IDENTIFICATION AND CHARACTERIZATION OF POTENT ODORANTS IN SELECTED BEET ROOT (*BETA VULGARIS)* PRODUCTS

BY

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THESIS

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ABSTRACT

The beet, in its various forms, has been an important agricultural commodity for millennia; it was first mentioned in writing in 8th century BC Mesopotamia. Although the sugar beet contributes to a quarter of sugar production worldwide, the red beet root is an equally, if not more, important product. Not only is the beet root consumed in a culinary setting, it is highly valued for its betalain pigments, which provide a natural source of colorant for food and pharmaceutical use. Despite the beet's impact on our food supply, the majority of the flavor research on beets concentrates on contaminating aromas in beet sugar. A small number of studies have been performed on beet roots themselves; however, the focus is solely placed on the volatile compounds, which may or may not be aroma-active or impactful to the overall product. In order to fill these gaps in our understanding, a complete aroma analysis of the beet root was performed in the present study.

Potent odorants were characterized by the use of aroma extract dilution analysis (AEDA) coupled with gas chromatography-olfactometry (GCO), as well as supported analytically by the use of gas chromatography-mass spectrometry (GC-MS). Initial analyses were performed on four different types of beets: boiled, oven-roasted, canned, and colorant. Extraction of the aroma-active components was accomplished by direct solvent extraction (DSE) with ether as solvent, paired with solvent-assisted flavor evaporation (SAFE). Extracts were fractionated into acidic and neutral-basic components to improve accuracy of identification. During screening analyses using the four different beet preparations, twenty-one different compounds were detected, one of which was unidentified. Canned beets served as the primary focus in each subsequent analysis because they provided the most odor-active and consistent samples. Forty-one aroma compounds

were identified and characterized by the use of AEDA, with some of the most important being 2-acetyl-1-pyrroline, geosmin, methional, furaneol, *p*-vinylguaiacol, and vanillin due to their very high odor potency. Three 3-alkyl-2-methoxypyrazines (isopropyl, *sec*-butyl, and isobutyl), as well as 1-octen-3-one, phenylacetic acid, and eugenol, were also identified at moderate odor potencies.

To detect compounds that might have otherwise been lost during extraction or co-eluted with ether during GCO, a static headspace technique for odor analysis was used. Decreasing volumes of headspace from gently heated canned beet mixtures were analyzed using GCO. Some compounds detected, such as geosmin and methional, were found in previous odor analyses. However, two potent compounds that were previously undetected, including methanethiol and dimethyl sulfide, which was a particularly potent odorant. This technique enabled the discovery of previously undetected compounds, which may be important for subsequent creation of a beet odor model system.

However, knowledge of the specific compounds in the beets does not provide complete information for the understanding of the beet's odor profile. Therefore, it was necessary to perform quantification of compounds both in the solvent extract and headspace of the canned beets, using the technique stable isotope dilution analysis (SIDA). In the headspace, methanethiol and dimethyl sulfide were targeted for quantification using headspace solid-phase microextraction (HS-SPME). Twenty-four individual aroma-active compounds were selected for analysis by DSE-SAFE. After using SIDA to determine the concentration for each individual compound, odor activity values (OAVs) were determined. OAVs were calculated by dividing the concentration of a compound by its odor threshold. An OAV provides an estimate of a compound's odor potency and its potential importance to the overall aroma of the product. Of

the 26 aroma compounds analyzed in the headspace and solvent extracts, dimethyl sulfide, geosmin, *E*-4,5-epoxy-[*E*]-2-decenal, methanethiol, *p*-vinylguaiacol, and β-damascenone possessed the highest OAVs and, therefore, contributed the most to the overall aroma profile of the canned beets. Meanwhile, octanal, *Z*-1,5-octadien-3-one, nonanal, acetic acid, 2-isopropyl-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, 2-*sec*-butyl-3-methoxypyrazine, *E*, *Z*-2,6-nonadienal, and vanillin were of moderate odor potency.

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CHAPTER 1

INTRODUCTION

The beet root, in its many forms, has been an important crop for millennia, with its first written appearance in 8th century Mesopotamia (Attokaran, 2011). The beet descended from the sea beet, which grows in the Mediterranean Sea. From there, cultivation spread across North Africa and the Mediterranean regions of Europe. While in modern times the tuberous root portion is commonly consumed, it was originally grown for its foliage. Although the ancient Romans were the first to cultivate beets for human consumption, for hundreds of years the root was primarily used as animal feed. However, in sixteenth century Europe, the beet root grew to be a popular vegetable (Neelwurne, 2013). Another hallmark moment in the story of the beet root was the 1747 discovery by Andreas Marggraf. He helped to uncover that the tuber was a concentrated source of sugar, in quantities tantamount to sugar cane. Based on this new research, Napoleon averred that the beet should be used in place of sugar cane for the production of table sugar, and even established specific schools to teach this practice. This discovery helped to foster the popularity of beets as well as encouraged improvements in cultivation. Today, the sugar beet contributes a quarter of the world's sugar production.

While the beet is grown and consumed worldwide, the largest areas of production are Europe and North America, the USA in particular. World production is approximately 227 million tons (Neelwurne, 2013). Popular methods of beet consumption include boiling, steaming, roasting, and pickling. Much of the beet root's popularity as a vegetable is due to its myriad of health benefits. Its low caloric density is suitable for calorie-controlled diets; the beet also contains significant levels of magnesium, selenium, folate, and phytosterols. The beet

pigments themselves are also believed to have positive effects on human health. Various studies have suggested that the pigments in beets possibly serve anti-carcinogenic, antioxidant, and cardiovascular functions (Attokoran, 2011).

One defining characteristic of the beet root is its vibrant red color. This hue is due to the abundance of betalain pigments, which are derived from the amino acid tyrosine. Its general structure is composed of a sugar moiety and a chromophore (Neelwurne, 2013). The betalains can be divided into two subgroups: betacyanins and betaxanthins, which are red and yellow respectively. The red appearance is due to the 3:1 ratio of betacyanins to betaxanthins (Goldman and Navazio, 2008). Although the use of beet pigment for food use has increased in popularity in recent years, the first application of betalain pigments to color food dates back to the 18th century. Because of the instability of betalains under heat, beet pigment is generally used in products such as ice cream, chilled fruit juices, and pharmaceuticals (Attokoran, 2011). Production of pigment from beets is carried out using a hydraulic press coupled with macerating enzymes to increase yield. The pigment is extracted with water, generally with the addition of an ethanol or methanol solution (Neelwurne, 2013). Fermentation, filtration, and pasteurization steps follow, and the end product can range from whole liquid extract to spray-dried powder or encapsulation. While the beet provides an excellent alternative to synthetic dyes, there are a variety of functionality issues related to their use. With changes in pH or temperature, the pigment can be altered, producing an often unwanted color change. Furthermore, the pigment itself can produce aroma compounds that are undesirable.

A second major feature of the beet is its distinct flavor, which is often polarizing in terms of liking of the vegetable. Geosmin, the chemical that causes the characteristic earthy aroma of beets, was discovered by Acree et. al. in 1975. Three different types of 3-alkyl-2-

methoxypyrazines (isopropyl, *sec*-butyl, and isobutyl) were also noted as aroma contributors in beets (Murray and Whitfield , 1975). Two years later a more complete analysis of beet aroma compounds was conducted; the researchers characterized twenty-two volatiles using GC-MS (Parliment et. al., 1977). Major findings were that 4-methylpyridine, pyridine, dimethyl sulfide, isovaleraldehyde, and furfural appeared in great abundance. Geosmin and 2-methoxy-3-*sec*-butylpyrazine were found in lower amounts; however, with the knowledge that these two compounds exhibit low odor thresholds, it was hypothesized that these contribute heavily to the overall flavor impact of the beet. Outside of these studies, the majority of aroma analyses with regards to beets concentrate on odors as contaminants in the production of beet sugar. While these studies collectively provide a good backbone of the aroma chemistry of the beet, they are limited in their scope. In order to truly understand the beet's flavor chemistry, it is essential to perform not only a comprehensive volatile analysis, but also olfactometric measurements to determine the roles and importance of the odor-active compounds.

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CHAPTER 2

LITERATURE REVIEW

I. The beet

The beet root or table beet (*Beta vulgaris* subsp. *vulgaris*) is a member of the Amaranthaceae family, which includes a multitude of cultivated varieties such as the sugar beet and Swiss chard (Goldman 2003). The beet root is a biennial plant, producing every two years, that grows best in temperate climates. Conversely, the sugar beet prefers tropical environments (McGrath et. al. 2007). Beets have leafy stems which range from 1-2 m containing 5-20 cm heart-shaped leaves with a brightly pigmented root tuber, which is the main part of the beet that is consumed (Attokaran 2011). The root is colored red due to the presence of betalain pigments, mainly betacyanins and betaxanthins.

Similarly to many other members of the *Beta* genus, the beet can trace its cultivation origins back thousands of years to Northern Africa and the Mediterranean regions of Europe. The commonly acknowledged ancestor of the modern beet is the sea beet, which grows in the Mediterranean. In eighth-century BC Mesopotamian text, the beet was mentioned for the first time in recorded history. While in modern times the root of the beet is regarded as the most important, beets were originally cultivated for their foliage. However, it was the Romans who first grew beet roots for human consumption. After hundreds of years as use primarily as animal feed, beets became a popular vegetable in Europe during the sixteenth century (Neelwurne, 2013). In 1747, after Andreas Marggraf discovered that the beet was a concentrated source of sugar in similar amounts to sugarcane, the root surged in popularity. This discovery led to a

variety of advancements in cultivation of the beet species. In fact, the sugar beet is responsible for a quarter of the world's sugar production for human consumption.

Today, most cultivated varieties are classified as leaf beets, table beets, fodder beets, or sugar beets. Worldwide beet production totals 227,158,114 tons, with the United States being one of the major contributors and supplying approximately 27 million tons (Neelwurne, 2013). One reason for the beet's success as a vegetable dish can be attributed to its high nutritional value. Although the beet has a low caloric value, it contains high amounts of magnesium, selenium, folate, and phytosterols (Attokaran, 2011). However, these amounts can be altered based on the cooking method chosen. Common cooking techniques include boiling, steaming, oven roasting, and pickling.

II. Flavor chemistry of beets

Although the beet has been consumed by humans for thousands of years, characterization studies of the aroma compounds in the beet root are few and rather limited in their scope. Some of the earliest papers written on the subject include the discovery of various 3-alkyl-2-methoxypyrazines as well as geosmin in beet roots (Murray and Whitfield 1975; Acree et. al. 1975). In 1977, Parliment et. al. completed a more comprehensive analysis of the volatile components in raw beet roots. Using a continuous steam distillation extraction, volatile components were removed from the beet matrix for analysis by gas chromatography-mass spectrometry (GC-MS). Twenty-two volatile compounds were identified, with 4-methylpyridine and pyridine constituting 60% of the total volatiles present; the high levels of these were hypothesized to be due to the prevalence of dihydropyridine-containing betalains. Other compounds that were present at significant levels included dimethyl sulfide, isovaleraldehyde,

and furfural. Combined with the knowledge that geosmin and 2-methoxy-3*sec*butylpyrazine are known to possess very low aroma thresholds and fact that both compounds were found in small amounts in the beet samples, Parliment et. al. hypothesized that these two compounds might be responsible for much of the characteristic beet aroma (1977). However, this hypothesis was not confirmed by the use of gas chromatography-olfactometry or sensory studies.

Outside of the aforementioned studies, the majority of others have centered on aromas in beet sugar extractions, which are considered undesirable. One such study was conducted by Dean Foods, aiming to characterize and quantify the chemical substances responsible for off-odors in beet sugar (Marsili et. al., 1994). Using close-looped stripping analysis and direct thermal desorption coupled with gas chromatography-olfactometry (GCO) and GC-MS twenty-six compounds were identified and characterized. However, only five of these substances were determined to be responsible for the characteristic malodor of beet sugar: geosmin (dirt), furfural (almond), isovaleric acid (sweaty feet), butyric acid (parmesan cheese), and 2,5-dimethylpyrazine (roasted nuts). This conclusion was made by comparing GCO results of the beet sugar with that of cane sugar, which is generally low in odor. While this study provides a piece of the puzzle, the major limitation is that it lacks depth because only the final product was analyzed. Another that was conducted in Sweden on beet sugar aroma compounds using not only the final product, but various points throughout the processing of sugar beets, helps to expound upon this idea (Pihlsgård et. al., 2001). Analyses were performed on raw beet juice, post-boiling refinery syrup, and final liquid sugar; this scheme allowed the researchers to understand the changes which happen during the processing steps. Sample preparation was performed on each sample using both headspace sampling and liquid-liquid extraction with diethyl ether. To identify compounds, GCO was coupled with GC-MS. Due to the multitude of

aromatic compounds, they were organized into five groups: sour/manure-like (I), earthy (II), caramel-like (III), floral/green (IV), and ester-like (V). Although groups I and IV did not show any clear-cut trends, the other three were more telling. Earthy aromas, such as geosmin, were present mainly in the raw juice fraction and decreased over the processing steps. Caramel-like odors, such as 2,5-dimethylpyrazine, increased over time, likely due to Maillard and caramelization reactions in the heating of the beet sugar. The fruity, ester-like compounds, including 2-methylpropyl acetate, appeared at high intensities in the raw juice, but had essentially disappeared by the second sampling. While these studies provide a greater understanding of the flavor chemistry of sugar beets, the results are not directly applicable to red beets because they are vastly different varieties.

In addition to studies of off-odor compounds in beet sugar, one important aroma analysis study centers on of the major aroma chemical in the beet root: geosmin. Geosmin, *E*-1,10-dimethyl-*E*-(9)-decalol, provides the characteristic earthy flavor to the beet root, which is often a factor in liking of this polarizing vegetable (Goldman and Navazio, 2008). Due to the fact that geosmin can be synthesized by a variety of soil microbes, it was originally hypothesized that microorganisms in the beets' growing environment produced geosmin that was then absorbed into the beet. Comparative analysis of geosmin content in the peel, body, and core portions of the beet supported this idea because the peel contained the highest amount of geosmin. While previous studies were unsuccessful in drawing further conclusions, Lu et. al. concluded that the origin of geosmin arose from the beets themselves rather than the uptake of geosmin in the soil produced by surrounding microbiota (2003). Two main experimental groups were used: control seeds grown in regular soil and sterilized seeds grown in an aseptic environment. In order to test the hypothesis of absorption of geosmin, a portion of the aseptic group was removed and grown

in environments containing added geosmin. Using GC-MS analysis of the aseptically-grown seedlings, geosmin was in fact detected; this suggests that the beet is able to synthesize geosmin endogenously. In addition, no significant difference in geosmin content between aseptically-grown seedlings and those grown with added geosmin was found, indicating that absorption from the environment was unlikely. While these studies help to provide good information about the aroma profile of beets, several opportunities for further research in this area remain that could help to give a more complete analysis.

