Deodorization of Garlic Breath Volatiles by Food and Food Components.

Thesis

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Abstract

The ability of foods and beverages to reduce allyl methyl disulfide, diallyl disulfide, allyl mercaptan, and allyl methyl sulfide on human breath after consumption of raw garlic was examined. The treatments were consumed immediately following raw garlic consumption for breath measurements, or were blended with garlic prior to headspace measurements. Measurements were done using selected ion flow tube mass spectrometry (SIFT-MS). Chlorophyllin treatment performed similarly to the control. Successful treatments may be due to enzymatic, polyphenolic, or acid deodorization. If occurring, enzymatic deodorization involved oxidation of polyphenolic compounds by enzymes, with the oxidized polyphenols causing deodorization. This may have been shown in raw apple, parsley, spinach, and mint treatments. Polyphenolic deodorization involved deodorization by polyphenolic compounds without enzymatic activity. This might have occurred for microwaved apple, green tea, and lemon juice treatments. Acid deodorization involves the inactivation of alliinase at a pH below 3.6, which causes garlic volatiles concentrations to be lowered. This deodorization mechanism is possible in soft drink and lemon juice breath treatments, and in pH-adjusted headspace measurements. Whey protein volatile concentrations were similar to the control's with lack of enzymes, polyphenolic compounds, and acidic pH as the possible cause. Headspace concentrations appear to have over-predicted the effectiveness of whey protein and chlorophyll, and appear to have under-predicted the effectiveness of other treatments.

Dedication

This thesis is dedicated to anyone trying to improve every day.

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Practical Application

The chemical make-up of specific foods can reduce the volatile compounds that are known to cause malodorous breath due to the consumption of garlic. The enzymatic activity, polyphenolic compounds, and acidity of specific foods allows for reduction of these volatiles, while chlorophyll does not cause a deodorization effect. The results of this study could lead to commercial application of successful food components in breath enhancers or deodorants, or to further research on the topics of deodorization by these mechanisms. Chlorophyll can potentially be removed from breath enhancement applications. Additionally, the positive health benefits of garlic could be enhanced with an increase in its consumption with practical, successful ways to alleviate its negative effect on breath.

Chapter 1: Introduction

The use of garlic (*Allium sativum* L.) dates back to 2600-2100 BC when Sumerians made the first written record of its use as a spice, medicine, and condiment. It is even believed to have already been in use before these accounts by civilizations in the Indus Valley (Hahn 1996). Garlic is a food that is notorious for having a profound effect on the human body. The food itself has an unmistakable odor and taste separating itself from other types of food. Furthermore, garlic is known to have a significant impact on consumers' breath. For all of these reasons, garlic has become a target for a number of scientific studies.

The four volatile organic compounds responsible for what is known as “garlic breath” are diallyl disulfide, allyl methyl sulfide, allyl mercaptan, and allyl methyl disulfide (Cai and others 1995; Suarez and others 1999; Rosen and others 2001; Tamaki and others 2008).

The formation of these four breath volatiles begins with the degradation of alliin to allylsulfenic acid by the enzyme alliinase. This does not happen until the garlic is crushed or chopped. Allylsulfenic acid is then rapidly condensed to allicin (allylthiosulfinate).

Once allicin is formed, it leads to the formation of garlic volatiles (Negishi and others 2002). Two of these volatiles are allyl methyl disulfide and diallyl disulfide. Allyl methyl disulfide is produced from allicin, is present in raw garlic when chopped, and does not further react to form other garlic volatiles examined in this experiment (Suarez and others 1999). Diallyl disulfide is also present in raw garlic stemming from the transformation of

allicin, but is reduced to form another garlic breath volatile, allyl mercaptan (Iciek and others 2009). While all four garlic breath volatiles are present in raw garlic, allyl methyl sulfide forms outside and inside of the body (Yu and others 1989; Kim and others 1995; Lawson 1998; Hansanugrum and Barringer 2010). In the body, S-adenosylmethionine methylates allyl mercaptan forming allyl methyl sulfide (Lawson 1998). Once allyl methyl sulfide is created, it is metabolized slower than the other volatiles measured. Due to its different formation and metabolism, allyl methyl sulfide's concentration is higher for an extended period of time, which causes it to be the primary volatile associated with malodorous garlic breath (Suarez and others 1999).

There have been a multitude of studies attempting to reduce the volatile organic compounds associated with garlic and its consumption. These studies have attempted volatile deodorization with food and supplemental compounds that can be found in food. Some of the foods that have been proven to deodorize volatile compounds from garlic in-vitro are beverages including green tea, ku-ding-cha tea, raw fruits and vegetables such as apple, prune, kiwi, banana, blueberry, eggplant, spinach, lettuce, parsley, basil, asparagus, yam, cooked rice, cow's milk, bovine serum albumin, egg, pear, peach, nectarine, plum, apricot, cherry, grape, orange, lemon, grapefruit, avocado, cabbage, chicory, celery, peppermint, carrot, potato, ginger, tomato, pepper, cucumber, melon, (Yasuda and Arakawa 1995; Negishi and Negishi 1999; Negishi and others 2002; Negishi and others 2004). It has been proposed that the deodorization effect is caused by enzymatic deodorization.

Other foods have been examined by researchers for their deodorizing ability in-vivo to remove garlic volatiles from human breath after their consumption. Foods that have been shown to deodorize human breath are ku-ding-cha, green tea, apple, pear, prune, chicory, and cow's milk (Negishi and Negishi 1999; Negishi and others 2004; Lodhia and others 2008; Hansanugrum and Barringer 2010). It has been proposed that chlorophyll and its derivatives have the ability to act as an internal deodorant in the human body (Kephart 1955). However, there have been no recent studies supporting this. While foods that are high in chlorophyll have been shown to reduce volatile levels on human breath, it is unknown if it is due to enzymatic deodorization or its chlorophyll content. The objective of this study was to identify foods which deodorize garlic breath volatiles and propose possible mechanisms for their perceived effects.

Chapter 2: Literature Review

2.1 Garlic

2.1.1 Garlic Chemistry

Garlic (*Allium sativum* L.) is a widely-consumed food that is one of the oldest of all cultivated plants. Its unique flavor and folk-like medicinal uses have caused it to be popular and very intriguing. From a food chemistry standpoint, garlic's distinctive odor, taste, and physiological impact on the human body have made it a target for numerous studies (Block 1985). While garlic is technically considered a vegetable, it has considerable differences in its composition compared to other vegetables. One main difference is the content of water in garlic. The water content is only about 65%, whereas most vegetables and fruits are near the 80-90% level. The remaining content of garlic includes mainly fructose-containing carbohydrates ($\approx 26\text{-}30\%$), sulfur compounds, protein, and free amino acids (Lawson 1996).

A major difference between garlic and other fruits and vegetables is its high sulfur content. Fresh garlic contains slightly over 3 mg of sulfur for 1 g of total weight. This level is substantially higher than most other fruits and vegetables which have sulfur levels slightly under 1 mg of sulfur for 1 g of total weight (Lawson 1996). The most important and studied class of sulfides studied in garlic are thiosulfates. The first thiosulfinate identified from garlic was allyl 2-propenethiosulfinate (allicin). Since allicin was only

found in crushed garlic, it was decided that there must be a stable precursor from which allicin is formed when crushed. This precursor is an oxygenated sulfur amino acid known as S-allylcysteine sulfoxide (alliin) which is converted to allicin by an enzyme named alliinase (alliin lyase). Alliin has two asymmetric atoms which allows for four diastereomeric isomers. The predominant isomer that occurs is (+)-S-allyl-L-cysteine sulfoxide (S-2-propenyl-L-cysteine sulfoxide). Also, (+)-S-methyl-L-cysteine sulfoxide (methiin) and (+)-S-*trans*-1-propenyl-L-cysteine sulfoxide (isoalliin) are found in lower levels. While alliin and allicin were the beginning of research on garlic sulfur compounds, the parent compounds of alliin and isoalliin were discovered later on. Garlic was found to contain nine different γ -glutamylpeptides, with six of those containing cysteine. The two most abundant of these γ -glutamylpeptides are γ -glutamyl-S-allylcysteine and γ -glutamyl-S-*trans*-1-propenylcysteine which precede alliin and isoalliin (Lawson 1996).

The total amount of sulfur in garlic is approximately 1.0% of its dry weight. Known sulfur compounds make up approximately 86% of the total sulfur of garlic. Alliin, allicin, and the two previously mentioned γ -glutamylpeptides make up nearly 72% of the total sulfur content (Lawson 1996). Protein sulfur, both soluble and insoluble, accounts for 3% and 6% (9% total) of the total sulfur content (Lawson 1993). Overall, whole garlic cloves have been shown to contain sixteen nonprotein organosulfur compounds with only three compounds not containing cysteine. Crushed garlic cloves were found to contain more nonprotein organosulfur compounds with a total of twenty-three compounds (Lawson 1996).

