H ₃ O ⁺	100	118	3.3	$C_8H_8N^+$	Wang and others 2004b
isobutanoic and butanoic acid	O ₂ ⁺	20	88	2.5	(CH ₃) ₂ CHCOOH ⁺	Spanel and Smith 1998a
maltol	NO ⁺	50	156	2.5	$C_6H_6O_3.NO^+$	Syft 2009
methanol	O ₂ ⁺	50	31	1.0	CH ₃ O ⁺	Spanel and Smith 1997
methylbutanals	H ₃ O ⁺	94	87	3.7	C ₅ H ₁₀ O.H ⁺	Michel and others 2005; Spanel and others 2002
methylpropanals	O ₂ ⁺	70	72	3.0	$C_4H_8O^+$	Spanel and others 2002
n-amyl alcohol	NO ⁺	85	87	2.5	$C_5H_{11}O^+$	Spanel and Smith 1997

Continued

Table 3.1 continued

nonadienal	NO^+	50	168	2.5	$C_9H_{140}.NO^+$	Syft 2009
nonanal	O_2^+	15	138	3.2	$C_{10}H_{18}^{+}$	Syft 2009
nonenal	NO ⁺	80	139	3.8	$C_9H_{15}O^+$	Spanel and others 2002
phenylacetaldehyde	NO^+	60	120	2.5	$C_8H_8O^+$	Syft 2009
and acetophenone						
phenylacetic acid	NO^+	50	91	2.5	C ₇ H ₇ ⁺	Syft 2009
and phenylacetaldehyde				3.6		
tetramethylpyrazines	O ₂ ⁺	100	136	2.5	$C_8H_{12}N_2^+$	Syft 2009
toluene	NO ⁺	100	92	1.7	$C_{7}H_{8}^{+}$	Spanel and Smith 1998c
trimethylpyrazines	NO ⁺	95	122	2.5	$C_7 H_{10} N_2^+$	Syft 2009
vanillin	O_2^{+}	100	152	2.8	$C_8H_8O_3^+$	Syft 2009
γ-octalactone and 2- nonanone	NO^+	100	172	2.5	$C_8H_{14}O_2.NO^+$	Syft 2009
δ-decalactone	NO ⁺	100	200	2.5	$C_{10}H_{18}O_2.NO^+$	Syft 2009

Chapter 4: Results and Discussion

4.1 Conching studies

In the three types of chocolate (unsweetened dark (UDC), dark (DC), and unsweetened milk chocolate (UMC)), the volatiles showed a similar pattern during 48h of conching where the volatiles initially increased then decreased (Figure 4.1, 4.2). From 0-4h, all volatiles increased to their highest concentration. This increase was caused by the effect of mixing, equilibration into the headspace, and the increase in temperature. Volatiles release from food into the headspace to achieve equilibrium because of the concentration difference, and mixing increases the rate of volatile release by increasing the mass transfer rate (Dutta 2007). During conching, frictional heat is generated in chocolate which increases the volatility of the volatiles. As temperature increases, molecules have more energy to be released from the medium, which results in a higher concentration in the gas phase (Hall and Andersson 1983). The initial temperature of unsweetened dark chocolate was 29°C, and increased to 34°C in the first 2h then maintained at that temperature (Figure A.2). Similarly, dark chocolate increased from 30 to 39°C, and unsweetened milk chocolate from 36 to 43°C.



Figure 4.1 Volatile concentrations (above 1000ppb at 0h) during 48h conching in unsweetened dark chocolate. (Acetic acid is plotted on the secondary axis)



Figure 4.2 Volatile concentrations (below 1000ppb at 0h) during 48h conching in unsweetened dark chocolate.

During conching, the three types of chocolate lost 42%, 65% and 75% of the total volatiles after 8h, 28h, and 48h due to volatilization. There was no significant difference in percent volatiles lost at 8 and 48 hour between the three types of chocolate. At 28h, the percent lost in unsweetened dark chocolate was significant lower than dark and unsweetened milk chocolate. The majority of the volatiles loss was during the first 28h in all types of chocolate. Similar results were reported by Maniere and Dimick (1979), who observed a 79% decrease in total volatiles during 38h conching with the major loss, 77%, in the first 16h in dark chocolate. An increase in most volatiles due to the Maillard reaction and decrease of some Strecker aldehydes due to evaporation and chemical reaction was found in dark chocolate during conching at 70-80 °C (Counet and others 2002). In this study, no volatile increase was seen, because the temperature was not high enough to generate volatiles.

4.1.1 Changes of top ten volatiles during conching

The volatiles with the highest initial concentrations, at 1h of conching, in unsweetened dark, dark, and unsweetened milk chocolate were methanol, acetic acid, methylbutanals, 2, 3-butanedione, nonanal, isobutanoic and butanoic acid, benzaldehyde, furfuryl pyrrole, acetone, 2-nonanone, methylpropanals, and benzyl alcohol (Table A.1). Most of these compounds are the products of fermentation and roasting (Lopez and Quesnel 1973; Frauendorfer and Schieberle 2008; Gill and others 1984). Half of these, including acetic acid, methylbutanals, 2, 3-butanedione, nonanal, butanoic acid, benzaldehyde, furfuryl pyrrole, and methylpropanals have been reported as important volatile compounds in

chocolate flavor because of their high flavor dilution value or odor activity (Schnermann and Schieberle 1997; Counet and others 2002).

4.1.1.1 Methanol

Methanol was present at the highest concentration, 50% of the total volatiles in all three types of chocolate. Its loss during conching produced 46-60% of the total lost. Methanol is widely used as a solvent for extraction, thus it is difficult to detect by many analysis methods. The human odor detection threshold of methanol is around 100ppm (Leonardos and others 1969), thus the concentration of methanol in the chocolate is not high enough to contribute to the aroma. Therefore, the decrease of methanol during conching is not important.

4.1.1.2 Acetic acid

Acetic acid was the second highest volatile compound in the headspace, and it was also the highest acid. Acetic acid is the most dominant acid with highest concentration in chocolate and its products (Frauendorfer and Schieberle 2006; Brian and others 1987). The odor of acetic acid is classified as sharp and vinegary (Hoskin and Dimick 1979). Acetic acid is one of the volatile fatty acids which may affect the final flavor of chocolate and are responsible for dull, insipid off-flavors (Lopez and Quesnel 1973). Because of its high concentration in chocolate and undesired odor, acetic acid is important to the quality of chocolate.

4.1.1.3 Other compounds in the top 10

Methylbutanals, methylpropanals, 2, 3-butanedione, benzaldehyde, and furfuryl pyrrole are products of the Maillard reaction, which is reacting protein and reducing sugar and important to chocolate flavor formation during the roasting process. Methylbutanals, methylpropanals, and benzaldehyde are Strecker aldehydes which come from amino acid, leucine, isoleucine, valine, phenylglycine. 2, 3-Butanedione was formed from the cleavage of glucose in the Maillard reaction (Yaylayan and Keyhani 1999). Furfuryl pyrrole is one of nitrogen heterocycles, and pyrroles may come from the participation of proline and hydroxy proline in the Strecker degradation or reaction of an amino acid with the corresponding furan (Hodge and others 1972; Rizzi 1974). Methylbutanals and methylpropanals have been described as chocolate like and malty, 2, 3-butanedione as buttery, acetic acid as sour, butanoic acid as buttery and rancid, nonanal as soapy, and furfuryl pyrrole as roasted, chocolate, green (Schnermann and Schieberle 1997).

After 48h conching, these volatiles were still in the top ten volatiles, except furfuryl pyrrole in all samples, benzyl alcohol in unsweetened dark chocolate and dark chocolate, and acetone in dark chocolate. 3-Methylbutanoic acid became one of the highest volatiles after conching in all samples, as well as phenylacetic acid and phenylacetaldehyde in unsweetened dark chocolate and dark chocolate, and azine in dark chocolate (Table A.2). These compounds moved into the top 10 volatiles due to their low percent lost during conching.

Most volatiles decreased 80-90% during conching in all three types of chocolate. A few volatiles showed less decrease, acetic acid, 3-methylbutanoic acid, phenylacetic acid and phenylacetaldehyde, and azine were 16-61%, 43-60%, 58-75%, and 40-75% (Table A.3). The percent lost of acetic acid (16-61%) during conching was the lowest compared with other compounds in three types of chocolate (Table A.3). The low percent was also reported by Hoskin and Dimick (1979), which approximately 18% acetic acid lost during 44h conching by using Suchard conche.

There was a high correlation between the percent of the total concentration and percent of total lost during conching at 48h in all samples ($R^2 = 0.99$ in UDC and UMC, 0.95 in DC). The only compound where the concentration was not approximately equal to total lost, was acetic acid (Table A.1). Since acetic acid contributes a sour flavor, the loss of acetic acid is desired. However, acetic acid only contributed 3-12% to total loss (Table A.1).

3-Methylbutanoic acid and phenylacetic acid were formed from fermentation and phenylacetaldehyde is the product of Strecker degradation in the Maillard reaction (Frauendorfer and Schieberle 2008; Lopez and Quesnel 1973). All of these compounds have been reported as important compounds in chocolate products, and they all contributed a sweet aroma (Schnermann and Schieberle 1997). Other compounds which had low percent lost are 5-methylfurfural (59-68%), azine (40-75%), heptanal (62-73%), and namyl alcohol (72-77%) (Table A.3).

4.2 Storage

During storage, oil separation was observed in 50 $^{\circ}$ C in the first week, and volatiles decreased over time in both dark chocolate and unsweetened milk chocolate (Figure 4.3, Table A.4). The greatest loss of volatile was observed in the first 3 weeks in both types of chocolate. Dark chocolate at all three storage temperatures (4 $^{\circ}$ C, 25 $^{\circ}$ C, and 50 $^{\circ}$ C) had higher total volatile concentration than unsweetened milk chocolate. It may be due to sugar, replacing the milk powder which is an additional solute, increase the volatility of volatiles in dark chocolate due to a salting out effect (Godshall 1995). The differences of volatiles in dark and unsweetened milk chocolate may also due to the interaction between milk protein and volatiles. Protein in the milk has been proven that may bind with volatiles and cause the reduction of volatile (Ebeler and others 1988; Landy and others 1995).



Figure 4.3 The total volatile in dark chocolate (DC) and unsweetened milk chocolate (UMC) at 4 °C, 25 °C, and 50 °C.