III. Color chemistry of beets

One major factor in the popularity of beets as a commercial crop is the abundance of natural red pigment that it provides. Not only do these pigments provide rich color, there is evidence to show that they may have health benefits, including anti-inflammatory and anti-cancer properties. These water-soluble, nitrogenous pigments are classified as betalains, of which there are two main types: betacyanins and betaxanthins (Attokoran, 2011). The betacyanins appear red, while the betaxanthins contain a yellow color; it is the ratio of these constituents which determines the final hue (Neelwurne, 2013). In most beets, the ratio of betacyanins to betaxanthins is 3:1 (Goldman and Navazio, 2008). However, yellow cultivars exist that solely contain betaxanthins. The general structure of a betalain, which is derived from the amino acid tyrosine, contains a chromophore and a sugar moiety. Structurally different, the betacyanins are condensed with a glucose group whereas betaxanthins contain an amino acid. During the life of the beet, the amount of color present in the tuber increases; therefore, more mature beets are generally used for pigment extraction. One major issue in the color chemistry of the beet is the instability of these natural pigments. A variety of factors can cause degradation of the pigments, including

light, oxygen, pH, temperature, and enzymes. The individual types of pigments themselves display varying degrees of stability; betacyanins have been found to be more stable than their betaxanthin counterparts, both at room temperature and during heating.

IV. Production of industrial pigments

While the use of these pigments may appear to be a new phenomenon, the earliest recorded use of betalains to color food dates back to the 18th century in pancakes. Other historical examples include the use of beets in hamburgers and red wine. Although there are a variety of plant sources, such as red rice and the cactus pear, for betalain-based pigments, beet root is the only source currently approved for food use in the United States and the European Union. Due to the public's penchant for the use of natural substances in food and pharmaceutical products, many companies are turning to the beet root to provide red pigments rather than use an FD&C color. Because of the instability of beet pigments with heat, it is often used in chilled foods, such as ice cream and fruit juice drinks (Attokoran, 2011). The extraction process begins with peeling and chopping of the beets then blanching them in order to inactivate endogenous enzymes, including polyphenol oxidases, which can cause a loss of color. Although the removal of the peel causes a loss of 30% of the pigment, it is beneficial because there is a greater abundance of enzymes located in the peel (Neelwurne, 2013). Beets are then further macerated using a hydraulic press and pigment is extracted using water, usually with the addition of a 20-50% methanol or ethanol solution in order to ensure complete extraction. Ascorbic is generally added as an acidulant to lower the pH in order to stabilize the pigment and prevent enzymatic oxidation. Fermentation with lactic acid bacteria species then takes place in order to degrade carbohydrates and nitrogenous material; this aids in enhancing the betanin concentration. The

extract is then filtered and then pasteurized to protect against microbial contamination before the use of various concentration and drying methods, including encapsulation and spray drying. However, in many instances the whole beet pigment extract is sold; the presence of high amounts of pectin help to stabilize the pigments in storage. As previously discussed, betalain pigments are subject to a variety of instability-inducing forces; however, the industry attempts to combat these with preservatives and new technologies. Combining traditional extraction methods with newer technologies such as pulsed electric field treatment, ultrasound, and irradiation aids in stabilization against high temperatures and oxidation. The antioxidant vitamin E has also been shown to protect against pigment degradation. Chelating agents, such as citric acid and EDTA, sequester metal ions that can cause oxidative changes in the pigments, especially during storage. However, even under the best conditions with storage at 5°C, after 60 days the beet pigment is estimated to be at most approximately 46.9% of its original value (Neelwurne, 2013).

V. Aroma analysis techniques

Foods are composed of a variety of constituents, such as fat, protein, carbohydrate, water, and ash; however, it is the volatile components that make up the aroma profile of that food item. These volatile substances are those that reach the olfactory bulb either by entering through the air breathed in by the nostrils, orthonasally, or by being released through chewing and traveling up the throat, retronasally (Marsili, 2002). However, volatility alone does not determine whether or not the compound produces an aroma, and additionally how important a single aroma compound is to the overall profile of the food product. While GC-MS is an excellent technique to quantitatively identify chemical compounds, it lacks the ability to determine whether a compound actually provides an aroma. Additionally, in many cases, odorants that contribute

most to a food's aroma profile occur at the lowest concentration. This is due to the odorant's low threshold of detection. GCO couples the resolving ability of gas chromatography with the acute sensitivity of the human nose. As compounds are separated by the column, the human nose serves as a detector, and retention time, intensity, and odor characteristics are noted with each aroma. Retention indices are then calculated with the use of standard alkanes; these can be compared to a database of known RIs to aid in determining the identity of a compound. However, it is not enough to simply characterize the aroma compounds in a food; analyzing the importance of each specific aroma compound to the overall odor is necessary as well. A technique called aroma extract dilution analysis (AEDA) is one such method of doing so (Marsili, 2002). This method involves sniffing the same sample at increasing dilutions to determine at which dilution a specific odorant can no longer be detected. To express these results, a flavor dilution (FD) factor is determined; the FD factor is the maximum dilution at which a particular aroma compound can be detected. In addition to GCO of the flavor extract, a complete odor analysis often includes a headspace component. Some compounds, that may inevitably prove necessary to the overall odor of a product, may be lost during the distillation component of a solvent extraction process due to their high volatility. Another issue is the likelihood of co-elution with the solvent used, which would impede detection by GCO. Static headspace is one technique that is used to remedy these limitations of solvent extract GCO. The process simply involves removing a predetermined volume of headspace gas from above a heated sample to inject into the GCO. No solvent is used and distillation does not take place, helping to improve resolution of these specific compounds. A technique similar to AEDA can be applied to headspace GCO, where decreasing volumes are removed and then subsequently analyzed by GCO to determine dilution factors for compounds of interest.

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CHAPTER 3

IDENTIFICATION OF POTENT ODORANTS IN BEETS AND BEET PRODUCTS

I. ABSTRACT

Aroma-active compounds in beet products were identified and characterized using gas chromatography-olfactometry (GCO) gas chromatography-mass spectrometry (GC-MS). Aroma extract dilution analysis (AEDA) was used to screen for potent odorants in four different preparations of the common garden beet (*Beta vulgaris*): boiled, oven-roasted, canned and colorant. Due to their high odor potency and consistent sample quality, canned beets were utilized for further flavor analysis. Of the forty-one odorants detected in the solvent-extracted beets, 2-acetyl-1-pyrroline, geosmin, methional, furaneol, *p*-vinylguaiacol, and vanillin were the most potent odorants. Three 3-alkyl-2-methoxypyrazines were also identified at moderately potent levels, as well as 1-octen-3-one, phenylacetic acid, and eugenol. Static headspace GCO was also performed on the canned beets, which revealed two previously undetected compounds, methanethiol and dimethyl sulfide, to be present and potent.

II. KEYWORDS

Beet root; beet colorant; gas chromatography-olfactometry; aroma extract dilution analysis; static headspace

III. INTRODUCTION

The beet root (*Beta vulgaris subsp. vulgaris*), in its many forms, has been an important crop for millennia, with its first written appearance occurring in 8th century Mesopotamia

(Attokaran, 2011). The beet we know today descended from the sea beet, which grows in the Mediterranean Sea; cultivation then spread across North Africa and the Mediterranean regions of Europe. While the beet was mainly utilized as animal fodder for hundreds of years, in sixteenth century Europe, the beet root grew to be a popular vegetable for human consumption (Neelwurne, 2013). Worldwide beet production totals over 227 million tons, with the United States being one of the major contributors and supplying approximately 27 million tons. One reason for the beet's success as a vegetable dish can be attributed to its high nutritional value, containing high amounts of magnesium, selenium, folate, and phytosterols. However, these amounts can be altered based on the cooking method chosen. Common cooking techniques include boiling, steaming, oven roasting, and pickling.

A major feature of the beet is its distinct earthy flavor, which is often polarizing in terms of liking of the vegetable. Discovery of geosmin, one of the main aroma components that cause this characteristic earthy quality of beets, was performed by Acree et. al. in 1975. Despite the popularity of the beet in cuisine and industrial use, minimal research has been performed on its aroma components. The prevalence of various 3-alkyl-2-methoxypyrazines, dimethyl sulfide, and furfural in beets has been demonstrated in several studies using gas chromatography-mass spectrometry techniques (Murray and Whitfield, 1975; Parliment et. al., 1977); however, these studies lack depth due to the absence of any odor analysis techniques. The majority of additional aroma analysis studies involving beets focus on odors as contaminants during the process of extracting sucrose from sugar beets. Although some of these odor compounds may be shared, sugar and red beet root varieties are significantly different crops; one main contrast is the lack of pigment in the sugar beet. This aspect is important because some researchers have hypothesized that certain aroma compounds may originate with the betalain pigment molecules (Parliment et.

al., 1977). While these studies help to provide important information about the aroma profile of beets, several opportunities for further research in this area remain that could help to provide a more complete analysis.

In order to better establish an exhaustive profile of the aroma-active compounds in beets and their subsequent products, gas chromatography-olfactometry (GCO) is a vital tool because it utilizes the human nose in addition to instrumental analysis. It is important to not simply identify the main odorants, but to determine which are the most potent, and therefore contribute more significantly to the final aroma of the product. To fulfill this need, aroma extract dilution analysis (AEDA) is often used (Grosch, 1993, 1994). AEDA involves stepwise dilution of aroma extracts to determine relative intensity when analyzing using GCO. From AEDA, flavor dilution (FD) factors can be determined based on the last dilution where a certain odorant is detected; a higher FD factor indicates a more potent odorant.

In the present study, four different types of beet products (oven-roasted, boiled, canned, and colorant) were preliminary screened with the use of GCO and gas chromatography-mass spectrometry (GC-MS). AEDA was used to determine the most potent odorants contained in these four samples. Due to its potency and ability to provide consistent samples, canned beets were chosen with which to pursue further analysis. With the canned beets, AEDA was again performed on a more concentrated solvent extract, and was paired with GCO of decreasing headspace volumes. The results of these studies provide a foundation for greater understanding of the flavor chemistry of the beet.

IV. MATERIALS AND METHODS

Materials

Raw beet roots as well as store brand canned beets were purchased from a local market (Urbana, IL). Liquid beet colorant was obtained from Sensient Technologies (St. Louis, MO). Deodorized, distilled water was prepared by boiling glass-distilled, deionized water by one third from its original volume.

Chemicals

n-Alkane standards, 2-methyl-3-heptanone (internal standard for neutral-basic fraction), and 2-ethylbutyric acid (internal standard for acidic fraction) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Sodium sulfate (99%), sodium bicarbonate, hydrochloric acid (36.5%), diethyl ether (anhydrous, 99.8%), sodium chloride (99%), methanol (99.9%) were obtained from Fisher Scientific (Fairlawn, NJ). Ultra-high purity (UHP) nitrogen, liquid nitrogen, and UHP helium were purchased from S.J Smith (Davenport, IA).

Reference standard compounds. The standard compounds used to confirm the odor properties and retention indices of the aroma compounds listed in **Tables 3.1-3.3** were supplied by companies in parentheses: compounds nos. **2, 4-6, 9, 14, 16-18, 20, 23, 26, 28-29, 31, 34, 39-41, 43-44** (Sigma-Aldrich, St. Louis, MO); **19, 24, 38, 42** (Alfa Aesar, Lancashire, United Kingdom); **21** (Fluka, Bluchs, Switzerland); **32** (Firmenich, Princeton, NJ); **35** (Bedoukian, Danbury, CT).

Syntheses

E, E, Z-2,4,6-Nonatrienal was synthesized as previously reported by Schuh et. al. (2005). *Z*-1,5-Octadien-3-one was synthesized using the method of Genthner and Cadwallader. (2010). *E*-4,5-Epoxy-[*E*]-2-decenal was synthesized according to the method of Jianming et. al. (1999).

Sample Preparation

Raw beets were prepared for roasting in a 190°C oven or boiling by removing residual soil by rinsing in tap water then by slicing stems. For roasting, beets were sliced in half and placed cut side up in a glass Pyrex baking dish and baked for one hour, until beets were tender. For boiling, whole beets were placed in a 2000 mL glass beaker and deodorized water was added to the 1500 mL level. Beets were boiled for 30 minutes until tender. Canned beets and liquid beet colorant required no sample preparation step.

Isolation of Volatile Compounds by Direct Solvent Extraction (DSE)

Screening

For each beet variety, 100 g of beets were measured into a blender with 150 mL of deodorized water and 90 g of sodium chloride. The mixture was blended until homogenous and then split in half between two Teflon centrifuge bottles, which were each sealed with Teflon caps. Twenty µL of an internal standard mixture (containing 2-methyl-3-heptanone and 2-ethylbutyric acid in methanol) were added and allowed to sit for 5 minutes. Diethyl ether (30 mL) was added to each bottle and the mixtures were agitated for 5 minutes on an orbital shaker at 226 rpm (VWR Scientific Products, DS-500, Radnor, PA). The mixture was then centrifuged at 4000 rpm for 10 minutes in order to separate the solvent phase, which was then pipetted into a round-bottom flask. This protocol was repeated twice more, using 20 mL of diethyl ether each time. The solvent phases were then condensed to 50 mL by distillation using a Vigreux column in a 43°C water bath. The volatile compounds from this extract were isolated using solvent-assisted flavor evaporation (SAFE) (Engel et. al, 1999) according to the procedure of Watcharananun et al. (2009). The aroma extract was then fractionated into acidic (aqueous phase)

and neutral-basic (organic phase) fractions by using 0.5 M $NaHCO_3$ (2 x 30 mL). The aqueous layer was acidified using 4 N HCl to a pH of approximately 4.0 and subsequently extracted using diethyl ether (3 x 20 mL). The organic layer was washed with a saturated NaCl solution (2 x 10 mL) to remove excess sodium bicarbonate; the aqueous portion was then discarded. Both fractions were then concentrated to 1 mL each using Vigreux column distillation and drying over anhydrous Na_2SO_4. The solvent extract was further concentrated to 500 µL using a nitrogen stream and stored at -70°C until further analysis.

Canned

All parameters remained the same except for the following: 200 g of canned beets were blended with 300 mL of deodorized water and 150 g sodium chloride, and the mixture was split between 5 Teflon bottles, with each bottle receiving 10 µL of the internal standard mixture and following the same solvent extraction scheme with diethyl ether. Extraction was followed by SAFE and fractionation, following the previously described procedures.

Identification of Aroma-Active Compounds

All aroma extracts were analyzed using gas chromatography-olfactometry (GCO) and gas chromatography-mass spectrometry (GC-MS).

GCO. GCO was performed using 6890 GC (Agilent Technologies, Inc., Santa Clara, CA) coupled with a flame ionization detector (FID) and an olfactory detection port (DATU Technology Transfer, Geneva, NY). 1 µL of aroma extract per run was injected using cool, on-column mode (+3 °C temperature tracking mode) in order to prevent formation of artifacts due to heat, loss of highly volatile compounds, and injection bias. Separations were performed on both a nonpolar RTX®-5 column and polar Stabilwax® column (both 15 m × 0.32 mm i.d. × 0.5 µm df; Restek, Bellefonte, PA). Effluent leaving the column was equally split between the olfactory

detection port and the FID by the use of deactivated fused silica tubing (1 m x 0.25 mm i.d.: Restek). The detector temperatures were both set to 240°C. The oven temperature was programmed from 35° to 225°C at a rate of 10°C/min with initial and final hold times of 5 and 15 minutes respectively, then to 230°C with a hold time of 15 minutes. The carrier gas used was helium, at a constant flow rate of 9.5 mL/min.