Early research on garlic showed that allicin is the cause of the food's antibacterial properties in addition to its distinctive odor (Cavallito and Bailey 1944). Allicin is just one of the thiosulfinates found in garlic. It makes up approximately 70% of the total thiosulfinate content of the food. While thiosulfinates are a very important constituent of garlic, they are not directly present in it. Initially, the two main γ -glutamylcysteines (γ -glutamyl-S-allylcysteine and γ -glutamyl-S-trans-1-propenylcysteine) are transformed into cysteine sulfoxides by increased synthesis or availability of γ -glutamylpeptidase and an oxidase. The three cysteine sulfoxides found in garlic are alliin, isoalliin, and methiin at 85%, 5%, and 10% abundance. These cysteine sulfoxides are extremely rare in nature. Methiin is found commonly in cruciferous vegetables, but the other two cysteine sulfoxides are native almost exclusively in the Alliaceae family in the *Allium* genus. The cysteine sulfoxide of most abundance (alliin) is only found in a handful of *Allium* plants. The cysteine sulfoxides have no known functions and exist only to be transformed enzymatically (Lawson 1996).

The transformation of the cysteine sulfoxides occurs very rapidly due to the unusually large amount of the enzyme alliinase (≈ 10 mg/g fresh weight). Alliinase can be found in multiple plants of the *Allium* genus and garlic alliinase is a pyridoxal 5'-phosphate-dependent glycoprotein made up of two subunits. The enzyme shows its optimal effectiveness at a pH range of 5-8, and can be inhibited by an acidic pH (pH of 3.5 or lower) (Lawson 1996). Each alliinase subunit consists of 448 amino acids and has a molecular weight of 51,500 Da. Alliinase works by breaking the C-S bond of the sulfoxide. Specifically, it breaks down alliin into allicin while forming pyruvate and

ammonia (Kuettner and others 2002). Two variations of the enzyme are seen within garlic. One acts on alliin and isoalliin, while the other acts specifically on methiin. The methiin-affecting variation has yet to be isolated, but there is sufficient evidence to know its presence (Lawson and Hughes 1992). It is believed that the cysteine sulfoxides and alliinase are stored in separate compartments of the garlic as whole garlic is free of the thiosulfinates that give it odor. The result of the lysis of cysteine sulfoxides is the formation of eight thiosulfinates. One of which is allicin, the flavor for which garlic is known (Lawson 1996).

2.1.2 Garlic Volatiles

Garlic's thiosulfinates are colorless, liquid volatiles with extremely strong and distinctive odors. They are also very reactive compounds with a plethora of different routes that can be taken when in different solvents or media. The major volatiles formed are that of diallyl trisulfide, diallyl disulfide, and allyl methyl trisulfide (in order of abundance). After these three components, there are a vast number of minor volatiles present in garlic. The appearance of different volatiles from garlic comes from various pathways beginning with the two major thiosulfinates formed. These are allicin and allyl methanethiosulfinate. One thiosulfinate reaction pathway involves water or steam distillation of these two compounds. The oil of steam-distilled chopped garlic has yielded 24 different sulfide compounds, but predominantly allylsulfides and disulfides. A second pathway that exists is that of pure allicin transforming into other compounds. Pure, liquid allicin is an unstable compound that will spontaneously transform into other compounds. When given time to transform, pure allicin will predominantly turn into vinyldithiins and

ajoenes. Vinylthiins and ajoenes are also formed in a third pathway when allicin is in an organic solvent. The product formed depends on the polarity of the solvent. Low-polarity solvents will form primarily vinylthiins with lesser amounts of ajoenes and allyl sulfides. However, high-polarity solvents will turn allicin into mainly diallyl trisulfide, diallyl disulfide, and ajoene with no vinylthiins. Furthermore, when left in a basic solution, thiosulfates will instantly experience hydrolysis and transform into a disulfide. Finally, allicin has shown that it will react quickly with cysteine. The products of this reaction are mercaptocysteines, one of which is S-allylmercaptocysteine. S-allylmercaptocysteine has shown the ability to form allyl mercaptan in the blood (Lawson 1996).

Yu and others in 1989 analyzed volatile components of crushed garlic that were obtained by water, steam, and Likens-Nickerson distillation/solvent extraction using gas chromatography-mass spectrometry (GC-MS). The results of this study demonstrated the presence of propene, propenethiol, 1,2-epithiopropene, allyl methyl sulfide, dimethyl disulfide, 2,4-dimethylfuran, 2-propen-1-ol, diallyl sulfide, tetrahydro-2,5-dimethylthiophene, allyl sulfide, methyl propyl disulfide, 3-methyl-2-cyclopentene-1-thione, trans-1-propenyl methyl disulfide, allyl methyl disulfide, 1,3-dithiane, aniline, 1-hexanol, dimethyl trisulfide, propyl allyl disulfide, allyl disulfide, diallyl disulfide, 1,2-dimercaptocyclopentane, 4-methyl-5-vinylthiazole, allyl methyl trisulfide, 2-methylbenzaldehyde, 3,5-diethyl-1,2,4-trithiolane, isobutyl isothiocyanate, 3-vinyl-4H-1,2-dithiin, diallyl trisulfide, and 2-vinyl-4H-1,3-dithiin in garlic when tested according to their method. This study also pointed out that the major compounds of garlic can be

classified as either sulfur-containing or oxygen-containing compounds. Sulfur-containing compounds could be further classified by the number of sulfurs they contained (one, two, or three). This classification system came to be from the decomposition and rearrangement of diallyl thiosulfinate. The reactions with diallyl thiosulfinate are thought to be the predominant forces in the final stages of formation for compounds (Yu and others 1989).

In 1989, Laakso and others also analyzed the volatile garlic odor compounds using combined headspace gas chromatography-mass spectrometry. They analyzed the garlic volatiles found in sliced garlic, as well as from garlic powder and crushed garlic incubated in vegetable oil. The volatiles present in the sliced garlic were acetaldehyde, acetone, acetic acid, diallyl disulfide, trans-1-propenyl methyl disulfide, allyl methyl disulfide, dimethyl trisulfide, diallyl disulfide, allyl methyl trisulfide, diallyl trisulfide, allyl mercaptan, dimethyl sulfide, cis-1-propenyl methyl disulfide, cis-1-propenyl allyl disulfide, and trans-1-propenyl allyl disulfide. The volatiles produced by the garlic powder and crushed garlic incubated in the vegetable oil did not include all of the volatiles listed from the sliced garlic. The main difference in the incubated garlic was the absence/decrease of the di- and trisulfides. This was attributed to poor stability of allicin in a lipophilic atmosphere. Furthermore, no dithiines or ajoenes were present in this study as they most likely decomposed during the headspace-vial incubation at high temperature (Laakso and others 1989).

A study done by Kim and others in 1995 analyzed the garlic volatiles present in stir-fried garlic. This was done by extraction with supercritical carbon dioxide, then purge

and trap injection (PTI) into a sample flask along with simultaneous distillation and extraction (SDE) to be measured using gas chromatography-mass spectrometry and high-performance liquid chromatography. The extraction by supercritical carbon dioxide was done to concentrate the volatiles of the stir-fired garlic that were dissolved in the soybean oil. Volatiles reported in this study were allyl methyl sulfide, 2-propen-1-ol, diallyl sulfide, trans-1-propenyl methyl disulfide, allyl methyl disulfide, dimethyl trisulfide, propyl allyl disulfide, diallyl disulfide, allyl methyl trisulfide, 3-vinyl-4H-1,2-dithiin, diallyl trisulfide, allyl mercaptan, dimethyl sulfide, 2-vinylcyclopropanal, cis-1-propenyl methyl disulfide, 3-methyl thiopropanal, cis-1-propenyl allyl disulfide, trans-1-propenyl allyl disulfide, allyl thiopropanal, allyl propyl trisulfide, and 2-vinyl-4H-1,2-dithiin. The main volatiles isolated by PTI were dimethyl sulfide, allyl alcohol, diallyl sulfide, allyl methyl disulfide, and diallyl disulfide, while SDE isolated diallyl disulfide, dithiins, and diallyl trisulfide. The PTI method was not always advantageous over SDE as headspace gas analysis is not an ideal method for identifying peaks with high boiling points like allicin. Allicin did not show a high yield as it possibly dissolved in the soybean oil which would lead to non-detection (Kim and others 1995).

Overall, 43 compounds have been identified with their presence in garlic verified. The reoccurrence of a volatile in multiple studies shows that is constant in the food and not dependent upon the method of analysis. Table 2.1 shows the common volatiles found in garlic in the previously mentioned studies. This table also shows the abundance of each volatile as noted by Kim and others in 1995. A requirement for each volatile to be included in this table is that it must be present in multiple studies. This table shows the

Table 1 Garlic Volatiles Present in Common Studies

Volatile	Yield (mg/100g of raw garlic)^a
allyl methyl sulfide	0.37 ^b
2-propen-1-ol	1.00 ^b
diallyl sulfide	1.04 ^{bc}
trans-1-propenyl methyl disulfide	0.81 ^{bc}
allyl methyl disulfide	8.16 ^{bc}
dimethyl trisulfide	1.08 ^{bc}
allyl propyl disulfide	0.03 ^b
diallyl disulfide	48.6 ^{bc}
allyl methyl trisulfide	12.8 ^{bc}
3-vinyl-4H-1,2-dithiin	0.77 ^b
diallyl trisulfide	71.7 ^{bc}
allyl mercaptan	0.06 ^c
dimethyl sulfide	0.27 ^c
cis-1-propenyl methyl disulfide	0.25 ^c
cis-1-propenyl allyl disulfide	3.07 ^c
trans-1-propenyl allyl disulfide	7.67 ^c

^a Volatile level in garlic homogenates (Kim and others 1995).