During storage, a greater loss of volatiles at higher temperature was expected, since when chocolate is stored at higher temperature; it should cause more volatile loss due to the temperature increases increasing compound volatility. There was no significant difference found between samples stored at 50°C, 25°C, and 4°C in both types of chocolate on day 1. In both types of chocolate, volatiles decreased over time and started to show significant difference between different temperatures on day 35, which the volatiles in 50°C samples were significant higher than other temperatures (Figure 4.3). On day 56, dark chocolate lost 33%, 50%, and 59% of total volatile at 50°C, 25°C, and 4°C (Figure 4.3). In unsweetened milk chocolate, total volatile lost 3%, 55%, and 65% at 50°C, 25°C, and 4°C (Figure 4.3). Both samples showed at 25°C and 4°C, the percent lost of total volatile were significant higher than samples stored at 50°C both types of chocolate, and no significant difference was found in 25°C and 4°C samples. The results were unexpected, since the samples stored at higher temperature (50°C) decreased less than lower temperatures (25°C and 4°C). However, some chemical reactions may occur faster at higher temperature and cause compound generation which leads to low decrease at 50°C.

	DC % lost			UMC % lost		
	50°C	25°C	4°C	50°C	25°C	4°C
(E)-2-octenal	55 ^a	76 ^a	76 ^a	19 ^b	59 ^a	69 ^a
2,3-butanedione	51 °	68 ^b	76 ^a	23 °	43 ^b	64 ^a
2,3-diethyl-5-methylpyrazine	54 ^b	68 ^{ab}	88 ^a	52 ^b	77 ^a	85 ^a
2-acetylpyrrole	42 ^a	51 ^a	70 ^a	29 ^b	54 ^a	59 ^a
2-heptanol	55 ^b	63 ^{ab}	73 ^a	38 ^b	47 ^b	60 ^a
2-heptanone	75 ^b	62 ^{ab}	70 ^a	57 ^a	55 ^a	69 ^a
methylpropanals	15 ^b	56 ^a	69 ^a	-51 ^b	35 ^a	53 ^a
2-nonanone	54 ^a	83 ^a	84 ^a	-22 ^a	67 ^a	80 ^a
2-phenylethanol and phenylacetaldehyde	27 ^b	46 ^{ab}	65 ^a	9 ^b	50 ^a	59 ^a
3-methylbutanoic acid	39 °	59 ^b	72 ^a	36 ^b	56 ^a	63 ^a
3-methylindole	56 ^a	77 ^a	77 ^a	20 ^a	50 ^{ab}	75 ^a
5-methylfurfural	58 ^a	57 ^a	66 ^a	33 ^b	48 ^{ab}	64 ^a
acetic acid	18 ^b	26 ^{ab}	43 ^a	19 ^b	61 ^a	65 ^a
acetone	-54 ^b	27 ^a	48 ^a	-295 ^b	-18 ^a	12 ^a
azine	24 ^b	34 ^{ab}	50 ^a	9 ^b	57 ^a	63 ^a
benzaldehyde	47 ^b	68 ^{ab}	80 ^a	19 ^b	65 ^a	70 ^a
benzonitrile	58 ^b	68 ^{ab}	80 ^a	49 ^b	65 ^a	73 ^a
benzyl alcohol	40 ^a	48 ^a	60 ^a	34 ^a	53 ^a	52 ^a
decadienals	56 ^b	76 ^{ab}	84 ^a	63 ^b	78 ^b	88 ^a

Table 4.1 The percent lost of volatiles on day 56 at different temperature in dark chocolate (DC) and unsweetened milk chocolate (UMC)

Continued

Table 4.1 continued

dimethylpyrazines	57 ^b	66 ^{ab}	78 ^a	47 ^b	56 ^{ab}	65 ^a
furaneol and sotolone	79 ^a	69 ^a	69 ^a	45 ^a	62 ^a	68 ^a
furfural	57 ^a	59 ^a	66 ^a	26 ^b	38 ^{ab}	59 ^a
furfuryl pyrrole	69 ^b	72 ^b	84 ^a	53 ^b	71 ^a	80 ^a
guaiacol	51 ^a	53 ^a	56 ^a	-10 ^a	19 ^a	13 ^a
heptanal	59 ^a	62 ^a	69 ^a	38 ^b	31 ^{ab}	61 ^a
indole	64 ^b	74 ^{ab}	85 ^a	10 ^b	61 ^a	73 ^a
isobutanoic and butanoic acid	51 ^b	67 ^a	75 ^a	26 ^b	51 ^a	65 ^a
maltol	51 ^a	56 ^a	10 ^a	-12 ^a	53 ^a	65 ^a
methanol	47 ^b	69 ^a	71 ^a	-27 ^b	45 ^a	63 ^a
methylbutanals	55 ^b	73 ^a	80 ^a	25 ^b	56 ^a	70 ^a
n-amyl alcohol	70 ^a	62 ^a	70 ^a	46	37 ^b	57 ^a
nonadienal	33 ^a	72 ^a	44 ^a	42 ^a	28 ^a	77 ^a
nonanal	41 ^b	71 ^{ab}	83 ^a	35 ^b	68 ^{ab}	75 ^{ab}
nonenal	59 ^a	65 ^a	76 ^a	35 ^a	68 ^a	68 ^a
phenylacetaldehyde and acetophenone	58 ^b	56 ^{ab}	71 ^a	43 ^b	63 ^a	72 ^a
phenylacetic acid and phenylacetaldehyde	35 ^b	41 ^{ab}	54 ^a	29 ^b	55 ^a	61 ^a
tetramethylpyrazines	27 ^b	66 ^a	76 ^a	34 ^c	57 ^b	70 ^a
toluene	37 ^a	47 ^a	54 ^a	17 ^b	57 ^a	68 ^a
trimethylpyrazines	46 ^c	58 ^b	68 ^a	37 ^b	49 ^{ab}	62 ^{ab}
vanillin	48 ^a	51 ^a	83 ^a	42 ^b	87 ^a	90 ^a

Continued

Table 4.1 continued

γ-octalactone and 2- nonanone	23 ^b	64 ^a	70 ^a	28 ^a	43 ^a	56 ^a
δ-decalactone	100	N/A	100	100	11	N/A

Concentrations in the same row with different superscripts are significant different

The percent lost of most volatiles were significantly lower at 50°C than other temperatures and had no significant between 25 and 4°C samples (Table 4.1). The significance of δ-decalactone cannot be calculated due to data absent. The percent lost of all Strecker aldehydes (methylbutanals, methylpropanals, benzaldehyde, and phenylacetaldehyde) was significant lower in samples stored at 50°C than 4°C in dark and unsweetened milk chocolate (Table 4.1). The concentration of Strecker aldehydes on the day 1 was no significant difference between samples stored at 50, 25, and 4°C in both types of chocolate. All samples had the greatest decrease in the first two weeks; however, the 50°C samples of dark and unsweetened milk chocolate had lowest percent lost during storage and started to show significant higher concentration on day 14 than other temperature. Moreover, on day 56, a significant increase of Strecker aldehydes was observed in unsweetened milk chocolate stored at 50°C (Figure 4.4). The low loss of Strecker aldehydes in 50°C samples may indicate the occurrence of the Maillard reaction.



48

Figure 4.4 The change of Strecker aldehydes during storage in dark chocolate (DC) and unsweetened milk chocolate (UMC) at 4 $^{\circ}$ C, 25 $^{\circ}$ C, and 50 $^{\circ}$ C

Methylbutanals, methylpropanals, and benzaldehyde are Strecker aldehydes, which are one of main products of the Maillard reaction. They formed from Strecker degradation after Amadori and Heyns rearrangements in the Maillard reaction. In Strecker degradation, Strecker aldehydes are formed from dicarbonyls or hydroxycarbonyl intermediate which from reductones and dehydroreductones by deaminating and decarboxylating amino acids (Reineccius 2006). The increase or low loss of Strecker aldehydes may indicate compounds generated because of the Maillard reaction, and the compounds generating increased the total volatile at 50°C. An increase of browning caused by the Maillard reaction on day 15 to day 80 was reported in a preheated model system which had oil present during 180 days storage at 25°C (Mastrocola and Munari 2000). An ovalbumin-glucose system was also showed an occurrence of the Maillard reaction started on day 4 of storage stored at 50°C (Kato and others 1988).

Heptanal and nonanal were also found lower percent lost in 50°C samples than samples at other temperatures (Table 4.1). The low percent of heptanal and nonanal in 50°C samples may imply that besides the Maillard reaction, the lipid oxidation may also occur. Heptanal and nonanal are secondary oxidation products of lipids formed during storage (Frankel 1982). A significant increase of heptanal and nonanal was reported in cocoa butter during 12 weeks storage at room temperature (Hashim and others 1997). Heptanal and nonanal may be derive from the oxidation of oleic acid. Compounds such as heptanal, nonanal, ethanol, 1-octanol, hexanal, octanal were reported that increased significantly while the concentration of oleic and linoleic acid significantly decreased (Mexis and others 2010). In other volatiles associated with lipid oxidation, decadienals showed significant lower percent lost in 50 °C dark chocolate, 50 °C unsweetened milk chocolate and 25 °C unsweetened milk chocolate. The percent lost of (*E*)-2-octenal was also shown significant low percent lost in 50 °C unsweetened milk chocolate. The samples stored at 4 °C had highest percent lost of these compounds since low temperature may reduce the reaction rate of lipid oxidation; therefore, less volatiles form in the 4 °C samples (Table 4.1).

Not many studies reported the volatiles change in chocolate during storage. An increase in aldehydes, ketones, alcohols, and alkanes with a decrease in pyrazines was reported after 12 months storage of dark chocolate at 20°C (Mexis and others 2010). The increase in volatiles was referred to the lipid oxidation. Other study has reported an increase in aldehydes and ketones with a decrease in pyrazines during 6 months in cocoa substitute stored at room temperature (Fadel and others 2006). Both studies reported the volatiles increase during storage, which was opposite result to this study. However, the storage time of these researches were much longer than this study. In the Fadel and others' study (2006), most volatiles decreased in the first 2 months and then increased, this result was similar to us. Therefore, short term storage (less than 2 months) may not provide enough time to generate compounds more than loss.