GC-MS. The GC-MS system was composed of a 6890 GC (Agilent Technologies, Inc.) paired with a mass selective detector (Hewlett-Packard, Bloomington, IL). Separations were performed using 1 µL of extract on both a polar Stabilwax® DA column (30 m x 0.25 mm id x 0.25 µm df; Restek) and a non-polar SAC™-5 column (30 m x 0.25 mm id x 0.25 µm df; Supelco, Bellefonte, PA) capillary columns using cold, splitless mode. The detector temperature was set to 250°C. The oven temperature was programmed from 40°C to 225°C at a rate of 4°C/min with a hold time of 30 minutes. The carrier gas used was helium with a constant flow rate of 1.0 mL/min.

The retention index (RI) for each compound was calculated using its retention time (RT) compared to the RTs from standard *n*-alkanes (van den Dool and Kratz 1963). Mass spectra were compared against those in the NIST2008 mass spectral database, and retention indices were compared to literature values in order to create a tentative identity for each compound. Aroma-active compounds were positively identified based on comparison of their RI values on both polar and non-polar columns, odor descriptors, and mass spectra versus those of true standard compounds as described by Molyneux and Schieberle (2007). Without an authentic standard available, a compound was considered only tentatively identified.

Aroma Extract Dilution Analysis

Each aroma fraction, acidic or neutral-basic, was diluted stepwise with diethyl ether in sequential ratios of 1:3 (v/v) using the method previously described by Watcharananun et. al

(2009). Dilutions were each stored in a 300 µL glass insert in a 1 mL clear glass vial stored at -70°C until it was to be analyzed. Each dilution was performed by GCO on Stabilwax® column. Dilutions were sniffed sequentially until no odorants were detectable. The flavor dilution (FD) factor for each compound was determined by the highest dilution at which it could be detected by the human nose.

Static Headspace Analysis of Canned Beets

At room temperature, 200 g of canned beets were blended with 300 mL of deodorized water to form a homogenous mixture. The mixture was then placed into a 500 mL glass flask and immediately sealed with a silicon septum stopper. The flask was placed into a 40°C water bath and completely covered with aluminum foil, for 30 minutes. Gas-tight syringes (SGE Analytical Science Pty Ltd; Ringwood, Australia) were used to draw headspace samples for analysis by GCO. A fresh beet sample was used for each analysis.

Gas Chromatography-Olfactometry of Decreasing Headspace Volumes (GCOH)

GCOH was performed using 6890 GC (Agilent Technologies, Inc., Santa Clara, CA) coupled with a flame ionization detector (FID) and an olfactory detection port (ODP2, Gerstel, Germany). Headspace volumes of 25 mL, 5 mL, 1 mL, 0.200 mL, and 0.040 mL were analyzed. A CIS4 programmable temperature vaporizer (PTV) inlet (Gerstel) was used to cryofocus the headspace volatiles prior to injection. Separations were performed on a polar RTX®-Wax column (15 m × 0.32 mm i.d. × 0.5 µm df; Restek). The inlet was programmed to -120°C (0.1 min hold) with a ramp rate of 10°C/sec; final temperature was set to 260°C with a hold time of 10 minutes. The carrier gas used was helium, at a constant flow rate of 5 mL/min. The oven temperatures were programmed as follows: initial temperature, 35°C (5 min hold), ramp rate, 10°C/min, final temperature 225°C (20 min hold). In addition the same headspace volumes were also analyzed

using a RTX®-5 column (15 m length x 0.53 mm i.d. x 1 μm film thickness; Restek). Other GC conditions were the same as those used for AEDA.

V. RESULTS AND DISCUSSION

Initial analyses were performed on four different types of beet preparations: store-bought canned, boiled, oven-roasted, and liquid colorant. To isolate the aroma components from the non-volatile beet matrices, direct solvent extraction (DSE) and solvent-assistant flavor evaporation (SAFE) were used. The aroma extract collected from each of these beet product samples possessed characteristic, highly earthy beet-like odors, confirming that the extraction process was successful in obtaining the typical odor-active compounds from beets.

Screening of Aroma-Active Components

A total of 21 odorants were detected during preliminary analysis in the four different beet samples (**Table 3.1),** of which one was unidentified. Because these products did not contain added flavorings, all aroma compounds detected are assumed to have originated from the beets themselves. Geosmin, a predominant odorant in all samples, has a strongly earthy note. Three methoxypyrazine derivatives were detected in all samples, except for the beet colorant; these compounds provide vegetable-like, earthy odors (Green et. al., 2011). Many of the compounds detected are derived from lignin, a component of the beet's cell walls; these include vanillin, *p*-vinylguaiacol, guaiacol, and eugenol.

AEDA was used to determine which odorants contribute more significantly to the overall aroma by ranking them on their relative potency using flavor dilution (FD) factors. Geosmin ranked very highly in all four samples, likely due to its extremely low odor threshold, which ranges between 250 and 500 ppb in air (Grimm et. al., 2004). The geosmin in beet colorant

possessed a lower FD factor as compared to the other beet preparations. This may be due to the manufacturer's choice to use a low-geosmin variety of beet, which may help to minimize any noticeable flavor contribution to the food product to which it is added (Neelwarne, 2013). Filtration procedures may also have been utilized to reduce levels of not only geosmin, but other undesirable odorants. Methional, a cooked potato-like odor, contributed greatly as well. Two of the lignin-derived compounds, *p*-vinylguaiacol and vanillin, were potent in all four of the samples tested, *p*-vinylguaiacol having a very high FD factor in canned beets and vanillin in both canned and roasted beets. The three 3-alkyl-2-methoxypyrazine derivatives, isopropyl, isobutyl, and *sec*-butyl, were moderately potent in the three cooked beet samples, but were not detected in the colorant. Furaneol, a Maillard reaction-derived compound that possesses a caramel odor, was found with widely varying potency in three of the samples, being particularly strong in the canned beets.

While this screening experiment provided an adequate foundation from which to build on, only the canned beet samples were chosen to with which pursue further analyses. Overall, it was the most impactful in terms of aroma-active compounds. Furthermore, consistency of results would be better maintained by using cans from the same lot number. Using fresh beets introduced an element of uncertainty; differences in growing seasons and supplier could have a large impact on the aroma compounds detected. In addition, compounds were lost during the boiling and oven-roasting cooking processes more easily, particularly in regards to the most volatile odorants.

Predominant Aroma-Active Components of Canned Beets

A total of 41 odorants were detected by GCO from the DSE-SAFE extracts of the canned beet aroma extracts (**Table 3.2**). Thirty-six were positively identified, four were tentatively identified, and one was unidentified (unknown). Odorants were considered positively identified with detection from mass spectra or the use of a standard reference compounds. Odorants can be grouped by the use of the average flavor dilution factors, with a higher FD factor indicating a more potent odorant. From the variety of odorants present, their origins can be divided into three groups: odorants inherent to the raw beets, reaction products of enzymes released during cutting, or compounds formed during cooking of the beets.

Geosmin is perhaps the most important native aroma compound in the beet. Although it was previously believe to originate from microbial production in the soil, geosmin is, in fact, biosynthesized in the beet itself (Lu et. al., 2003). The three 3-alkyl-2-methoxypyrazines are also impactful odorants that originate from the beets themselves (Murray and Whitfield, 1975). These findings help to support the hypotheses of previous studies that suggested that 3-alkyl-2-methoxypyrazines were important in the overall odor impact of beets (Parliment et. al., 1977).

The presence of the green, hay, cucumber, and citrus-like aldehydes found in the canned beets are likely due to enzymes released during the cutting of the beets. When the plant tissue is damaged, enzymes such as lipoxygenases and hydroperoxide lyases are able to catalyze the formation of these aldehydes from fatty acids (Luning et. al., 1994, 1995; Gigot et. al., 2012). Although some enzymes are deactivated during blanching, some remain active. For example, 1-octen-3-one is formed via 1-octen-3-ol derived from linoleic acid (Kim et al., 2013). Based on the carbon chain lengths of the compounds detected, it can be inferred that C8 and C9 lipoxygenases are more active than C6 lipoxygenases. These C8 and C9 lipoxygenases are

responsible for creating odorants such as 1-octen-3-one, octanal, *E*-2-nonenal, and 2,4-nonadienal (Galliard and Phillips, 1976). C10 lipoxygenases are also present, forming compounds such as *E*-4,5-epoxy-[*E*]-2-decenal.

The majority of compounds found in the canned beets are a result of the cooking processes that beets are subjected to during the high-heat retorting process. Furaneol, maltol, and sotolon are all created during the Maillard reaction due to the presence of hexose sugars, such as fructose and glucose. The presence of a variety of amino acids contained in beets contributes to a variety of Maillard-derived odorants (Neelwarne, 2013). Methional, a potent potato-like odorant, is formed during thermal processing by the Strecker degradation segment of the Maillard reaction, originating from the amino acid methionine (Di, 2008). 2-acetyl-1-pyrroline, a potent compound with a popcorn-like odor, is formed when 1-pyrroline, a Strecker degradation product of the amino acid proline, undergoes acylation (Adams and de Kimpe, 2006). Two important compounds in the acid fraction, isovaleric acid and phenylacetic acid, are formed from leucine and phenylalanine, respectively. The vast array of odor-active compounds evolving from amino acids give evidence to the hypothesis that betalain pigments themselves may actually form odorants, causing off-odors in products colored with beet pigments. Heating of the beets causes a breakdown of lignin, one of the main structural components of the cell wall, giving rise to a myriad of different aroma compounds. Ferulic acid, a phenolic which is released from lignin during heating, can be further transformed into a variety of different important odorants. One of the most potent classes, the phenols, can be formed from ferulic acid, including odorants such as *p*-vinylguaiacol, guaiacol, eugenol, vanillin and *p*-cresol. Carotenoids can also be transformed to create odorants, such as β-damascenone and β-ionone.

Although AEDA GCO and headspace GCO are an important tools in characterizing the components of the odor profile of canned beets, their measurements are solely based on odor-activity in the air without taking into account matrix effects from the beets. To help correct for these limitations, odor-activity values (OAVs) will be determined using stable isotope dilution analysis (SIDA), with the results being presented in **Chapter 4** of this thesis.

Gas Chromatography-Olfactometry of Decreasing Headspace Volumes

Eleven odor-active compounds were identified in the headspace of canned beet, as shown in **Table 3.3.** While the majority of the compounds detected during headspace dilution analysis matched those from AEDA, two potent compounds emerged that were previously undetected: methanethiol and dimethyl sulfide, as shown in **Table 3.3**. Their previous absences in AEDA were likely due to their loss during the extraction process because of high volatility as well as co-elution with the ether solvent peak. Like methional, methanethiol is also formed from the amino acid methionine. However, methanethiol is formed in plants due to amino acid catabolism rather than by the Maillard reaction (Schmidt et. al., 1985). Dimethyl sulfide had been previously identified as a volatile in cooked beets by the use of GC-MS (Parliment et. al., 1977) and the present GCO results demonstrate its importance in the aroma profile It originates from S-methylmethionine, an intermediate formed from the amino acid methionine during heating processes (Scherb et. al., 2009). Although these compounds were not detected in the odor analyses from DSE-SAFE extracts, headspace dilution GCO demonstrates their importance to the overall aroma profile of canned beets, which must be taken into account for further studies of quantification and odor model building, which are presented in **Chapter 4** of this thesis.

Gas Chromatography-Mass Spectrometry of Solvent Extracts

Although the majority of the odor analyses on the canned beets relied mainly upon GCO methods, gas chromatography mass spectrometry (GC-MS) was performed as an analytical support system to the more subjective GCO. **Table 3.4** shows the odor-active compounds that could be detected on GC-MS scan mode. Compared to the forty-one compounds that were identified using GCO, only thirteen were detectable by GC-MS. Some of the most potent compounds detected by GCO are missing, and this is likely due to their low abundances and low odor thresholds. For example, the odor threshold for vanillin ranges from 20-200 parts per billion (ppb) while that for 2-isobutyl-3-methoxypyrazine ranges from 2-16 parts per trillion (ppt). The extremely low odor threshold for compounds such as 2-isobutyl-3-methoxypyrazine allows them to exhibit high odor potency while being undetectable under standard conditions on GC-MS. As previously mentioned in this chapter, geosmin too possess a low odor threshold; however, it is likely detectable by GC-MS due to its higher concentration in the sample.

In order to properly perform quantification, compounds must be able to be detected in some capacity by GC-MS. To help uncover compounds that may not reveal themselves in the regular scanning ion mode, selective ion mode (SIM) will be used. Every volatile compound breaks down into specific ions when passing through the mass selective detector. With the knowledge of these ions, the GC-MS system can be set to detect only certain ions, improving resolution. **Table 3.5** shows compounds of high importance to be analyzed with SIM mode for use in quantification. Compounds were chosen based on qualifications of high potency (FD factor ≥ 27) as well as feasibility of analysis. These results are presented in **Chapter 4** of this thesis.

VI. FIGURES AND TABLES

Table 3.1 Aroma-active compounds found by screening in four preparations of beets

	RI[b]					FD Factor[e]			
No.[a]	wax	RTX-5	F[c]	Compound	Odor Description[d]	C[f]	B	R	Co
1	1290	-	NB	Acetoin	Cheesy, buttery	-	3	3	<3
2	1293	1006	NB	Octanal	Pungent, citrus	<3	-	9	-
3	1327	931	NB	2-acetyl-1-pyrroline	Popcorn	<3	<3	-	<3
4	1364	982	NB	Dimethyl trisulfide	Green bean	3	3	<3	-
5	1419	1097	NB	2-Isopropyl-3-methoxypyrazine	Dirt	243	81	27	-
6	1449	911	NB	Methional	Cooked potato	81	243	27	81
7	1450	-	A	Acetic acid	Vinegar	9	-	<3	9
8	1514	1174	NB	2-*sec*-butyl-3-methoxypyrazine	Earth	27	27	9	-
9	1513	1168	NB	2-Isobutyl-3-methoxypyrazine	Green bell pepper	27	9	3	-
10	1578	1185	NB	Methylisoborneol	Musty, green	-	-	-	<3
11	1618	-	A	Butyric acid	Cheesy	<3	-	-	-
12	1657	832	A	Isovaleric acid	Cheesy	9	<3	<3	81
13	1819	1415	NB	Geosmin	Earth	243	81	81	27
14	1859	1065	A	Guaiacol	Spice	9	-	-	-
15	1997	-	NB	unknown	Bread, sweet	27	3	9	81
16	2029	-	A	Furaneol	Caramel, sweet	729	<3	27	-
17	2159	-	NB	Eugenol	Clove	243	-	<3	-
18	2200	-	A	Sotolon	Honey	-	-	-	81
19	2196	1326	NB	*p*-vinylguaiacol	Spice	729	27	9	81
20	2546	-	A	Phenylacetic acid	Honey	9	-	-	27
21	2560	1397	A	Vanillin	Vanilla	729	27	243	81

Table 3.1 (continued)

[a]Numbers correspond to those in **Tables 3.1-3.5**. [b]Retention indices determined on two stationary phases (Stabilwax® and RTX®-5). [c]Fraction: acidic (A); neutral-basic (NB). [d]Odor quality determined by GCO. [e]Flavor dilution factors were determined on Stabilwax® column. [f]Beet preparations: canned (C); oven-roasted (R); boiled (B); colorant (Co).