^b Yu and others 1989

^c Laakso and others 1989

most abundant volatile found in garlic is diallyl trisulfide, which is followed by diallyl disulfide and then allyl methyl trisulfide. These three volatiles are the major compounds present in garlic (Lawson 1996). Of the minor compounds found in garlic, the highest levels belong to allyl methyl disulfide, trans-1-propenyl allyl disulfide, and cis-1-propenyl allyl disulfide.

2.1.3 Garlic Bioavailability and Breath Volatiles

Garlic has several bioavailable components that cause numerous reactions within the human body. There is very little data available from preclinical and clinical studies describing the absorption, metabolism, and distribution of garlic-derived compounds. One of the only water-soluble bioavailable components found in garlic is S-allyl-L-cysteine (SAC). The concentration of SAC increases through a long-term extraction in an aqueous medium. After oral intake of garlic, SAC can be detected in plasma, as well as the liver and kidney. The compound has shown high bioavailability in mice (103.0%), rats (98.2%), and in dogs (87.2). In humans, SAC is believed to be N-acetyl-S-allyl-L-cysteine as this compound has been found in human urine. SAC is commonly used for standardization of prepared garlic products as it found in many preparations. Presently, SAC is the only reliable marker used to detect garlic consumption in human subjects (Amagase and others 2001; Amagase 2006).

The majority of organosulfur compounds found in garlic are oil-soluble. The oil-soluble compounds are allicin, sulfides, ajoene, and vinylthiins. Having a small amount of data about garlic bioavailability is due to the fact that all of these compounds are not found in blood or urine after consumption of garlic (Amagase and others 2001). Studies

done on alliin in mice and rats have shown that alliin is never converted to allicin in vivo and is actually metabolized into various organosulfur compounds such as diallyl disulfide by enzymes in the liver. Concerning allicin, there have been no definitive studies outlining the absorption of allicin in the digestive tract. It has been reported that allicin is metabolized into other compounds and released into the breath (Amagase 2006).

History has shown the difficulty in finding garlic metabolites in blood and urine; analysis of human breath has been done to further analyze garlic and its reactions in the human body. In 1989, Minami and others tested the mouths of subjects immediately following garlic consumption. A glass microhematocrit capillary tube was sealed with clay and the needle of a gas-tight syringe was inserted into the sealed end of the capillary tube. With the capillary tube in the mouth, each subject expired 5.0 mL of mouth air into the tube. The air was then analyzed by gas chromatography-mass spectrometry. Measurements were taken at the time of consumption and 1, 2, and 3 hours after consumption. The results of this study were that dimethyl disulfide, dipropyl disulfide, allyl mercaptan, allyl methyl disulfide, and diallyl disulfide were present in human breath after garlic consumption. It was also reported that allyl mercaptan is the major garlic volatile found on human breath after consuming garlic (Minami and others 1989).

A more recent study done by Cai and others in 1995 used atomic emission detection in conjunction with gas chromatography to identify organoselenium compounds in human breath after the ingestion of garlic. Gas chromatography-atomic emission detection has proven the ability to perform multielement analysis simultaneously. This has allowed them to detect mixed organoselenium-sulfur compounds. The major

compounds found in breath after 1 hour of garlic consumption were allyl methyl sulfide, allyl methyl disulfide, diallyl sulfide, diallyl disulfide, allyl mercaptan, dimethyl selenide, allyl methyl selenide, methanesulfenoselenoic acid methyl ester, 2-propenesulfenoselenoic acid methyl ester, dimethyl disulfide, diallyl trisulfide, and dimethyl selenide. Of these, allyl methyl sulfide, allyl methyl disulfide, diallyl sulfide, diallyl disulfide, allyl mercaptan, dimethyl disulfide, and diallyl trisulfide were the organosulfur compounds (Cai and others 1995).

For an additional perspective other than just chromatography, Tamaki and others in 2008 also used electronic nose to measure garlic odor in vitro and in vivo in 2008. They developed a semiconductor-based electronic nose which used multiple metal oxide elements to respond to different odor molecules. These metal oxide semiconductors have an advantage that they are sensitive to a low vapor concentration, resistant to change in humidity, and possess a wide range of selectivity due to the abundance of electrons on the surface of the semiconductor. This study found that methyl mercaptan, allylthiol, diallyl disulfide, and allyl methyl sulfide were present from in vitro tests (Tamaki and others 2008).

For possible explanations as to how volatiles are formed in the body and noticed in the breath, studies were done on how garlic volatiles react in the human body. A combination of both garlic bioavailability and breath analysis was done by Rosen and others in 2001. They analyzed various components of garlic, and aged garlic extract in human breath and plasma, as well as in simulated human gastric fluid by using gas-chromatography-mass spectrometry and high performance liquid chromatography-mass

spectrometry. The results of this study found that allicin is not formed at high levels in the simulated stomach acid or decomposes at a low pH. Furthermore, it has been proven that allicin is stable at a pH level similar to human intestines, but not at a pH level found in the human stomach. Also, S-allylcysteine was located in human plasma. The breath analysis of volatiles showed the presence of D-limonene, *p*-cymene, diallyl disulfide, diallyl sulfide, and allyl methyl sulfide in human breath after garlic consumption. The predominant volatile formed was allyl methyl sulfide. It is believed that allicin is metabolized to allyl mercaptan in human blood. Then, allyl mercaptan is metabolized to S-adenosylmethionine by methyltransferase. Finally, S-adenosylmethionine is further metabolized into allyl methyl sulfide. It is also believed that D-limonene and *p*-cymene are formed as conjugates of glycosides or amino acids found in garlic (Rosen and others 2001).

For further evidence of allyl methyl sulfide differentiation from other breath volatiles, one can turn to another investigation into the formation of breath volatiles was done by Suarez and others in 1999. Analysis of mouth and alveolar air and urine was done with gas-chromatography-mass spectrometry. After garlic ingestion, the mouth showed elevated levels of methyl mercaptan, allyl mercaptan, allyl methyl sulfide, allyl methyl disulfide, and allyl disulfide. Lung measurements showed that only allyl methyl sulfide was present in a higher concentration in the lungs than in the mouth. Additionally, allyl methyl sulfide was present in larger concentrations for a longer period of time (up to four hours), with its peak concentration being one hour after garlic consumption. This states that allyl methyl sulfide is formed differently than the other volatiles (in the gut)

and not as rapidly metabolized by the body as the other volatiles (Suarez and others 1999).

In total, 13 volatiles have been shown to be present in human breath after the consumption of garlic. The most recent study done in 2011 by Hansanugrum and Barringer used five volatiles when measuring garlic breath volatiles. These included allyl mercaptan, methyl mercaptan, allyl methyl sulfide, allyl methyl disulfide, and diallyl disulfide. They indicated that the concentration level of methyl mercaptan on the subject's breath was undetectable by the SIFT-MS instrument. Therefore, it was not included in the study (Hansanugrum and Barringer 2011). Table 2.2 shows the four volatiles used in this study. These volatiles include allyl mercaptan, allyl methyl disulfide, diallyl disulfide, and allyl methyl sulfide. Also in Table 2.2, the odor thresholds are given for these volatiles. These odor thresholds are the lowest detectable limit in air. Allyl mercaptan has the lowest odor threshold which means that a smaller concentration of this volatile is needed in order for the volatile to be detected. Allyl methyl sulfide has the highest threshold, which means it requires the highest concentration to be detected. Additionally, there is no odor threshold at this time for diallyl disulfide (Henry and Gehr 1980; Nagata and Takeuchi 1990).

Table 2 Volatiles Present in Human Breath after Garlic Consumption with Odor Thresholds in Air

Volatile	Odor Threshold (ppb)
allyl mercaptan ^{abcf}	0.05 ^g
allyl methyl sulfide ^{bcdef}	0.14 ^h
allyl methyl disulfide ^{abcf}	N/A ⁱ
diallyl disulfide ^{abdef}	0.22 ^h

^a Minami and others 1989

^b Cai and others 1995

^c Suarez and others 1999

^d Rosen and others 2001

^e Tamaki and others 2008

^f Hansanugrum and Barringer 2011

^g Henry and Gehr 1980

^h Nagata and Takeuchi 1990

ⁱ Not Available

Figure 1 Possible Formation Pathway of Garlic Breath Volatiles from Alliin in Raw Garlic

2.2 Garlic Volatile Deodorization

2.2.1 Chlorophyll

Chlorophyll and its derivatives have long been used in traditional and therapeutic medicine. Of all the natural pigments, chlorophyll is the most abundant in nature. It is responsible for the color of all green plants and is found at such high levels as 1000 to 2000 ppm (wet weight) (Ferruzzi and Blakeslee 2007). Approximately 95% of chlorophyll in plants is found in the leaf and its primary role is maintaining photosynthesis which keeps the plant alive (Kephart 1955). In the human body chlorophyll derivatives are released from the food matrix and exposed to the acidity of gastric digestion. This causes a conversion of chlorophyll to pheophytin. In the small intestine, these pheophytins partition into bile salt lipid micelles which cause solubilization of the pheophytins. Once solubilized, these micelles move into the enterocyte by passive diffusion and possibly into circulation. The extent of how much of this gets into the circulation is unknown (Ferruzzi and Blakeslee 2007).