4.3 Blooming studies

4.3.1 Effect of blooming on different types of chocolate

Comparing unbloomed and bloomed samples, the concentrations of most volatiles showed no significant difference in three types of chocolate (cocoa mass, unsweetened dark chocolate, and dark chocolate). However, the concentration of acetic acid was significant higher in unbloomed than bloomed samples in all three types of chocolate. The acetic acid had the highest concentration in all sample and contributed approximately 44-65% of total volatile to each sample (Figure 4.5, 4.6, 4.7).

Most volatiles were not effected by fat blooming, except acetic acid. Acetic acid was the highest volatile and significant reduced by fat blooming in all samples (Figure 4.5, 4.6, 4.7). The second highest volatile, methanol, was not found significant difference in any sample. This result suggests that the difference was caused by only acetic acid, but not because of its highest concentration. None of previous research reported volatile changes caused by blooming. However, cocoa fat is the continuous phase surrounding sugar crystals and nonfat cocoa constituents in chocolate, and distribution of volatiles between lipid and polar phases can affect release of volatiles (Keeney 1972). Fat blooming occurs due to the polymorphic transformation of cocoa butter from relatively unstable form (β V) to stable form (β VI) (Wille and Lutton 1966). During the storage of chocolate, the more liquid-like triglycerides in cocoa butter migrate from center to the surface of chocolate, and these triglycerides recrystallize on the surface due to fluctuation in solid fat content and the change of solubility of triglycerides caused by temperature fluctuation (Bricknell and Hartel 1998). Once the crystals formed, they gradually increase in size and extend over time, and finally the blooming appears. The migration of the triglycerides may change the homogeneous distribution of cocoa butter and affect the volatile compound release. Acetic acid is one of volatile fatty acids, which are easily vaporized hydrocarbon compounds with single, polar carboxylic acid groups, and also have low molecular weight (Hoskin and Dimick 1979). Isobutanoic and butanoic acid, which was found significant higher in unbloomed cocoa mass and unsweetened dark chocolate, is another volatile fatty acid with log P value below one. Therefore, it is possible that the volatile fatty acids with low log P values and low carbon numbers may be more easily affected by recrystallization during blooming (Table 4.10).



Figure 4.5 Volatile concentrations in unbloomed and bloomed cocoa mass



Figure 4.6 Volatile concentrations in unbloomed and bloomed unsweetened dark chocolate



Figure 4.7 Volatile concentrations in unbloomed and bloomed dark chocolate.

4.4 Real time volatile release in chocolate

4.4.1 Headspace

In the headspace, most volatiles in the unsweetened dark chocolate were significant higher than in dark and unsweetened milk chocolate due to the higher amount of cocoa mass in unsweetened dark chocolate (Table 4.2, 4.3). The compound with highest concentration is acetic acid. Many previous studies have reported acetic acid as an important volatile and the most plentiful acid in chocolate (Schnermann and Schieberle 1997; Frauendorfer and Schieberle 2008; Frauendorfer and Schieberle 2006; Krings and others 2006). Benzaldehyde, 2-methylbutanal, 2-methylpropanal, 3-methylbutanal, and 2, 3-butanedione, which had high concentration in all types of chocolate, are the products of the Maillard reaction and commonly discovered in chocolate products.
Table 4.2 Volatile concentration (ppb) in headspace (HS) in unsweetened dark chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: O_2^+)

	UDC	DC	UMC
2, 3-diethyl-5-methylpyrazine	20 ^a	12 ^b	9.0 ^c
2, 5-dimethylpyrazine	75 ^a	55 ^b	51 ^b
2-methylbutanal	490 ^a	310 ^b	380 ^b
2-methylpropanal	130 ^a	79 ^b	80 ^b
2-nonanone	13 ^a	10 ^{ab}	8.0 ^b
2-phenylethanol	160 ^a	120 ^b	110 ^c
3-methylbutanal	1700 ^a	1100 ^b	1300 ^b
benzaldehyde	180 ^a	140 ^b	140 ^b
methanol	7900 ^a	5100 ^b	5500 ^b
nonanal	120 ^a	110 ^{ab}	87 ^b
tetramethylpyrazines	120 ^a	94 ^b	92 ^b
vanillin	28 ^a	16 ^b	10 ^c

Table 4.3 Volatile concentration (ppb) in headspace (HS) in unsweetened dark chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: NO⁺)

	UDC	DC	UMC
2, 3-butanedione	690 ^a	460 ^b	5700 ^c
2-heptanol	100 ^a	76 ^b	79 ^b
3-methylbutanoic acid	330 ^a	260 ^b	240 ^b
acetic acid	12000 ^a	8900 ^b	6900 ^c
acetophenone	21 ^a	15 ^b	12 ^c
benzonitrile	51 ^a	39 ^b	35 ^b
benzyl alcohol	340 ^a	270 ^b	240 °
methional	120 ^a	77 6	71 ⁶
phenylacetic acid	260 ^a	190 ^b	160 °

Concentrations in the same row with different superscripts are significant different

The mouthspace before swallowing to headspace ratios varied from 0.00033 to 0.17. The volatiles in headspace were much higher than in mouthspace (Table 4.4, 4.5), and the nosespace to headspace ratio (NS/HS) varied from 0.0012 to 0.17, and most ratios were below 0.05 which were around 3 to 10 times lower than the mouthspace to headspace ratios (Table 4.6, 4.7). The differences between mouthspace, nosespace and headspace were expected, since the mouthspace and nosespace analysis was under dynamic conditions, and headspace analysis was under equilibrium condition (Taylor and Linforth 1996; Diaz 2004). The volatile measurement in the mouthspace and nosespace is complicated, because

many factors can affect the release of volatile in the mouth and nose, such as temperature,

dilution effect, length of time the food in the mouth, melting of the fat, and inversion of

emulsion phases (Taylor and Linforth 1996).

Table 4.4 The mouthspace before swallowing to headspace (HS) ratio in unsweetened dark chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: O_2^+)

	MSbs/HS				
	UDC	DC	UMC		
2, 3-diethyl-5-methylpyrazine	0.050 ^a	0.14 ^a	0.11 ^a		
2, 5-dimethylpyrazine	0.075 ^b	0.096 ^{ab}	0.13 ^a		
2-methylbutanal	0.053 ^a	0.065 ^a	0.028 ^a		
2-nonanone	0.14 ^a	0.22 ^a	0.17 ^a		
2-phenylethanol	0.11 ^a	0.16 ^a	0.17 ^a		
3-methylbutanal	0.030 ^a	0.038 ^a	0.026 ^a		
benzaldehyde	0.076 ^a	0.079 ^a	0.076 ^a		
methanol	0.015 ^a	0.030 ^a	0.016 ^a		
2-methylpropanal	0.00033 ^b	0.046 ^a	0.057 ^a		
nonanal	0.084 ^a	0.084 ^a	0.12 ^a		
tetramethylpyrazines	0.11 ^a	0.11 ^a	0.12 ^a		
vanillin	0.013 ^a	0.035 ^a	0.067 ^a		

 Table 4.5 The mouthspace before swallowing to headspace (HS) ratio in unsweetened dark chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: NO⁺)

 MSbs/HS

	M308/113		
	UDC	DC	UMC
2, 3-butanedione	0.036 ^a	0.033 ^a	0.018 ^a
2-heptanol	0.087 ^a	0.056 ^a	0.067 ^a
3-methylbutanoic acid	0.087 ^a	0.081 ^a	0.074 ^a
acetic acid	0.064 ^a	0.065 ^a	0.050 ^a
acetophenone	0.10 ^a	0.084 ^a	0.12 ^a
benzonitrile	0.097 ^a	0.097 ^a	0.056 ^a
benzyl alcohol	0.053 ^a	0.035 ^a	0.054 ^a
methional	0.11 ^a	0.14 ^a	0.092 ^a
phenylacetic acid	0.059 ^a	0.072 ^a	0.060 ^a

Table 4.6 The nosespace (NS) to headspace (HS) ratios in unsweetened dark chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: $O_2^+)$

	NS/HS		
	UDC	DC	UMC
2, 3-diethyl-5-methylpyrazine	0.037 ^a	0.036 ^a	0.047 ^a
2, 5-dimethylpyrazine	0.0090 ^a	0.0064 ^a	0.014 ^a
2-methylbutanal	0.0052 ^a	0.012 ^a	0.0058 ^a
2-methylpropanal	0.0012 ^b	0.065 ^a	0.0069 ^b
2-nonanone	0.075 ^a	0.12 ^a	0.16 ^a
2-phenylethanol	0.0093 ^c	0.048 ^a	0.030 ^b
3-methylbutanal	0.0076 ^a	0.012 ^a	0.0087 ^a
benzaldehyde	0.0067 ^a	0.013 ^a	0.012 ^a
methanol	0.0046 ^a	0.0086 ^a	0.010 ^a
nonanal	0.018 ^a	0.026 ^a	0.038 ^a
tetramethylpyrazines	0.015 ^a	0.024 ^a	0.016 ^a
vanillin	0.015 ^a	0.020 ^a	0.035 ^a

Table 4.7 The nosespace (NS) to headspace (HS) ratios in unsweetened dark chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: NO⁺)

	NS/HS		
	UDC	DC	UMC
2, 3-butanedione	0.0042 ^a	0.0046 ^a	0.0035 ^a
2-heptanol	0.017 ^a	0.013 ^a	0.010 ^a
3-methylbutanoic acid	0.0093 ^a	0.014 ^a	0.0094 ^a
acetic acid	0.020 ^a	0.015 ^a	0.011 ^a
acetophenone	0.019 ^a	0.028 ^a	0.024 ^a
benzonitrile	0.020 ^a	0.032 ^a	0.016 ^a
benzyl alcohol	0.010 ^a	0.0067 ^a	0.011 ^a
methional	0.015 ^b	0.044 ^a	0.019 ^b
phenylacetic acid	0.010 ^a	0.019 ^a	0.011 ^a

Concentrations in the same row with different superscripts are significant different

4.4.2 Volatile release in the mouthspace during eating

In the real time volatile release study, the data was obtained from only one person. To compare the volatile release during eating in the mouthspace and nosespace, data received from only one person can minimize variations since research has shown that the eating pattern of one person is consistent (Haring 1990). Twenty one volatiles were analyzed in the mouthspace, and the volatiles showed the similar trend between mouthspace before swallowing and mouthspace after swallowing (Figure 4.8). Before swallowing, the volatile concentration decreased over time; however, after swallowing, the concentration maintained a constant level (Figure 4.8). The decrease of volatile concentration during mouthspace before may due to the dilution effect.