Table 3.2 Aroma-active compounds in canned beets

	Ri[b]						
No.[a]	**wax**	**RTX-5**	**F[c]**	**Compound**	**Odor[d]**	**FD Factor[e]**	**Identification[f]**
22	<1000	<700	NB	3-methylbutanal	Dark chocolate	3	RI, O
23	1083	793	NB	Hexanal	Green, grassy	<3	RI, O, MS, S
2	1293	997	NB	Octanal	Citrus	9	RI, O, MS, S
24	1296	973	NB	1-octen-3-one	Mushroom, metallic	243	RI, O, S
3	1327	931	NB	2-acetyl-1-pyrroline	Popcorn	729	RI, O, S
4	1364	982	NB	Dimethyl trisulfide	Green bean	<3	RI, O, S
25	1371	979	NB	*Z*-1,5-octadien-3-one	Metallic	81	RI, O, S
26	1388	1098	NB	Nonanal	Citrus	27	RI, O, MS, S
5	1419	1097	NB	2-Isopropyl-3-methoxypyrazine	Earth, pea	6561	RI, O, S
6	1449	911	NB	Methional	Cooked potato	6561	RI, O, S
7	1450	<700	A	Acetic acid	Vinegar	729	RI, O, MS
8	1514	1174	NB	*2-sec*-butyl-3-methoxypyrazine	Earth	729	RI, O, S
27	1508	1142	NB	*Z*-2-nonenal	Hay	3	RI, O
9	1513	1168	NB	2-Isobutyl-3-methoxypyrazine	Bell pepper	729	RI, O, S
28	1531	1155	NB	*E*-2-nonenal	Cucumber, hay	<3	RI, O, S
29	1583	1149	NB	*E,Z*-2,6-nonadienal	Cucumber	81	RI, O, S
11	1618	-	A	Butyric acid	Cheesy	27	RI, O, MS
12	1657	832	A	Isovaleric acid	Cheesy	243	RI, O, MS
30	1661	1041	NB	unknown	Rose, floral	2187	O
31	1704	1208	NB	*E, E*-2,4-nonadienal	Fried, fatty	<3	RI, O, S
13	1819	1415	NB	Geosmin	Earth	2187	RI, O, S, MS
32	1848	-	NB	β-damascenone	Apple, sweet	81	RI, O, S
14	1859	1065	A	Guaiacol	Smoky	81	RI, O, S, MS
33	1877	1267	NB	*E,E,Z*-2,4,6-nonatrienal	Oats	9	RI, O, S
34	1921	1110	NB	2-phenylethanol	Bread, sweet	3	RI, O, S
35	1974	-	NB	β-ionone	Hay-like, saffron	3	RI, O, S
36	1993	-	A	Maltol	Caramel	3	RI, O

Table 3.2 (continued)

No.[a]	Ri[b] wax	Ri[b] RTX-5	F[c]	Compound	Odor[d]	FD Factor[e]	Identification[f]
37	2006	-	NB	*E*-4,5-epoxy-[*E*]-2-decenal	Metallic, unripe	243	RI, O, S
16	2029	1071	A	Furaneol	Caramel	6561	RI, O, MS, S
38	2058	1277	NB	4-ethylguaiacol	Cloves	243	RI, O, MS, S
39	2077	1075	A	*p*-cresol	Musty, dung	9	RI, O, S
40	2115	-	NB	*m*-cresol	Inky, phenolic	27	RI, O, S
17	2160	1352	A	Eugenol	Clove	729	RI, O, S
19	2196	1326	NB	*p*-vinylguaiacol	Spice	6561	RI, O, MS, S
18	2200	-	A	Sotolon	Honey, maple	9	RI, O, S
41	2230	1302	NB	*o*-aminoacetophenone	Grape	9	RI, O, S
42	2381	-	NB	*E*-isoeugenol	Spice	27	RI, O
43	2449	1444	NB	Coumarin	Herbaceous, sweet	<3	RI, O, S
44	2462	-	NB	Skatole	Fecal, mothball	3	RI, O, S
20	2546	1252	A	Phenylacetic acid	Honey	729	RI, O, S
21	2560	1397	A	Vanillin	Vanilla	6561	RI, O, MS, S

[a]Numbers refer to those in **Tables 3.1-3.5** [b]Retention indices determined on two stationary phases (Stabilwax® and RTX®-5). [c] Fraction: acidic (A); neutral-basic (NB). [d]Odor quality determined by GCO. [e]Flavor dilution factors were determined on Stabilwax® column. [f]Identification criteria: retention index (RI); odor quality (O); mass spectra (MS); reference standard compound (S).

Table 3.3 Aroma-active compounds in headspace of canned beets

No[a]	RI[b] wax	RI[b] RTX-5	Compound	Odor Description[c]	FD Factor[d]
45	545	<500	Methanethiol	Rotten vegetable, sulfur	5
46	576	<500	Dimethyl sulfide	Canned corn	125
23	808	515	3-methylbutanal	Dark chocolate	5
47	914	565	2-methylbutanal	Dark chocolate	5
3	1313	943	2-acetyl-1-pyrroline	Popcorn	1
4	1376	978	Dimethyl trisulfide	Garlic salt, cabbage	1
5	1439	-	2-Isopropyl-3-methoxypyrazine	Earthy	1
6	1454	915	Methional	Cooked potato	5
9	1501	1185	*2-sec*-butyl-3-methoxypyrazine	Earthy	5
10	1530	1195	2-Isobutyl-3-methoxypyrazine	Bell pepper	5

Table 3.3 (continued)

No[a]	RI[b]		Compound	Odor Description[c]	FD Factor[d]
	wax	RTX-5			
14	1822	1434	Geosmin	Earthy	1

[a]Numbers refer to those in **Tables 3.1-3.5**. [b]Retention indices determined on two stationary phases (RTX®-wax and RTX®-5).[c]Odor quality determined by GCO. [d]Flavor dilution factors were determined on RTX®-wax column, and were equal to the highest headspace volume tested (25 mL) divided by the lowest headspace volume tested by GCO.

Table 3.4 Compounds detected by GC-MS in scan mode

No.[a]	Compound
23	Hexanal
2	Octanal
26	Nonanal
7	Acetic acid
11	Butyric acid
12	Isovaleric acid
14	Geosmin
14	Guaiacol
16	Furaneol
38	4-ethylguaiacol
19	*p*-vinylguaiacol
20	Phenylacetic acid
21	Vanillin

[a]Numbers refer to those in **Tables 3.1-3.5**.

Table 3.5 Compounds to be targeted for quantification by GC-MS in selective ion mode (SIM)

No.[a]	Compound	Ions[b]
24	1-octen-3-one	55, 70
25	*Z*-1,5-octadien-3-one	55[c]
3	2-acetyl-1-pyrroline	41, 43
5	2-Isopropyl-3-methoxypyrazine	137, 152
6	Methional	48, 104
8	*2-sec*-butyl-3-methoxypyrazine	124, 138
9	2-Isobutyl-3-methoxypyrazine	124
29	*E,Z*-2,6-nonadienal	41, 70
32	β-damascenone	69, 121
37	*E*-4,5-epoxy-[*E*]-2-decenal	68, 81[c]
17	Eugenol	103, 164

Table 3.5 (continued)

[a]Numbers refer to those in **Tables 3.1-3.5**. [b]Target ions determined from NIST database. [c]Target ions determined from mass spectra of known standard compounds

VII. REFERENCES

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CHAPTER 4

QUANTIFICATION OF CANNED BEETS USING STABLE ISOTOPE DILUTION ANALYSIS

I. ABSTRACT

Twenty-six aroma-active compounds in canned beets were accurately quantified using stable isotope dilution analysis (SIDA). Both headspace solid-phase microextraction (HS-SPME) and direct solvent extraction/solvent-assisted flavor evaporation (DSE-SAFE) methods were used to extract compounds for analysis. Using the quantification results, an odor activity value (OAV) was calculated for each by dividing the concentration of a compound by its odor threshold in water. Of the 26 aroma compounds chosen for quantification, dimethyl sulfide, geosmin, *E*-4,5-epoxy-[*E*]-2-decenal, methanethiol, *p*-vinylguaiacol, and β-damascenone possessed the highest OAVs and, therefore, contributed the most to the overall aroma profile of the canned beets. Octanal, *Z*-1,5-octadien-3-one, nonanal, acetic acid, 2- isopropyl-3methoxypyrazine, 2-isobutyl-3methoxypyrazine, 2-*sec*-butyl-3-methoxypyrazine, *E, Z*-2,6-nonadienal, and vanillin were also moderately potent odorants.

II. KEYWORDS

Beet root; gas chromatography-mass spectrometry; odor activity value; stable isotope dilution analysis; solid-phase microextraction, solvent-assisted flavor evaporation

III. INTRODUCTION

Beet roots have been consumed by humans for thousands of years, with current yearly production at 227,158,114 tons (Neelwurne, 2013). Despite their widespread consumption and use in commercial colorants, studies on the flavor chemistry of beets are limited in number and scope of research. In the previous study of this thesis, 41 odorants were identified using gas-chromatography olfactometry (GCO) coupled with aroma extract dilution analysis (AEDA) in canned beets (**Chapter 3**). In addition, 11 compounds were identified by GCO in the headspace of canned beets. Some of the most potent odorants in the canned beets are geosmin, three distinct 3-alkyl-2-methoxypyrazines (earthy); methional (cooked potato); furaneol (caramel); *p*-vinylguaiacol (clove); and vanillin (vanilla). Other potent odorants include 1-octen-3-one (mushroom), acetic acid (vinegar), isovaleric acid (cheesy), and phenylacetic acid (honey). In the headspace, the two most important compounds are methanethiol and dimethyl sulfide.

Although AEDA is effective as a screening tool for the determination of potent odorants in a product, it does possess some limitations. This technique does not take into account synergy between the matrix and the odorants detected. One useful method that accounts for these disadvantages that AEDA possess is the calculation of odor activity values (OAVs). This calculation relates the potency of a certain compound to its effect in the whole matrix. To determine the OAV of a specific compound, the concentration of a target odorant is divided by its odor threshold. Those compounds possessing an OAV greater than 1 are considered to be odor-active (Grosch, 2001). In order to determine concentration for each compound of interest, stable isotope dilution analysis (SIDA) serves as a highly accurate method. This technique involves adding an isotope-labeled version for each compound of interest to the sample in order to determine the concentration of each compound specified. These results can then be used to determine OAVs. For certain compounds possessing very low odor thresholds, and are subsequently present in very low concentrations, the mass spectrometer can be adjusted to select ion mode (SIM) in order to selectively detect ions of interest within a certain time period. This technique increases sensitivity of detection, allowing compounds, such as methional and 2-isobutyl-3-methoxypyrazine, to be detected where they would otherwise appear to be absent using traditional scan mode.

IV. MATERIALS AND METHODS

Materials

Canned beets were purchased from a local market (Urbana, IL). Deodorized, distilled water was prepared by boiling glass-distilled, deionized water by one third from its original volume.

Chemicals

n-Alkane standards, 2-methyl-3-heptanone, 2-ethylbutyric acid, 6-undecanone, ethynylmagnesium bromide, anhydrous tetrahydrofuran (THF), 0.5M solution in THF, ammonium chloride, ethyl ether, pyridinium chlorochromate, lithium aluminum deuteride (96% atom%D, 1.0 M solution in THF), and ethyl maltol were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Sodium sulfate (99%), sodium bicarbonate, hydrochloric acid (36.5%), diethyl ether (anhydrous, 99.8%), dichloromethane (99.9%), sodium chloride (99%), and methanol (99.9%) were obtained from Fisher Scientific (Fairlawn, NJ). (Z)-3-hexanal was obtained from Bedoukian Research, Inc. (Danbury, CT). Ultra-high purity (UHP) nitrogen, liquid nitrogen, and UHP helium were purchased from S.J Smith (Davenport, IA).

Standard Compounds. The standard compounds used to determine the responses factors for the corresponding isotopes were obtained from the companies in parentheses: hexanal. 2-*sec*-butyl-3-methoxypyrazine, 2- isopropyl-3-methoxypyrazine, 2- isobutyl-3-methoxypyrazine, *E*, Z-2,6-nonadienal, and geosmin (Sigma-Aldrich); 1-octen-3-one (Alfa Aesar, Lancashire, United Kingdom). *Z*-1,5-Octadien-3-one was synthesized using the method of Genthner and Cadwallader (2010). *E*-4,5-Epoxy-[*E*]-2-decenal was synthesized according to the method of Jianming et. al. (1999).

Isotope Standard Compounds. The following isotopically-labeled standards were obtained from the commercial sources listed in parentheses: [$^2H_3$]-acetic acid, *[$^2H_3$]*-geosmin,

[$^{2}H_6$]-dimethyl sulfide (Sigma-Aldrich; St. Louis, MO**);** [$^{2}H_3$]-guaiacol, *[$^{2}H_7$]*-butyric acid, 2-isobutyl-3-methoxy-*[$^{2}H_3$]*-pyrazine (CDN Isotopes Inc.; Pointe-Claire, Quebec, Canada); [$^{13}C_2$]-phenylacetic acid (Isotec, Miamisburg, OH)

Syntheses

The following isotopically-labeled standards were synthesized according to the methods in parentheses: *[$^{2}H_4$]*-hexanal (Steinhaus et. al., 2009**)**, *[$^{2}H_4$]*-octanal, *[$^{2}H_4$]*-nonanal, *[$^{2}H_3$]*-eugenol (Lorjaroenphon and Cadwallader, 2012), *[$^{2}H_2$]*-1-octen-3-one **(**Lin et. al., 1999**),** *[^{13}C]*-2-acetyl-1-pyrroline **(**Feng and Cadwallader, 2013), *[$^{2}H_3$]*-methional **(**Sen and Grosch, 1991), [$^{2}H_2$]-isovaleric acid (Steinhaus and Schieberle, 2005), *[$^{13}C_2$]*-Furaneol (Blank et. al., 1997**),** *[$^{2}H_3$]*-vanillin (Schnieder and Rolando, 1992), *[$^{2}H_4$]*-β-damascenone (Kotseridis et. al., 1998), 4-*[$^{2}H_5$]*-ethyl-2-methoxyphenol (Lahne and Cadwallader, 2010), 2-*[$^{2}H_3$]*-methoxy-4-vinylphenol (Sheidig et. al., 2007)

[^{2}H]-Methanethiol. The synthesis was performed according to the method previously reported, with one modification of methanol being used as the solvent (Guth and Grosch, 1994).