One of the functional uses of chlorophyll has been deodorization of the human body. Although there is a lack of recent evidence, natural chlorophyll and commercial-grade derivatives such as sodium copper chlorophyllin have been investigated as a method of internal deodorization (Ferruzzi and Blakeslee 2007). In 1955, Kephart reported that chlorophyll has the ability to effectively neutralize obnoxious odors in the mouth resulting from food, beverages, tobacco, and metabolic changes. This theory was tested in 1950 by researchers at Quaker Oats. They reported that dog food containing chlorophyllin improved breath odor in dogs within three to six hours. Furthermore, in

1952 Wall and Harrison Research Laboratories, in association with the Department of Pharmacology at the Philadelphia College of Pharmacy and Science did a study with human subjects. They found that the addition of 4 mg of water-soluble chlorophyll to gum better effectively reduced breath odor following ingestion of onions than that of gum without chlorophyll. This also held true with mouth lozenges (Kephart 1955).

Chlorophyllins are semi-synthetic food-grade molecules derived from chlorophyll. They are made by hydrolysis of the phytol tail found on chlorophyll and replacement of magnesium in the center of the porphyrin ring by another metal (commonly Copper). This removal of the phytol tail allows chlorophyllin to be hydrophilic. It is believed that chlorophyllin has the ability to capture free radicals to reduce the bioactivity of carcinogens (de Vogel and others 2005). It is believed that chlorophyllins would not have different reactions than chlorophyll with thiols. Furthermore, natural health gurus have echoed these claims, with no scientific data (Adams 2009; Walling 2011). Cadbury has even added Actizol to its Clorets gum stating that chlorophyll in Actizol can enhance breath freshening (Cadbury 2010).

2.2.2 Foods

One of the most popular methods of deodorization is utilization of other food products to remove or mask garlic's sulfur volatiles. In 1999, Negishi and Negishi attempted to deodorize methyl mercaptan (a compound sometimes present in garlic breath) with enzymatic deodorization of raw fruits, vegetables, and mushrooms. It is widely accepted that polyphenolic compounds such as tea catechins have the ability to deodorize. The mechanism for this deodorization is that oxidation of polyphenolic

compounds in air forms radicals. These radicals then mix with methyl mercaptan with a resulting compound that is odorless or has a different smell. Polyphenolic compounds are also known to oxidize when the catalyst polyphenol oxidase is present to initiate the oxidation reaction. When polyphenol oxidase is present, the deodorizing capacity of polyphenolic compounds is enhanced. Polyphenol oxidase is known to be present in different raw fruits, vegetables, and mushrooms.

The experiment was conducted by crushing food materials and mixing them with water and methyl mercaptan to analyze the deodorization activity. The effectiveness of the variables was tested on human breath using sensory analysis and with a metal oxide semi-conductor. In all, there were 31 different kinds of fruit, 33 vegetables, and 2 kinds of mushrooms tested. The food types that showed the highest rate of deodorization (at least 70%) were apple (except Kougyoku variety), pear (except Kousui and Housui varieties), loquat, peach, plum, prune, apricot, cherry, grape, lettuce, chicory, udo, perilla, peppermint, basil, burdock, potato, eggplant, and mushroom (*Agaricus bisporus*). The main polyphenolic compounds present in apple, pear, plum, grape, eggplant, lettuce, chicory, and herbs are epicatechin and chlorogenic acid. The deodorization in these foods occurs when their tissues are damaged by chewing. The polyphenolic compounds are then oxidized by polyphenol oxidase. In this oxidation, hydrogen atoms from phenolic hydroxyl groups form negative phenoxide ions. From these phenoxide ions, an electron is lost to form a radical(s). These radicals delocalize and react with sulfur-containing radicals from methyl mercaptan. The conjugates of this reaction have a different smell or less smell than methyl mercaptan. In addition to this information, apple proved useful in

removing bad breath after garlic consumption. After consumption of a raw apple, the odor was shown to decrease with time. This was shown with the odor sensor and sensory results. Furthermore, a microwave-cooked apple did not show deodorization effects. This was likely due to the heat-inactivation of polyphenol oxidase (Negishi and Negishi 1999).

A second study was done by Negishi and others in 2002 on the removal of volatile sulfur compounds by foods. In a continuation of the aforementioned study, raw fruits, vegetables, and mushrooms were analyzed for their ability to deodorize volatile sulfur compounds specific to the *Allium* species. The first part of the study looked at thiol-capturing activities of fruits and vegetables for methyl mercaptan, propyl mercaptan, and allyl mercaptan. It was found that prune, burdock, basil, eggplant, and mushroom (except *Lentinus edodes*) had 100% capturing activity, with apple ranging from 35%-47% capturing activity, blueberry 37%-53%, and kiwi fruit 15%-57%. This proved that *Allium* odors could be removed by these foods. Additionally, diallyl disulfide and diallyl sulfide were tested for their removal. Diallyl disulfide was more effectively reduced than diallyl sulfide. Foods that dramatically reduced diallyl disulfide (at least 70% capturing activity) were kiwi fruit, spinach, cutting lettuce, parsley, basil, mushrooms, cow's milk, raw egg, boiled rice, and 1% bovine serum albumin. Only cow's milk and raw egg dramatically lowered diallyl sulfide (Negishi and others 2002).

The deodorizing effect of foods on the two sulfide compounds measured is attributed to the likelihood of physical and chemical interactions of the volatiles with chlorophyll, proteins, lipids, or enzymes in the food. The mechanism for deodorization by food materials is done by either an addition reaction, degradation reaction, or physical

and chemical interaction. The addition reaction removes thiols by binding to quinone products formed from polyphenolic compounds. This reaction can be accelerated by the polyphenol oxidase in different foods. This route of deodorization is enzymatic deodorization (Negishi and others 2002).

The degradation reaction breaks down disulfides by heat. The extent of this reaction is limited unless the reaction occurs at high temperatures. The products of this degradation are thiols which are then removed by an addition reaction to form a quinone similar to that found in the addition reaction. The last mechanism is that of physical and chemical interaction between volatile sulfur compounds and foods. This is initiated by an affinity of molecules or a trapping of polymers found in foods. The results of this experiment demonstrate that disulfides and thiols formed in the mouth and gut can be removed with potential for allyl methyl sulfide to be reduced (Negishi and others 2002). Additionally, in 2004, Negishi and others showed that the amount of allyl methyl sulfide decreased significantly 1 h after garlic ingestion when an apple was eaten for internal deodorization. However, from 2 to 4 h after garlic ingestion, the level of allyl methyl sulfide increased (Negishi and others 2004).

The ability of mushrooms and milk to deodorize garlic volatiles has led to research of these two foods' ability to deodorize malodorous breath after the consumption of garlic. In 2007, Tamaki and others investigated the effect of mushroom (*Agaricus bisporus*) extract on breath after garlic extract-induced oral malodor. A garlic rinse was prepared using varying amounts of garlic and distilled water, and a mushroom rinse was made with distilled water and mushrooms. Breath analysis was done comparing breath

after garlic rinse use with and without mushroom extract afterwards. The breath samples were collected and analyzed by comparative gas chromatography and sensory evaluation.

Using principal component analysis and sensory results, it was found that oral malodor was correlated with volatile sulfur compound concentration in the mouth. Furthermore, rinsing with the mushroom extract reduced the volatile sulfur compound levels in the breath, which in turn reduced the malodor. Specifically, the concentration of thiol compounds was found to be markedly reduced by the mushroom extract rinse. It is believed that the mechanism for this deodorization involves the addition of thiol compounds to polyphenol compounds in the mushroom extract. This suppresses the production of volatile sulfur compounds. Monophenolic compounds in plants and fruits are hydroxylated to o-diphenols when in the presence of oxygen and polyphenol oxidase. These o-diphenols are then oxidized to o-quinones which can be polymerized with other phenolic compounds to cause browning. This mechanism has been shown to deodorize with other plant and fruit extracts by preventing polymerization. Specifically in *Agaricus bisporus*, γ -L-glutaminy-4-hydroxybenzene (GHB) is involved in melanogenesis which is catalyzed by tyrosinase or phenolase. Tyrosinase catalyzes hydroxylation of the phenolic compounds to o-diphenols, and then oxidation of those diphenols to o-diquinones when oxygen is present (Tamaki and others 2007).

Hansanugrum and Barringer in 2010 examined the effect of milk on the deodorization of malodorous breath resulting from garlic consumption. This study specifically looked at levels of diallyl disulfide, allyl methyl disulfide, allyl mercaptan, allyl methyl sulfide, and methyl mercaptan in garlic headspace, mouthspace, and

nosespace after garlic ingestion with analysis by selected ion flow tube mass spectrometry (SIFT-MS). Pertaining to the in vivo breath analysis, chopped raw or blanched garlic was added to water, fat-free milk, whole milk, or 10% sodium caseinate. Furthermore, garlic was chewed and swallowed with milk or nothing drank 1 min after consumption. Exhaled air from the nose and mouth were exhaled into the SIFT-MS machine up to 1 h after garlic consumption (Hansanugrum and Barringer 2010).