Figure 4.8 Real time concentration change of acetic acid between mouthspace before and after swallowing.

High correlations between volatiles in mouthspace before swallowing and headspace were found (R^2 =0.80 in UDC, 0.91 in DC, and 0.79 in UMC), as well as mouthspace after swallowing (R^2 = 0.99 in UDC, 0.94 in DC, and 0.84 in UMC).

The concentrations of volatiles in mouthspace before swallowing varied from 6.4 to 680 ppb between different volatiles and different types of chocolate (Table 4.8, 4.9). In the mouthspace, the volatile changes, such as hydration, dilution, and increase surface area may occur, and these changes can affect the release of volatiles which will transfer to nose (Taylor 1996). Mouthspace measurement provides the first understanding of volatile change during eating. Few of research have reported the volatile changes during chocolate eating. A correlations between 2-methylbutanal in breath and perceived intensity of chocolate flavor was reported, and the highest perceived intensity was found 15s after swallowing (Legger and Roozen 1994). None of previous has reported the volatile concentrations of chocolate in mouthspace.

In the mouthspace before swallowing, methanol, 2-methylbutanal, 3-methylbutanal, methional, acetic acid, and 2, 3-butanedione had show significant higher in unsweetened dark chocolate than unsweetened milk chocolate (Table 4.8, 4.9). It may due to high cocoa mass amount in the unsweetened dark chocolate and the interaction between volatiles and protein in milk solid. The protein in milk solids may interact with volatiles in the food and causes the decrease of volatile concentration in the headspace (Ebeler and others 1988). There was no significance found between unsweetened dark chocolate and dark chocolate (Table 4.8, 4.9). Unsweetened dark chocolate contained higher amount of cocoa mass than dark chocolate; however, most volatiles were not found significance between them, which may due to the interaction between sugar and the volatiles resulting in increase of volatiles in dark chocolate. Additional sugar in the food may change the volatility of the compounds and result in the increase or decrease of volatile concentration (Roberts and others 1996).

Table 4.8 Volatile concentration in mouthspace before (MSb) and after swallowing (MSa) in unsweetened dark chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: O_2^+)

	MSb (ppb)		N	ASa (ppb)	
	UDC	DC	UMC	UDC	DC	MC
2-methylbutanal	26 ^a	16 ^{ab}	7.9 ^b	5.0 ^a	5.0 ^a	4.6 ^a
2-phenylethanol	16 ^a	19 ^a	18 ^a	0.74 ^b	6.0 ^a	2.2 ^b
3-methylbutanal	49 ^a	41 ^{ab}	32 ^b	6.0 ^a	4.1 ^a	5.7 ^a
benzaldehyde	14 ^a	11 ^a	9.7 ^a	0.70 ^a	0.56 ^a	1.3 ^a
methanol	104 ^{ab}	140 ^a	81 ^b	64 ^a	82 ^a	71 ^a
tetramethylpyrazines	13 ^a	10 ^a	11 ^a	3.1 ^a	3.3 ^a	3.5 ^a

Table 4.9 Volatile concentration in mouthspace before (MSb) and after swallowing (MSa) in unsweetened dark chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: NO⁺)

	MSb (ppb)			Ν	ASa (ppb)
	UDC	DC	UMC	UDC	DC	MC
2, 3-butanedione	23 ^a	14 ^b	9.6 ^b	2.4 ^a	1.2 ^a	1.6 ^a
3-methylbutanoic acid	28 ^a	20 ^a	18 ^a	1.8 ^a	3.1 ^a	2.7 ^a
acetic acid	680 ^a	580 ^{ab}	330 ^b	78 ^{ab}	93 ^a	42 ^b
benzyl alcohol	17 ^a	9.3 ^a	13 ^a	2.1 ^a	1.6 ^a	1.7 ^a
methional	12 ^a	10 ^{ab}	6.4 ^b	1.2 ^b	3.7 ^a	1.1 ^b
phenylacetic acid	14 ^a	14 ^a	9.1 ^a	2.3 ^{ab}	3.2 ^a	1.5 ^b

In the mouthspace after swallowing, 2-phenylethanol, methional, phenylacetic acid, and acetic acid had showed significant difference between different types of chocolate (Table 4.8, 4.9). The first two compounds were significant higher in dark chocolate than unsweetened and unsweetened milk chocolate. The last two compounds were significant higher in dark than unsweetened milk chocolate. The cocoa mass content in the dark chocolate and unsweetened milk chocolate were the same, so the differences may due to the interaction between volatiles and sugar or milk solids. The log P values of these four compounds are 1.57, 0.71, -0.17, and -0.45, which were relatively lower than other compounds, and it indicated that these four compounds were relative more polar than other compounds (Table 4.10). The polarity of the volatiles may drive the results of volatile concentration changes, which the more polar volatiles increase when sucrose concentration increases (Roberts and others 1996). Dark chocolate contained higher sucrose than unsweetened milk chocolate, so it may have higher volatile concentrations.

Compounds	LogP	Compounds	LogP
(E)-2-nonenal	3.26	benzonitrile	1.67
(E)-2-octenal	2.76	benzyl alcohol	1.23
(E,E)-2, 4-decadienal	3.41	delta-decalactone	2.76
(E,Z)-2, 6-nonadienal	2.81	furaneol	0.30
2, 3-butanedione	-0.45	furfural	0.60
2, 3-diethyl-5-methylpyrazine	1.87	furfuryl pyrrole	1.73
2, 5-dimethylpyrazine	0.57	gamma-octalactone	1.73
2-acetylpyrrole	0.75	guaiacol	1.40
2-heptanol	2.39	heptanal	2.45
2-heptanone	2.04	indole	2.12
2-methylbutanal	1.30	isobutanoic acid	0.71
2-methylpropanal	0.82	maltol	-0.02
2-nonanone	3.13	methanol	-0.56
2-phenylethanol	1.57	Methional	0.71
3-methylbutanal	1.30	n-amyl alcohol	1.47
3-methylbutanoic acid	1.17	nonanal	3.52
3-methylindole	2.50	phenylacetaldehyde	1.71
5-methylfurfural	0.92	phenylacetic acid	1.50
acetic acid	-0.17	tetramethylpyrazines	1.30
acetone	0.07	toluene	2.50
acetophenone	1.76	trimethylpyrazines	0.94
azine	0.72	vanillin	1.17
benzaldehyde	1.64		

 Table 4.10 The logarithm of the partition coefficient

The mouthspace before to after swallowing ratios indicates the lingering of the volatiles after swallowing, and the value varied from 0.042 to 49 in this study (Table 4.11, 4.12).

Between three different types of chocolate, only methional and 2-phenylethanol had shown the significant difference in the mouthspace before to after swallowing ratio (MSbs/MSas), and it indicated that methional and 2-phenylethanol significant lingered more in unsweetened dark chocolate than dark chocolate in the mouth (Table 4.11, 4.12). None of previous research has reported methional as an important volatile in chocolate; however, 2-phenylethanol had been identified as the most odor active volatile in milk chocolate, and its aroma was described as sweet and yeast-like (Schnermann and Schieberle 1997).

Table 4.11 The mouthspace before (MSbs) to mouthspace after (MSas) swallowing ratio in unsweetened dark chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: O_2^+)

	MSbs/MSas			
	UDC	DC	UMC	
2, 3-diethyl-5-methylpyrazine	2.1 ^a	2.3 ^a	1.1 ^a	
2, 5-dimethylpyrazine	4.8 ^a	5.7 ^a	8.8 ^a	
2-methylbutanal	5.1 ^a	3.3 ^a	1.7 ^a	
2-methylpropanal	0.042 ^a	0.54 ^a	5.3 ^a	
2-nonanone	2.1 ^a	2.2 ^a	0.67 ^a	
2-phenylethanol	20 ^a	3.5 ^b	9.7 ^{ab}	
3-methylbutanal	21 ^a	12 ^a	6.0 ^a	
benzaldehyde	23 ^a	40 ^a	11 ^a	
methanol	1.9 ^a	1.7 ^a	1.6 ^ª	
nonanal	6.7 ^a	1.4 ^a	2.6 ^a	
tetramethylpyrazines	4.4 ^a	3.1 ^a	3.1 ^a	
vanillin	0.73 ^a	1.2 ^a	0.30 ^a	

Table 4.12 The mouthspace before (MSbs) to mouthspace after (MSas) swallowing ratio in unsweetened dark chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: NO⁺)

	MSbs/MSas			
	UDC	DC	UMC	
2, 3-butanedione	10 ^a	14 ^a	8.2 ^a	
2-heptanol	7.5 ^a	6.5 ^ª	9.3 ^a	
3-methylbutanoic acid	15 ^a	9.8 ^a	7.6 ^ª	
acetic acid	8.7 ^a	6.9 ^a	7.9 ^ª	
acetophenone	4.5 ^a	2.6 ^a	3.0 ^a	
benzonitrile	13 ^a	3.8 ^a	2.7 ^a	
benzyl alcohol	16 ^a	7.1 ^ª	10 ^a	
methional	15 ^a	2.9 ^b	5.9 ^{ab}	
phenylacetic acid	6.5 ^a	5.1 ^a	6.9 ^a	

Concentrations in the same row with different superscripts are significant different

Compared volatiles in each type of chocolate, benzaldehyde, 2-phenylethanol, 3methylbutanal, benzyl alcohol, 3-methylbutanoic acid, and 2, 3-butanedione had high MSbs/MSas value in all types of chocolate except 2-phenylethanol in dark chocolate (Table 4.11, 4.12). The higher MSbs/MSas ratio implied these volatiles retained more in the mouth after swallowing (Table 4.11, 4.12). The persistence of volatile in breath associated with air-water partition coefficient. Volatiles with a higher air-water partition coefficient decay faster than volatiles with lower air-water partition coefficient (Hodgson and others 2004). However, there was no correlation found between volatile lingering and air-water partition in this study (Table 4.10, 4.11, 4.12). The results from pure volatile solution in the mouth may not be consistent to chocolate which is a more complex system.