*[$^{2}H_2$]-Z-1,5-Octadien-3-one***.** Thirty mL of an ethynylmagnesium bromide solution in THF (0.5M in THF; 0.015 mol) was added to a 100-mL three neck flask equipped with nitrogen purge, reflux condenser and magnetic stirrer. The solution was cooled to 0 °C in an ice-water bath and then 1.0 g of (*Z*)-3-hexanal (0.010 mol, neat) was added dropwise to the vigorously stirred solution. The reaction was allowed to proceed for 6 h (at °C) and then 20 mL of saturated **NH_4Cl** solution (aqueous) was added to the flask. The mixture was extracted with ether and the solvent removed by Vigreux column distillation (43 °C). The residue was distilled under vacuum (2 x 10^{-2} Torr) to yield Octa-1-yn-(*Z*)-5-en-3-ol (0.8 g; 65% yield). 1-Octyl-1-(Z)-5-

ene-3-one (0.73 g; 0.0058 mmol) was treated with $LiAl^2H_4$ using the same procedure described by Lin et al. (1999) for the synthesis of [1,2]-$^2H_2$-octen-3-one from 1-octyn-3-ol. Yield of [1,2]-$^2H_2$-(*Z*)-1,5-Octadien-3-one (0.51 g; 69%).

Stable Isotope Dilution Analysis

The stable isotopically-labeled compounds for selected odorants were prepared in solutions of dichloromethane, methanol, or heptane in order to dilute to working concentrations as well as to prevent decomposition of sensitive compounds. Isotopes were directly added to the beet mixture before extraction with diethyl ether or absorption by the solid-phase microextraction fiber. Amounts were preliminarily determined by ratios of peak areas of internal standards (2-ethylbutyric acid or 2-methyl-3-heptanone) to selected compounds, based on previous GC-MS chromatograms, and adjusted accordingly.

Headspace-Solid Phase Microextraction (HS-SPME) of Methanethiol and Dimethyl Sulfide

Canned beets (200 g) were blended with 300 mL of deodorized, distilled water until homogeneous. Beet homogenate (1 g), 0.50 g of NaCl, and 5µL of either isotope-labeled internal standard were added to glass screw-top headspace vials with a Teflon-lined silicon closure. Three separate cans were used for each analysis, with three samples coming from each can (n=9). Each sample vial was incubated at 40°C for 20 minutes with the agitator set to 250 rpm. The SPME fiber (50/30µm DVB/Carboxen™/PDMS StableFlex™; Supelco, Bellefonte, PA) pierced the septum of the vial and was exposed to the headspace volatiles for 10 minutes at 40°C. The absorbed volatile compounds on the SPME fiber were desorbed into a hot, splitless injection port, set to 260°C, of the GC-MS for 20 minutes with a 4 minute valve delay. The GC-MS system was composed of a 6890 GC (Agilent Technologies, Inc.) paired with a mass

selective detector (Hewlett-Packard, Bloomington, IL). Separations were performed on a polar Stabilwax® DA column (30 m x 0.25 mm id x 0.25 µm df; Restek) capillary column. The detector temperature was set to 250°C. The oven temperature was programmed from 35°C to 225°C at a rate of 8°C/min with a hold time of 30 minutes. The carrier gas used was helium, with a constant flow rate of 0.7 mL/min.

Direct Solvent Extraction of Odor-Active Compounds

All other odor-active compounds were extracted using the same direct solvent extraction (DSE) procedure that appears in **Chapter 3** of this thesis. The volatile flavor compounds were extracted using diethyl ether and then nonvolatile material was removed using solvent-assisted flavor evaporation (SAFE). However, fractionation into acidic and neutral-basic fractions was not performed in this study. After SAFE, the extract was condensed to 1 mL using Vigreux column distillation and then filtered through sodium sulfate. The extract was further condensed to 100 µL under a nitrogen stream and stored at -70°C until further analysis.

For analysis, one microliter of extract was injected into the gas chromatography-mass spectrometry (GC-MS) system. The system consisted of a 6890 GC (Agilent Technologies, Inc.) paired with a mass selective detector (Hewlett-Packard, Bloomington, IL). Separations were performed on both a polar Stabilwax® DA column (30 m x 0.25 mm id x 0.25 µm df; Restek, Bellefonte, PA) capillary column, injected using cold, splitless mode. The detector temperature was set to 250°C. The oven temperature was programmed from 40°C to 225°C at a rate of 4°C/min with a hold time of 30 minutes. The carrier gas used was helium with a constant flow rate of 1.0 mL/min. The extract was first analyzed using full scan mode. (35-300 a.m.u., scan rate 5.27 scans/s, interface temperature 280°C, and ionization energy 70 eV). However, as previously mentioned in **Chapter 3**, some compounds required the use of selective ion mode (SIM). This

method improves sensitivity and resolution by monitoring only pre-determined ions at specific time intervals. Ions for each compound of interest were chosen on the basis of abundance and uniqueness. In order to determine the proper parameters to set the MS to, both unlabeled and isotope-labeled standard versions of target compounds were run in scan mode to calculate retention times and target ions. For all compounds except for *Z*-1,5-octadien-3-one, two ions each from the unlabeled and labeled isotopes were chosen to locate in SIM mode; however, only one ratio between the ions was used for calculation of concentration for each compound.

Calibration of Stable Isotopes

Although many of the isotopes used in this experiment possessed previously-determined response factors, some compounds required calibration to ensure accurate calculation of concentrations. In order to obtain these values, standard curves were created using ratios of labeled vs. unlabeled compounds of known concentration. The compounds requiring these response factors included: hexanal, 1-octen-3-one, *Z*-1,5-octadien-3-one, 2-isopropyl-3-methoxypyrazine, 2-*sec*-butyl-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, *E*,*Z*-2,6-nonadienal, geosmin, and *E*-4,5-epoxy-[*E*]-2-decenal. A solution was made in methanol containing approximately 0.1 mg/mL of each of the previously-listed unlabeled compounds. Another solution was created with 50 µL of diethyl ether and 5 µL *[$^2H_4$]*-hexanal, 20 µL *[$^2H_2$]*-1-octen-3-one, 20 µL *[$^2H_2$]*-*Z*-1,5-octadien-3-one, 10 µL 2-isobutyl-3-methoxy-*[$^2H_3$]*-pyrazine, 10 µL 6-undecanone, 10 µL *[$^2H_3$]*-geosmin. The ether solution containing the labeled isotopes was sequentially spiked with 2 µL, 5 µL, 10 µL, and 20 µL of a solution containing approximately 0.1 mg/mL of all unlabeled compounds. Each solution was analyzed using a cold, splitless injection on an RTX®-wax column, with separate runs being performed in SIM and scan modes. For each compound, the selected mass ions were integrated using Enhanced Data

Analysis Software (Agilent Technologies, Inc.). In order to create the standard curve, Microsoft Excel was used to plot the actual mass ratio of each labeled to unlabeled compound against the ratio integrated areas of labeled/unlabeled compounds from the chromatogram. From these points, the linear equation was calculated, with the slope being the response factor. These response factors were then used with chromatogram integration results from runs using the beet solvent extract in order to calculate concentrations for each compound (**Appendix**)

Calculation of Concentration

In order to determine the concentration of each compound, the peak areas of selected ions for both the compounds and the isotopes were integrated using Enhanced Data Analysis Software (Agilent Technologies, Inc.). The following equation was used to calculate the final concentration:

$$\frac{\frac{area_{target\ ion}}{area_{isotope\ ion}} \text{ x } R_f\ x\ mass_{isotope\ added}}{beet\ weight} = mass_{target}$$

V. RESULTS AND DISCUSSION

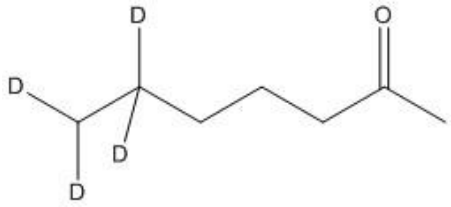
All 26 compounds of interest were accurately quantified using stable isotope dilution analysis. SIDA is a highly accurate technique because it utilizes a stable, isotopically-labeled version of the compound of interest as the internal standard. However, for some compounds in the present experiment, it was necessary to make modifications to this technique. For example, 2-isobutyl-3-*[$^2H_3$]*-methoxypyrazine was employed for the quantification of not only 2-isobutyl-3-methoxypyrazine, but also2- isopropyl-3-methoxypyrazine and 2-*sec*-butyl-3-

methoxypyrazine, because of the similarities in the structures of the target compounds as well as the increased simplification of analysis. In addition, *E,Z*-2,6-nonadienal and *E*-4,5-epoxy-[*E*]-2-decenal were both quantified with the use of 6-undecanone as the internal standard.

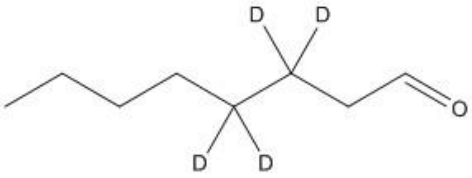
Of the 26 aroma compounds chosen for quantification, dimethyl sulfide, geosmin, *E*-4,5-epoxy-[*E*]-2-decenal, methanethiol, *p*-vinylguaiacol, and β-damascenone possessed the highest OAVs and, therefore, contributed the most to the overall aroma profile of the canned beets. Octanal, nonanal, acetic acid, 2-isopropyl-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, 2-*sec*-butyl-3-methoxypyrazine, *Z*-1,5-octadien-3-one, *E, Z*-2,6-nonadienal, and vanillin were also moderately potent. These results correlate well with results from the odor characterization experiments in **Chapter 3**; those compounds exhibiting high flavor dilution (FD) factors also tended to possess high odor-activity values (OAVs) as well. These results suggest that the two techniques are similar in their identification of potent odor-active compounds. However, there are some notable exceptions to this trend, such as furaneol. In **Chapter 3**, the FD factor of furaneol was determined to be 6561; however, in SIDA its OAV was calculated as 0.81, indicating that it was not significantly impactful to the overall odor of canned beets. These discrepancies can be explained by a variety of factors, not limited to the sampling bias of certain extraction techniques as well as varied sensitivities to certain odorants exhibited by the person performing GCO. Conversely, methanethiol only exhibited a FD factor of 5, but possessed an OAV of 136. However, using these complementary two techniques, AEDA and SIDA, together is an effective way to reduce extraction biases while increasing the overall power and accuracy of the results. These quantification experiments provided comprehensive base knowledge about the concentrations and OAVs of the potent odorants in the canned beet samples. Using these values, the creation of an aroma model would be possible and would serve as a practical

experiment to evaluate how the odorants perform synergistically, particularly when compared to an actual sample of canned beets.

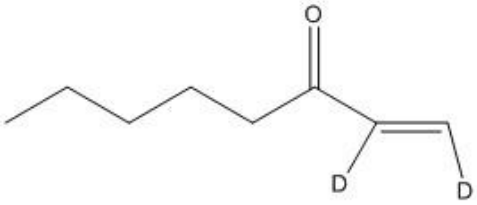
VI. FIGURES AND TABLES



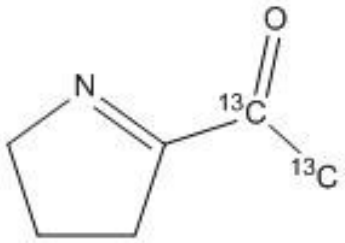
I-23 *[$^{2}H_{4}$]*-Hexanal



I-2 *[$^{2}H_{4}$]*-Octanal



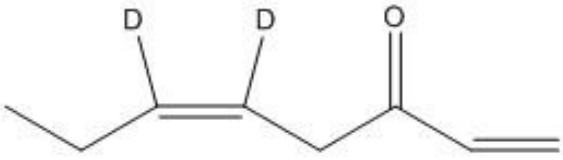
[$^{2}H_{2}$]-1-octen-3-one



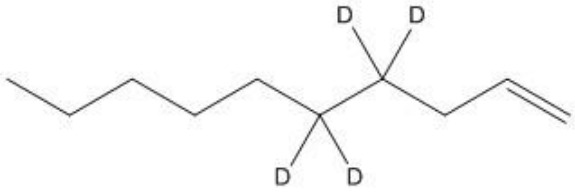
I-24

I-3 *[^{13}C]*-2-acetyl-1-pyrroline

Figure 4.1 Chemical structures of isotope standards.
Numbers correspond to those in **Tables 4.1** – **4.2** and **Chapter 3**.

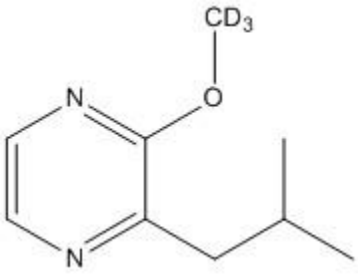


I-25 *[$^{2}H_{2}$]-Z*-1,5-octadien-3-one



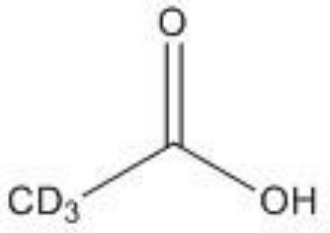
I-26 *[$^{2}H_{4}$]*-Nonanal

Figure 4.1 (continued)

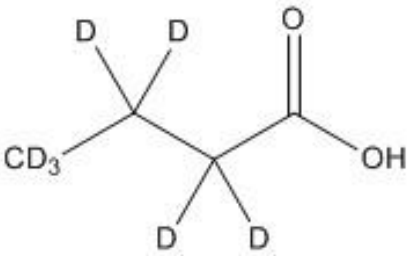


I-9 2-isobutyl-3-*[$^2H_3$]*-methoxypyrazine

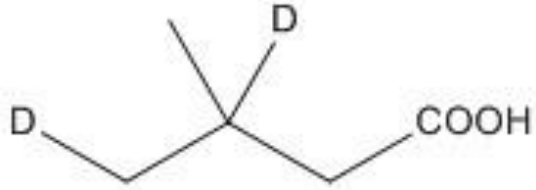
I-6 *[$^2H_3$]*-Methional



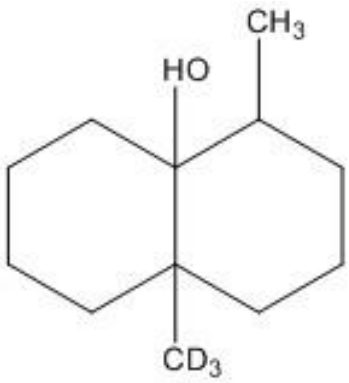
I-7 *[$^2H_3$]*-Acetic acid



I-11 *[$^2H_7$]*-Butyric acid

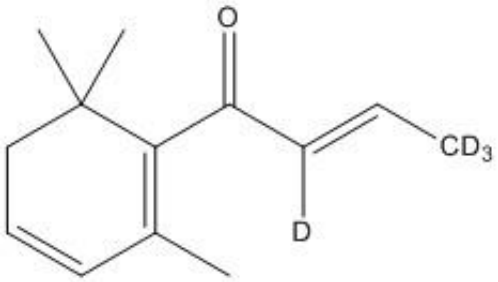


I-12 *[$^2H_2$]*-Isovaleric acid

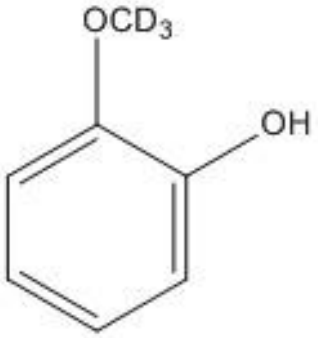


I-13 *[$^2H_3$]*-Geosmin

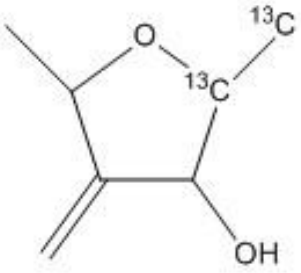
Figure 4.1 (continued)