It was found that the addition of water, fat-free milk, whole milk, and 10% sodium caseinate to chopped garlic before ingestion reduced the concentrations of all analyzed volatile compounds in the mouth. Water has the ability to reduce volatile levels due to partitioning of volatiles between phases. When in water, garlic volatiles partition between garlic, water, and gas phases. Oil's ability to deodorize garlic volatiles is dependent upon the volatile. Oil was more effective than water for diallyl disulfide and allyl methyl sulfide, but less effective for the other volatiles measured. Diallyl disulfide and allyl methyl sulfide are hydrophobic and dissolve better in oil. The other three volatiles are more hydrophilic and have a higher headspace concentration in oil than in water. With no treatment, diallyl disulfide and allyl mercaptan were at levels approximately 2% to 8% of their initial concentrations with allyl methyl sulfide at approximately 50%. Milk reduced the allyl methyl sulfide levels more effectively than that of water and 10% sodium caseinate (Hansanugrum and Barringer 2010).

Reduction of garlic volatiles was likely due to chemical and physical interaction with milk which reduces the availability diallyl disulfide with the phase II enzyme glutathione. Allyl methyl sulfide is formed by diallyl disulfide interacting with

glutathione. Furthermore, when garlic was mixed with milk before consumption, the volatile levels were reduced more than when milk was consumed after garlic consumption. This is due to additional reactions that may occur when milk is directly mixed with garlic. The main component responsible for deodorization in all treatments used was water. However, fat proved to be effective for additional deodorization as seen when comparing whole milk with fat-free milk as whole milk was more effective in volatile reduction. Like the oil, fat in the milk better deodorized the more hydrophobic volatiles (diallyl disulfide and allyl methyl sulfide). Drinking beverages or foods with high water and fat content may help reduce malodorous garlic breath (Hansanugrum and Barringer 2010).

Ikeura, in 2012, investigated the ability of *Houttuynia cordata* Thunb. (Dokudami) to mask garlic odor. There have been a number of studies done attempting to reduce garlic volatiles, but little work has been done to mask the garlic volatile odor. Dokudami was tested along with other plants such as dandelion, spiny sowthistle, thyme, rosemary, and green tea. Garlic was placed in a beaker with powdered samples of the plants added with additional volatile or non-volatile dokudami extract. Dokudami showed the highest deodorizing activity of all the plants tested, and increased as dose increased. Furthermore, the volatile solution of dokudami more effectively deodorized garlic than the non-volatile dokudami. Further research is needed to declare the mechanism of deodorization for dokudami (Ikeura 2012).

Dating back to 2000 BC, cardamom has been used not only as a flavor, but as a functional food capable of medicinal-like properties. One of these uses has been the

enhancement of breath. There are multiple theories as to why cardamom could reduce breath malodor. One theory is that cardamom has a high level of flavonoids and antioxidants (Charles 2013). In 1998, Nair and others reported that cardamom has 50-100 mg of flavonoids per 100 g of cardamom. This content is the summation of quercetin, kaempferol, luteolin, and pelargonidin (Nair and others 1998). It is these flavonoids that are believed to have the ability to freshen breath. Additionally, in 1991, Kubo and others found that the ten most abundant volatiles found in cardamom essential oil had a limited antimicrobial activity. While this potential is small, it is still enough for cardamom to be used as an antimicrobial agent in cosmetic products (Kubo and others 1991). The potential to act as a microbial agent, as well as impart a generally positive aroma, could qualify cardamom as a breath enhancer after garlic consumption.

2.2.3 Tea

Tea catechins EGCg, (-)-epicatechin gallate (ECg), (-)-epicatechin (EC), epigallocatechin (EGC), and gallic acid (GA) in green tea have been shown to deodorize the thiol compound methyl mercaptan by oxidation of the catechin (by atmospheric oxygen) to an *ortho*-quinone which undergoes 1,4-addition or 1,6-addition to the methylthio group (of methyl mercaptan) which has an odor not associated with the odor of methyl mercaptan (Yasuda and Arakawa 1995). Particular polyphenolic compounds have the ability to have specific reactions with garlic breath volatiles in order to deodorize breath.

Investigation of tea-type beverages has been done by researchers searching for a way to reduce malodorous breath from the consumption of garlic. Negishi and others in

2004 tested deodorization with Ku-ding-cha that contained a large amount of caffeoyl quinic acid derivatives. Ku-ding-cha is a tea-like beverage generally consumed in China. Ku-ding-cha was thought to be an effective deodorizer due to the fact that it contains caffeoylquinic acid (CQA) derivatives. It was believed that CQA derivatives could play a role in deodorization of garlic volatiles in conjunction with enzymatic deodorization (Negishi and others 2004).

The result of this study was that ku-ding-cha and other products containing CQA showed no deodorization when used alone to lower garlic volatile concentration. However, when the ku-ding-cha was combined with acetone powder (prepared and stored at -20°C) from that of an apple, there was complete deodorization. Pear acetone powder also showed slight deodorization properties. Furthermore, different derivatives of CQA were extracted from the various products tested. It was found that 3,5-dicaffeoylquinic acid (3,5-diCQA) and 5-caffeoylquinic acid (5-CQA) had a deodorization effect. Similar to the products tested, 3,5-diCQA and 5-CQA only showed the deodorization effect when used with apple acetone powder. The proposed mechanism for this reaction is an addition reaction between the CQA derivatives and garlic volatiles that is catalyzed by polyphenol oxidase. When tested *in vivo*, ku-ding-cha tea was responsible for a reduction approximately 10 ppb less than the control for allyl methyl sulfide in the 3 to 5 h range. Polyphenolic compounds in ku-ding-cha most likely have the ability to capture thiols that have been produced by sulfides and disulfides in the human intestine. The result of this capturing is a halt in reduced level of allyl methyl sulfide (Negishi and others 2004).

A similar study was done with green tea and volatile sulfur compounds in mouth air by Lodhia and others in 2008. The deodorizing ability of green tea was compared to toothpaste (with brushing as a positive control), mints, chewing gum, and parsley oil both in vitro and in vivo. In vivo, green tea initially showed immediate reduction of volatile sulfur compounds in mouth air. The other variables tested (with the exception of toothpaste) showed no reduction in volatile sulfur compounds. Although green tea was effective initially, when measured at 1, 2, or 3 h after garlic consumption there was no significant reduction in volatile sulfur compounds. For the in vitro test a saliva putrefaction study was done. To do this, paraffin-stimulated saliva from subjects was used. Each person's saliva was filtered with a cheese cloth and analyzed. Then, one of the treatments was applied and further investigation was done after 24 h (Lodhia and others 2008).

In this portion of the study, toothpaste, mints, and green tea strongly inhibited volatile sulfur compounds when compared to no treatment. However, chewing gum and parsley oil showed no effect. Throughout the study, green tea proved to be the most effective inhibitor of volatile sulfur compounds. It is thought that the tea catechin of (-)-epigallocatechin (EGCg) is responsible for deodorization by a proposed pathway where it reacts with methanethiol and with the hydroxyl groups of its B ring. This could be proved by an observation in the study. It was noticed that deterioration of the green tea compounds caused a decrease in deodorization. It is hypothesized that the tea catechins are oxidized and transform the green tea into another type of tea that is fermented. This oxidation alters the B ring, which could prevent it from reacting with the volatile sulfur

compounds. While not yet tested, this theory could demonstrate why green tea is effective in deodorization (Lodhia and others 2008).

A more recent study by Zeng and others, 2010, investigated the effect of green tea extract on removal of sulfur-containing volatiles (methanethiol and hydrogen sulfide). In addition to the investigation of green tea volatiles, the study also looked at how pH in the mouth can affect the effectiveness of the green tea extract. An in vitro analytical method of measurement of volatile sulfur compounds was done using solutions of green tea extract with and without acetone powder. Using this method, an optimum pH for the reaction of green tea extract with volatile sulfur compounds was obtained. Using this pH and physiological body temperature, the optimum green tea extract concentration was calculated for reaction with thiols of interest with and without polyphenol oxidase and peroxidase containing vegetable acetone powders. Additionally, a base was added to chewing gum in order to make saliva have the optimum pH for green tea extract to effectively react with volatile sulfur compounds. An in vivo test was also done to analyze the addition of a base (sodium bicarbonate) to chewing gum to obtain a higher saliva pH (Zeng and others 2010).

It was found that an increase in pH from 7.5 to 8.0 significantly improved the effectiveness of green tea extract to remove volatile sulfur compounds in vitro. This is thought to occur as a result of the formation of dimethyl disulfide in place of methanethiol or hydrogen sulfide. Furthermore, the optimum pH for green tea effectiveness was between 8.1 and 8.4 at 37°C for 5 min. When pH was adjusted above this optimum level a decrease in effectiveness was shown. Also, when the samples' pH

was not adjusted ($\text{pH} \approx 6.5$), there was no decrease in methanethiol or hydrogen sulfide concentrations. Sodium bicarbonate proved to be effective for buffering saliva pH to 8.0 for 10 min of chewing. However, a discoloration and bitterness was observed in the gum. The discoloration appeared as a brown color appearing during storage. This was thought to occur due to polymerization of green tea polyphenols at alkaline pH. In order to apply this method to chewing gum, it would be necessary to encapsulate the green tea extract to avoid contact with the sodium bicarbonate (Zeng and others 2010).

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

2.3.1 Principles of SIFT-MS

Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) is a method of analysis that uses chemical ionization in combination with mass spectrometry to detect and quantify volatile organic compounds. SIFT-MS is a powerful tool due the ability to identify and quantify multiple trace gases simultaneously, and in real time from the whole, untreated gas of a sample (Harper and others 2011). In SIFT-MS as an analytical tool, positive ions are created by an ion source. This mixture of ions produces a current of ions which are given a mass-to-charge ratio by a quadrupole mass filter (Smith and Španěl 2005).