4.4.3 Nosespace

In nosespace, volatile concentrations varied from 0.16 to 210 ppb, and 2phenylethanol, 2-methylpropanal, methional, and acetic acid had been found significance, that all of them were significant higher in dark chocolate than other types of chocolate, except acetic acid which only significant higher in unsweetened dark, and dark chocolate than unsweetened milk chocolate (Table 4.13, 4.14). Similarly, 2-phenylethanol, 2methylpropanal, and methional had also found significant higher in dark chocolate than other types of chocolate in mouthspace after swallowing (Table 4.8, 4.9), and it may imply the effects of sugar on these compounds in mouthspace after swallowing may have the same effects on nosespace. The compounds with higher concentrations in nosespace were also high in mouth. High correlations were found between the concentrations of volatiles in nosespace and mouthspace before swallowing in all types of chocolate ($R^2 = 0.998$ in UDC. 0.996 in DC, 0.872 in UMC). The volatile concentration in nosespace is much lower than headspace; however, measuring the volatiles in the nosespace is the closest analysis to actual perception, because it is what consumer perceived during eating (Xu and Barringer 2010). Some volatiles lost when they transferred from mouthspace to nosespace and also swallowing. During delivering of volatiles, the gas-phase dilutes volatiles and volatiles also are absorbed by nasal epithelia when exhaled (Linforth and others 2002). These resulted in decrease of volatile concentration in the nosespace.

Table 4.13 Volatile concentration (ppb) in nosespace (NS) in unsweetened dark chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: O_2^+)

	UDC	DC	UMC
2, 3-diethyl-5-			
methylpyrazine	0.65 ^a	0.39 ^a	0.36 ^a
2, 5-dimethylpyrazine	0.65 ^a	0.35 ^a	0.70 ^a
2-methylbutanal	2.6 ^a	3.7 ^a	2.2 ^a
2-methylpropanal	0.16 ^b	4.9 ^a	0.55 ^b
2-nonanone	0.62 ^a	0.79 ^a	1.22 ^a
2-phenylethanol	1.4 ^b	5.8 ^a	3.1 ^b
3-methylbutanal	12 ^a	13 ^a	10 ^a
benzaldehyde	1.1 ^a	1.9 ^a	1.6 ^a
methanol	34 ^a	41 ^a	47 ^a
nonanal	2.0 ^a	2.7 ^a	3.3 ^a
tetramethylpyrazines	1.7 ^a	2.1 ^a	1.5 ^a
vanillin	0.37 ^a	0.29 ^a	0.32^{a}

Table 4.14 Volatile concentration (ppb) in nosespace (NS) in unsweetened dark chocolate (UDC), dark (DC), and unsweetened milk chocolate (UMC) (precursor: NO⁺)

	UDC	DC	UMC
2, 3-butanedione	2.8 ^a	1.5 ^a	1.9 ^a
2-heptanol	1.7 ^a	0.75 ^a	0.82 ^a
3-methylbutanoic acid	2.9 ^a	2.8 ^a	2.2 ^a
acetic acid	210 ^a	110 ^{ab}	72 ^b
acetophenone	0.36 ^a	0.38 ^a	0.27 ^a
benzonitrile	1.0 ^a	0.99 ^a	0.55 ^a
benzyl alcohol	3.3 ^a	1.8 ^a	2.6 ^a
methional	1.6 ^b	2.7 ^a	1.3 ^b
phenylacetic acid	2.1 ^a	3.0 ^a	1.7 ^a

4.5 Conclusion

During conching, volatile changes in three types of chocolate (unsweetened, dark, and unsweetened milk chocolate) were similar, which volatiles reached the highest concentrations in the first 4h and then concentrations of all volatiles decreased. The mean loss of the volatiles occurred during the first 28h in all types of chocolate. Most volatiles reduced 80-90% of their concentration except 3-methylbutanoic acid, 5-methylfurfural, acetic acid, azine, heptanal, n-amyl alcohol, and phenylacetic acid and phenylacetaldehyde. The results suggested that if only for flavor reason, conching can be finished in 28h for unsweetened dark, dark, and unsweetened milk chocolate. However, if more loss of 3-methylbutanoic acid, 5-methylfurfural, acetic acid, azine, heptanal, n-amyl alcohol, and phenylacetic acid and phenylacetaldehyde is desired, longer conching time may be needed.

During storage, volatiles decreased over time at 4, 25, and 50 °C in dark and unsweetened milk chocolate. However, because of high temperature of the chocolate stored at 50 °C, chemical reactions, such as lipid oxidation and the Maillard reaction, occurred, and it may cause defect of flavor quality of chocolate. Also, oil separation was observed in the first week of storage in the 50 °C sample. There was no significant difference found between 4 and 25 °C. Therefore, the results suggested that the best storage temperature should be at 25 °C, which had less chemical reactions occurred than stored at 50 °C and less energy needed than stored at 4 °C.

The result of blooming test showed only acetic acid was significant decreased when blooming occurred. The recrystallization may influence the release of acetic acid. Understanding how the recrystallization affected acetic acid may be further applied to reduce acetic acid in chocolate since it contributed undesired aroma.

In the real time volatile release results, the effects of sugar and milk solid on volatile release during eating were not significant. The difference observed between headspace, mouthspace and nosespace may be a reference for headspace studies in the future. Some volatiles, such as benzaldehyde, 2-phenylethanol, 3-methylbutanal, benzyl alcohol, 3-methylbutanoic acid, and 2, 3-butanedione were lingering longer than other compounds.

There was no correlation found between air-water partition and volatile release in headspace, mouthspace, and nosespace. Therefore, there should have some other factors can affect the release of volatiles during eating. It may be interesting to investigate why those compounds can retain in the mouth and apply it to retain more volatiles in the mouth.

References

Akhtar M, Koutsidis G, Chan PSK, Kanjee U, Clayton G. 2010. Physical and chemical properties of dark chocolate processed using spinning disc reactor technology – effects of disc temperature and residence time. Food Manufacturing Efficiency. 3(1):7-14.

Baigrie BD, Rumbelow SJ. 1987. Investigation of flavor defects in Asian cocoa liquors. J. Sci. Food Agric. 39:357-368.

Bainbridge J. S. Davies S. H. 1912. The essential oil of cocoa. J. Chem. Soc. 101 (1912): 2209–2220.

Beckett S.T. 1994. Industrial chocolate manufacture and use. 2nd ed. Glasgow: Blackie A&P. p. 1

Bonvehi JS. 2005. Investigation of aromatic compounds in roasted cocoa powder. Eur Food Res Technol. 221:19-29.

Bricknell J, Hartel RW. 1998. Relation of fat bloom in chocolate to polymorphic transition of cocoa butter J. Am. Oil Chemists' Soc. 75(11):1609-1615.

Counet C. Callemien D. Ouwerx C. Collin S. 2002. Use of gas chromatographyolfactometry to identify key odorant compounds in dark chocolate. Comparison of samples before and after conching. J. Agric. Food Chem. 50(8):2385-2391.

De Melo LLMM, Bolini HMA, Efraim P. 2009. Storage time study of sugar-free and reduced calorie milk chocolates. J Food Quality. 32:577-589.

Dimick P.S. Hoskin J.M. 1981. Chemico-physical aspects of chocolate processing-a review. Can Inst. Food Sci. Technol. J. 14(4):269-282

Diaz ME. 2004. Comparison between orthonasal and retronasal flavor perception at different concentrations. Flavour Fragr J. 19:499-504.

Dutta BK. 2007. Principles of mass transfer and separation processes. New Delhi: Prentice-Hall of India. P7-59.

Ebeler S.E. Pangborn R.M. Jennings W.G. 1988. Influence of dispersion medium on aroma intensity and headspace concentration of menthone and isoamyl acetate. J. Agric. Food Chem. 36(4):791-796.

Fadel HHM, Abdel Mageed MA, Abdel Samad AKME, Lotfy SN. 2006. Cocoa substitute: evaluation of sensory qualities and flavor stability. Eur Food Res Technol. 223:125-131.

Flink J, Karel M. 1970. Retention of organic volatiles in freeze-dried solutions of carbohydrates. J. Agric. Food Chem. 18(2):295-297.

Frankel E. (1982). Volatile lipid oxidation products. Progress in Lipid Research. 22(1):1-33.

Frauendorfer F. Schieberle P. 2006. Identification of the key aroma compounds in cocoa powder based on molecular sensory correlations. J. Agric. Food Chem. 54(15):5521-5529.

Frauendorfer F. Schieberle P. 2008. Changes in key aroma compounds of Criollo cocoa beans during roasting. J. Agric. Food Chem. 56(21): 10244-10251.

Gill MS, Macleod AJ, Moreau M. 1984. Volatile components of cocoa with particular reference to glucosinolate products. Phytochemistry. 23(9): 1937-1942.

Godshall M.A. 1995. Role of sucrose in retention of aroma and enhancing the flavor of foods. In: Mathlouthi M and Reiser P, editors. Sucrose, properties and application. New York : Blackie Academic & Professional. p.249-263

Gremli HA. 1974. Interaction of flavor compounds with soy protein. J. Am. Oil Chemists' Soc. 51:95A-97A.

Guinard J.X. Mazzucchelli R. 1999. Effects of sugar and fat on the sensory properties of milk chocolate: descriptive analysis and instrumental measurements. J. Sci. Food Agric. 79:1331-1339.

Hall G, Andersson J. 1983. Volatile fat oxidation products 2. Influence of temperature on volatility of saturated, mono- and di-unsaturated aldehydes in liquid media. Lebensm. Wiss. –Technol. 16:362-366.

Haring PG. 1990. Flavor release: from product to perception. In: Bessiere Y and Thomas AF, editors. Flavor Science and Technology. Wiley: Chichester, U.K. p.351-354.

Hashim L, Hudiyono S, Chaveron H. 1997. Volatile compounds of oxidized cocoa butter. Food Research International. 30(3-4):163-169.

Huang Y, Barringer SA. 2010. Alkylpyrazines and other volatiles in cocoa liquors at pH 5 to 8, by Selected ion flow tube-Mass spectrometry (SIFT-MS). J Food Sci. 75(1): C121-127.

Hodge JE, Mills FD, Fisher BE. 1972. Compounds of browned flavor derived from sugar-amine reactions. Cereal Sci. Today. 17(2): 34-38.