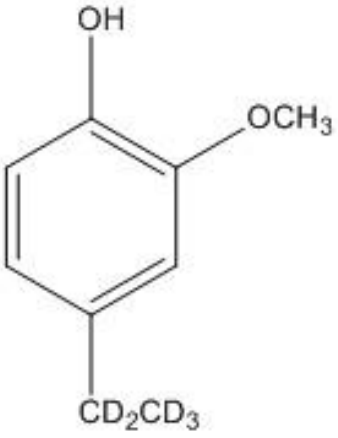
I-31 *[$^{2}H_{4}$]*-β-damascenone



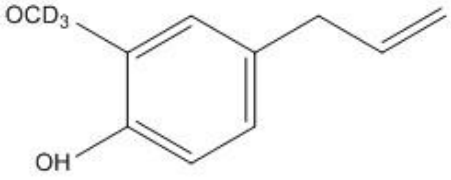
I-14 *[$^{2}H_{3}$]*-Guaiacol



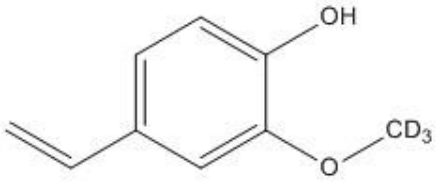
I-16 *[$^{13}C_{2}$]*-Furaneol



I-38 4-*[$^{2}H_{5}$]*-ethyl-2-methoxyphenol

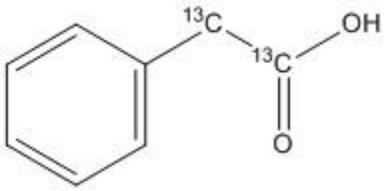


I-17 *[$^{2}H_{3}$]*-Eugenol

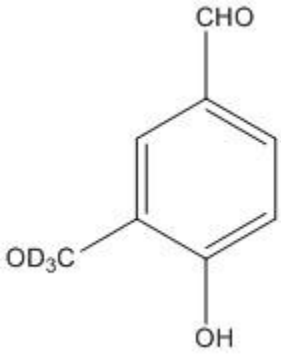


I-19 2-*[$^{2}H_{3}$]*-methoxy-4-vinylphenol

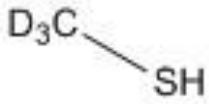
Figure 4.1 (continued)



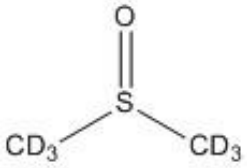
I-20 *[$^{13}C_2$]*-Phenylacetic acid



I-21 *[$^{2}H_3$]*-Vanillin



I-45 *[^{2}H]*-methanethiol.



I-46 *[$^{2}H_6$]*-dimethyl sulfide

Table 4.1 Concentrations, odor detection thresholds, and odor activity values for selected odorants in the canned beet solvent extracts

		Selected ion (m/z)			Concentration	Threshold	
no.[a]	**Compound**	**unlabeled**	**labeled**	**R_f[b]**	**(ng/g; ppb)[c]**	**(ppb)[d]**	**OAV[r]**
23	Hexanal	82	76	0.52	6.14	5[e]	1.23
2	Octanal	110	114	0.49	4.99	0.7[e]	7.1
24	1-octen-3-one	55	58	0.57	0.0191	0.05[f]	0.38
3	2-acetyl-1-pyrroline	111	113	0.82	0.0125*	0.1[e]	0.13
25	*Z*-1,5-octadien-3-one	55	57	4.12	0.0334	0.0012[g]	27.8
26	Nonanal	114	116	0.44	6.01	1[h]	6.0
5	2-Isopropyl-3-methoxypyrazine	137	127	1.27	0.110	0.004[g]	28
6	Methional	104	107	1.74	0.00545	0.2[f]	0.027
7	Acetic acid	60	63	1.12	97300	22000[f]	4.4
8	*2-sec*-butyl-3-methoxypyrazine	138	127	0.59	0.0588	0.003[g]	20
9	2-Isobutyl-3-methoxypyrazine	124	127	1.03	0.0653	0.002[i]	33
29	*E,Z*-2,6-nonadienal	70	99	18.62	0.571*	0.01[j]	57
11	Butyric acid	60	63	1.34	13.8	240[f]	0.058
12	Isovaleric acid	87	89	1.35	19.0	250[k]	0.076
13	Geosmin	112	115	10.32	2.29	0.0038[l]	603
32	β-damascenone	190	194	1.50	0.154	0.002[h]	77
14	Guaiacol	124	127	2.19	1.55	3[e]	0.52
37	*E*-4,5-epoxy-[*E*]-2-decenal	81	99	277.78	20.4	0.12[m]	170
16	Furaneol	128	130	1	46.7	6[n]	0.78
38	4-ethylguaiacol	152	157	1.17	0.0869	50[o]	0.002
17	Eugenol	164	167	1.06	0.245	6[p]	0.041
19	*p*-vinylguaiacol	150	153	1.33	390	3[e]	130
20	Phenylacetic acid	136	138	1.1	120	1000[q]	0.12
21	Vanillin	151	155	1.12	430	25[o]	17

[a]Numbers refer to those in **Table 4.1, 4.2** and **Chapter 3**. [b]Response factor of unlabeled compound to isotope-labeled compound. [c]Calculated as an average from peak areas on a Stabilwax® column ($n \geq 3$). [d]Odor threshold in water. [e]Buttery et. al., 1988. [f]Buttery and Ling, 1998. [g]Mansanetz and Grosch, 1998. [h]Buttery et. al., 1989. [i]Buttery et. al., 1969. [j]Josephson et. al., 1983. [k]Buttery et. al., 1990. [l]Young et. al., 1996. [m]Kerler and Grosch, 1996. [n]Buttery et. al., 1994. [o]Semmelroch et. al., 1995. [p]Buttery et. al., 1987. [q]Maga and Lorenz, 1973. [r]Odor activity values.*Indicates use of n<3 to calculate average concentration

Table 4.2 Concentrations, odor detection thresholds, and odor activity values of headspace compounds

		Selected ion (m/z)			Concentration	Threshold	
no.[a]	**Compound**	**unlabeled**	**labeled**	**R_f[b]**	**(ng/g; ppb)[c]**	**(ppb)[d]**	**OAV[g]**
45	Methanethiol	51	47	0.882	27.28*	0.2 [e]	136
46	Dimethyl sulfide	68	62	0.879	2710.7	0.3 [f]	9040

[a]Numbers refer to those in **Table 4.1, 4.2** and **Chapter 3**. [b]Response factor of unlabeled compound to isotope-labeled compound. [c]Average concentration calculated from peak area on a Stabilwax® column (n=9) . [d]Odor threshold in water. [e]Guth and Grosch, 1994. [f]Buttery et. al., 1990. [g]Odor activity values.*Methanethiol concentration was calculated using values from 2 of the 3 cans (n=6)

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CHAPTER FIVE

SUMMARY AND CONCLUSIONS

The beet root (*Beta vulgaris)* is consumed as a vegetable worldwide, and has maintained its popularity over several millennia. In addition, the beet serves as an important agricultural commodity for the production of betalain pigments, which are utilized as natural sources of red and purple colors for foods and pharmaceuticals. Despite the widespread consumption and industrial significance of the beet root, existing flavor research concerning the beet is limited in scope and depth. Most centers on aromas as contaminants in the extraction of sucrose from sugar beets, a close relative of the table beet; other studies simply analyze the volatile components of the beet root, revealing compounds that may not actually be odor-active or contribute significantly to the overall aroma of beets. Off-odors have also been previously noted in beet-derived colorants; however, it is difficult to determine their origin without a complete understanding of the flavor chemistry of beets themselves. Therefore, the main objective of the study was to gain a deeper understanding of which compounds exist in the aroma profile of the beets, as well as their relative potencies. Based on previous research, it was hypothesized that the aroma profile of the beets would contain geosmin, various 3-alkyl-2-methoxypyrazines, and Maillard- and lignin-derived aroma compounds. It was first necessary to establish which compounds were contained and odor-active in the beets. Next, potency and abundance for odorants were determined.

Preliminary screening experiments revealed that of the four beet preparations studied, boiled, roasted, canned, and colorant, that canned beets would serve as the most consistent and

potent sample type. Forty-one odorants were detected in the canned beets using gas chromatography-olfactometry (GCO) and gas chromatography-mass spectrometry (GC-MS). In order to compare compounds for relative odor potency, aroma extract dilution analysis (AEDA) was used. The most potent odorants included: geosmin (soil), 2-isobutyl- isopropyl- and 2-*sec*-butyl -3-methoxypyrazines (earthy), 2-acetyl-1-pyrroline (popcorn), methional (potato), furaneol (caramel), *p*-vinylguaiacol (clove-like), and vanillin (vanilla). Headspace GCO was additionally performed in order to detect compounds that may have been lost due to distillation or co-eluted on the chromatogram with the solvent used. Two previously unidentified compounds were present at high potencies: methanethiol (sulfurous) and dimethyl sulfide (canned corn). Stable isotope dilution analysis (SIDA) was used to quantify the amount of twenty-six odor-active compounds. SIDA was performed on both the headspace and solvent extracts. Of the aroma compounds chosen for quantification, dimethyl sulfide, geosmin, *E*-4,5-epoxy-[*E*]-2-decenal, methanethiol, *p*-vinylguaiacol, and β-damascenone possessed the highest OAVs and, therefore, contributed the most to the overall aroma profile of the canned beets. Octanal, *Z*-1,5-octadien-3-one, nonanal, acetic acid, 2-isopropyl-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine, 2- *sec*-butyl-3-methoxypyrazine, *E, Z*-2,6-nonadienal, and vanillin were also moderately potent.

In addition to the previously-discussed studies, there exists additional work that could aid in contributing to greater understanding of the flavor chemistry of beet roots. Although quantification of the potent odorants and their corresponding odor-activity values (OAVs) was completed, sensory experiments would be an advantageous next step. Using the OAVs calculated, a model system could be created and tested against beets themselves. Assembly of a sufficient model would improve understanding of how the flavor compounds interact with each other and the beet matrix to create the overall odor impression.

Another additional study that would have extremely useful implications for the food industry would be determination of how odorants form in beet-derived colorants. While beets provide a natural source of red-purple color, they have a tendency to impart off-odors to the foods they are used in. When comparing the structures of the betalain colorants to those odors that evolve in the colorants, one major similarity stands out. Both are structured in ways that bear high resemblances to amino acids. Knowledge that many of the odor-active compounds contained in beets evolve from amino acids, either due to pathways in the Maillard reaction or amino acid catabolism, it has been hypothesized that these off-odors evolve from the colorants themselves. Furthermore, high amounts of ascorbic acid are used during the process of extracting color from beets. With the high amounts of amino acid-structured colorants and ascorbic acid, which may be acting as a reducing sugar, a Maillard-type pathway may be occurring. Understanding of the mechanism of these reactions may encourage alterations in the colorant extraction processes or addition of inhibitory ingredients. Lowered abundance of off-flavors may encourage more widespread use of the colorant as well as increased consumer satisfaction.

APPENDIX A

AMOUNTS OF LABELED ISOTOPES FOR STABLE ISOTOPE DILUTION ANALYSIS

No.[a]	Target analyte	Internal standard	Concentration (mg/mL)	Volume (µL)[b]
23	Hexanal	*[$^{2}H_4$]*-Hexanal	0.509	5
2	Octanal	*[$^{2}H_4$]*-Octanal	1.19	5
24	1-octen-3-one	*[$^{2}H_2$]*-1-octen-3-one	0.00091	2
3	2-acetyl-1-pyrroline	*[^{13}C]*-2-acetyl-1-pyrroline	0.0001975	10
25	*Z*-1,5-octadien-3-one	*[$^{2}H_2$]*-*Z*-1,5-octadien-3-one	0.001069	2
26	Nonanal	*[$^{2}H_4$]*-Nonanal	1.18	5
5	2-Isopropyl-3-methoxypyrazine	2-isobutyl-3-*[$^{2}H_3$]*-methoxypyrazine	0.00890	10
8	2-*sec*-Butyl-3-methoxypyrazine			
9	2-Isobutyl-3-methoxypyrazine			
6	Methional	*[$^{2}H_3$]*-Methional	0.00562	1
7	Acetic acid	*[$^{2}H_3$]*-Acetic acid	Neat	10
29	*E*,*Z*-2,6-nonadienal	6-undecanone	0.0011545	10
37	*E*-4,5-epoxy-[*E*]-2-decenal			
11	Butyric acid	*[$^{2}H_7$]*-Butyric acid	1.30	5
12	Isovaleric acid	*[$^{2}H_2$]*-Isovaleric acid	1.28	5
13	Geosmin	*[$^{2}H_3$]*-Geosmin	0.004824	10
31	β-damascenone	*[$^{2}H_4$]*-β-damascenone	0.00982	5
14	Guaiacol	*[$^{2}H_3$]*-Guaiacol	0.109	5
16	Furaneol	*[$^{13}C_2$]*-Furaneol	1.10	50
38	4-ethylguaiacol	4-*[$^{2}H_5$]*-Ethyl-2-methoxyphenol	0.0251	5
17	Eugenol	*[$^{2}H_3$]*-Eugenol	0.0102	10
19	*p*-vinylguaiacol	2-*[$^{2}H_3$]*-Methoxy-4-vinylphenol	2.62	25
20	Phenylacetic acid	*[$^{13}C_2$]*-Phenylacetic acid	1.01	5
21	Vanillin	*[$^{2}H_3$]*-Vanillin	1.17	50
45	Methanethiol	*[^{2}H]*-methanethiol	0.0128	5
46	Dimethyl sulfide	*[$^{2}H_6$]*-dimethyl sulfide	0.085	5

[a]Numbers refer to those in **Chapters 3-4** and **Appendix B**. [b]Volume of internal standard spiked into beet solution before extraction by DSE-SAFE or SPME

APPENDIX B

QUANTIFICATION DATA

Hexanal (23)