The SIFT-MS process begins with a microwave resonator that generates reagent ions. These ions are precursor ions and they must be unreactive with the major air/breath components while remaining reactive with the gases to be analyzed in the sample. The precursor (reagent) ions used with SIFT-MS are H_3O^+ , NO^+ , and O_2^+ . These ions do not

undergo bimolecular reactions with compounds in air. The precursor ions are injected into helium which acts as a fast-flowing carrier gas (Smith and Španěl 2005). The carrier gas and precursor ions then react with the sample that has been introduced to the system. There are a few different reactions that can occur between the precursor ions and the sample. These reactions take place at well-defined conditions. Following the reactions, the products and unreacted reagent ions enter a downstream chamber where they are analyzed by a second quadrupole mass filter. The ions are detected by a particle multiplier at a selected mass. From this information, a count rate is obtained and processed by the machine's computer. This count rate is directly proportional to the number of product ions divided by the number of reagent ions (Harper and others 2011).

There are 5 classes of reactions that occur between the reagent ions and the analyte in the sample being measured. The first class of reaction is the proton transfer. In this type of reaction hydrogen is transferred to the analyte. The product then is the analyte plus one proton.

The second class of reaction is the charge transfer reaction which occurs when the reagent removes a charge from the analyte. The product is then the analyte with a positive charge.

The third class of reaction that can occur is the dissociative charge transfer reaction where a charge transfer takes place with fragments formed due to the reaction.

This reaction mainly occurs with O_2^+ , but will sometimes be seen with NO^+ when those compounds have low ionization energy. The fourth class of reaction seen is an association reaction. In this circumstance, there is a three body collision between the reagent ion, analyte, and either the carrier gas (helium), nitrogen, or oxygen (symbolized by M).

This type of reaction is common with NO^+ but can be seen with H_3O^+ . The last class of reaction seen between the reagent ions and sample analyte is a hydride extraction. In this case, negative hydrogen ion is removed by the reagent ion.

As seen in the examples, the type of reaction is dependent upon the reagent ion and the chemical make-up of the analyte. For best results, a mass scan is necessary for every suspected compound in the sample (Smith and Španěl 2005; Harper and others 2011).

A valuable asset when using SIFT-MS is that of the kinetics database. This database is based on kinetics that occurs from known reactions of analytes with the reagent ions. The partial pressures of the individual trace gases are calculated with

information on count rates of the precursor ions with the relevant analyte ions, appropriate rate coefficient, measured flow rate, flow tube pressure, mass discrimination and differential diffusion factors. All of this information is used to make a kinetics database which has reaction information for numerous compounds. It is known that different compounds have different reaction rate coefficients. These coefficients have an important impact on the calculation of concentrations present and must be known in order to identify any compound (Španěl and Smith 2011).

The SIFT-MS instrument allows for two modes of operation. The first mode of operation is full-scan mode (FS). When the instrument is used in this manner it sweeps the detection quadrupole ion over a selected mass-to-charge ratio for a set amount of time while the sample analyte is introduced to the system. The result of this scan is a complete mass spectrum of the analyte. From the number of counts and total sampling time for each ion the count rate is calculated. The count rate is stored and displayed as a linear/semi-logarithmic scale, and the mass spectra are interpreted by relation of product ion peaks to trace gas samples of the analyte. The second mode of operation available to use with SIFT-MS is the multiple ion monitoring mode (MIM) or selected ion mode (SIM). When in this mode, the count rates of the precursor ions and those of selected product ions are monitored. In order to do this, the downstream mass spectrometer is rapidly switched between the masses of all the primary ions and the selected product ions. Then, each of these masses is examined for a predetermined amount of time. The machine's specifically-designed interface driver computer program performs the switching between different ions and count rates (Smith and Španěl 2005). Furthermore,

SIFT-MS has been used on multiple occasions to analyze human breath effectively (Španěl and Smith 2011).

2.4 Acetone as Breath Marker

Acetone is one of many constituents that make up human breath. Fluctuation in the concentration of acetone has been used as a non-invasive breath marker for biological conditions such as diabetes, congestive heart failure, and ketosis (Kundu and others 1993; Kupari and others 1995; Musa-Veloso and others 2002; Wang and others 2009). Furthermore, breath measurement of acetone is used by Syft Technologies in order to demonstrate the ability of SIFT-MS to analyze human breath (Syft 2013). It is known that consumption of standardized garlic preparations, allicin, and allicin-derived compounds can lead to increased levels of acetone (Lawson and Wang 1995). Despite normal fluctuation and increase of concentration due to garlic consumption, acetone has been used as the marker of breath exhalation for previous garlic breath study (Hansanugrum and Barringer 2010).

Chapter 3: Materials and Methods

3.1 SIFT Analysis

Headspace and breath measurements were taken using selected ion mode (SIM) with a selected ion flow tube-mass spectrometer (SIFT-MS) (Voice 200, Syft Technologies Ltd., Christchurch, New Zealand). Selected compounds were measured on human breath after garlic consumption and in headspace analysis with Syft VOICE-200 software (v.1.4.9.17754, Syft Technologies Ltd., Christchurch, New Zealand). The volatiles examined for breath measurement were acetone, diallyl disulfide, allyl methyl sulfide, allyl methyl disulfide, and allyl mercaptan, all of which have known kinetic parameters (Hansanugrum and Barringer 2010). Breath measurements had a scan time of 120 sec. Acetone was measured as a signal of breath exhalation. The volatiles selected for headspace measurement along with SIFT-MS settings are listed in Appendix A (Tables A.1 and A.2). Headspace measurement scan times were 95 sec.

Breath measurements of one subject were obtained using 20.3 cm straws (Red Slim Straws, Dixie Consumer Products LLC., Atlanta, GA) cut to a length of 5.08 cm. These straws were positioned around the SIFT passivated needle encompassing the entire needle and tight to the end to prevent air escape. Breath scan patterns of 15 sec exhailes and 5 sec inhales were used for the span of the breath measurement. Six individual

breaths were present in every breath scan. The settings for SIFT-MS scans can be found in Appendix A.

Figure 2 SIFT-MS Breath Measurement Positioning

Figure 3 Red straw SIFT-MS configuration for breath measurements

3.2 Breath Measurements: Treatments consumed after garlic

3.2.1 Control

Raw garlic was obtained from a local market (Kroger Co., Cincinnati, OH). The garlic was peeled by hand and $20.00 \pm 1.00\text{g}$ was blended (Magic Bullet Express, Emson, New York, NY) with 50 mL of water for 1 min. The solid blended garlic mixture was strained and $9.00 \pm 0.10\text{ g}$ of strained garlic applied to one slice of bread. The slice of bread was consumed immediately followed by 591 ml of water. Breath measurements

with SIFT-MS followed after consumption at 5, 10, 20, 30, 40, 50, and 60 minutes from the start of consumption.

3.2.2 Treatments

Treatments were consumed immediately after consumption of the strained garlic on the bread slice. Whey protein treatment was 591 of water mixed with 23.3 g of whey protein (Natural Flavor 100% Whey Protein, Biochem Sports, Country Life, LLC, Hauppauge, NY). Lemon juice treatment consisted of a 591 ml lemon juice drink using a 1:1 ratio of water and lemon juice (100% Lemon Juice, Kroger Co., Cincinnati, OH). Green tea treatment was 591 ml of green tea (Decaffeinated Green Tea, Lipton-Unilever, Englewood Cliffs, NJ). Sodium copper chlorophyllin treatment was 591 ml of water mixed with 1 tbsp. of chlorophyllin (Liquid Chlorophyll 100 mg, World Organics Corp., Huntington Beach, CA). Soft drink treatment was 355 ml of the beverage (7UP, Dr Pepper Snapple Group, Plano, TX). Raw apple treatment was $100.00 \text{ g} \pm 5.00 \text{ g}$ of sliced apple (Pink Lady apple, Rainier Fruit Co., Selah, WA). Apple ($100.00 \text{ g} \pm 5.00 \text{ g}$ sliced apple) was microwaved for 10 min on high setting (600 W, Model KOR-6115, Daewoo Electronics, Seoul, South Korea). Parsley and spinach treatments used were $10.00 \pm 0.10 \text{ g}$ of fresh leaves (Kroger Co., Cincinnati, OH). Mint treatment used $10.00 \pm 0.10 \text{ g}$ of fresh leaves (Whole Foods Market, Austin, TX).

3.3 Headspace Measurements: Control and treatments blended with garlic before analysis.

3.3.1 Control

Raw garlic was peeled, blended with 50 ml of water, and poured into a 500 ml Pyrex media storage bottle (Corning Incorporated, Tewksbury, MA). The blended mixture was covered with an open top cap coupled with polytetrafluoroethylene (PTFE)-faced silicone septa, equilibrated for 1 h at 40°C in a hot water bath (Precision Scientific, Winchester, VA), and analyzed with a SIFT-MS headspace measurement.