Hodgson M, Parker A, Linforth RST, Taylor AJ. 2004. *In vivo* studies on the long-term persistence of volatiles in the breath. Flavour Fragr. J. 19:470-475.

Hoskin J.M. Dimick P.S. 1979. Volatile fatty acid changes during the conching of chocolate. Proc. Ann. Prod. Conf. of Pa. Manuf. Confectioner's. 33:23-31.

Kato Y, Matsuda T, Kato N, Nakamura R. 1988. Browning and protein polymerization induced by amino-carbonyl reaction of ovalbumin with glucose and lactose. J Agric Food Chem. 36: 806-809.

Keeney PG. 1972. Various interactions in chocolate flavor. J. Am. Oil Chemists' Soc. 49:567-572.

King CJ. 1983. Physical and chemical properties governing volatilization of flavor and aroma components. In: Peleg M, Bagley E, editors. Physical properties of foods. Westport, CT: AVI Publishing Co. p399–421.

Krings U, Zelena K, Wu S, Berger RG. 2006. Thin-layer high-vacuum distillation to isolate volatile flavor compounds of cocoa powder. Eur Food Res Technol. 223:675-681.

Landy P. Druaux C. Voilley A. 1995. Retention of aroma compounds by proteins in aqueous solution. Food Chem. 54(4):387-392.

Legger A, Roozen JP. 1994. Gas chromatography and sensory analysis of chocolate flavor: intensity changes in time. In: Maarse H, van der Heij DG, editors. Trends in Flavour Research. Amsterdam, The Netherlands: Elsevier Science. p.287-291

Leonardos G, Kendall D, Barnard N. 1969. Odor threshold determinations of 53 odorant chemicals. J. Air Pollut Control Assoc. 19(5): 91-95.

Linforth R, Martin F, Carey M, Davidson J, Taylor AJ. 2002. Retronasal transport of aroma compounds. J. Agric. Food Chem. 50:1111-1117.

Lopez A, Quesnel V. 1973. Volatile fatty acid production in cacao fermentation and the effect on chocolate flavor. J Sci Food Agric. 24:319-326.

Maniere FY. Dimick PS. 1979. Effects of conching on the flavor and volatile components of dark semi-sweet chocolate. LWT-Food Sci and Technol. 12(2): 102-107.

Mastrocola D, Munari M. 2000. Progress of the Maillard reaction and antioxidant action of Maillard reaction products in preheated model systems during storage. J Agric Food Chem. 48:3555-3559.

Massaldi HA, King CJ. 1973. Simple technique to determine solubilities of sparingly soluble organics: solubility and activity coefficients of *d*-limonene, *n*-butylbenzene, and *n*-hexyl acetate in water and sucrose solutions. J Chem Eng Data. 18(4): 393-397.

Mexis SF, Badeka AV, Riganakos KA, Kontominas MG. 2010. Effect of active and modified atmosphere packaging on quality retention of dark chocolate with hazelnuts. Innov Food Sci Emerg Technol. 11:177-186.

Michel E, Schnoon N, Amelynck C, Guimbaud C, Catoire V, and Arijs E. 2005. A selected ion flow tube study of the reactions of H_3O^+ , NO^+ , and O_2^+ with methyl vinyl ketone and some atmospherically important aldehydes. Int J Mass Spectrom. 244(1): 50-59.

Mohr W. 1959. The investigation of cocoa flavor by gas chromatograph; with special reference to chocolate conching. International chocolate review. 14: 371-375.

Nawar WW. 1971. Some variables affecting composition of headspace aroma. J Agr Food Chem. 19(6): 1057-1059.

Nebesny E, Rutkowski J. 1998. Effect of cocoa bean enrichment and chocolate mass conching on the composition and properties of chocolates. Pol J Food Nutr Sci. 4: 673-681.

Pickenhagen W. 1999. Flavor chemistry-the last 30 years. In: Teranishi R, Wick EL, Hornsten I. Flavor chemistry: thirty years of progress. New York: Kluwer Academic/Plenum. P 75.

Piggott JR, Schaschke CJ. 2001. Release cells, breath analysis and in-mouth analysis in flavor research. Biomol Eng 17:129-36.

Plumas B, Hashim L, Chaveron H. 1996. Measurement of the olfactive intensity of chocolates by differential olfactometry. Food Control. 7(3):117-120.

Pontillon J. 1995. La fabrication du chocolat. In: Pour la Science. Paris: Editions Belin. P118-126.

Praag MV, Stein HS, Tibbetts MS. 1968. Steam volatile aroma constituents of roasted cocoa beans. J Agric Food Chem. 16(6): 1005-1008.

Pratt-Johnson WH. 1987. Factors affecting chocolate quality. The Manufacturing confectioner. 67(5):52-56.

Prawira M, Barringer SA. 2009. Effects of conching time and ingredients on preference of milk chocolate. J Food Process Pres. 33:571-589.

Ramli N. Hassan O. Said M. Samsudin W. Idris N.O. 2005. Influence of roasting conditions on volatile flavor of roasted Malaysian cocoa beans. J. Food Process Pres. 30:280-298.

Reineccius G. 2006. Flavor chemistry and technology. 2nd ed. Boca Raton: Taylor & Francis. 33-66p. 104-105p

Rizzi GP. 1974. Formation of N-alkyl-2-acyl-pyrroles and aliphatic aldimines in model nonenzymatic browning reactions. J Agric Food Chem. 22(2): 279-282.

Robert A, Martin JR. 1987. Chocolate. In: Chichester, editor. Advances in food research. San Diego, CA: Academic Press, INC. 31:213-330.

Roberts DD, Elmore JS, Langley KR, Bakker J. 1996. Effects of sucrose, guar gum, and carboxymethylcellulose on the release of volatile flavor compounds under dynamic conditions. J Agric Food Chem. 44: 1321-1326.

Saint-Eve A, Martin N, Levy C, Souchon I. 2006. How can protein ration affect aroma release, physical properties and perceptions of yoghurt? Flavor Science: Recent Advances and Trends. 43:391-394.

Schnermann P. Schieberle P. 1997. Evaluation of key odorants in milk chocolate and cocoa mass buy aroma extract dilution analyses. J Agric Food Chem. 45(3): 867-872

Smith D, Wang T, Spanel P. 2003. Analysis of ketones by selected ion flow tube mass spectrometry. Rapid Commun Mass Sp. 17: 2655-2660.

Spanel P, Smith D. 1997. SIFT studies of the reactions of H_3O^+ , NO^+ , and O_2^+ with a series of alcohols. Int J Mass Spectrom and Ion Processes. 167/168: 375-388.

Spanel P, Smith D. 1998a. SIFT studies of the reactions of H_3O^+ , NO^+ , and O_2^+ with a series of volatile carboxylic acids and esters. Int J Mass Spectrom and Ion Processes. 172: 137-147.

Spanel P, Smith D. 1998b. Selected ion flow tube studies of the reactions of H_3O^+ , NO^+ , and O_2^+ with several amines and some other nitrogen-containing molecules. Int J Mass Spectrom. 176:203-211.

Spanel P, Smith D. 1998c. Selected ion flow tube studies of the reactions of H_3O^+ , NO⁺, and O_2^+ with several aromatic and aliphatic hydrocarbons. Int J Mass Spectrom. 181:1-10.

Spanel P, Smith D. 1999. Selected ion flow tube – mass spectrometry: detection and real-time monitoring of flavours released by food products. Rapid Commun Mass Spectrom. 13:585-596.

Spanel P, Van Doren JM, Smith D. 2002. A selected ion flow tube study of the reactions of H_3O^+ , NO^+ , and O_2^+ with saturated and unsaturated aldehydes and subsequent hydration of the product ions. Int J Mass Spectrom. 213:163-176.

Spanel P, Ji Y, Smith D. 1997. SIFT studies of the reactions of H_3O^+ , NO^+ , and O_2^+ with a series of aldehydes and ketones. Int J Mass Spectrom and Ion Processes. 165/166:25-37.

Stauffer M. 2000. The flavor of milk chocolate changes caused by processing. The Manufacturing Confectioner. 80(9): 113-118.

Syft Technologies Inc. 2007. SIFT-MS technology overview [online]. New Zealand: Syft Technologies Inc, 2007 [cite 16 January 2010]. Available from World Wide Web:

http://www.syft.com/images/Documents/Technology/syfttechnologyoverview1.3.pdf

Syft Technologies Inc. 2009. Kinetics library database. Christchurch, New Zealand: Syft Technologies Inc.

Taylor AJ. 1996. Volatile flavor release from foods during eating. Food Sci Nutr. 36(8): 765-784.

Taylor AJ, Linforth RST. 1996. Flavour release in the mouth. Trends Food Sci Tech. 7:444-448.

Wang T, Smith D, Spanel P. 2004a. Selected ion flow tube, SIFT, studies of the reactions of H_3O^+ , NO^+ , and O_2^+ with compounds released by Pseudomonas and related bacteria. Int J Mass Spectrom. 233:245-251.

Wang T, Spanel P, Smith D. 2004b. A selected ion flow tube, SIFT, study of the reactions of H_3O^+ , NO^+ , and O_2^+ ions with several N- and O-containing heterocyclic compounds in support of SIFT-MS. Int J Mass Spectrom. 237:167-174.

Wang T, Spanel P, Smith D. 2004c. A selected ion flow tube study of the reactions of H_3O^+ , NO^+ , and O_2^+ with some phenols, phenyl alcohols and cyclic carbonyl compounds in support of SIFT-MS and PTR-MS. Int J Mass Spectrom. 239:139-146.

Welty WM, Marshall RT, Grun IU, Ellersieck MR. 2001. Effects of milk fat, cocoa butter, or selected fat replacers on flavor volatiles of chocolate ice cream. Am Dairy Sci Assoc. 84:21-30.

Wientjes AG. 1968. The influence of sugar concentrations on the vapor pressure of food odor volatiles in aqueous solutions. J Food Sci. 33(1):1-2.

Wille RL, Lutton ES. 1966. Polymorphism of cocoa butter. J. Am. Oil Chemists' Soc. 43:491-496.

Xu Y. Barringer SA. 2010. Comparison of volatile release in Tomatillo and different varieties of tomato during chewing. J Food Sci. 75(4):C352-C358.

Yaylayan V, Keyhani A. 1999. Origin of 2,3-Pentanedione and 2,3-butanedione in D-Glucose/L-Alanine Maillard model systems. J Agric Food Chem. 47:3280-3284.