Can	Concentration (ng/g)
3	2.650121697
4	11.99363881
5	3.937981241
6	2.571315418
7	9.544188144
Average	6.139449062
Standard deviation	4.347908392

Octanal (2)

Can	Concentration (ng/g)
2	4.283045933
3	4.722916792
4	5.496779534
5	5.440437743
Average	4.985795001
Standard deviation	0.586164488

1-octen-3-one (24)

Can	Concentration (ng/g)
3	0.024144735
4	0.015901651
5	0.017500516
Average	0.019182301
Standard deviation	0.004371317

2-acetyl-1-pyrroline (3)

Can	Concentration (ng/g)
3	0.012471217

Z-1,5-octadien-3-one (25)

Can	Concentration (ng/g)
3	0.009900919
4	0.009896836
5	0.105160393
7	0.008583164
Average	0.033385328
Standard deviation	2.88726E-06

Nonanal (26)

Can	Concentration (ng/g)
2	6.488972784
3	3.852230321
4	5.081384118
5	6.451366331
Average	6.007241077
Standard deviation	0.047854063

2-isopropyl-3-methoxypyrazine (5)

Can	Concentration (ng/g)
3	0.131087957
4	0.116958034
5	0.079627646
Average	0.109224545
Standard deviation	0.026587517

Methional (6)

Can	Concentration (ng/g)
2	0.005831949
3	0.005242592
4	0.004772514
5	0.005942599
Average	0.00567238
Standard deviation	0.000376297

Acetic acid (7)

Run	Concentration (ng/g)
2	99808.16497
3	93866.02808
4	116915.0293[a]
5	98369.51827
Average	97347.90377
Standard deviation	3100.003203
Average (with outlier)	102239.6851
Standard deviation (with outlier)	10105.68059

[a]Indicates outlier

2-*sec*-butyl-3methoxypyrazine (8)

Can	Concentration (ng/g)
3	0.058744185
4	0.056244429
5	0.061328591
Average	0.058772402
Standard deviation	0.002542199

2-Isobutyl-3methoxypyrazine (9)

Can	Concentration (ng/g)
3	0.067572402
4	0.072033054
5	0.056318044
Average	0.065307834
Standard deviation	0.008098555

E,*Z*-2,6-nonadienal (29)

Can	Concentration (ng/g)
3	1.249596009[a]
4	0.224239913
5	0.23724953
7	0.5723092
Average	0.033385328
Standard deviation	0.047854063

Butyric acid (11)

Can	Concentration (ng/g)
1	13.39467705
2	12.29067102
3	16.96159209
4	13.60710165
5	12.75948987
Average	13.80270634
Standard deviation	1.841049952

Isovaleric acid (12)

Can	Concentration (ng/g)
2	21.33076724
3	19.12159346
4	18.20179364
5	17.40643109
Average	19.01514636
Standard deviation	1.695379848

Geosmin (13)

Can	Concentration (ng/g)
3	2.382842287
4	2.402667113
5	2.095973682
Average	2.293827694
Standard deviation	0.171633078

β-damascenone (32)

Can	Concentration (ng/g)
2	0.184890945
3	0.144749491
4	0.21063588
5	0.074961979
Average	0.153809574
Standard deviation	0.059144763

Guaiacol (14)

Can	Concentration (ng/g)
2	2.154298232
3	1.744851607
4	0.901180589
5	1.401629737
Average	1.550490041
Standard deviation	0.531075294

E-4,5-epoxy-[*E*]-2-decenal (37)

Can	Concentration (ng/g)
3	20.56111727
4	21.57628202
5	19.00532272
Average	20.38090733
Standard deviation	1.294918776

Furaneol (16)

Can	Concentration (ng/g)
2	49.20167439
3	40.8216654
4	47.82429776
5	48.77310045
Average	46.6551845
Standard deviation	3.931367433

4-ethylguaiacol (38)

Can	Concentration (ng/g)
2	0.065867431
4	0.108530143
5	0.086291754
Average	0.086896442
Standard deviation	0.021337783

Eugenol (17)

Can	Concentration (ng/g)
3	0.306766421
4	0.240912672
5	0.185831676
Average	0.244503589
Standard deviation	0.060547289

p-vinylguaiacol (19)

Can	Concentration (ng/g)
2	387.7963545
3	371.8680025
4	399.973022
5	348.4508507[a]
Average	386.545793
Standard deviation	14.09418171
Average (with outlier)	377.0220574
Standard deviation (with outlier)	22.25391643

[a]Indicates outlier

Phenylacetic acid (20)

Can	Concentration (ng/g)
1	114.2408894
2	126.3801536
3	120.6073253
4	114.3420799
5	122.622831
Average	119.6386558
Standard deviation	5.302853327

Vanillin (21)

Run	Concentration (ng/g)
2	410.2998547
3	439.9760446
4	435.3771065
5	425.6557333
Average	427.8271848
Standard deviation	13.12146604

Methanethiol (45)

Can 2[a]

Run	**Concentration (ng/g)**
A	43.23719501
B	44.15860736
C	46.81936186
Average	44.73838808
Standard deviation	1.860131469

Can 3

Run	**Concentration (ng/g)**
A	26.70218503
B	25.92538989
C	29.16916553
Average	27.26558015
Standard deviation	1.693688474

Can 4

Run	**Concentration (ng/g)**
A	25.04307966
B	25.66739867
C	23.38005427
Average	24.6968442
Standard deviation	1.182326245

[a]Indicates outliers

Total:

Average	25.98121218
Standard deviation	1.919924923
Average (with outliers)	32.23360414
Standard deviation (with outliers)	9.54603342

Dimethyl sulfide (46)

Can 3	
Run	**Concentration (ng/g)**
A	2728.911068
B	2445.759656
C	2616.985933
Average	2597.218886
Standard deviation	142.6069166

Can 4	
Run	**Concentration (ng/g)**
A	2830.599798
B	2538.183238
C	2962.51589
Average	2777.099642
Standard deviation	217.1664103

Can 5	
Run	**Concentration (ng/g)**
A	2786.261358
B	2817.714114
C	2669.345027
Average	2757.7735
Standard deviation	78.1793777

Total:

Average	2710.697
Standard deviation	160.3622

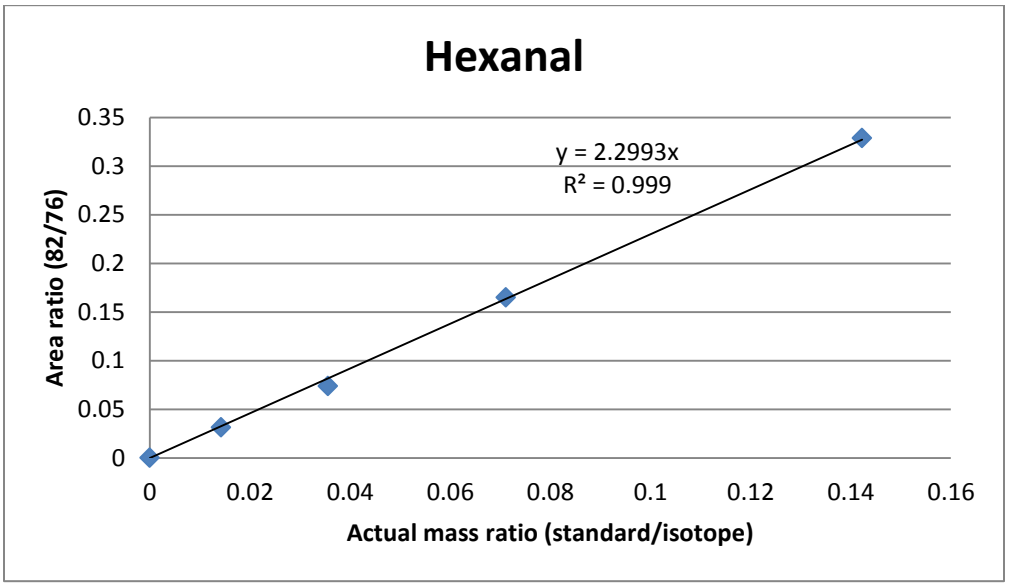
APPENDIX C

CALIBRATION CURVES

Response factor of Hexanal to *[$^2H_4$]*-Hexanal

Standard:	Isotope *[$^2H_4$]*-Hexanal	Unlabeled Hexanal

Selected ion:		**82**	**76**	**Ratio**
Mass ratio:	0.014224	30859	982614	0.031405
	0.035560	56972	772889	0.073713
	0.071120	118698	720521	0.164739
	0.142240	186244	566790	0.328594



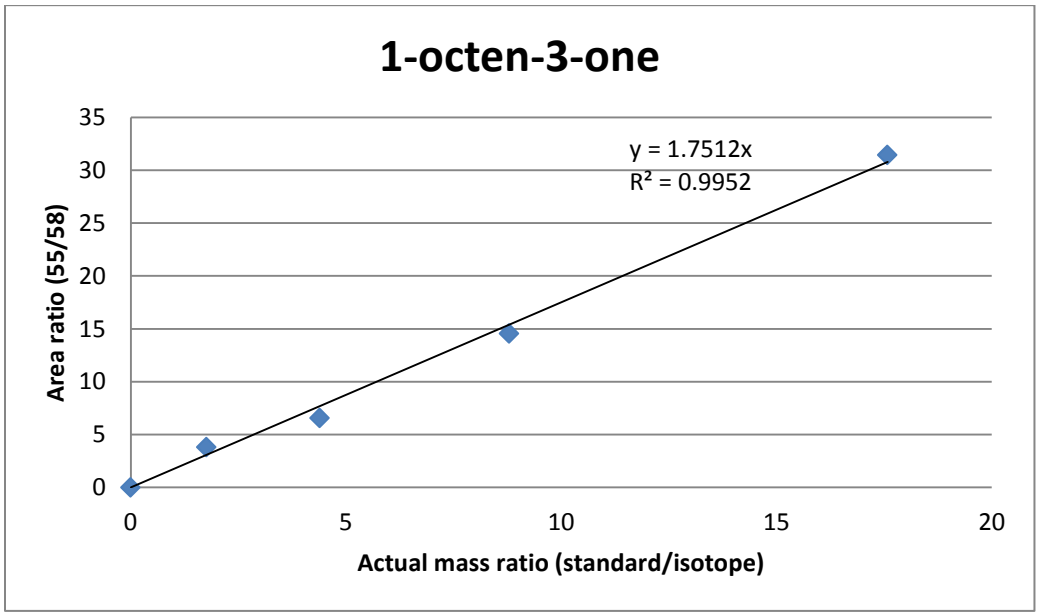
Slope: 2.2993

Response factor: 0.435

Response factor of 1-octen-3-one to *[$^2H_2$]*-1-octen-3-one

Standard:	Isotope *[$^2H_2$]*-1-octen-3-one	Unlabeled 1-octen-3-one

Selected ion:		**55**	**58**	**Ratio**
Mass ratio:	1.758242	284354	74638	3.809775
	4.395604	460483	70315	6.548859
	8.791209	1014484	69687	14.55772
	17.58242	1944629	61885	31.42327

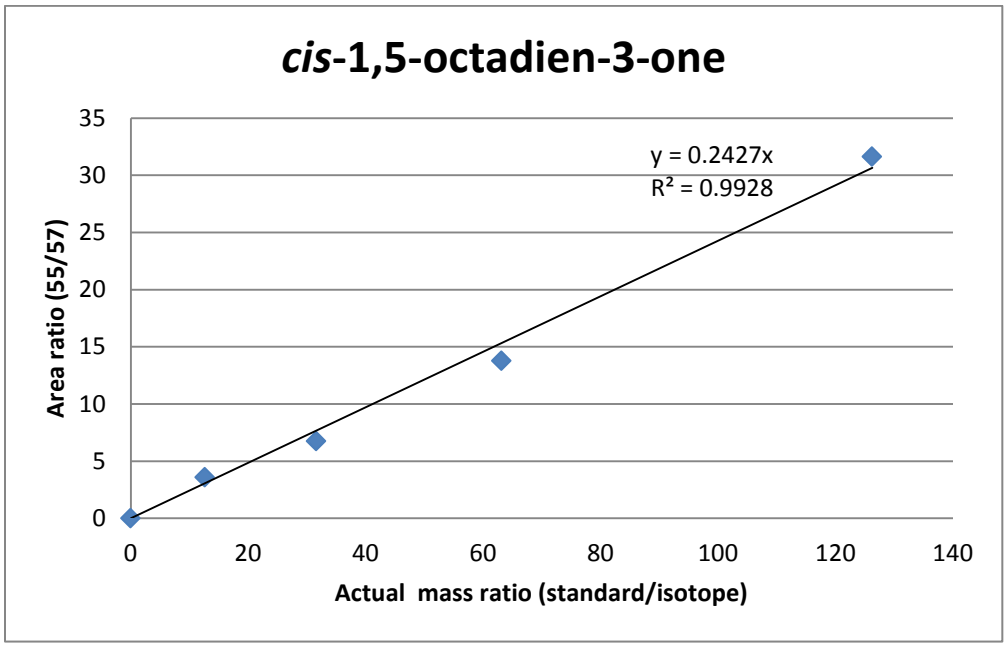


Slope: 1.7512

Response factor: 0.57

Response factor of *Z*-1,5-octadien-3-one to *[$^2H_2$]-Z*-1,5-octadien-3-one

Standard:	Isotope *[$^2H_2$]-Z*-1,5-octadien-3-one	Unlabeled *Z*-1,5-octadien-3-one	
Selected ion:	**55**	**57**	**Ratio**
Mass ratio:	12.628625	271271	76174	3.561202
	31.571562	385671	57220	6.740143
	63.143124	695044	50512	13.75998
	126.286249	1419077	44879	31.62007

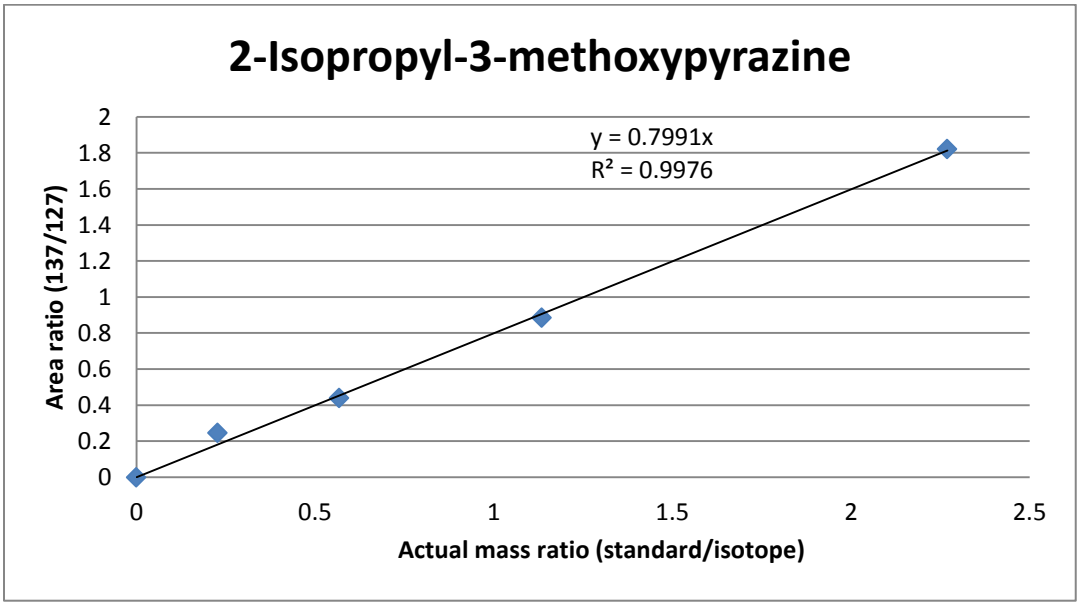


Slope: 0.2427

Response factor: 4.12

Response factor of 2-Isopropyl-3-methoxypyrazine to 2-isobutyl-3-*[$^2H_3$]*-methoxypyrazine