3.3.2 Treatments

Treatments were added to the water before blending. Treatments used for this study included 23.3 g whey protein isolate, 100 ml lemon juice, 100 ml green tea, 100 ml soft drink, 100 ml of sodium copper chlorophyllin, 10.00 ± 0.10 g and 25.00 ± 0.10 g of fresh parsley, 10.00 ± 0.10 g fresh spinach, 10.00 ± 0.10 g fresh mint, and 10.00 ± 0.10 g of fresh apple. Whey protein, lemon juice, soft drink, and green tea were added to 50 mL of water as seen with the control, whereas the other treatments were added to 100 mL of water. The increase in water used for the solid food treatments ensured complete blending as complete blending was not possible with 50 mL of water. Additionally, headspace measurements were made with neutralized lemon juice and soft drink and treatments as well as acidified green tea and whey protein treatments. Approximately 50-100 ml of 1 M citric acid or NaOH was added to these treatments to adjust pH to desired levels.

Blending was done with resulting volume which ranged from 100-200 ml.

3.4 pH Measurement and Adjustment

Measurement and adjustment of pH was done following manufacturer's protocol for one point standardization with automatic temperature compensation (Accumet Model 10 pH Meter, Thermo Fisher Scientific, Waltham, MA). The pH was adjusted done for lemon juice, soft drink, whey protein, and green tea treatments. Lemon juice and soft drink treatments were adjusted with 1 M NaOH (Certified A.C.S. Sodium Hydroxide Electrolytic Pellets, Fisher Chemicals, Fair Lawn, NJ) until a pH of 7.00 ± 0.05 was obtained. Whey protein and green tea treatments were adjusted with 1 M citric acid to a pH of 2.5 ± 0.05 (Sigma-Aldrich Co., St. Louis, MO). Additionally, 1 ml of antifoam was

used to prevent foam formation from protein denaturation during blending when acidified whey protein was tested (Antifoam A Concentrate, Sigma-Aldrich Co., St. Louis, MO).

3.5 Data Analysis

Breath scan measurements had a scan time of 120 sec, with 6 individual breaths lasting approximately 15 sec each. Acetone was used for this study in order to signal breath exhalation. For each breath, only time points that fell within the top 10% of the maximum acetone level were used for each volatile. Time points outside of this range were discarded. With the remaining time points, an average was calculated. The average of each breath was used as the measurement level for the volatile for that point in time. This was done for all volatiles. Headspace data was measured and averaged. This average was used as the volatile concentration level. In an attempt to utilize consistent raw

ingredients, interquartile ranges were calculated to test for outliers. The formulas used to find the range of acceptable data were:

$$25^{\text{th}} \text{ percentile} - (1.5 * \text{Interquartile Range})$$

$$75^{\text{th}} \text{ percentile} + (1.5 * \text{Interquartile Range})$$

For headspace and breath measurements, any data points that fell outside of this range were considered to be outliers and not used (Stapel 2012). Less than 5 outliers were found and removed from the data.

Chapter 4: Results and Discussion

4.1 Odor Thresholds, Control, and Effect of Sodium Copper Chlorophyllin on Garlic Breath Volatiles

Allyl methyl disulfide, diallyl disulfide, allyl mercaptan, and allyl methyl sulfide have been identified as the volatiles that make up malodorous breath due to garlic consumption (Cai and others 1995; Suarez and others 1999; Rosen and others 2001). Of these volatiles, allyl mercaptan has the lowest odor threshold in air (0.05 ppb), followed by allyl methyl sulfide (0.14 ppb), and diallyl disulfide (0.22 ppb), while allyl methyl disulfide's threshold is unknown (Henry and Gehr 1980; Nagata and Takeuchi 1990). These thresholds indicate the lowest concentration necessary for detection of the volatile in air. In this experiment, the concentrations for all four breath volatiles in the control were above their odor thresholds for the entire hour of testing, meaning they have a considerable impact on the odor of breath after consumption of garlic (Figure 3).

It also must be noted that consumption of garlic caused fluctuation of acetone as the breath exhalation marker. This is similar to previous reports of acetone fluctuation after garlic consumption (Lawson and Wang 1995). However, the manner in which acetone was used in this experiment, and in previous garlic breath study, was as a marker for intervals of breath (Hansanugrum and Barringer 2010). These intervals yielded the most consistent values for target volatiles.

In the control, garlic followed by water, three of the volatiles, allyl methyl disulfide, diallyl disulfide, and allyl mercaptan decreased dramatically in the first 30 min, then slowly decreased for the next 30 min (Figure 3). Allyl mercaptan had the highest concentration, followed by diallyl disulfide and allyl methyl disulfide. The disulfides and allyl mercaptan are rapidly formed from cutting/crushing of the raw garlic and then decrease quickly. The highest final concentration belonged to allyl methyl sulfide, which increased and peaked from 5 to 10 min, then decreased gradually over the next 50 min. At one hour, allyl methyl sulfide still had a concentration approximately 9 ppb higher than the next closest volatile (allyl mercaptan). Allyl methyl sulfide is poorly metabolized by the body causing its concentration levels on the breath to remain elevated for a prolonged period of time (Suarez and others 1999). Allyl methyl sulfide increases in concentration immediately after garlic consumption and is the predominant garlic volatile on human breath after 3 hr (Suarez and others 1999; Negishi and others 2004).

It has long been claimed that chlorophyll and its derivative chlorophyllin have the potential to freshen breath and could be used as an internal deodorant. An early sensory study concluded that 65-200 mg of chlorophyllins had the potential to freshen human and dog breaths (Kephart 1955). Despite this claim, there have been no recent studies to support these statements. Parsley, spinach, and mint treatments were effective in deodorization of garlic breath volatiles in comparison to the control (Figure 3). Allyl methyl disulfide appears to be deodorized for the first several minutes, while diallyl disulfide and allyl mercaptan are deodorized longer into the hour. Allyl methyl sulfide showed deodorization throughout the entire hour.

These three foods are known to be high in chlorophyll, with an approximate 6 to 15 mg of chlorophyll per 10 g of sample that was consumed, thus a chlorophyll derivative, chlorophyllin was analyzed for its role in deodorization. A chlorophyllin drink treatment (100 mg) was consumed in place of water immediately after consumption of garlic in order to analyze its deodorization effect. When compared to the control, chlorophyllin treatment appears similar to control volatile concentration levels for all volatiles throughout the hour (Figure 3). Chlorophyllin is a semi-synthetic, water-soluble derivative of chlorophyll which is thought to be more bioactive (de Vogel and others 2005). While not certain, the deodorization effect of chlorophyllin could be better than chlorophyll due to bioactivity level. With the lack of deodorization displayed by the chlorophyllin treatment, it is possible to question the effectiveness of chlorophyll (or its derivatives) in the parsley, spinach, and mint treatments.

4.2 Enzymatic Deodorization

One possible mechanism for deodorization of garlic breath volatiles is enzymatic deodorization. In order to analyze this, raw and microwaved apple treatments were tested for their deodorization ability. Raw apple has been previously shown to deodorize garlic breath volatiles in vivo (Negishi and Negishi 1999; Negishi and others 2004). Negishi and others (2004) proposed that deodorization occurs due to polyphenol oxidase oxidizing polyphenolic compounds which contain *ortho*-diphenols, into *ortho*-quinones. Thiol compounds bind with the newly-formed *ortho*-quinones and form new compounds with a different odor (Negishi and Ozawa 1997; Negishi and others 2002; Negishi and others 2004). Thus allyl mercaptan loses its own odor and allyl methyl sulfide is reduced

inside the body. They also proposed that the deodorization of allyl methyl disulfide and diallyl disulfide is due to degradation enzymes such as reductase which turn disulfides into thiols which are then captured by *ortho*-quinones (Negishi and others 2002). If food treatments in this study are enzymatically deodorizing garlic breath, it would be due to this mechanism.

Comparing raw and microwaved apple, a similar food matrix is present, but heating has likely inactivated the enzymes. Raw apple treatment deodorized more effectively than microwaved apple treatment in the beginning of the hour of breath measurements for allyl methyl disulfide, diallyl disulfide, and allyl mercaptan, while there is separation in concentration level shown for allyl methyl sulfide throughout the hour (Figure 4). If enzymatic deodorization is occurring, it is likely that the presence of enzymatic activity in the raw apple likely that caused more deodorization. The deodorization effect of parsley, spinach, and mint could potentially be due to enzymatic activity (Figure 3). While no previous studies have tested these foods for breath deodorization, these treatments have demonstrated the ability to deodorize garlic volatiles in vitro (Negishi and Negishi 1999; Negishi and others 2002).

Headspace deodorization is likely easier to achieve than breath deodorization. Garlic volatiles are believed to form quickly in body and room temperature as allicin reaches 97% of its maximum yield in approximately 18 sec at 37°C and in 30 sec at 23°C (Lawson and Hughes 1992). Once allicin present, garlic volatiles form in a rapid manner. Headspace treatment was introduced before blending, which allowed it to immediately deodorize volatiles as they formed during blending or early while incubating. In contrast,

breath deodorization treatments were not consumed until after garlic consumption. This may have caused the deodorization effect of the treatment to be lessened in comparison to the headspace measurements as the breath deodorization likely occurred in the mouth and stomach (Negishi and others 2004).