Appendix A



Figure A.1 Needle position of sampling volatiles in mouthspace or nosespace



Figure A.2 The temperature changes of unsweetened dark (UDC), dark (DC), and unsweetened milk chocolate (UMC) during 48h conching

 Table A.1 The contribution to total concentration at 1h and contribution to total lost at 48h of top 10 volatiles in different types of chocolate

Unsweetened dark chocolate					
Top 10 volatiles	Percent of total concentration	Percent of total lost at 48h			
methanol	50	46			
acetic acid	10	5.3			
2, 3-butanedione	7.2	8.6			
methylbutanals	5.1	6.2			
isobutanoic and butanoic acid	2.5	3.2			
methylpropanals	2.4	3.3			
acetone	2.4	3.2			
benzaldehyde	2.2	2.7			
furfuryl pyrrole	2.1	2.7			
nonanal	1.7	2.0			
Dark chocolate					
Top 10 volatiles	Percent of total concentration	Percent of total lost at 48h			
methanol	53	60			
acetic acid	14	2.9			
methylbutanals	4.8	5.8			
2, 3-butanedione	4.6	5.2			
nonanal	2.4	2.6			
isobutanoic and butanoic acid	2.3	2.7			

Table A.1 continued

benzaldehyde	1.9	2.2
furfuryl pyrrole	1.6	1.9
methylpropanals	1.5	1.9
acetone	1.5	1.8
Unsweetened mill	chocolate	
Top 10 volatiles	Percent of total concentration	Percent of total lost at 48h
methanol	48	50
acetic acid	16	12
2, 3-butanedione	4.5	4.7
methylbutanals	3.9	4.5
nonanal	3.2	3.5
isobutanoic and butanoic acid	2.6	2.8
benzaldehyde	2.3	2.6
furfuryl pyrrole	1.7	1.9
acetone	1.7	1.7
benzyl alcohol	1.3	1.3

Unsweetened dark chocolate						
Top 10 volatiles at 1h	Concentration (ppb)	Top 10 volatiles at 48h	Concentration (ppb)			
methanol	92000	methanol	31289			
acetic acid	19000	acetic acid	12102			
2,3-butanedione	13000	2,3-butanedione	1827			
methylbutanals	9300	methylbutanals	1096			
isobutanoic and butanoic acid	4600	3-methylbutanoic acid	495			
methylpropanals	4500	isobutanoic and butanoic acid	436			
acetone	4400	nonanal	429			
benzaldehyde	3900	benzaldehyde	427			
furfuryl pyrrole	3800	phenylacetic acid and phenylacetaldehyde	296			
nonanal	3100	acetone	263			
Dark chocolate						
Top 10 volatiles at 1h	Concentration (ppb)	Top 10 volatiles at 48h	Concentration (ppb)			
methanol	15000	acetic acid	32963			
acetic acid	39000	methanol	14089			
methylbutanals	14000	3-methylbutanoic acid	1211			
2,3-butanedione	13000	2,3-butanedione	1141			
nonanal	6800	nonanal	977			

Table A.2 The top 10 volatiles and their concentration at 1 and 48h

Table A.2 continued

isobutanoic and butanoic acid	6600	2-methylbutanal	707	
		phenylacetic acid and		
benzaldehyde	5500	phenylacetaldehyde	556	
furfuryl pyrrole	4600	benzyl alcohol	541	
methylpropanals	4400	isobutanoic and butanoic acid	528	
acetone	4300	azine	527	
Unsweetened milk	chocolate	I		
Top 10 volatiles at 1h	Concentration (ppb)	Top 10 volatiles at 48h	Concentration (ppb)	
methanol	88000	acetic acid	11622	
acetic acid	30000	methanol	11258	
2,3-butanedione	8200	2,3-butanedione	989	
methylbutanals	7200	3-methylbutanoic acid	573	
nonanal	5900	nonanal	499	
isobutanoic and butanoic acid	4700	acetone	447	
benzaldehyde	4300	methylbutanals	393	
furfuryl pyrrole	3200	benzyl alcohol	343	
acetone	3100	isobutanoic and butanoic acid	325	
benzyl alcohol	2400	benzaldehyde	321	

	UDC	UMC	DC
(E)-2-octenal	90	91	88
2, 3-butanedione	86	88	91
2, 3-diethyl-5-methylpyrazine	96	98	94
2-acetylpyrrole	98	87	82
2-heptanol	87	85	85
2-heptanone	86	78	77
2-nonanone	93	94	92
2-phenylethanol and phenylacetaldehyde	89	89	85
3-methylbutanoic acid	60	67	43
3-methylindole	97	98	97
5-methylfurfural	65	68	59
acetic acid	36	61	16
acetone	94	86	93
azine	40	75	51
benzaldehyde	89	92	91
benzonitrile	92	91	87
benzyl alcohol	90	86	80
decadienals	91	95	84
delta-decalactone	92	92	93
dimethylpyrazines	85	90	84
furaneol and sotolone	80	80	65

Table A.3 Percent lost of volatiles in unsweetened dark chocolate (UDC), unsweetened milk (UMC), and dark chocolate (DC) after conching

Continued

Table A.3 continued

furfural	86	90	88
furfuryl pyrrole	95	96	91
guaiacol	99	80	76
heptanal	62	73	67
indole	95	96	96
isobutanoic and butanoic acid	91	93	92
maltol	87	72	70
methanol	66	87	91
methylbutanals	88	95	95
methylpropanals	96	92	95
n-amyl alcohol	72	79	77
nonadienal	91	80	78
nonanal	86	92	86
nonenal	85	93	84
phenylacetaldehyde and acetophenone	83	87	77
phenylacetic acid and phenylacetaldehyde	74	75	58
tetramethylpyrazines	87	90	87
toluene	93	87	85
trimethylpyrazines	70	80	70
vanillin	92	97	89
γ -octalactone and 2-nonanone	96	90	91

	DC 50°C					
Days	1	7	14	35	42	56
nonenal	18	10	10	12	10	7.1
(E)-2-octenal	10	7.7	6.8	5.1	5.4	4.1
decadienals	30	21	14	16	13	13
nonadienal	10	10	7.5	10	6.8	5.7
2, 3-butanedione	1100	1100	940	820	600	548
2, 3-diethyl-5-methylpyrazine	61	46	35	44	32	26
dimethylpyrazines	210	140	120	110	96	90
2-acetylpyrrole	120	76	75	85	70	66
2-heptanol	160	120	100	110	80	74
2-heptanone	65	48	42	27	24	16
methylbutanals	1100	930	790	710	460	486
methylpropanals	300	300	280	310	220	253
2-nonanone	49	35	27	42	20	22
2-phenylethanol and phenylacetaldehyde	390	300	270	350	290	283
3-methylbutanoic acid	390	300	280	290	250	240
3-methylindole	36	28	22	26	18	15
5-methylfurfural	40	25	24	22	17	17
acetic acid	21000	19000	17000	20000	18000	17217
acetone	270	410	380	480	350	418
azine	890	670	630	720	630	670
benzaldehyde	450	330	300	310	220	235
benzonitrile	140	100	84	86	63	60
benzyl alcohol	260	170	150	170	160	154
δ-decalactone	3.9	2.1	0	1.2	1	0
furaneol and sotolone	110	61	46	39	27	22
furfural	110	82	64	60	54	49
furfuryl pyrrole	190	130	95	90	60	57
γ-octalactone and 2-nonanone	19	19	17	15	13	14
guaiacol	19	15	13	11	10	9.2
heptanal	28	23	21	21	14	12
indole	46	29	25	22	15	18

Table A.4 The volatile concentrations during storage in dark chocolate (DC) and unsweetened milk chocolate (UMC).

91

Continued

Table A.4 continued

• 1 4 • 11 4 • • 1	770	580	480	480	340	370
isobutanoic and butanoic acid	170	10	460	400	340	370
maltol	17000	12	11	5./	8.9	0.8
methanol	17000	16000	14000	11000	8000	9146
n-amyl alcohol	330	230	190	150	98	100
nonanal	260	210	190	220	140	147
phenylacetaldehyde and acetophenone	110	72	60	70	53	47
phenylacetic acid and phenylacetaldehyde	590	460	410	470	370	383
tetramethylpyrazines	173	161	162	166	119	124
toluene	75	56	54	58	50	47
trimethylpyrazines	170	140	130	120	95	93
vanillin	110	79	66	82	62	61
Total	47227	42535	37622	37837	31164	31626
	DC 25	C				
Days	1	7	14	35	42	56
nonenal	20	20	14	9.4	5.7	6.5
(E)-2-octenal	9.3	8.5	7.2	4.3	3	2.2
decadienals	35	26	22	19	9.3	7.9
nonadienal	12	13	6.5	4.7	8.8	2.5
2, 3-butanedione	1200	830	600	570	460	384
2, 3-diethyl-5-methylpyrazine	80	60	44	28	15	21
dimethylpyrazines	250	180	140	110	72	83
2-acetylpyrrole	130	100	89	78	49	62
2-heptanol	180	120	100	84	58	67
2-heptanone	70	49	41	34	24	26
methylbutanals	1200	760	530	460	340	323
methylpropanals	340	270	190	190	140	139
2-nonanone	60	42	33	19	7.6	7.9
2-phenylethanol and phenylacetaldehyde	400	350	280	260	180	208
3-methylbutanoic acid	430	350	280	240	160	175
3-methylindole	57	28	23	21	11	11
5-methylfurfural	39	28	27	19	14	16
acetic acid	21000	21000	19000	18000	15000	15657