Standard:		Isotope 2-isobutyl-3-*[$^2H_3$]*-methoxypyrazine	Unlabeled 2-Isopropyl-3-methoxypyrazine	
Selected ion:		**137**	**127**	**Ratio**
Mass ratio:	0.226966	357132	1452049	0.24595
	0.567416	517333	1175643	0.440043
	1.134831	1077044	1216200	0.885581
	2.269663	2056699	1129278	1.821251

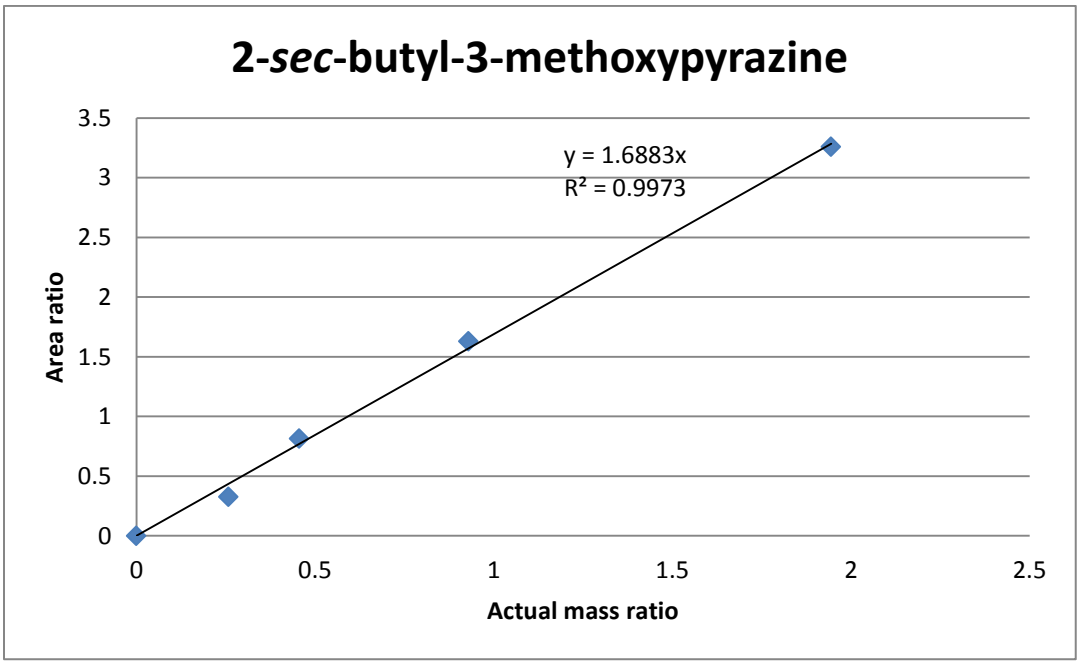


Slope: 0.7991

Response factor: 1.25

Response factor of 2-*sec*-butyl-3-methoxypyrazine to 2-isobutyl-3-*[$^2H_3$]*-methoxypyrazine

Standard:		Isotope 2-isobutyl-3-*[$^2H_3$]*-methoxypyrazine	Unlabeled *2-sec*-butyl-3-methoxypyrazine	
Selected ion:		**138**	**127**	**Ratio**
Mass ratio:	0.325843	374216	1452049	0.257716
	0.814607	536198	1175643	0.456089
	1.629213	1130021	1216200	0.929141
	3.258427	2196117	1129278	1.944709



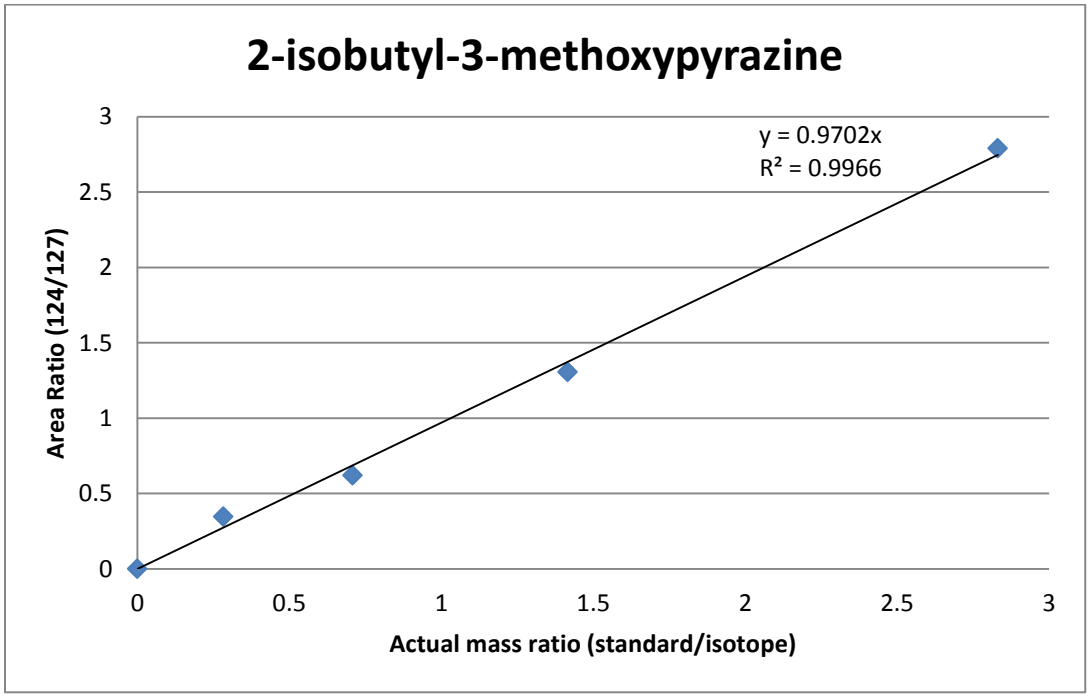
Slope: 1.6883

Response factor: 0.59

Response factor of 2-Isobutyl-3-methoxypyrazine to 2-isobutyl-3-*[$^2H_3$]*-methoxypyrazine

Standard:	Isotope 2-isobutyl-3-*[$^2H_3$]*-methoxypyrazine	Unlabeled 2-Isopropyl-3-methoxypyrazine

Selected ion:		**124**	**127**	**Ratio**
Mass ratio:	0.283146	503978	1452049	0.347081
	0.707865	728371	1175643	0.619551
	1.41573	1587281	1216200	1.305115
	2.831461	3151510	1129278	2.79073

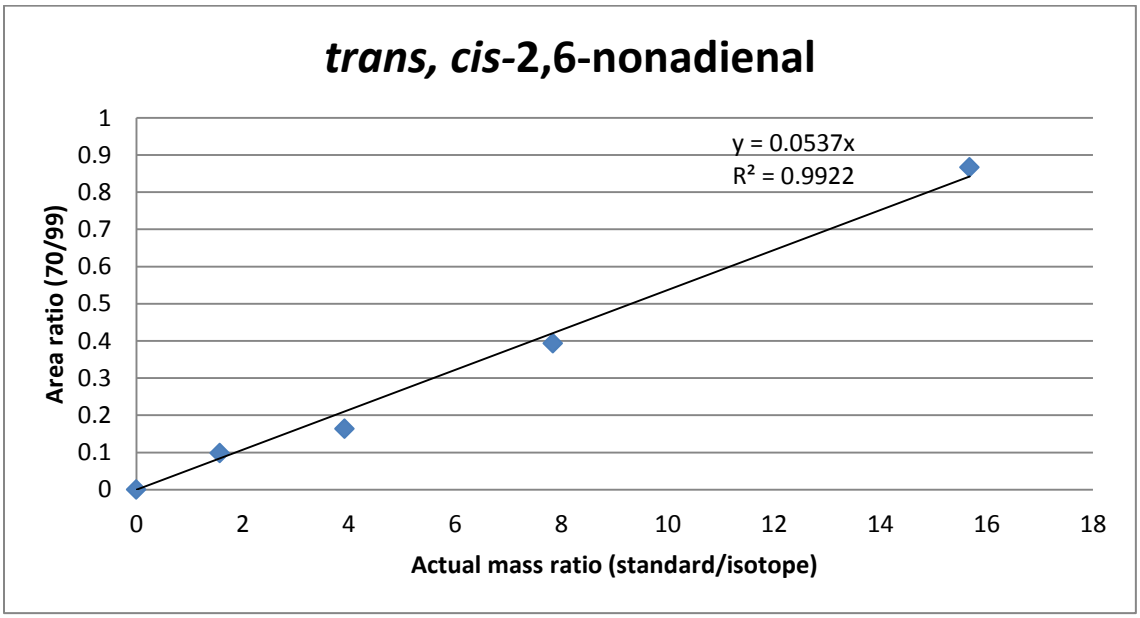


Slope: 0.9702

Response factor: 1.03

Response factor of *E,Z*-2,6-nonadienal to 6-undecanone

		Isotope		Unlabeled
Standard:		6-undecanone		*E,Z*-2,6-nonadienal
Selected ion:		**70**	**99**	**Ratio**
Mass ratio:	1.567917	60701	615899	0.098557
	3.919792	83211	507812	0.163862
	7.839584	196426	498654	0.393912
	15.67917	398018	459347	0.866487



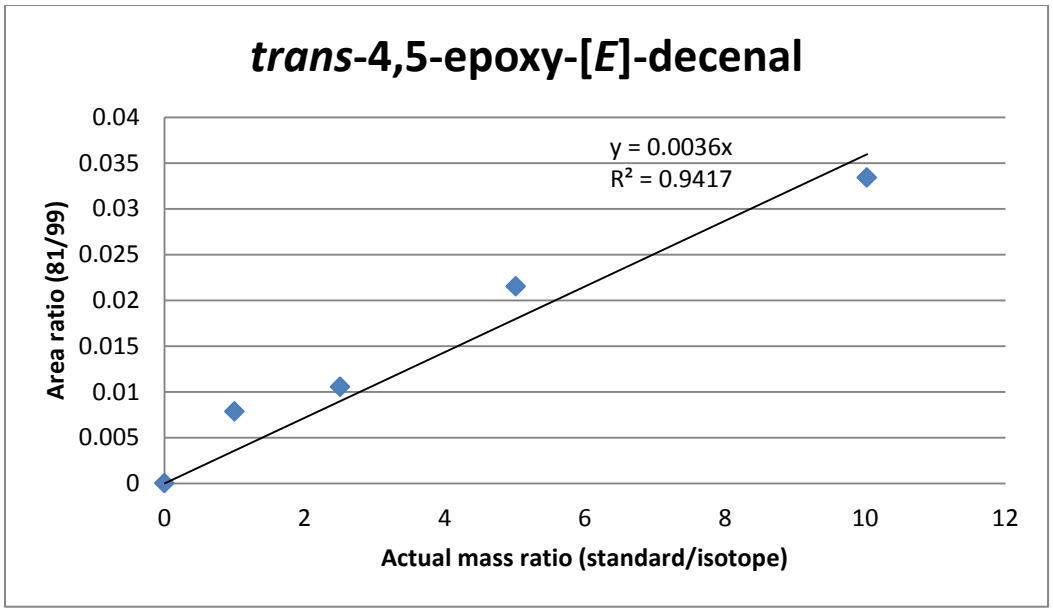
Slope: 0.0537

Response factor: 18.62

Response factor of *E*-4,5-epoxy-[*E*]-2-decenal to 6-undecanone

	Isotope	Unlabeled
Standard:	6-undecanone	*E*-4,5-epoxy-[*E*]-2-decenal

Selected ion:		**81**	**99**	**Ratio**
Mass ratio:	1.002789	4830	615899	0.007842
	2.506973	5356	507812	0.010547
	5.013945	10728	498654	0.021514
	10.02789	15346	459347	0.033408

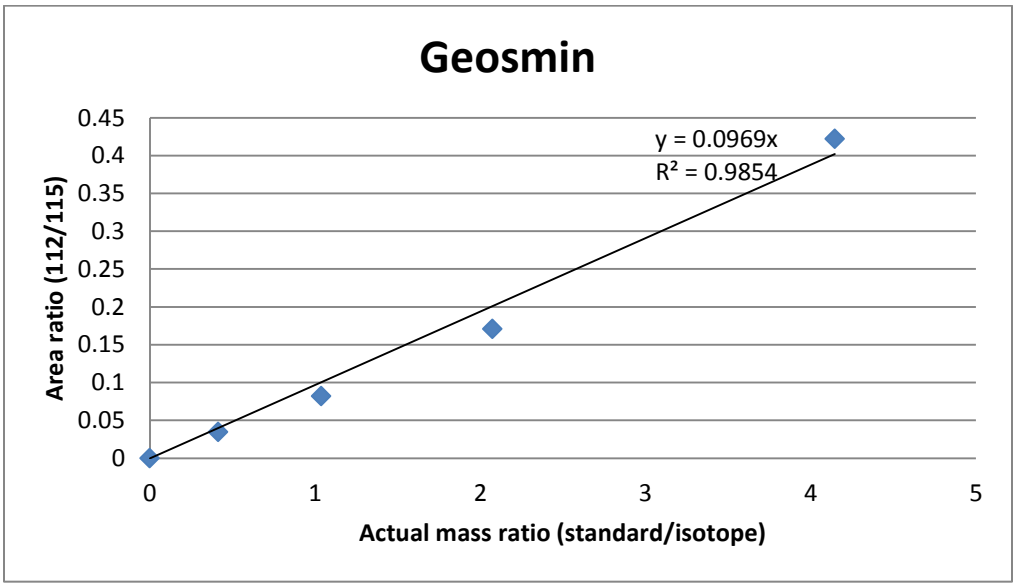


Slope: 0.0036

Response factor: 277.78

Response factor of Geosmin to *[$^2H_3$]*-Geosmin

Standard:	Isotope *[$^2H_3$]*-Geosmin		Unlabeled Geosmin	
		112	**115**	**Ratio**
Selected ion:	0.414594	253677	7285781	0.034818
Mass ratio:	1.036484	640197	7788181	0.082201
	2.072968	1328147	7769160	0.170951
	4.145937	3789041	8978758	0.422001



Slope: 0.0969

Response factor: 10.32