4.3 Polyphenolic Deodorization

Microwaved apple, lemon juice, and green tea treatments appeared to have an effect on garlic breath volatiles in this experiment even though they likely did not have enzymatic activity (Figure 4, 5). Polyphenolic compounds have shown the ability to deodorize without oxidative enzymes as polyphenolic compounds can also be oxidized by atmospheric oxygen (Yasuda and Arakawa 1995). Microwaved apple, which was cooked in an attempt to inactivate polyphenol oxidase, showed deodorization compared to the control for the first few minutes for three of the volatiles, and possibly the entire hour for allyl methyl sulfide (Figure 4). Lemon juice and green tea treatments also underwent a heat process capable of inactivating enzymes. Green tea and lemon juice showed a similar amount of deodorization in comparison to the control with allyl methyl sulfide showing the greatest reduction of the four volatiles (Figure 5).

Differences in the type and amount of polyphenolic compounds present in the treatments could have accounted for differing extents of deodorization. Chlorogenic acid is a polyphenolic compound that has been shown to effectively deodorize garlic breath volatiles in the beverages ku-ding-cha and green tea (Negishi and others 2004). Lemons contain 93 μg of chlorogenic acid per 1 g of fruit (Wang and others 2007). Thus, it is possible that chlorogenic acid caused some of the deodorization effect demonstrated by

Figure 4 Effect of chlorophyll, enzymatic activity, and polyphenolics on garlic breath volatiles when chlorophyll, parsley, spinach, and mint treatments were consumed after garlic

Figure 5 Effect of enzymatic activity and polyphenolics on garlic breath volatiles when raw and microwaved apple treatments were consumed after garlic

Figure 6 Effect of polyphenolic compounds and acidity on garlic breath volatiles when whey protein, soft drink, green tea, and lemon juice treatments were consumed after garlic

lemon juice and green tea (Figure 5).

4.4 Effect of pH on Garlic Breath Volatile Deodorization

While enzymatic activity and polyphenolic content appear to have an effect on garlic breath deodorization, an acidic pH might also be responsible for some of the deodorization. A pH below 3.6 immediately and irreversibly destroys the enzyme alliinase which minimizes the formation of other garlic volatiles (Lawson and Hughes 1992; Negishi and others 2002). Lemon juice breath treatment had a pH of 3.0 which possibly could have added to its deodorization effect (Figure 5). The soft drink treatment had a pH of 3.3 and contained little polyphenolic compounds, but still showed some deodorization. Volatile concentration levels appear to be lower for the first 20-30 min of breath measurement. The whey protein treatment contained no enzymatic activity or polyphenolic compounds with a pH of 6.3, and it did not show great reduction in volatile concentration level (Figure 5). To further test the effect of pH on garlic volatiles, treatments were applied in headspace measurements and compared to neutralized and acidified versions of the liquids. The results of this show that lower pH level generally caused lower volatile concentration level (Figure 6).

While the impact of an acidic pH might be present in breath and headspace measurements, the inactivation of alliinase is not likely occurring in breath measurements as it has been shown that allicin forms in less than 1 min (Lawson and Hughes 1992). In this experiment, blending lasted for 1 min, and allicin would have already formed. However, in breath measurements, soft drink treatment did demonstrate some ability to deodorize in comparison to the control even though it does not have a substantial amount

of polyphenolic compounds or enzymatic activity (Figure 5). Therefore, without inactivation of alliinase, acidity possibly has an effect on breath deodorization. In addition to the success of soft drink treatment, whey protein demonstrated very little deodorization in comparison to the control (Figure 5). Like soft drink treatment, whey protein has little to zero enzymatic activity and polyphenolic compounds. It was also likely ineffective due to a nearly neutral pH at 6.4. The mechanism for acid breath deodorization is unknown, but comparison of these two treatments suggests that low pH may have a breath deodorization effect.

Figure 7 Effect of acidity on headspace concentrations when treatments were blended with garlic

Chapter 5: Conclusion

Garlic breath volatiles (allyl methyl disulfide, diallyl disulfide, allyl mercaptan, and allyl methyl disulfide) were reduced by parsley, spinach, mint, raw and microwaved apple, soft drink, green tea, and lemon juice treatments in comparison to water (control) on SIFT-MS breath measurements, but were not as effectively reduced by chlorophyllin and whey protein treatments. Enzymatic deodorization could have occurred in parsley, spinach, mint, and raw apple treatments. This involves oxidation of *ortho*-diphenols (in polyphenolic compounds) into *ortho*-quinones by oxidative enzymes (polyphenol oxidase and peroxidase) which then bind to thiols present in garlic and cause a change in odor. The deodorization effect of microwaved apple, lemon juice and green tea could be due to polyphenolic deodorization. This process is similar to enzymatic deodorization, but the *ortho*-diphenols are oxidized into *ortho*-quinones by atmospheric oxygen. Microwaved apple showed some breath deodorization, but was not as effective as raw apple. If enzymatic and polyphenolic deodorization are occurring, enzymatic may be a more effective deodorization mechanism on human breath. Acidic pH may have been involved in the deodorization of lemon juice and soft drink breath experiments. Lemon juice did not contain enzymatic activity, and was highly effective in deodorization in comparison to the control. Soft drink treatment had no enzymes or polyphenolic compounds, but

resulted in lower volatile concentration than the control. Soft drink was not as effective as lemon juice which suggests that acid has some ability to deodorize garlic breath volatiles, but polyphenolic deodorization may add to the deodorization level. Whey protein demonstrated little garlic breath deodorization with breath measurement and its ineffectiveness could possibly be attributed to lack of enzymatic activity, polyphenolic compounds, and its neutral pH.

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Appendix: Tables and Figures

Table 3 Volatile Compounds Measured in Headspace Scan

Volatile Compound	Ion Product	Precursor Ion	<i>m/z</i>	Reaction Rate (<i>k</i>) (10 ⁻⁹ cm ³ /s)
Methyl Mercaptan ^a	CH ₄ S.H ⁺	H ₃ O ⁺	49	1.8
Allyl Methyl Sulfide ^b	C ₄ H ₈ S.H ⁺	H ₃ O ⁺	89	2.6
Diallyl Sulfide ^c	(C ₃ H ₅) ₂ S.H ⁺	H ₃ O ⁺	115	2.9
Allicin ^d	C ₆ H ₁₀ OS ₂ H ⁺	H ₃ O ⁺	163	2.4
Allyl Methyl Tetrasulfide ^d	C ₄ H ₈ S ₄ .H ⁺	H ₃ O ⁺	185	2.6
Diallyl Tetrasulfide ^d	C ₆ H ₁₀ S ₄ .H ⁺	H ₃ O ⁺	211	2.6
Dimethyl Thioether (Dimethyl Sulfide) ^c	(CH ₃) ₂ S ⁺	NO ⁺	62	2.2
Allyl Mercaptan ^b	C ₃ H ₆ S	NO ⁺	74	2.4
Acetone ^b	NO ⁺ .C ₃ H ₆ O	NO ⁺	88	1.2
Dimethyl Disulfide ^c	(CH ₃) ₂ S ₂ ⁺	NO ⁺	94	2.4
Dimethyl Thiosulfinate ^d	C ₂ H ₆ OS ₂ ⁺	NO ⁺	110	2.4
Allyl Methyl Disulfide ^d	C ₄ H ₈ S ₂	NO ⁺	120	2.4
Dimethyl Trisulfide ^c	C ₂ H ₆ S ₃ ⁺	NO ⁺	126	1.9
Allyl Methyl Thiosulfinate ^d	C ₄ H ₈ S ₂ O	NO ⁺	136	2.4
2-or 3-vinyl-4H-1 2or3-dithiin ^d	C ₆ H ₈ S ₂ ⁺	NO ⁺	144	2.4
Diallyl Disulfide ^{bc}	(C ₃ H ₅) ₂ S ₂ ⁺	NO ⁺	146	2.4
Allyl Methyl Trisulfide ^d	C ₄ H ₈ S ₃ ⁺	NO ⁺	152	2.4
Diallyl Trisulfide ^d	C ₆ H ₁₀ OS ₂	NO ⁺	178	2.4

^a Williams and others 1998

^b Hansanugrum and Barringer 2010

^c Španěl and Smith 1998

^d Syft 2012

Table 4 Settings for SIFT-MS Measurements

Carrier Flow (torr L/sec)	5.1
Sample Flow (torr L/sec)	0.35
Tube Pressure (torr)	0.65
Tube Temperature (°C)	21
Downstream Intensity (pA)	1.8×10^2
Upstream Intensity (nA)	6.8
Reaction Time (ms)	2.8
Dilution Factor	1
Sample Needle Size (gauge),(cm)	18,1.9
Pressure Needle Size (gauge), (cm)	14,15

Table 5 Chlorophyll Content of Treatments as used in Experiment

Treatment	Chlorophyll Content
Parsley (10g)	6.3 mg ^a
Spinach (10g)	7.9 mg ^a
Mint (10g)	15 mg ^b
Chlorophyll	100 mg

^a Higdon 2005

^b Hsu and others 2010

Table 6 Polyphenolic Content of Treatments

Treatment	Polyphenolic Content^a
Green Tea	61.9 ^b
Parsley	89.3 ^c
Lemon Juice	180.0 ^c
Apple	201.5 ^c
Mint	246.9 ^c
Spinach	248.7 ^c

^a Rothwell and others 2013 (As determined by Folin Assay)

^b mg/100 ml

^c mg/100 g Fresh Weight

Figure 8 Correlations of Polyphenolic Content and Breath Concentration

Figure 9 Effect of Spinach, Parsley, Raw Apple, Mint, Chlorophyll, and Microwaved Apple Treatments on Headspace Concentrations of Garlic Breath Volatiles when Blended with Garlic