Continued
Table A.4 continued

acetone	320	340	260	330	260	230
azine	880	740	680	640	510	574
benzaldehyde	530	380	270	250	150	155
benzonitrile	170	120	94	77	43	49
benzyl alcohol	280	240	180	180	120	141
δ-decalactone	0	3.9	0	0	1.5	0
furaneol and sotolone	110	66	60	40	35	34
furfural	130	100	78	74	49	54
furfuryl pyrrole	230	160	120	97	59	62
γ-octalactone and 2-nonanone	26	18	15	14	7.5	9.2
guaiacol	22	17	14	15	10	10
heptanal	30	22	17	18	13	12
indole	55	27	21	19	7.7	12
isobutanoic and butanoic acid	820	610	490	410	320	263
maltol	9	13	8.7	7.8	5.3	7.4
methanol	18000	13000	9600	8900	6900	5366
n-amyl alcohol	350	240	200	170	150	129
nonanal	320	260	220	170	80	87
phenylacetaldehyde and acetophenone	100	80	65	50	40	43
phenylacetic acid and phenylacetaldehyde	590	520	450	410	320	345
tetramethylpyrazines	233	172	143	113	61	78
toluene	78	65	52	46	39	41
trimethylpyrazines	180	140	120	97	67	76
vanillin	120	110	89	68	38	49
Total	49095	41677	34673	32347	25843	25026
	DC 4°C					
Days	1	7	14	35	42	56
nonenal	21	15	9.4	10	-	5.0
(E)-2-octenal	11	8.4	4.2	4	-	2.2
decadienals	37	30	20	16	-	5.7
nonadienal	9	8	7.8	3.8	-	3.1
2, 3-butanedione	1200	900	630	540	-	300
2 3-diethyl-5-methylpyrazine	90	69	32	32	-	11

Table A.4 continued

dimethylpyrazines	250	190	130	110	-	56
2-acetylpyrrole	140	120	84	66	-	43
2-heptanol	170	140	95	79	-	46
2-heptanone	72	53	45	38	-	22
methylbutanals	1300	910	610	470	-	256
methylpropanals	340	310	200	200	-	105
2-nonanone	70	56	31	20	-	11
2-phenylethanol and phenylacetaldehyde	430	370	270	250	-	151
3-methylbutanoic acid	450	340	240	220	-	124
3-methylindole	46	33	23	20	-	9.3
5-methylfurfural	37	29	23	20	-	13
acetic acid	22000	20000	18000	17000	-	12757
acetone	350	360	290	370	-	179
azine	900	750	650	630	-	446
benzaldehyde	540	410	260	240	-	107
benzonitrile	170	130	78	72	-	33
benzyl alcohol	290	230	160	170	-	113
δ-decalactone	2.3	1.8	1.9	1	-	0
furaneol and sotolone	99	81	59	52	-	30
furfural	130	110	71	69	-	42
furfuryl pyrrole	230	180	110	87	-	37
γ-octalactone and 2-nonanone	26	20	16	11	-	7.8
guaiacol	20	18	11	10	-	8.4
heptanal	29	25	17	16	-	9.0
indole	52	43	23	17	-	7.7
isobutanoic and butanoic acid	850	620	460	410	-	214
maltol	11	12	8.9	7.9	-	4.9
methanol	19000	16000	12000	8500	-	5434
n-amyl alcohol	360	260	200	170	-	107
nonanal	380	310	200	180	-	64
phenylacetaldehyde and acetophenone	110	92	58	54	-	33
phenylacetic acid and phenylacetaldehyde	610	530	420	420	-	284

Table A.4 continued

tetramethylpyrazines	210	179	137	102	-	51
toluene	71	63	50	50	-	32
trimethylpyrazines	180	150	100	93	-	58
vanillin	130	110	79	67	-	22
Total	51425	44267	35912	30902	-	21241
	UMC 50 °C					
Days	1	7	14	35	42	56
nonenal	16	11	8.9	8.5	9.3	10
(E)-2-octenal	6.4	6	6.4	6.3	6.2	5.1
decadienals	18	12	11	7.9	10	6.7
nonadienal	12	6.5	8.4	7.4	7	6.1
2, 3-butanedione	880	930	770	650	530	675
2, 3-diethyl-5-methylpyrazine	43	25	24	18	22	20
dimethylpyrazines	160	120	99	80	83	84
2-acetylpyrrole	86	73	56	55	52	62
2-heptanol	120	110	96	83	73	75
2-heptanone	49	35	34	23	21	21
methylbutanals	780	790	600	490	410	588
methylpropanals	220	250	230	260	230	336
2-nonanone	29	26	26	26	19	29
2-phenylethanol and phenylacetaldehyde	290	220	210	220	220	265
3-methylbutanoic acid	310	250	220	200	200	201
3-methylindole	29	26	16	19	15	23
5-methylfurfural	28	23	22	19	18	19
acetic acid	15000	12000	11000	12000	12000	12339
acetone	250	390	450	620	570	976
azine	550	460	390	390	410	495
benzaldehyde	350	260	220	230	210	278
benzonitrile	100	75	65	62	54	53
benzyl alcohol	210	140	140	130	150	136
δ-decalactone	2.1	1.5	4.1	1.5	2	3.1
furaneol and sotolone	75	43	48	41	36	41
furfural	83	67	67	71	53	61

Table A.4 continued

furfuryl pyrrole	120	90	71	57	53	56
γ-octalactone and 2-nonanone	15	17	18	10	9.3	9.4
guaiacol	29	27	31	36	29	32
heptanal	21	21	20	16	13	13
indole	22	22	16	12	11	21
isobutanoic and butanoic acid	510	440	360	320	280	376
maltol	13	2.9	9.1	17	11	14
methanol	11000	10000	9000	8500	7600	13637
n-amyl alcohol	230	220	180	140	120	125
nonanal	220	140	150	130	130	146
phenylacetaldehyde and acetophenone	64	51	41	32	37	36
phenylacetic acid and phenylacetaldehyde	420	330	290	300	280	299
tetramethylpyrazines	170	150	130	110	100	114
toluene	46	44	41	39	37	38
trimethylpyrazines	140	120	110	100	89	87
vanillin	57	36	25	30	28	33
Total	32773	28063	25313	25568	24235	31845
	UMC 25 °C	C				
Days	1	7	14	35	42	56
nonenal	17	10	9.5	11	7.6	4.9
(E)-2-octenal	8.8	7.2	4.8	7.1	4.6	3.7
decadienals	19	15	11	7.4	5.6	4.0
nonadienal	8.5	10	8.3	8.5	5.6	4.1
2, 3-butanedione	850	810	600	510	470	475
2, 3-diethyl-5-methylpyrazine	45	28	19	17	14	10
dimethylpyrazines	150	130	94	100	81	65
2-acetylpyrrole	89	68	60	57	48	40
2-heptanol	120	110	94	78	68	62
2-heptanone	46	45	36	22	26	20
methylbutanals	750	700	450	330	270	311
methylpropanals	210	200	170	170	150	128
2	37	29	25	20	13	10

Days	1	7	14	35	42	56
	UMC 4°C					
Total	31723	27868	22697	19882	14688	14311
vanillin	62	34	22	21	13	8.1
trimethylpyrazines	140	130	100	97	76	70
toluene	50	40	33	32	25	21
tetramethylpyrazines	160	140	120	96	72	69
phenylacetic acid and phenylacetaldehyde	410	350	300	280	230	183
phenylacetaldehyde and acetophenone	67	40	43	38	36	25
nonanal	220	160	120	120	100	71
n-amyl alcohol	230	250	180	150	130	141
methanol	10000	10000	7000	5500	3400	5075
maltol	13	6.4	10	12	6.6	4.5
isobutanoic and butanoic acid	510	450	340	310	280	243
indole	24	22	16	8.5	8.6	8.1
heptanal	19	20	17	17	15	12
guaiacol	31	32	35	38	35	25
γ -octalactone and 2-nonanone	19	13	15	12	10	10
furfuryl pyrrole	120	100	79	62	48	35
furfural	89	78	70	77	69	55
furaneol and sotolone	68	51	48	41	38	26
δ-decalactone	1.1	0.7	1.8	2	1	0.9
benzyl alcohol	210	140	140	170	150	98
benzonitrile	110	85	67	65	54	39
benzaldehvde	350	260	210	190	140	119
azine	560	480	370	330	270	237
acetone	250	270	300	430	340	289
o-meurynunural	15000	12000	11000	10000	7600	5995
5 mothylfurfurel	23	20	22	20	17	14
3-methylbutanoic acid	25	270	18	15	170	143
phenylacetaldehyde	330	270	240	220	100	145
2-phenylethanol and	280	230	200	190	160	142

Table A.4 continued

Table A.4 continued

nonenal	17	10	10	10	11	5.1
(E)-2-octenal	10	5.1	3.9	6.7	6.5	2.9
decadienals	20	14	8	8.6	7.5	2.5
nonadienal	12	6.5	6.9	6.1	3.6	1.9
2, 3-butanedione	1000	720	610	520	460	355
2, 3-diethyl-5-methylpyrazine	49	29	21	18	11	6.5
dimethylpyrazines	170	110	93	85	73	58
2-acetylpyrrole	94	69	49	48	46	39
2-heptanol	130	98	84	72	66	53
2-heptanone	57	41	36	22	20	17
methylbutanals	870	670	490	360	300	254
methylpropanals	240	190	160	160	160	112
2-nonanone	61	27	24	15	24	11
2-phenylethanol and phenylacetaldehyde	310	220	190	170	150	124
3-methylbutanoic acid	340	250	220	190	150	126
3-methylindole	36	21	17	15	11	9.0
5-methylfurfural	31	23	21	17	15	11
acetic acid	16000	13000	11000	8800	7100	5461
acetone	290	250	270	410	410	252
azine	550	470	380	310	260	207
benzaldehyde	380	250	210	180	160	113
benzonitrile	120	74	63	54	48	31
benzyl alcohol	220	150	140	170	170	103
δ-decalactone	0	0	2.8	1	0.9	1.5
furaneol and sotolone	78	53	50	47	35	25
furfural	100	76	65	67	59	40
furfuryl pyrrole	138	89	69	56	46	27
γ-octalactone and 2-nonanone	18	14	13	10	10	7.9
guaiacol	33	26	25	36	32	28
heptanal	25	20	17	15	14	10
indole	24	17	13	11	11	6.4
isobutanoic and butanoic acid	600	460	370	310	270	203
maltol	13	5.8	7.9	9.4	9	4.7

Table A.4 continued

methanol	12000	11000	8100	5200	4200	4111
n-amyl alcohol	260	230	180	140	130	110
nonanal	260	180	130	120	87	61
phenylacetaldehyde and acetophenone	74	47	45	30	37	21
phenylacetic acid and phenylacetaldehyde	430	340	290	260	210	165
tetramethylpyrazines	190	130	99	79	60	57
toluene	53	38	36	29	25	17
trimethylpyrazines	150	120	97	78	72	56
vanillin	63	36	23	18	15	6.3
Total	35515	29579	23738	18162	14986	12314